UNIVERSITY OF TORONTO
FACULTY OF APPLIED SCIENCE & ENGINEERING

Report on

BioZone

Centre for Applied Bioscience and Bioengineering

Spring 2012

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Report on BioZone
May 2012

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Table of Contents

Director's Message

Elizabeth Edwards
Professor and Director

Welcome to BioZone, a Centre for Applied Bioscience and Bioengineering at the University of Toronto.

This inaugural Report on BioZone highlights many accomplishments over the past year for which we should all be very proud. But if truth be told, highlights for me tend to revolve around the little things: BioZone tea time, the camping trip, laughter in the offices and labs, and other opportunities for relaxed, yet meaningful discussions…

When I joined the University of Toronto in 1997, I was welcomed into the labs of Grant Allen (Chemical Engineering), Barbara Sherwood Lollar (Geology), Brent Sleep (Civil Engineering), Scott Mabury (Chemistry) and the Pulp and Paper Centre (Doug Reeve), among others. Their collegiality and openness immediately provided a glimpse of what life would be like at the University of Toronto, and helped create an environment that spurred many new research ideas and lots of fun. And by fun I mean the rush you get when you really think your work can make a difference. My experiences prior to coming to U of T, at Stanford, at McMaster and in industry, were similarly welcoming and exciting, and these experiences solidified for me the importance of collaboration to have an impact, particularly when tackling complex, real-world problems that no one person could ever hope to solve alone.

Why BioZone? We want to create and apply novel technologies to minimize the magnitude of humankind's impact on the planet. In BioZone, we saw great opportunities to combine our practical skills as engineers with the scientific and technological breakthroughs in post-genomic biology, particularly as applied to energy, environment and health.

BioZone was founded for very pragmatic reasons:

1. Much of the equipment we need for research is expensive. Let's **share equipment** to make our dollars go further.

2. Proper use of equipment often requires extensive training and users benefit substantially from the experience of others. Let's **pool human resources**.

3. You never know where an idea may come from – often it is from unstructured exchange among passionate people with different perspectives. Let's **increase opportunities** for such exchanges by creating common spaces.

Creating a culture of excellence firmly committed to freely sharing ideas, expertise, know-how, and equipment is the vision for BioZone. Our mission is to ensure that our work will have a lasting and positive impact on our environment and on society. Welcome to BioZone.

Elizabeth A. Edwards

Elizabeth Edwards, Director

Vision & Mission

BioZone's vision is to be a multidisciplinary and internationally recognized centre for bioengineering research, technology transfer, outreach and training focused on urgent societal needs in sustainable energy, the environment and human health.

A particular strength will be our ability to effectively translate the most appropriate and up-to-date molecular and cellular discoveries and tools into industrial practice.

BioZone's mission is:

- to attract the best and brightest minds from a diverse range of fields, including chemical and process engineering, microbiology, genomics, biochemistry, medicine, computer science, economics and public policy
- to create a focal point for collaborative applied and environmental bioengineering research leading to the development of innovative new technologies that address urgent challenges and foster the long-term sustainability of our planet and its inhabitants
- to provide students with the knowledge and ability to debate public policies and influence political decisions that affect the environment and health, based on sound scientific principles
- to provide a state-of-the-art facility and exciting opportunities for research
- to foster innovation, creativity, and imagination
- to foster leadership and excellence, humility and collegiality
- to have fun
- to have a lasting and positive impact on our environment and society

Executive Summary

BioZone is a multidisciplinary bioscience and bioengineering research centre at the University of Toronto focusing on urgent societal needs in energy, environment and health. The centre has strong links to many research institutions and industry partners on- and off-campus, and supports collaborative programs in bioengineering within the University of Toronto's Faculty of Applied Science and Engineering.

BioZone was officially designated as a centre in December 2010, though its roots go back many years. This report describes BioZone, with metrics provided for our achievements during the first official annual reporting period of September 2010 to August 2011.

BioZone's collaborative approach aims to turn ideas into viable applications by carrying out basic bioscience research in the context of real technological, economic and public policy constraints that engineers can provide. Our goal is to **train creative thinkers** who will succeed in the new global knowledge-based economy by being skilled at working collaboratively with people outside their area of expertise and open to new ideas and approaches.

Our research capitalizes on dramatic progress made in recent years in biotechnology, particularly in genome science, to develop innovative solutions to pressing real world problems. This goal has been facilitated by the creation of **1,836 m^2 of contiguous collaborative space** in the Wallberg Building, creating a hub where people can easily learn from one another and exchange ideas. This shared space fosters cross-disciplinary collaborations that would otherwise be unlikely to happen.

Currently, BioZone comprises nine faculty members and their research groups and many close collaborators, bringing together skilled people with diverse backgrounds to brainstorm and work on common problems. **BioZone is about sharing** lab benches, instruments, offices, skills, expertise, and ideas on a daily basis. With access to state-of-the-art equipment and permanent technical staff, the only limitations are passion and imagination.

Our collective expertise spans a broad range of application areas from medicine, health, nutrition and food, to bioproducts, environment, energy and policy. Common to these varied applications is the need for **quantitative analysis of enzyme biocatalysts**, biochemical processes, bioproducts, cell growth and metabolism. In addition to providing opportunities for exciting research on a particular topic, BioZone's mission is to provide our students and postdoctoral fellows with the ability to think critically and creatively about the grand challenges we face as a society and to appreciate cooperation, collegiality and openess that will lead to a better collective future.

In our official inaugural year, BioZone received over $13M in cash funding, as well as valuable generous in-kind contributions from our many collaborators. Over 30% of the cash funding came from outside of Canada. Significant funding was obtained from organizations uncommon to engineering programs, such as Genome Canada and the U.S. Dept. of Energy Joint Genome Institute.

During our official inaugural year, **BioZone was home to 77 students** at all levels. Sixteen graduate degrees were awarded, seven postdoctoral fellows completed their terms and forty-five peer-reviewed papers were published. Our partners in industry, government and at other institutions near and far provided tremendous support, and mechanisms to transition our work to practice. BioZone hosted 6 outside speakers, and ran a number of technical and social events that altogether made for a fantastic year.

We are tremendously grateful to all of our current partners and seek to make BioZone an increasingly valuable resource. We welcome your feedback and advice as we move enthusiastically forward.

Executive Committee Report

BioZone's culture of achieving research excellence through collaboration and sharing has already reaped many rewards. The centre's success can be measured by its ability to provide an outstanding learning and research environment for the development of viable technological innovation in energy, environment and health. Although it is still a very young centre, BioZone is becoming known nationally and internationally for its high calibre of research and the breadth of opportunities enjoyed by its team members. This report, assembled by BioZone's Executive Committee (see the *"Leadership"* section, pg. 12), highlights our achievements during the September 2010 - August 2011 reporting period, unless otherwise indicated.

Research Funding

BioZone researchers worked on 36 funded research projects in 2010 - 2011, many of which involved collaborations between multiple research groups. Fig. 1 illustrates the total research cash funding to the core BioZone principal investigators (PIs) since BioZone's inception.

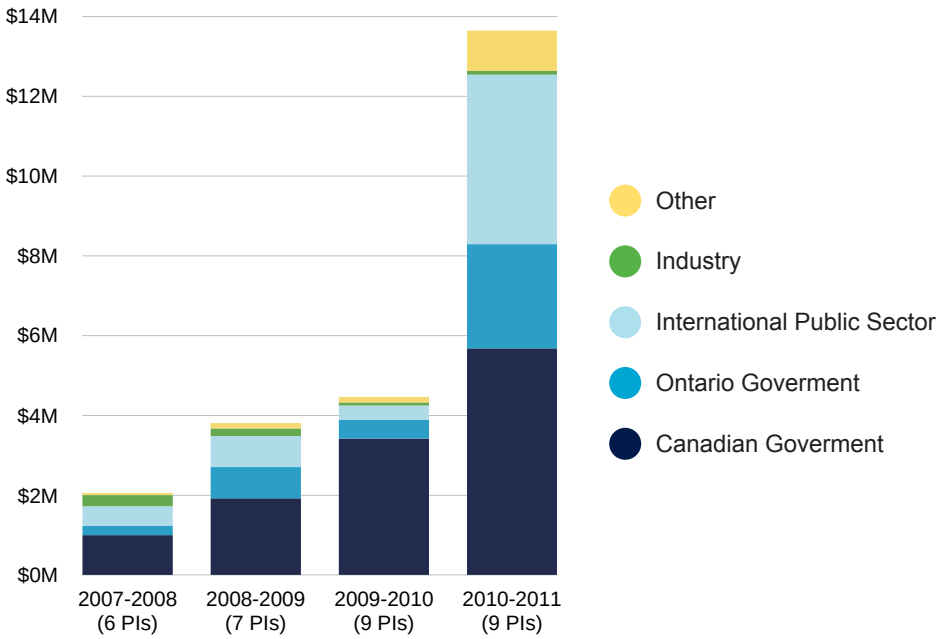
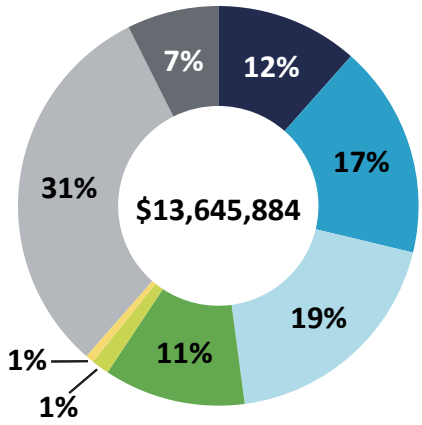


Fig 1. BioZone cash funding received per year (Sept-Aug). Includes operating, capital, and equipment grants, a Killam Fellowship, overhead, and amounts sub-awarded to other University of Toronto Professors when a BioZone PI leads the project. Does not include scholarships (<15% of total funds), in-kind contributions, or other leveraged funds.

	Canadian Government	Ontario Government	International Public Sector	Industry	Other	Total
2007 / 2008	$999,848	$238,144	$486,799	$275,899	$57,065	**$2,057,754**
2008 / 2009	$1,921,299	$784,218	$778,088	$190,568	$135,512	**$3,809,685**
2009 / 2010	$3,420,240	$474,808	$354,655	$75,000	$140,715	**$4,465,418**
2010 / 2011	$5,681,149	$2,611,153	$4,251,247	$93,155	$1,009,180	**$13,645,884**



Source	2010-2011 funding
NSERC	$1,573,271
CFI	$2,351,030
MRI	$2,611,153
Genome Canada	$1,560,347
Other Canadian Government	$196,500
Industry	$93,155
International Public Sector	$4,251,247
Nonprofit Organizations	$1,009,180
Total	**$13,645,884**

Fig 2. Sources of cash funding to BioZone Professors between September 1, 2010 and August 31, 2011. Inclusions and exclusions are listed in the caption for Fig. 1.

Major research initiatives in BioZone typically involve collaborations between multiple BioZone PIs, researchers at other institutions in Canada and abroad, and private sector partners. Thus, total project values for major research initiatives are often significantly higher than the cash awards as they include leveraged contributions from other institutions and private sector partners. Major awards held in 2010-2011 resulting in over $1M cash to U of T during this interval included:

BEEM: Bioproducts and Enzymes from Environmental Metagenomes (Edwards, Mahadevan, Master, Savchenko, Saville, Yakunin and 9 collaborators)
Contributions:
Genome Canada (2009-2013): $5M
Ontario Ministry of Research and Innovation: $3.3M
EU-funded MAMBA project, Geosyntec Consultants, Tembec Inc., US DoD, and others: $3.4M

BioZone: A Bioengineering Research Facility for Energy, Environmental and Economic Sustainability, also known as BioZone Phase II Infrastructure (All PIs)
Contributions:
Canada Foundation for Innovation (2009-2013): $2.5M
Ontario Ministry of Research and Innovation: $2.5M
Dept. of Chemical Engineering and Applied Chemistry: $890K
Equipment vendors' in-kind contributions: $380K

Center for Structural Genomics of Infectious Diseases (Savchenko and international collaborators)
Contributions:
U.S. NIH National Institute of Allergy and Infectious Diseases (NIAID) (2010-2012): $2.5M

Midwest Center for Structural Genomics, also known as Protein Structure Initiative III (Savchenko and international collaborators)
Contributions:
Argonne National Laboratory (2010-2012): $1.4M

In 2012, a new large project will begin in BioZone led by Professor Master in collaboration with Professors Saville, Savchenko, Yakunin, and 7 researchers at other institutions. *Forest FAB: Applied Genomics for Functionalized Fibre and Biochemicals* will bring $2.1M in cash from the Ontario government via an ORF-RE award and an additional $4.2M in cash and in-kind contributions from institutional and private sector partners.

Cash funding for BioZone research in 2010-2011 came from a mix of domestic and international sources, as illustrated in Fig. 2. International research grants provided 31% of total cash funding to BioZone in 2010-2011. As listed above, research projects valued at over $1M CAD were sponsored by the U.S. NIH National Institute of Allergy and Infectious Diseases (NIAID) and Argonne National Laboratory. A FiDiPro Fellowship, also valued at over $1M CAD, was awarded and started in September 2011.

Industry Support

BioZone acknowledges support from the wide range of private sector partners listed on the inside back cover of this report, whose contributions have included personnel time, expertise, samples, equipment and research funding. In addition, BioZone PIs have developed longstanding research relationships with several industrial collaborators that have led to technology transfer (see *"Collaborators"* on pg. 31 for more detail).

Philanthropic Support

BioZone expresses its gratitude for Dorothy Szymaszek's contribution on behalf of her late husband, Jan Walter Szymaszek, towards the new videoconference room, WB407.

BioZone is also grateful to Chemical Engineering alumnus Tai-Wing Ng, who donated funds for research and BioZone graduate student scholarships.

BioZone Team Members

Recruiting the Best and Brightest

BioZone is home to a very diverse and talented pool of students, researchers, staff and PIs from a wide range of disciplines. Since the centre's inception, we have attracted personnel and trainees from 28 countries on 5 continents (Fig. 3).

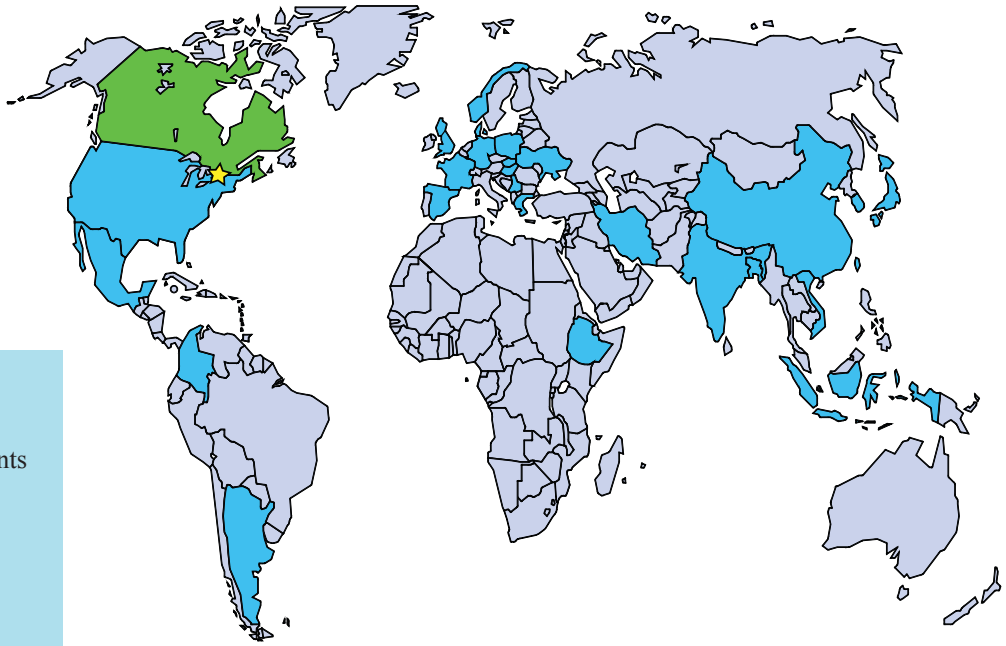


Fig 3. Countries of origin of BioZone staff, students and researchers outside of Canada are shown in blue.

BioZone's interdisciplinary personnel represent departments from across the Faculty and University:

- Cell & Systems Biology
- Biochemistry
- Chemical Engineering and Applied Chemistry
- Civil Engineering
- Mechanical Engineering
- Institute of Biomaterials & Biomedical Engineering
- Geology
- Medicine
- Geography

In the reporting period, BioZone was home to over 100 graduate students, postdoctoral fellows, staff and PIs (Fig. 4). In addition, 17 undergraduate students completed summer internships in BioZone's laboratories, and five undergraduate students theses were supervised by our PIs.

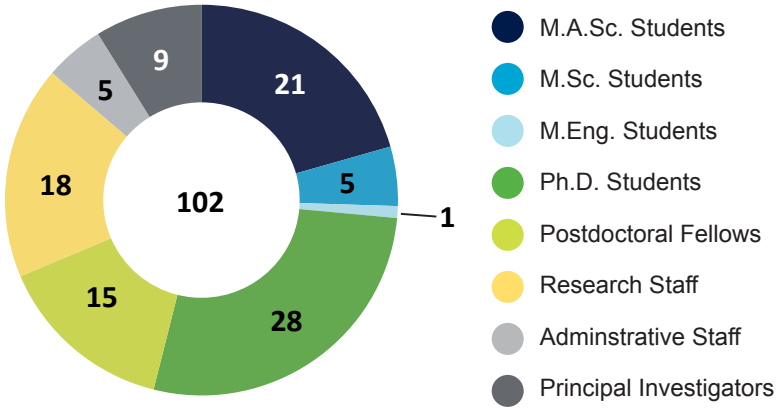


Fig 4. Headcount of BioZone personnel by category on Aug 31, 2011.

Faculty Recruitment

In 2009, Assistant Professor Alison McGuigan, a former Ph.D. student of ChemE/IBBME Professor Michael Sefton, returned to the Department to start her own research in BioZone after completing postdoctoral fellowships at Harvard University and Stanford Medical School.

In 2010, Alexander Yakunin and Alexei Savchenko, who lead world-renowned enzymology and structural proteomics groups at the University Health Network and the Best Institute, were appointed as Associate Professors. They will move their protein production and characterization pipeline into BioZone's facilities in the Wallberg Building in mid-2012.

BioZone Alumni

In 2010-2011, a total of 16 graduate degrees were awarded to students in BioZone (8 M.A.Sc., 4 M.Eng., 1 M.Sc., and 3 Ph.D.). In addition, 7 postdoctoral fellows completed their fellowships and 17 undergraduate students completed summer internships.

Since the inception of BioZone in 2007, a total of 46 graduate degrees have been awarded to students in BioZone, and 11 postdoctoral fellows have completed their fellowships. Alumni from BioZone have gone on to professional and academic positions in Canada and abroad. Fig. 5 shows the breakdown by sector.

Updates from many of our alumni detailing the exciting work in which they are currently involved can be found in the *"Alumni Updates"* section on pg. 126.

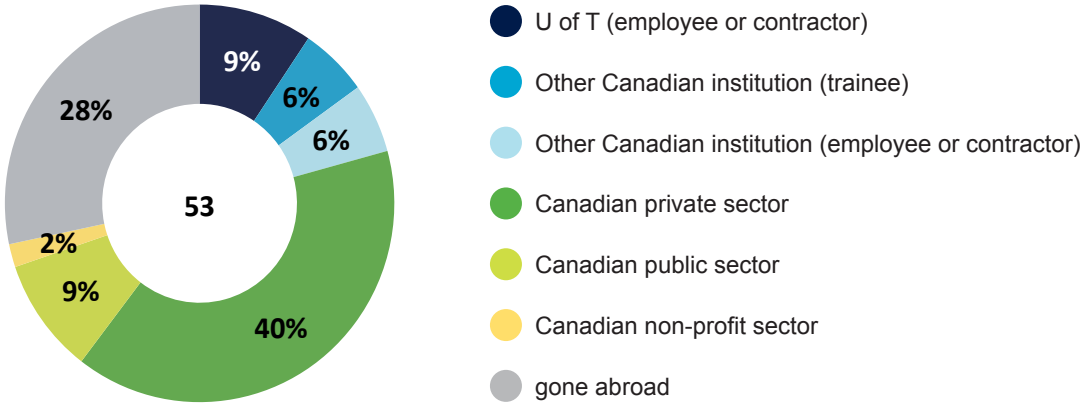


Fig 5. Breakdown of industry sectors reached by BioZone alumni, including former graduate students, postdoctoral fellows, research associates and technicians. Graph based on known information for 53 former BioZone members.

The BioZone Experience

BioZone aims to provide its team members with a richly diverse, multidisciplinary learning experience. This is manifested through coursework, teaching, and research, as well as through a variety of events.

Innovation in Education

The University of Toronto offers a very wide variety of graduate courses across many disciplines. One of BioZone's long-term goals is to streamline and focus graduate courses to be as relevant as possible to our students, building on and linking to others courses offered across the University. Currently we offer courses that introduce microbial ecology and bioenergetics (JCC1313: *Environmental Microbiology* – Edwards/Master), and build on these concepts with genomics and proteomics (CHE1135: *Advances in Bioengineering* - Savchenko). In 2012, two additional courses, *Modeling Optimization of Chemical and Biological Networks* (CHE1125; Mahadevan) and *Liquid Biofuels* (CHE 1123; Saville) will complement existing courses. We will also develop courses targeting applications of specific techniques, such as novel uses of mass spectrometry and bioreactor design and operation, as new equipment comes on-line.

By pooling resources to hire permanent and dedicated research staff to work alongside students and postdocs, BioZone provides continuity and consistency in training. This is particularly critical for high precision complex equipment and analyses that are key to research in modern biology. One feature of BioZone is that all researchers participate on a rotating basis in duties to maintain laboratories, equipment, hardware and software in good running order, and to train new recruits, providing direct hands-on training on all laboratory equipment.

International Exchanges

BioZone's many international collaborations have led to several student and postdoctoral fellow exchanges, resulting in valuable learning experiences and lifelong memories for both visitors and their hosts.

Since its inception in 2007, BioZone hosted:

- a postdoctoral fellow from Germany who spent two years of his Marie Curie Fellowship in BioZone developing molecular biological tools and conducting metabolomics experiments
- a postdoctoral fellow from Japan who learned techniques to characterize wood degradation by white-rot fungi
- a postdoctoral fellow from Guadeloupe who learned anaerobic microcosm preparation and helped expand BioZone's bioremediation research to banana plantations in the Caribbean
- a student from Japan who learned tissue engineering
- 1-2 students each year from France who learned food process engineering
- a student from Poland who learned high throughput enzyme screening techniques

During this time, BioZone sent:

- students to food engineering pilot plants around the globe to scale up processes developed in the lab. These included:
  - Brazil & South Africa (through the Program for Appropriate Technologies for Health)
  - USA (Texas A&M University and Glatt Air Techniques, NJ)
- two students to New Zealand to conduct lab scale bioreactor experiments and understand the fate of colour in pulp mill wastewater treatment lagoons
- a student to Sweden to learn how to characterize plant fibre chemistry through immunolocalization and electron microscopy
- a student to Finland to develop novel protein separation techniques
- a student to Germany to learn proteomics techniques for reductive dehalogenase analysis

Promoting integrity, ethics, sharing, collaboration, development and collegiality

Our alumni, as well as our current team members, agree that the most memorable part of their BioZone experience is the sense of spirit and shared passion that each member brings to the group. This spirit is manifested in a wide range of internal and external events designed to not only provide technical training and venues for the exchange of scientific ideas, but to further the social engagement of researchers both within BioZone and the broader community.

Events ranging from large, formal research meetings to small discussion groups, informal weekly tea socials, student-led camping trips, charitable fundraisers and outreach events have been organized by BioZone students and personnel during the reporting period. A more detailed description, including the participation of BioZone students in a Guinness World Record, can be found in the *"Events"* section on pg. 139.

Each of these events, as well as the design of our shared workspaces, provides opportunities for conversations that will spawn new ideas. The friendships that are fostered are also critical to the high level of mutual respect required for the smooth operation of our shared research facilities.

Research Accomplishments

Awards

The high calibre of work done by BioZone students and post-doctoral fellows was recognized with 37 awards in 2010-2011, including 21 scholarships:

Canada Graduate Scholarship (2)
Canada Graduate Scholarship - Michael Smith Foreign Study Supplement
Consejo Nacional de Ciencia y Tecnología (Mexico) Scholarship
Doctoral Completion Award
Frances Bradfield Graduate Fellowship in Environmental Engineering
Graduate Student Endowment Fund Scholarship
IMS Entrance Award
Marie Curie Postdoctoral Fellowship
Mitacs Elevate Postdoctoral Fellowship
NSERC Postgraduate Scholarship
NSERC Undergraduate Summer Research Award
Ontario Graduate Scholarship (2)
Ontario Graduate Scholarship in Science & Technology (5)
W. H. Rapson Memorial Award
Yoshio Masui Prize in Developmental, Molecular or Cellular Biology

See *"Awards, Grants & Scholarships"* on pg. 137 for details.

Publications

Our team published 45 peer-reviewed articles in international journals, and made over 95 oral and poster presentations at over 45 mostly international conferences. For a full list of all publications and presentations, including student theses, please see the *"Publications"* section on pg. 129.

Technology Transfer

For BioZone research to benefit society and the environment, strong relationships are required with industry, government, and other potential end users of the knowledge, tools, expertise, and technologies developed in our labs. Our Commercialization Committee (see *"Leadership"* on pg. 12) assists us with identifying and acting upon technology transfer opportunities that arise through our research. We have longstanding strong research partnerships with several corporations (see *"Collaborators"* on pg. 31 for more detail) and are actively seeking new partners for collaborative research. We also participated in outreach opportunities to regulators, such as Genomics on the Hill and Genomics in the Park events organized by Genome Canada and the Ontario Genomics Institute, respectively.

Between September 2010 and August 2011, BioZone researchers:

- filed 4 intellectual property disclosures with the University
- filed 1 provisional patent
- filed 1 full U.S. patent
- won 1 grant from MaRS Innovation to fund commercialization research for a specific set of novel enzymes

In addition, our ongoing relationships with our alumni are often BioZone's best "vectors" for technology transfer, through their careers and other endeavours.

International Recognition

BioZone is fulfilling its mission to be recognized internationally as a nexus for advanced biotechnological tools and expertise. Since 2007, we have attracted top talent from across the globe as trainees, staff, and Principal Investigators (Fig. 3), participated in many international exchanges, and have communicated our results at international conferences and in research publications.

BioZone's international reach extends to our growing list of research collaborations with other academic institutions as well as industrial partners. We welcomed new academic collaborators this year at Western University, Mt. Sinai Hospital, Université des Antilles et de la Guyane, Stanford University, Aalto University, and Wroclaw University. For a complete list of our international partners and collaborators, please see *"Collaborators"* on pg. 31. These diverse collaborations have led to many successful research awards and grants representing nearly a third of BioZone's cash funding in 2010-2011, as listed above and in *"Awards, Grants & Scholarships"* on pg. 137.

Strong Growth and a Bright Future

The ambitous goals and high degree of research excellence within our current projects will continue to attract high-calibre team members and collaborators, as well as play a key role in winning future grants. In the coming year, we plan to strengthen our existing relationships with industry and initiate collaborations with new private sector partners.

In 2012, we will complete renovations and open new research spaces on three floors of the Wallberg Memorial Building, welcoming Professors Savchenko and Yakunin's groups currently located at the Best Building. We will also strive to develop more new graduate course offerings and develop a coordinated list of existing graduate courses across many Departments that will form a core curriculum for BioZone students. Finally, we will complete our internal website, which will become an invaluable forum for sharing ideas, course information, laboratory protocols and information, and best practices.

History

BioZone brings great minds to great causes

Origins

BioZone began as an informal, loosely organized collection of research groups from across campus that arose naturally from successful collaborations on groundwater bioremediation, biological waste treatment and bioprocess engineering.

Research intensity and equipment and space needs in the Wallberg Building expanded dramatically after Professors Emma Master and Radhakrishnan Mahadevan joined the Department of Chemical Engineering and Applied Chemistry in 2006, bringing expertise in lignocellulosic bioprocessing, environmental microbiology, genomics, bioreactor design, genome-scale metabolic modeling and enzyme discovery.

Growth

In 2007, Professors Edwards, Mahadevan and Master secured a total of $550,000 from the Canada Foundation for Innovation (CFI Leaders Opportunity Fund), with matching funds from the Province of Ontario and additional support from Geosyntec Consultants Inc. and equipment vendors, to begin renovations in the Wallberg Building to create a common research space for BioZone "Phase I". Renovations to five major labs and associated facilities were completed in 2008, providing state-of-the-art laboratory space, dedicated equipment rooms, and student workspaces shared among these three professors.

In 2009, over $6 million in CFI (New Initiatives), Ontario Ministry of Research and Innovation, and other matching funds were awarded to a core of nine professors for BioZone "Phase II". This funding supported the creation of new space in the Wallberg Building to incorporate the significant research groups of Professors Yakunin and

Savchenko who co-lead a world-class protein production and characterization facility (Centre for Structural Proteomics in Toronto, or SPiT) currently located in the University of Toronto Faculty of Medicine's Best Building. The BioZone expansion includes approximately 270 m^2 of new space in a rooftop addition on the 4th floor of the building, directly above the 3rd floor BioZone space connected conveniently by an existing stairwell.

We wish to thank the supporters who made BioZone Phase I, II and III possible.

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Table 1. Total research and work space renovated or constructed as part of BioZone Phase I, II and III construction projects.

Renovated Spaces	Net Assignable Square Metres (NASMs)
BioZone Phase I (2008)	643
BioZone Phase II (2010-2012)*	851
BioZone Phase III (Spring 2012)	123
Total BioZone Space**	**1836**

* *Includes rooms renovated with Departmental and other support as well as rooms within CFI/MRI project #19427*

** *Does not include Professor's offices and shared Departmental facilities, including the Jan Walter Szymaszek Conference Room, glasswashing and sterilization facility, radioactivity laboratory, and server rooms*

BioZone "Phase II" also provides funds to renovate additional existing space to accommodate BioZone's growth and to purchase new equipment for its research initiatives (Fig. 1). This includes mass spectrometers for metabolite analysis and a differential calorimeter for enzyme characterization. The Department of Chemical Engineering and Applied Chemistry, the Faculty and the SPiT group are also contributing funds to renovate basic laboratories to house autoclaves, dishwashing, centrifuges, culture cultivation and a new x-ray diffractometer for protein structure determination (BioZone "Phase III"). Construction began in December 2010 and is nearing completion, with expected occupancy in spring 2012. When all construction is complete, BioZone will occupy approximately 1,800 m^2 in the Wallberg Building (Table 1).

The idea of dedicating common space to share know-how and integrate research approaches has thus developed from a strong track record of highly successful and well-funded interdisciplinary bioengineering research efforts. In particular, genome and high-tech tools and techniques used to explore human biology are highly relevant to research in environmental biotechnology and there are many exciting possibilities for cross-fertilization of ideas and approaches. Though BioZone's focus is environment and energy, the connections to human health, nutrition and cancer, for example, are clear. That several of BioZone's professors are engaged in health research only serves to enhance awareness and innovation in all research endeavours.

Fig 1. Before and after pictures of the Structural Biology Laboratory, one of the labs currently under construction as part of BioZone Phase II.

Future

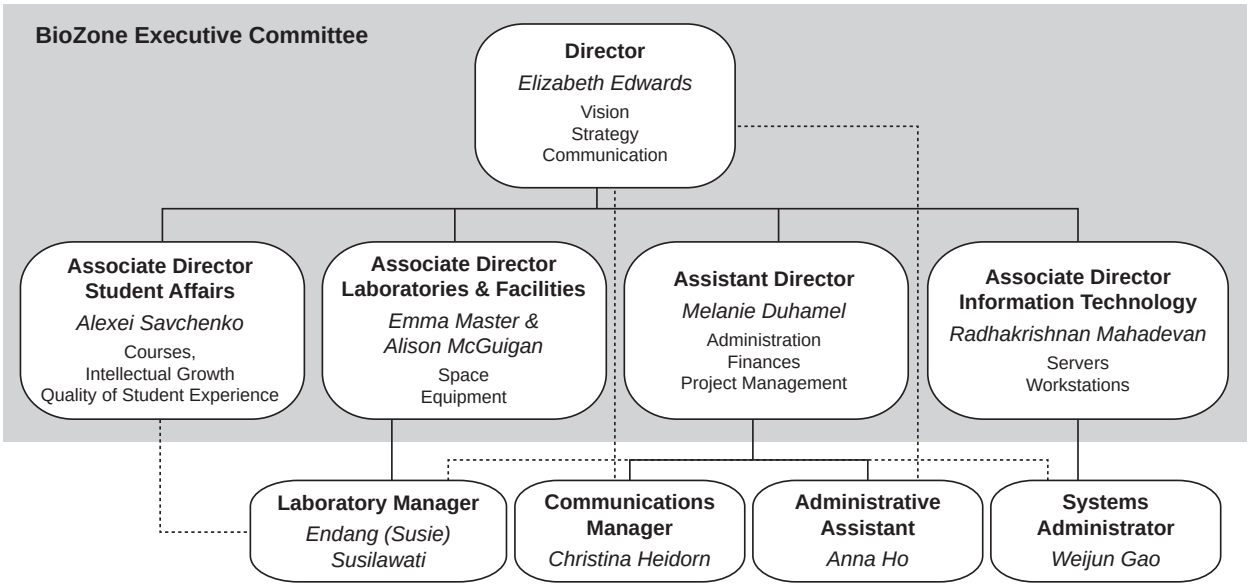
In January 2011, the University of Toronto recognized BioZone's impact as a research centre by granting it status as an Extra-Departmental Unit C (EDU:C). BioZone now has a mandate and structure to more effectively coordinate research, graduate teaching, and outreach in applied bioscience and bioengineering and related disciplines.

Leadership

BioZone's Director is appointed by the Dean of the University of Toronto's Faculty of Applied Science and Engineering, and reports administratively to the Dean. Elizabeth Edwards was appointed as BioZone's founding Director in January 2011 for a term ending in June 2014.

BioZone's strategic direction and day-to-day operations are overseen by the Centre's **Executive Committee**, consisting of the Director, three Associate Directors and the Assistant Director, each with separate portfolios. Membership in the Executive Committee rotates among BioZone professors. The current membership and the related areas of responsibility are shown in the organizational chart below.

The Executive Committee meets monthly to review new opportunities, provide an organizational framework for students and researchers, and oversee lab and computational resources. The committee also works closely with BioZone staff, as shown in the chart, to ensure that researchers' needs are met and their concerns promptly addressed.



An exciting recent development has been the establishment of BioZone's **Commercialization Committee** (CCOM), which provides important support toward the goal of taking cutting-edge research from the lab through to the development of effective, commercially viable real-world technologies.

The CCOM was initally formed as part of one of BioZone's biggest projects, *BEEM: Bioproducts and Enzymes from Environmental Metagenomes*, to help guide commercialization of technologies arising from its research. The committee has recently expanded its scope to include all of BioZone.

The CCOM includes:

- Frank Frantisak, Former Senior Vice-President, Noranda Inc.
- Michael May, CEO, Centre for Commercialization of Regenerative Medicine
- Peter Azmi, Business Development Officer, University of Toronto
- Rhonda Tannenbaum, Director of Business Development, Ontario Genomics Institute
- Doug Reeve, Professor, University of Toronto

The committee meets three times a year or as required to address specific issues that may be time-sensitive.

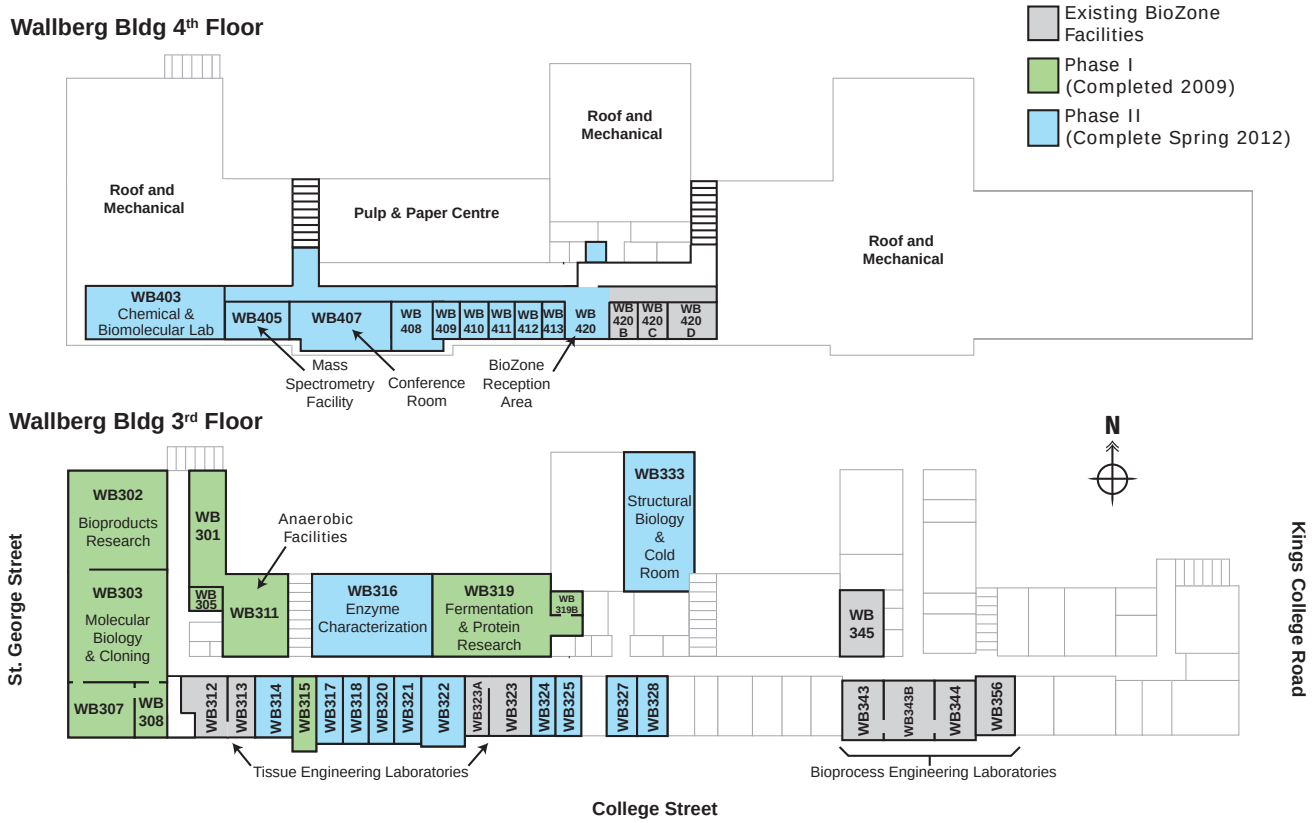
Facilities

BioZone is a focal point for biotechnology in the Faculty of Applied Science & Engineering

Overview

BioZone's facilities provide a **collaborative space and cross-disciplinary approach** that enable researchers to share knowledge, processes and equipment as they tackle difficult technical problems in environment, energy and health.

Facilities are located in the Wallberg Building at the University of Toronto and provide over 1,600 m^2 of laboratory and research workspace, including several large, bright, collaborative research labs. Further renovations are underway and will be completed in summer 2012 to provide futher research space.



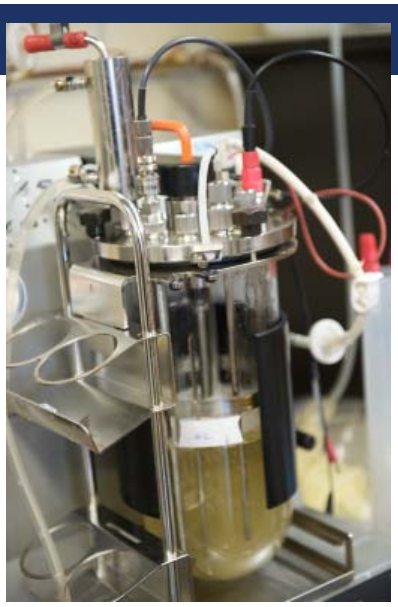
The labs house **a wide array of state-of-the-art analytical instruments** for molecular biology, protein purification and identification, enzyme kinetics, substrate and metabolite analysis, microscopy and cell growth.

The diversity of research within BioZone provides **exposure to a wide range of expertise and analyses** that can be useful for any given project, arming students and researchers with a broad array of state-of-the art equipment to tackle complex problems.

Featured equipment

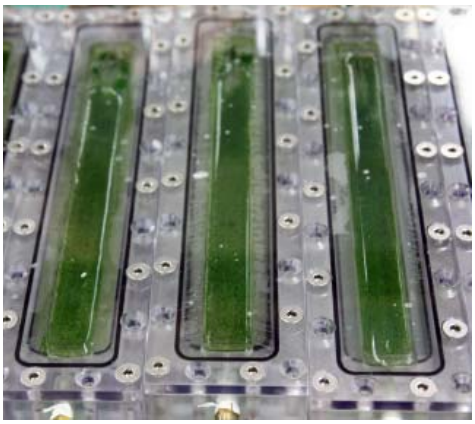
BioZone is structured around facilitating access to and training on analytical instruments. Some of the more sophisticated equipment housed in BioZone space is featured below. Moreover, BioZone is centrally located in close proximity to many other facilities on campus.

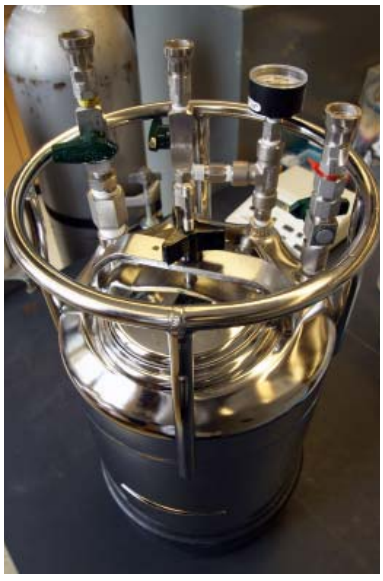
Bioreactors

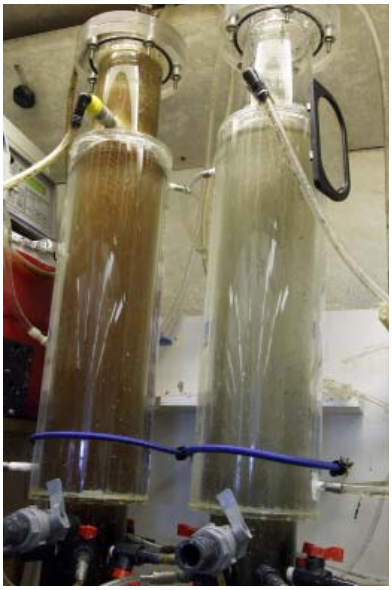


Bioreactors are at the heart of many of our projects. Therefore, a variety of **bioreactors** in a range of sizes are available for research on fermentation and biofiltration. These include:

1. Infors HT Minifors (5L)
2. Chemglass bioreactor (20L), suitable for hydrolysis, fermentations or any other type of aqueous phase reaction at temperatures up to about 70ºC. Used to explore hydrolysis of biomass at high solids - 15 to 25%.
3. Bench scale biofiltration system: continuous parallel bench scale bioreactors (app. 5 ft × 1 ft) for the study of biological gas cleaning. Their large size is suitable for testing various packing media. Gas and media sampling is possible for chemical, physical and microbial analysis. The system allows for introducing simulated air emissions with various air pollutants at controlled temperatures.
4. Reduced sulfur biofiltration system: continuous biofiltration units to study the treatment of reduced sulphur pollutants such as hydrogen sulphide and dimethyl sulphide.







X-ray Diffractometers

X-ray diffractometers are used to determine the 3-dimensional structure of proteins. X-rays are focused onto the protein crystal of interest, which scatters the rays and produces a diffraction pattern. The position and intensity of the individual spots of the diffraction pattern are read by an imaging plate at the back of the diffractometer. These data are then used to determine the structure i.e. the x, y, z co-ordinates of every (non-hydrogen) atom in the molecule.

1. Rigaku MICROMAX-007 x-ray generator with Rigaku R-axis 4++ image plate detector
2. Rigaku MICROMAX-007 x-ray generator with Rigaku HTC image plate detector

Mass Spectrometers

Our facility houses several options for **mass spectrometry**, with LC/MS and GC/MS systems catering to a variety of experimental needs, including:

1. ThermoScientific Exactive LC/MS coupled to Accela 1250 pump and autosampler
2. ThermoScientific LTQ XL LC/MS with EASY nLC / Proxeon
3. Varian Saturn 2100T GC/MS

The Exactive is ideal for identification and quantification of small molecules from complex metabolite pools or *in vitro* assays with an m/z range of 50-4000, high resolution and a mass accuracy in the sub 2ppm range.

The LTQ XL has an m/z range of 15-4000 and features selected ion monitoring (SIM), providing detailed analysis of specific ions and allowing for peptide sequence identification. Both of these mass spectrometers are attached to liquid chromatography systems allowing for analysis of liquid samples and provide countless options for compound separation, limited only by column and solvent choice. Both systems utilize solution based ionization and as such do not require extensive and lengthy derivatization of samples prior to analysis.

Liquid Handlers

Liquid handling robots are used for the automated dispensing of small volumes of reagents. BioZone has a TECAN Freedom evo.

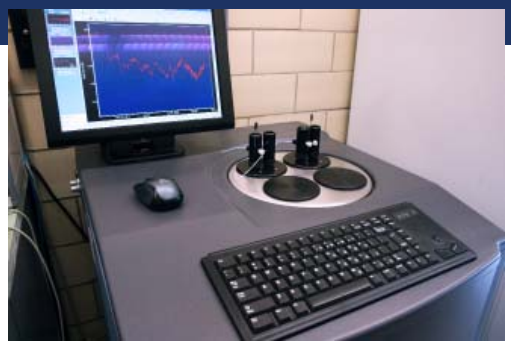
The Saturn 2100T has an m/z range of 20-650, is accurate to within 1 amu and features selected ion storage (SIS), providing MSn capability. It utilizes gas chromatography and is best suited for identification and quantification of volatile compounds and compounds that are not ionisable under solution chemistry.

BioZone will soon be acquiring additional mass spectrometry equipment to further expand our analytical capabilities.

Anaerobic Chambers

Anaerobic microbial transformations are an important part of our work on bioremediation, fermentation and anaerobic digestion. We have 4 **Coy anaerobic chambers** that are used for the manipulation and storage of microbial cultures and reagents.

Microcalorimeters



Microcalorimeters are used for stability screening, compatibility screening as well as for studying enzyme kinetics and molecular and binding reactions.

1. TAM III 3801 multi-channel microcalorimeter
2. Nano ITC 2G

Spray drier

The **spray drier** is used in food engineering to help add micronutrients to foods. It produces microcapsules that create a physical barrier between the added micronutrients and their environment in food, thus protecting the nutrients from degradation.

The drier is used to dry materials and produce a fine powder. A solution or suspension is sprayed through an atomizer and dried with a cocurrent air flow. This allows for the product to be dried at relatively low temperatures, which can improve product stability.

Membrane processing equipment

Our **membrane processing equipment** is used primarily for food engineering research. Its broad range of applications includes protein/enzyme concentration, acid purification, sugar concentration, desalination and antibiotic concentration.

The system is a SEPA CF II crossflow membrane filtration unit designed to work with low foulant fluids at pressures up to 1000 psig, and is suitable for microfiltration, ultrafiltration, nanofiltration and reverse osmosis operations. It accommodates any flat sheet membrane and offers an effective membrane area of 155 cm^2. It is connected to a Hydracell M-03 series diaphragm pump and a Baldor smartmotor with an inverter control, enabling work at flow rates up to 8 L/min.

PCR Thermocyclers

PCR thermocyclers are used to amplify DNA fragments using the polymerase chain reaction process.

1. MJ Research PTC-200 Gradient thermocyclers
2. BioRad C1000 with CFX 96 Real-Time System
3. Opticon 2 DNA Engine with continuous fluorescent detector for quantitative PCR

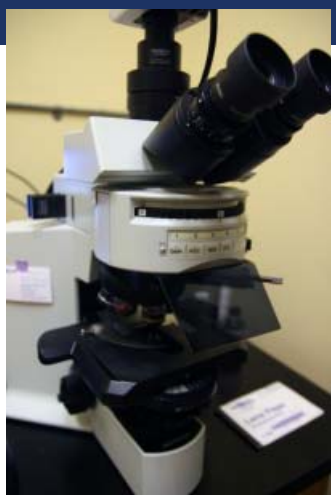
Computing Facilities

BioZone's computational server is an Oracle Sun Fire x86 server with 48 CPU processors and 256 GB RAM. It is powerful enough to support any kind of computational jobs within BioZone, such as metagenome assembly and metabolic modeling.

In addition, BioZone's web server hosts BioZone's public website as well as individual project sites and web applications. It also hosts internal research project sites for easy information sharing and online databases.

Software available includes Geneious, MATLAB, ProDesign and many others.

Microscopes



BioZone houses both upright and inverted **microscopes** that can take brightfield and fluorescence images. The automated microscope also has a stage top incubator to allow imaging of cells during cell culture for up to 5 days. Furthermore the automated microscope has a fully motorized stage allowing image stitching of images from adjacent fields of view. Microscopes are equipped with 4X, 10X, 20X, 40X, 63X and 100X lenses. In addition, the Image Express Micro is a high content screening microscope with environmental control. Images can be automatically collected in multiple locations within multiple wells of a well plate over time under conditions that allow cells to culture. Both brightfield images and fluorescence images can be collected.

1. Olympus CKX1 live cell inverted microscope
2. Olympus 120Q live cell inverted microscope
3. Olympus BX51 microscope
4. Leitz Laborlux S microscope
5. Molecular Devices Image Xpress Micro Widefield High Content Screening System

Local Collaborator Facilities

In addition to the equipment found within BioZone's labs, researchers and students have access to some of **our local collaborators' facilities**. These are within a short walk from BioZone and include:

- Centre for Analysis of Genome Evolution and Function
- Centre for Composites and Biomaterials Processing
- Donnelly Centre for Cellular and Biomolecular Research
- Graduate Collaborative Program in Genome Biology and Bioinformatics
- Institute for Biomaterials and Biomedical Engineering
- Nora Vaughan Laboratories in Civil Engineering
- Pulp and Paper Centre
- Stable Isotope Laboratory
- Surface Interface Ontario
- The Structural Genomics Consortium
- University Health Network Microarray and Proteomic Facilities

People

BioZone's strength lies with its people. Our talented pool of over 100 highly qualified personnel and trainees bring a wide range of expertise and experience to the table, and work towards challenging research goals in a collegial and supportive community.

Our personnel excels in research, teaching and leadership, and has extensive experience in collaborative work with industry.

The following pages include individual profiles of BioZone's members that outline their research interests.

- Principal Investigators
- Research Support Staff
- Collaborators
- Researchers
- Alumni

D. Grant Allen

Principal Investigator

Dr. Grant Allen is a Professor in the Dept. of Chemical Engineering and Applied Chemistry and began serving a 5-year term as Department Chair in July 2011. He previously served as Vice-Dean (Undergraduate) for the Faculty of Applied Science and Engineering, as well as the Department's Associate Chair for Graduate Studies (2003-2007). He was Director of the university's Pulp and Paper Centre from (2001-2003).

Dr. Allen completed his Ph.D. in Chemical Engineering at the University of Waterloo in 1987. Among his many professional accomplishments, he is a Fellow of the Chemical Institute of Canada, the Engineering Institute of Canada and the American Association for the Advancement of Science. He has published over 100 refereed publications and has led research consortia involving international partners from Brazil, Japan, New Zealand and the USA. His dedication to teaching has been recognized with the Chemical Engineering Teacher of the Year Award (2007).

Research Focus

Dr. Allen's research focuses on the field of **environmental bioprocess engineering** – using biological materials and biocatalysts for processes that benefit the environment. It is an exciting and growing field in which chemical engineering principles are applied to biologically-based processes. The field is interdisciplinary and highly collaborative, involving engineers, microbiologists, biologists and chemists. The range of applications includes the production of food, pharmaceuticals and chemicals, and the treatment of industrial wastes.

Algal cultures

One of Dr. Allen's main areas of interest is **biological wastewater treatment**, particularly as it relates to the pulp and paper industry. His work has included understanding and optimizing the treatment of bioactive compounds, organochlorines, thermophilic treatment and the role of disturbance. The research involves understanding how microbial processes adapt to environmental conditions as microbial flocs and biofilms, and improving treatment system robustness.

In addition, Dr. Allen's group has focused on **biologically producing value-added products** using mixed microbial communities that convert wastes into energy or biomaterials. This is especially important as we move towards a more sustainable future based on renewable resources. Taking full advantage of this requires that we develop efficient processes that can treat a wide range of compounds in various wastes (e.g. wastewater and biosolids) to produce useful byproducts (e.g. biofuels, adhesives, biopolymers).

Most recently, Dr. Allen has also been exploring the development of biofilm **photobioreactors** and growing microalgae for fuels or chemicals from waste carbon dioxide, sunlight and wastewater. Biological **treatment of waste gas streams** is another area of interest, including projects on treatment of VOCs, reduced sulphur compounds, bed design, kinetics, microbiology and modeling.

Food Engineering

Levente L. Diosady

Principal Investigator

Dr. Levente Diosady is a Professor in the Dept. of Chemical Engineering and Applied Chemistry at the University of Toronto, where he directs the Food Engineering Group. He received his Ph.D. from the Department in 1971. He is a Fellow of the Chemical Institute of Canada, Canadian Institute of Food Science and Technology, Canadian Academy of Engineering, the Hungarian Academy of Engineering, and the American Oil Chemists' Society.

Dr. Diosady's innovative work has been recognized with many professional awards, including the 2007 J. W. Eva Award for outstanding service to the Canadian Institute of Food Science and Technology through research and service, and the Babcock-Hart Award of the Institute of Food Technologists honouring an IFT member who has contributed to improved public health through nutrition. Dr. Diosady is the author of over 130 refereed publications, 18 patents, and he is a member of the Order of Ontario, the Province's highest civilian honour.

Research Focus

Food engineering applies the principles of chemical engineering and food chemistry to food processing to provide foods that are nutritious, safe and delicious. Dr. Diosady's main interest is the development of technologies for **food processing**. His current research areas are oilseed processing and micronutrient fortification.

Micronutrient deficiency diseases affect one third of the world's population, resulting in maternal mortality, reduced immunity to disease and impaired development in children. Dr. Diosady's research group is working with the Micronutrient Initiative and the Program for Appropriate Technologies for Health (PATH) to develop technologies for the introduction of key micronutrients into widely consumed food. His process for fortification of salt with iodine and iron **reduced cases of anemia by 1 million** in a test with 3.4 million children in India.

Fortified salt samples

His group is currently focused on **microencapsulation of micronutrients and nutraceuticals** for effective delivery through food.

Canada is a major producer and exporter of edible oils. The current interest in renewable energy sources has placed significant pressure on food supplies, as agricultural land and edible crops have been diverted to the production of ethanol and biodiesel. Dr. Diosady is working on the application of modern separation techniques for the simultaneous **production of biofuels, nutraceuticals and food-grade protein products** from conventional and unconventional triglyceride oil sources, including canola, mustard seeds and algae. This approach has the potential to contribute both fuel and food from crops that are not fully utilized.

Dr. Diosady is combining his interests in developing LIVE-ADE, a product combining nutritious protein, safe water and micronutrients in a carbonated beverage suitable for food aid and disaster relief.

Bioremediation of Groundwater Contaminants

Elizabeth Edwards
Principal Investigator

Dr. Elizabeth Edwards is the Director of BioZone and a Professor in the Dept. of Chemical Engineering and Applied Chemistry. She holds Bachelor's and Master's degrees in Chemical Engineering from McGill Univerisity and a Ph.D. in Environmental Engineering and Science from Stanford University.

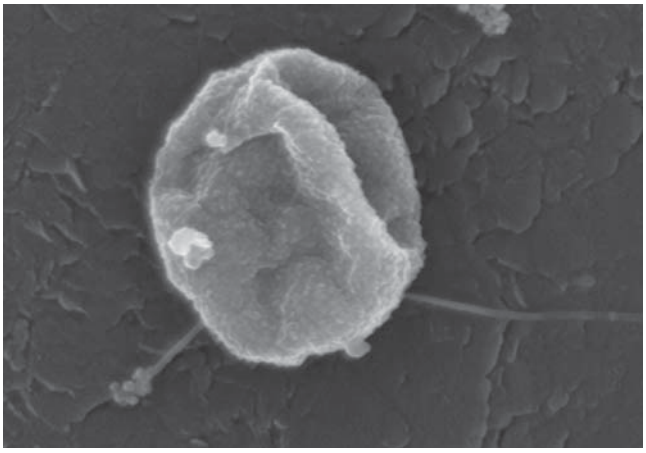
Dr. Edwards' research accomplishments have been recognized with an NSERC Women's Faculty Award, a Premier's Research Excellence Award, a Killam Research Fellowship (Canada Council for the Arts) and a 2011 Ontario Professional Engineers Award. She has been inducted into the Canadian Academy of Engineering and is a Fellow of the American Association for the Advancement of Science. In 2009 Dr. Edwards and Geosyntec Consultants, along with collaborators Barbara Sherwood Lollar and Brent Sleep, were recognized with an NSERC Synergy Award for their highly successful partnership and the commercialization of bioremediation technology.

Research Focus

Harmful chemical contaminants in groundwater, such as gasoline and industrial solvents, are a persistent environmental problem, a legacy of accidental spills and improper disposal that continues to pose a threat to environmental and human health. Dr. Edwards is an internationally renowned expert in **bioremediation** who has spent 20 years developing techniques that harness the power of microorganisms to help clean up these toxic pollutants.

Dr. Edwards' group has developed **anaerobic microbial cultures that can biologically degrade common and persistent groundwater pollutants**. One of the cultures developed in collaboration with Geosyntec, called KB-1®, is now a successful inoculant used to treat over 250 chlorinated solvent-contaminated sites around the world.

Anaerobic microbial processes, including methanogenesis, denitrification, and organohalide respiration, are key components of natural and engineered environments. A fundamental understanding of these biological processes is essential to improve remediation technologies. Dr. Edwards' group focuses on the **characterization of anaerobic microbial communities** in wastewater treatment plants and groundwater environments, and the use of molecular tools to detect gene and protein expression. Analytical chemistry, molecular biology, environmental genomics and microbiology, in combination with mass and energy balances and computational approaches, are used to unravel and model complex microbial physiology and metabolism to help understand and optimize their performance in remediation and energy generation.



A dechlorinating bacterium called Dehalococcoides

Metabolic Systems Engineering

Radhakrishnan Mahadevan

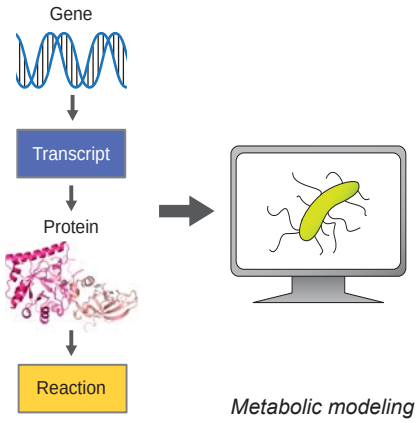
Principal Investigator

Dr. Radhakrishnan Mahadevan is an Associate Professor in the Dept. of Chemical Engineering and Applied Chemistry and serves as BioZone's Associate Director for Computational Resources. He is cross-appointed to the Institute of Biomaterials and Biomedical Engineering and holds a Ph.D. in Chemical Engineering from the University of Delaware. Prior to joining the university in 2006 he completed a postdoctoral fellowship at Genomatica Inc., and was a visiting researcher at the Universities of Massachusetts and California in San Diego.

Dr. Mahadevan's research was recognized in 2010 with the Jay Bailey Young Investigator Best Paper Award from Metabolic Engineering Journal, and he has received an Early Researcher Award for Research Excellence in Ontario and the David W. Smith Jr. Award for Outstanding Publication.

Research Focus

Recent advances in experimental and computational technologies have enabled the **detailed characterization of complex biological systems** at the level of genes, proteins and metabolites. Such a wealth of data is valuable for understanding the **fundamental design principles of biological networks and systems**. The Mahadevan lab focuses on developing integrated computational and experimental approaches to identify practically relevant organization principles of biological systems, with a particular emphasis on metabolism. This involves the iterative development of genome-scale mathematical models of metabolism to identify the principles, and algorithms that exploit these principles, for optimizing biological network function for applications in industrial and environmental biotechnology and medicine.



Metabolic modeling

Biorefineries: It is well recognized that the economics of biorefineries can be improved by manufacturing co-products, including value-added chemicals. Advances in genomics, bioinformatics, protein engineering, and mathematical modeling of metabolic pathways have opened a systematic approach to metabolic engineering for the overproduction of a range of biochemical compounds including non-natural difunctional chemicals.

Enviromental Biotechnology: Dr. Mahadevan's group has developed dynamic genome-scale models of microbial metabolism and physiology of individual members of microbial communities in two environments, namely, a metal reducing community relevant for uranium removal and a dechlorinating community. These models are linked to reactive transport models for optimizing uranium removal by manipulating the addition of nutrients.

Medicine: In many diseases, including diabetes, cancer and obesity, metabolism is drastically altered, motivating the investigation of metabolism for improved understanding of such disorders. Dr. Mahadevan's group is developing detailed metabolic models under these disease states to elucidate the underlying mechanisms and to identify therapeutic targets. Ultimately, such models could be tailored to individuals and could result in personalized nutrition and medicine.

Emma Master
Principal Investigator

Dr. Emma Master is an Associate Professor in the Dept. of Chemical Engineering and Applied Chemistry and is BioZone's Associate Director for Laboratories and Facilities, a position she shares with Dr. McGuigan. She holds a Ph.D. in Environmental Microbiology from the University of British Columbia and completed postdoctoral fellowships at the Royal Institute of Technology in Stockholm and Concordia University before joining the University of Toronto in 2006. She is cross-appointed to the Department of Cell and Systems Biology.

Dr. Master received a Finnish Distinguished Fellowship (FiDiPro) in 2010 and an Early Researcher Award from the Ontario Ministry of Research and Innovation in 2009. She is project leader for "Forest FAB: Applied Genomics for Functionalized Fibre and Biochemicals", a 4-year project funded by the Ontario Research Fund.

Research Focus

As we move from a petroleum-based economy to one based on renewable resources, plant biomass will become increasingly important not only for biofuels but also for many of the materials, such as plastics, which are currently produced from oil. Dr. Master's mission is to harness the specificity of enzyme-catalyzed reactions, and the enormous potential to discover and engineer new enzyme activities, to enable the synthesis of high-value biopolymers and specialized biochemicals that **provide renewable alternatives to petroleum-based compounds**.

Three benefits of applying enzymes for bioproduct synthesis include: 1) reaction specificity which allows reproducible modifications of heterogeneous and complex biomass substrates, 2) mild reaction conditions that retain the quality of biopolymers isolated from plant fibre, and 3) the renewability of enzyme catalysts, which helps ensure the sustainability of final bioproducts.

Fungal culture

Dr. Master's expertise lies in enzymology, protein engineering, proteomics, applied functional genomics, and lignocellulose chemistry. In addition to genomic and proteomic methods, techniques employed in her lab include protein engineering and recombinant protein production, high-throughput enzyme characterization and reaction optimization, plant transgenics and analytical characterization of plant fibre structure and composition.

Tissue Morphogenesis Engineering

Alison McGuigan
Principal Investigator

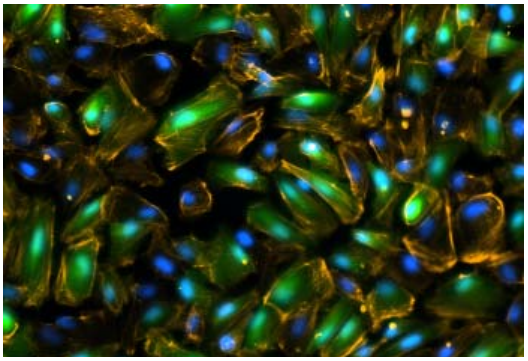
Dr. Alison McGuigan is an Assistant Professor in the Dept. of Chemical Engineering and Applied Chemistry and is BioZone's Associate Director for Laboratories and Facilities, a position she shares with Dr. Master. She joined the department in 2009 after receiving her Ph.D. in Chemical Engineering at the University of Toronto and completing postdoctoral fellowships at Harvard University and Stanford Medical School. She is cross-appointed to the Institute of Biomaterials and Biomedical Engineering.

Dr. McGuigan's innovative research into assembling engineered tissues has been recognized with the Wake Forest Institute for Regenerative Medicine Young Investigator Award in 2008, and an NSERC Discovery Accelerator supplement in 2009. She has authored 18 refereed publications, holds 2 patents and her work is funded by NSERC, the Heart and Stroke Foundation, and CIHR. The McGuigan research team is extremely multi-disciplinary with biologists, engineers, chemists, mathematicians and clinician scientists.

Research Focus

Regenerative medicine seeks to develop advanced health technologies to regenerate or replace damaged and diseased tissues. Dr. McGuigan's research focuses on **developing experimental systems to understand and control cellular morphogenesis (re-organization) for regenerative medicine applications.** The functionality of regenerated tissue depends critically on correct incorporation and re-organization of the cells during the regenerative process. Dr. McGuigan's research is addressing the central question "How do cells (re)organize themselves into functional tissues?"

Historically the signals that guide cell re-organization to produce tissue patterning have been studied extensively in developing embryos. However, the complexity of embryo morphogenesis makes this a challenging process to dissect. The McGuigan lab group is attempting to recreate in a Petri dish the signaling and cell-to-cell interactions that normally organize cells during tissue development in embryos. Her team is creating such systems using a combination of molecular biology techniques and cell patterning tools to control the organization of cells in two and three dimensions. These novel experimental systems will allow systematic analysis of the signals that direct cell organization. This work will fundamentally improve our understanding of morphogenetic processes such as wound healing, tissue regeneration, tissue integration, and tissue disorganization in disease.



Epithelial cells

Applying this fundamental information will provide new strategies for addressing problems in regenerative medicine such as engineering artificial tissues from stem cells, developing treatments for developmental diseases that result from incorrect tissue formation, and developing *in vitro* drug screening culture models to develop therapies for diseases like cancer or heart disease. For example, current research focuses on epithelial tissue engineering for creating engineered tracheas and endothelial tissue engineering for **creating engineered blood vessels.**

Another major focus of Dr. McGuigan's current work is the development of *in vitro* tissues for high-throughput screening to identify therapeutic strategies, such as drugs or biomaterials, for modulating and controlling the cell re-organization process.

Structural Proteomics

Alexei Savchenko
Principal Investigator

Dr. Alexei Savchenko is an Associate Professor in the Dept. of Chemical Engineering and Applied Chemistry and serves as BioZone's Associate Director for Students and Education. He is also an Assistant Professor in the Banting and Best Dept. of Medical Research and is Group Leader at Structural Proteomics in Toronto (SPiT). He received a Ph.D. in Molecular Biology and Microbiology from the University of Nantes in 1996 and joined the University of Toronto in 1999 after completing a postdoctoral fellowship in protein biochemistry at Michigan State University.

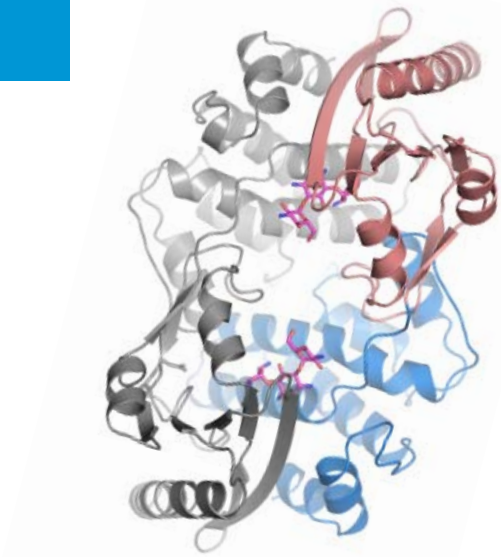
Dr. Savchenko has published over 75 peer-reviewed papers and reviews, and his research has been funded by NIH, CIHR, Genome Canada and NSERC.

Research Focus

Dr. Savchenko's work focuses on **characterization of protein function** using structural biology (primarily X-ray crystallography) and biochemistry. His Structural Genomics group is working on large-scale structural characterization of protein families whose 3-D molecular shape cannot be predicted from existing protein data.

Dr. Savchenko and his team developed the **high-throughput protein expression, purification and crystallization pipeline** that is central to SPiT and two major NIH funded structural genomics consortia – the Midwest Center for Structural Genomics (www.mcsg.anl.gov/) and the Center for Structural Genomics of Infectious Diseases (http://csgid.org/csgid/). This pipeline produces more than 80 novel protein structures per year and is one of the most efficient in the field of structural genomics. In collaboration with Dr. Alexander Yakunin, Dr. Savchenko has done pioneering work in enzyme discovery and biochemical characterization of novel microbial enzymes.

One of Dr. Savchenko's research interests is the characterization of **proteins involved in bacterial pathogenesis**, particularly the detailed characterization of enzymes involved in modification and inactivation of antibiotic molecules. Pathogenic bacteria use such enzymes to overcome antibiotic drug treatment, becoming a serious threat to human health. Understanding the enzymes' molecular mechanisms of action and developing potent inhibitors of their activity will pave the way for much-needed novel antimicrobial therapies.



Enzyme structure

Dr. Savchenko is also involved in the characterization of **novel enzymes for industrial applications**. His group applies structural biology methods to determine enzyme activity and provide vital information for structure-guided enzyme engineering. Enzyme families currently under investigation include esterases, hydrolases, dehalogenases and carboxylases.

Bioprocess and Enzyme Technology

Bradley A. Saville
Principal Investigator

Dr. Bradley Saville is a Professor in the Dept. of Chemical Engineering and Applied Chemistry where he leads the Bioprocess and Enzyme Technology research group. He joined the University of Toronto in 1989 after receiving his Ph.D. in Chemical Engineering from the University of Alberta, and he is cross-appointed to the Faculty of Pharmacy and the Institute for Biomaterials and Biomedical Engineering. He has authored over 40 refereed publications, 4 books and book chapters/monographs and over 45 technical reports, and he holds 25 patents.

Technology derived from Dr. Saville's research and patents related to novel hydrolytic enzymes has been field tested in several North American fuel ethanol plants, producing more than 400 million US gallons of ethanol since 2004. He has collaborated with SunOpta Bioprocess Inc., Mascoma, Greenfield Ethanol, BBI, Natural Resources Canada, Environment Canada, APEC, SDTC, and the North American Energy Working Group on various projects related to biofuels, bioenergy and bioproducts.

Research Focus

As we move from a petroleum-based economy to a more sustainable one, renewable resources such as plant biomass will become an important source of fuel and energy. Dr. Saville is an expert in **biofuels and bioenergy production and benchmarking**, and has been involved in the biofuels area from bench scale R&D to commercialization, economics and policy. His research interests focus on enzyme kinetics, enzyme reactors, and industrial applications of enzymes. In addition to applications to biofuels production, he is also interested in applications to pulp and paper processes, wastewater treatment, bioremediation and pharmaceutical production.

Bioreactor

Dr. Saville's research group is investigating **enzymatic hydrolysis of starchy and lignocellulosic substrates** for production of biofuels and bioproducts, and the characterization of hydrolytic enzymes and enzymes for biofuels and biopolymer synthesis. His group is also involved in bioreactor design and scale-up, as well as processing of high consistency biomass slurries.

In addition to the technical aspects of bioproducts, Dr. Saville has been very active in the **economic and life cycle analysis** of biofuels, bioenergy and bioproducts, as well as bioenergy policy. This work has involved advising government agencies, private companies and large lending institutions. Projects include strategic assessment of the impact of co-products on greenhouse gas emissions from lignocellusosic ethanol production, assessement of the impact of co-location strategies on emissions from biofuel production plants, and comparison of emissions from different modes of biofuel production.

Enzyme Genomics

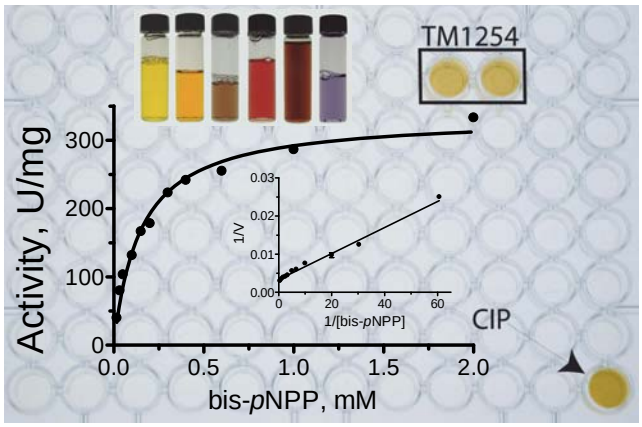
Alexander Yakunin
Principal Investigator

Dr. Alexander Yakunin is an Associate Professor in the Dept. of Chemical Engineering and Applied Chemistry. He is also Senior Research Associate and Assistant Professor in the Banting and Best Dept. of Medical Research, and Enzymology Group Leader at Structural Proteomics in Toronto (SPiT). Dr. Yakunin completed a M.Sc. in Molecular Biology at Moscow State University, and he holds a Ph.D. in Microbiology from the Institute of Microbiology of the Russian Academy of Sciences in Moscow. He was a postdoctoral fellow in the Institute of Soil Science and Photosynthesis in Pushchino (Russia) and at the University of Montreal before joining the University of Toronto in 2002.

Dr. Yakunin has broad expertise in microbial physiology, enzymology, and biotechnology, and has published over 100 peer-reviewed articles.

Research Focus

As a central element in the transformation of modern industrialized societies to sustainable economies, there is a global drive to promote "white" (or biocatalysis-based) biotechnology. Global genome and metagenome sequencing has produced millions of genes encoding unknown proteins, which represent a rich source of **novel enzymes**. Dr. Yakunin is mining this large pool of uncharacterized proteins to discover new industrial enzymes that catalyze novel reactions or are more active, more efficient, and more stable than those currently available.



Enzyme assay

These proteins must be experimentally characterized, because their functions cannot be computationally predicted on the basis of sequence homology to known proteins. Dr. Yakunin's research group uses enzymatic assays to directly test purified unknown proteins and metagenome gene libraries for catalytic activity. The approach is based on the **high-throughput screening, recombinant expression and biochemical characterization of purified proteins** with a focus on predicted hydrolases and oxidoreductases (dehalogenases, cellulases, esterases, dehydrogenases, and oxidases). Applications of these enzymes include biocatalysis, bioremediation and bioenergy.

Another project is focused on **Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs)** and associated, mostly uncharacterized proteins (Cas). These represent a novel microbial anti-viral defence system which functions to some extent analogously to eukaryotic RNA silencing machinery. The CRISPR system appears to contribute to cellular processes including biofilms, DNA repair, and gene expression. Many Cas proteins are predicted to have nuclease or helicase activity, and this project aims to reveal their biochemical activity and catalytic mechanism. This will contribute to further development of existing molecular tools for genetic engineering.

Research Support

Advanced research in a centre as large as BioZone relies on efficient and diligent administrative and technical support. BioZone has an experienced and knowledgeable research support team that ensures smooth day-to-day operations. Our support staff includes former researchers with backgrounds in science and engineering who understand the needs of students and investigators, and work hard to ensure that they have a productive and fulfilling experience.

Melanie Duhamel
Assistant Director

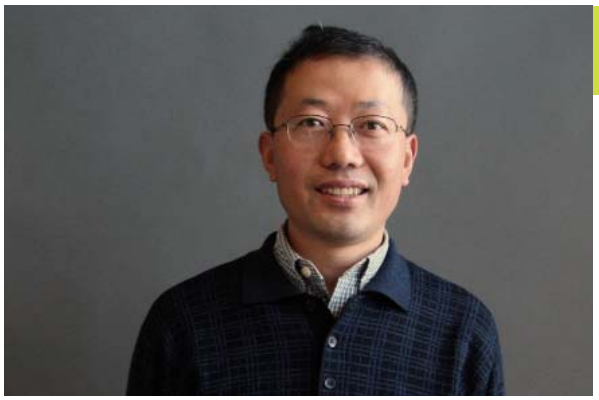
Melanie is responsible for research and financial management, including reports to sponsors, for large projects with a combined value of $24 million. She works closely with BioZone, departmental, and other university staff on sponsor agreements, human resources, project communications, and renovations coordination, including our current facility expansion.

Melanie holds a Ph.D. from the University of Toronto, with 10 peer-reviewed journal publications cited over 250 times in both the bioremediation and pulp and paper fields. She has previous experience in engineering consulting and is licensed by the Professional Engineers of Ontario.

Endang (Susie) Susilawati
Laboratory Manager

Susie is an experienced researcher who joined BioZone in 2009, bringing a wealth of technical experience to the task of managing lab operations. She received her Master's degree from the Crop and Soil Sciences Department at Michigan State University in 2003, where she subsequently worked as a Research Assistant in the Center for Microbial Ecology. Her work primarily related to biodegradation of contaminated soil, sediment and wastewater, looking for both aerobic and anaerobic degradation of organic pollutants and pharmaceutical products. Before moving to the United States to pursue her Master's degree, Susie was a researcher at the Indonesian Oil Palm Research Institute where she worked on improving palm oil quality in the Post Harvest Technology Department.

Susie manages daily lab activities in BioZone and provides students and researchers with information regarding safety, training, supplies and ordering. She is responsible for the maintenance of shared equipment, and coordinates student lab duties. She loves the colour pink.



Weijun Gao
Systems Administrator

Weijun was a lecturer and researcher in the Department of Computer Science and Engineering at the Northeastern University (China) before immigrating to Canada. He taught operating system and C/C++ programming courses, and conducted research into Chinese Natural Language Processing. He is a former software developer and research programmer who has supported teaching and research in Medicine and Bioinformatics. He holds a Bachelor's degree and a Master's degree in Computer Engineering, and previously worked in the Division of Academic Computing of the Faculty of Medicine and the Department of Electrical and Computer Engineering at the University of Toronto.

Weijun's current job is to provide IT support for BioZone's researchers and students. His responsibilities include managing networks, servers and workstations; developing web sites and web applications; providing IT technical support, as well as assisting researchers with their computational needs like programming, SQL databases, data analysis, data sharing, data and systems backup.

Christina Heidorn
Communications and Outreach Manager

Christina is a former researcher and television documentary producer who brings her combined experience in science and media to the task of bridging the gap between researchers and the public. She holds a B.A.Sc. in Engineering Science (Chem) and a M.Sc. in Botany from the University of Toronto, and has worked as a researcher, writer and producer for science and natural history television programs for broadcasters such as Discovery, CBC, TVO and National Geographic.

Christina's job is to foster collaboration among BioZone's members, manage outreach activities, and publicize the work of BioZone's researchers to a broad audience. She is responsible for internal and external communications, such as websites, discussion series, brochures and events, as well as outreach to high school and university students, donors and other stakeholders. She also provides communications training and writing support to researchers.

Monika Ignacak

Research Grant and Project Administrator

Monika was previously a lecturer and a scientist in the Department of Biochemistry and Molecular Biology at Poznan University of Medical Sciences in Poland. Her scientific research focused on the molecular biology of steroid hormones. As a university instructor, she taught molecular biology, biochemistry and clinical genetics courses, and was also involved in developing new curricula. She provided laboratory training to students focusing on theoretical and practical aspects of research.

Monika has been an invited guest lecturer at McMaster University on the topic of molecular aspects of Androgen Insensitivity Syndrome. She holds a Ph.D. in Molecular Biology from Poznan University of Medical Sciences and a M.Sc. in Biotechnology from Adam Mickiewicz University in Poland. While working as a visiting scientist at the University of Cincinnati she focused on regulation of hydroxylation and von Hippel-Lindau tumor suppressor protein dependent ubiquitylation of RNA polymerase II in response to oxidative stress.

As a former administrative assistant at the Research Innovation and Commercialization Centre and an experienced researcher, Monika has expertise in providing administrative assistance with research grants and projects.

Anna Ho

Administrative Assistant

Anna is a long-time member of the Faculty of Applied Science and Engineering with a wealth of experience in providing administrative support to professors and researchers. She obtained her secretarial diploma in 1977 and taught at a secondary school before joining the Computer Studies Department at the University of Hong Kong as departmental secretary. In 1987, she immigrated to Canada and joined the University of Toronto's Chemistry Department as research secretary for the Chemical Physics Theory Group. After spending several years overseas in the Philipines in the 1990s, Anna rejoined the university and became a secretary with the Pulp & Paper Centre in 1999. Since that time, she has provided administrative support to that centre, the Chair of the Department of Chemical Engineering and Applied Chemistry, as well as BioZone.

Anna's responsibilities include day-to-day secretarial tasks, assisting with event planning and travel arrangements, preparing printed materials for research meetings and symposia, and assisting in the preparation of grant applications.

Featured Partners & Collaborators

We would like to acknowledge all of our partners and collaborators, whose ongoing support is an integral part of the BioZone experience. A few individuals who have been particularly involved in the past year are highlighted below.

Robert Beiko

Faculty of Computer Science, Dalhousie University, Halifax, NS

Professor Robert Beiko is a Canada Research Chair and works extensively on the development and use of sophisticated computational tools for the comparative analysis of DNA and protein sequences between microorganisms to help determine the role of genes and proteins in the environment. He has collaborated with various groups in BioZone on methods for automatic annotation, metabolic network reconstruction and comparative analysis of metagenomes.

Lyle Biglow

Tembec Inc., Temiscaming, QC

Lyle Biglow is a Corporate Manager with Tembec Inc. in Temiscaming. Tembec is a model for the future biorefinery, making use of many biological processes in waste treatment and energy recovery, as well as converting biomass into a multitude of useful products. Currently Tembec is supporting research on anaerobic digestion for wastewater treatment and energy recovery.

Murray Burke

Mascoma Canada Inc., Mississauga, ON

Murray Burke is President and Chief Technology Officer of Mascoma Canada Inc. Mr. Burke has over 30 years of engineering experience and has been engineering, designing and building biomass conversion plants for over 20 years. He is a graduate from the University of Waterloo with a B.A.Sc. in Structural Engineering and is a member of Professional Engineers Ontario.

Mr. Burke and Mascoma Canada have supported R&D activities in BioZone related to the production of biofuels and bioproducts from biomass, spanning pretreatment, hydrolysis and fermentation, and have supported initiatives aimed at the production of value-added products from xylose and lignin.

Peter Golyshin

School of Biological Sciences, University of Bangor, UK

Professor Peter Golyshin is the Principal Investigator for the EU-funded project *MAMBA: Marine Metagenomics for New Biotechnological Applications*, a collaborative project that is looking for novel enzymes and metabolic pathways from extremophilic marine organisms and metagenomes. He and his team are working with several research groups in BioZone.

Susannah Green Tringe

Joint Genome Institute, Walnut Creek, CA

Susannah Green Tringe, Ph.D., is the Metagenome Program Lead at the DOE Joint Genome Institute (JGI) in Walnut Creek, California. She and others at JGI have been instrumental in BioZone's efforts at analyzing metagenome sequences of dechlorinating and benzene degrading consortia and the genome of the softwood-degrading fungus *Phanerochaete carnosa*.

Frank Löffler

Dept. of Microbiology, University of Tennessee, Knoxville, TN

Professor Frank Löffler is a leading expert in environmental microbiology, and collaborates extensively with BioZone researchers on the molecular biology and microbial physiology of organohalide-respiring microbes.

Heather MacLean

Dept. of Civil Engineering, University of Toronto

Professor Heather MacLean collaborates closely with BioZone on her work modeling the techno-economic and environmental performance of bioenergy systems using a life cycle approach. The research is inter-disciplinary, incorporating environmental, chemical and mechanical engineering, economics and public policy components.

David Major

Geosyntec Consultants Inc., Guelph, ON

David Major, Ph.D., is a Principal Scientist with Geosyntec Consultants Inc. and is an internationally known expert in the development and deployment of cost-effective remediation strategies for challenging contaminated sites. Geosyntec scientists have collaborated with research groups at the University of Toronto for many years. Geosyntec was a major contributor to Phase I of BioZone and is an ongoing BioZone partner.

Venkatesh Mannar

Micronutrient Initiative, Ottawa, ON

Venkatesh Mannar is President of the Ottawa-based Micronutrient Initiative. Prof. Levente Diosady's Food Engineering group has worked with the Micronutrient Initiative in developing technologies to introduce micronutrients into food for the prevention of micronutrient deficiency diseases.

Vladimiros Papangelakis

Dept. of Chemical Engineering and Applied Chemistry, University of Toronto

Professor Vladimiros Papangelakis leads the Aqueous Process Engineering and Chemistry Group to develop environmentally-responsible processes for the metals and minerals industry by applying fundamental aqueous process and engineering principles (hydrometallurgy). His group has recently partnered with BioZone on several bioleaching projects.

Douglas Reeve

Dept. of Chemical Engineering and Applied Chemistry, University of Toronto

Professor Douglas Reeve is the Director of the Institute for Engineering Leadership (ILead). As Chair of the Department of Chemical Engineering and Applied Chemistry from 2001 to 2011, he was instrumental in bringing the BioZone vision to life. Thank you, Doug!

Brent Sleep

Dept. of Civil Engineering, University of Toronto

Professor Brent Sleep has collaborated extensively with BioZone on experimental and computational analyses of biological and geochemical processes in soils and groundwater.

Honghi Tran

Dept. of Chemical Engineering amd Applied Chemistry, University of Toronto

Professor Honghi Tran is Director of the Pulp & Paper Centre at the University of Toronto. His research interests are in fouling and corrosion in recovery boilers and chemical recovery processes. Professor Tran's enthusiasm, experience and knowledge of the pulp and paper industry have contributed to active research efforts in biomass processing, anaerobic digestion. wastewater treatment and fibre modification.

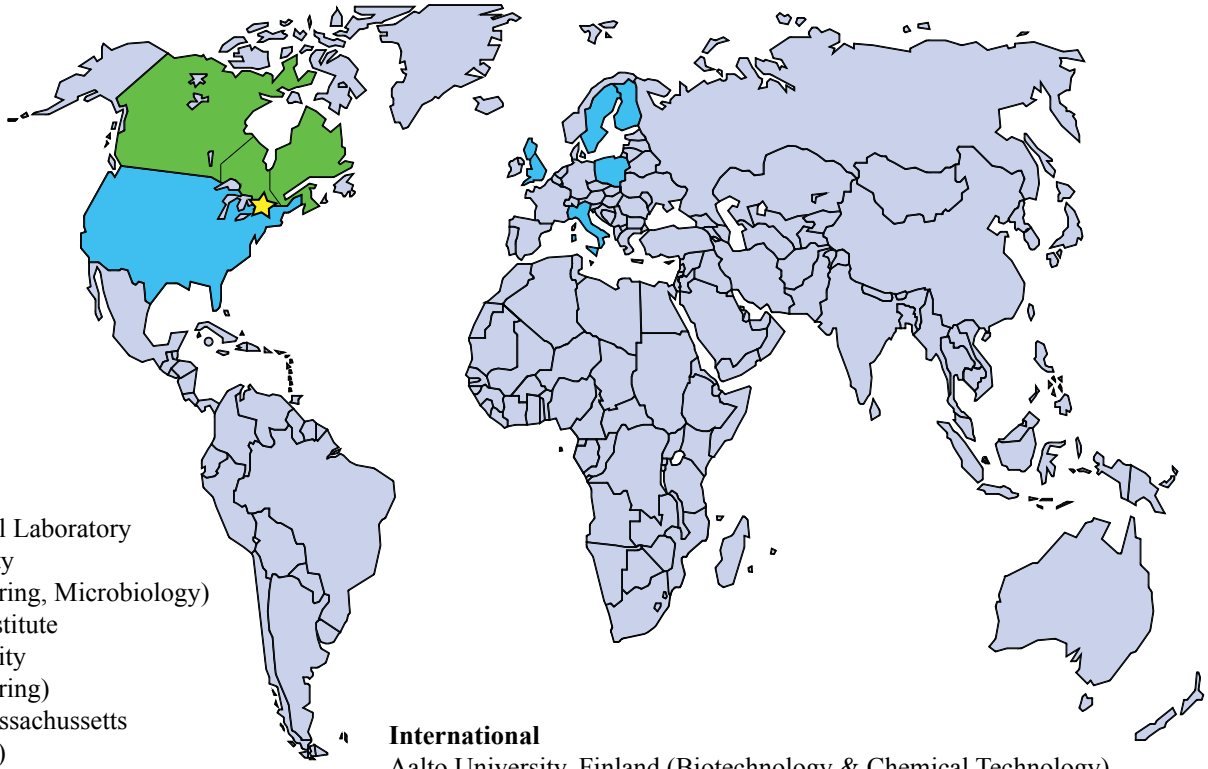
Institutional Collaborators

University of Toronto
Biological Sciences (Scarborough)
Cell & Systems Biology
Centre for Global Engineering
Chemistry
Civil Engineering
Geology
Institute for Leadership in Engineering
Institute of Biomaterials & Biomedical Engineering
Medical Biophysics
Molecular Genetics
Physical & Environmental Sciences (Scarborough)
Surface Interface Ontario

Toronto
Mt. Sinai Hospital
Ryerson University
Toronto General Hospital/MaRS
The Centre for Applied Genomics

Canada
Concordia University (Biology)
Dalhousie University (Computer Science)
McGill University (Civil Engineering)
University of British Columbia (Forestry)
University of Calgary (Biological Sciences)
University of New Brunswick (Chemical Engineering)

Ontario
Queen's University (Biochemistry, Chemistry, Geography)
University of Guelph (Environmental Sciences)
Western University (Civil Engineering)



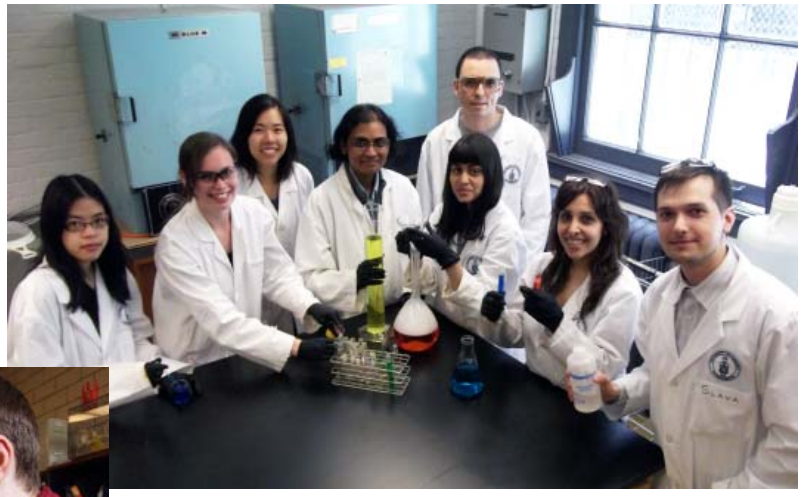
USA
Argonne National Laboratory
Cornell University (Civil Engineering, Microbiology)
Joint Genome Institute
Stanford University (Civil Engineering)
University of Massachussetts (Microbiology)
University of Tennessee (Microbiology)

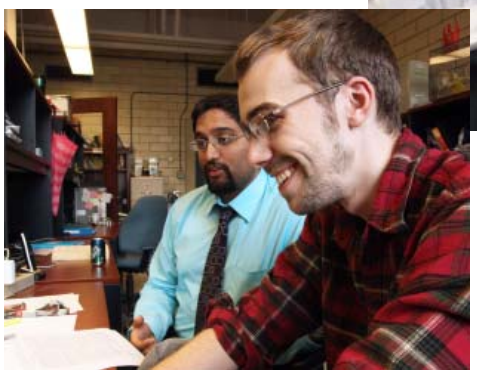
International
Aalto University, Finland (Biotechnology & Chemical Technology)
Instituto per l'Ambiente Marino Costiero, CNR, Italy
Université des Antilles et de la Guyane, Guadeloupe, France
University of Helsinki, Finland (Food & Environmental Sciences)
Umeä Plant Science Centre, Sweden
University of Bangor, United Kingdom (Biological Sciences)
Wroclaw University, Poland (Bioorganic Chemistry)

Researchers

BioZone is home to over 90 students, postdoctoral fellows, technicians and other researchers who strive for excellence and innovation in their work.

The following pages provide individual profiles that highlight their current research interests.





Research Highlights

Achampong, C. V. (2011). Metabolically engineering *Escherichia coli* for the production of pseudaminic acid. B.Sc. thesis, *Dept. of Chemistry*, University of Ottawa

Patent (pending): Radionuclide detection device for verification of an underground explosion (Application No. 2,724,143)

Recipient of Innocentive® Challenge Prize (Nuclear Test Monitoring and Verification Methods Challenge, 2011)

2011 Dr. Ouida Wright Memorial Scholarship recipient

Christine Achampong
M.A.Sc. Student

B.Sc., 2011, University of Ottawa

Supervisor:
Radhakrishnan Mahadevan
Co-Supervisor: Alexander Yakunin

Engineering microbial catalyses for the production of adipic acid

A great effort has been made by industrial chemists to establish a method of producing valuable chemicals via renewable processes. One of the proposed processes uses genetically modified microorganisms to catalyze inexpensive carbon sources into valuable chemical commodities. The use of microbial catalysts has been successful in the large scale production of amino acids, biofuels and pharmaceuticals - three classes of compounds that can be produced naturally by the host microorganism's metabolic network. The challenge lies, however, in producing chemicals that cannot be derived directly from the host organism's native metabolism.

Through the use of enzyme and metabolic engineering, **my project aims to produce a microbial strain that is capable of producing a non-naturally occurring compound called adipic acid.** Adipic acid (hexanedioic acid) is an organic diacid with an estimated market production exceeding 2 billion tonnes per year and is mainly used in the production of nylon.

Currently, the routes used to synthesize adipic acid require the by-products of hydrocarbon oxidation — an environmentally taxing process, or require the use of a metal catalyst — an economically taxing process. My Master's thesis eliminates these limitations by using a relatively harmless microorganism as a whole cell microbial catalyst to produce adipic acid.

Using a computational algorithm, EMILiO, we have determined the most viable routes for biosynthetic adipic acid production in our host organism. Further, because adipic acid cannot be made naturally by plants or other living organisms, we will screen enzymes used in similar processes and alter them in such a way that they will be specific for our engineered pathway. The enzyme screening and engineering portion of my project will be carried out in collaboration with the Structural Proteomics in Toronto (SPiT) centre. We also intend to genetically alter our host organism to ensure the proper adipic acid precursors are present and that these precursors are being led directly into adipic acid production. **Ultimately, this project will create a microbial strain that is capable of robust adipic acid synthesis with little detrimental economic or environmental impact.**

Parthiv Amin
M.A.Sc. Student

B.Sc.(Eng), 2011, Queen's University

Supervisor: Grant Allen

Research Highlights

Amin, P. (2011). A novel method for bio-diesel production using superacid pervaporative membranes. B.Sc. thesis, *Department of Chemical Engineering,* Queen's University

Investigating biosludge dewatering & combustion

Waste Activated Sludge (WAS), also known as Biosludge, represents a major challenge in wastewater processing in both an industrial and municipal setting. Of particular difficulty is efficiently de-watering sludges, and this process can often create an expensive bottleneck in water treatment. Furthermore, when the intent is to burn the cake solids for energy recovery, WAS also presents another challenge in that it has a poor heating value as compared to other biomass derived fuels.

In my masters thesis, **I will be investigating the practical interactions between various pulp and paper mill waste streams in an effort to identify potential improvements to dewatering, combustion, and potentially anaerobic treatability properties of WAS.**

Using a simple testing methodology, illustrated in Fig. 1, I aim to evaluate several pulp & paper mill waste streams and their ability to boost dewatering efficiency and combustion properties when mixed with WAS. These waste streams include sawdust, lignin powders, raw and dried primary sludge, and other readily available wastes such as shredded newspapers.

Dewatering efficiency will be evaluated through the use of a Crown Press, a device that can accurately simulate large scale belt press filtration, while combustion properties will be evaluated using bomb calorimetry and thermogravimetric analysis & differential scanning calorimetry.

The primary goal of this work is to **develop insights into the mechanisms of biosolids dewatering in order to provide practical improvements in wastewater treatment with a secondary goal of achieving efficient energy recovery from waste streams.**

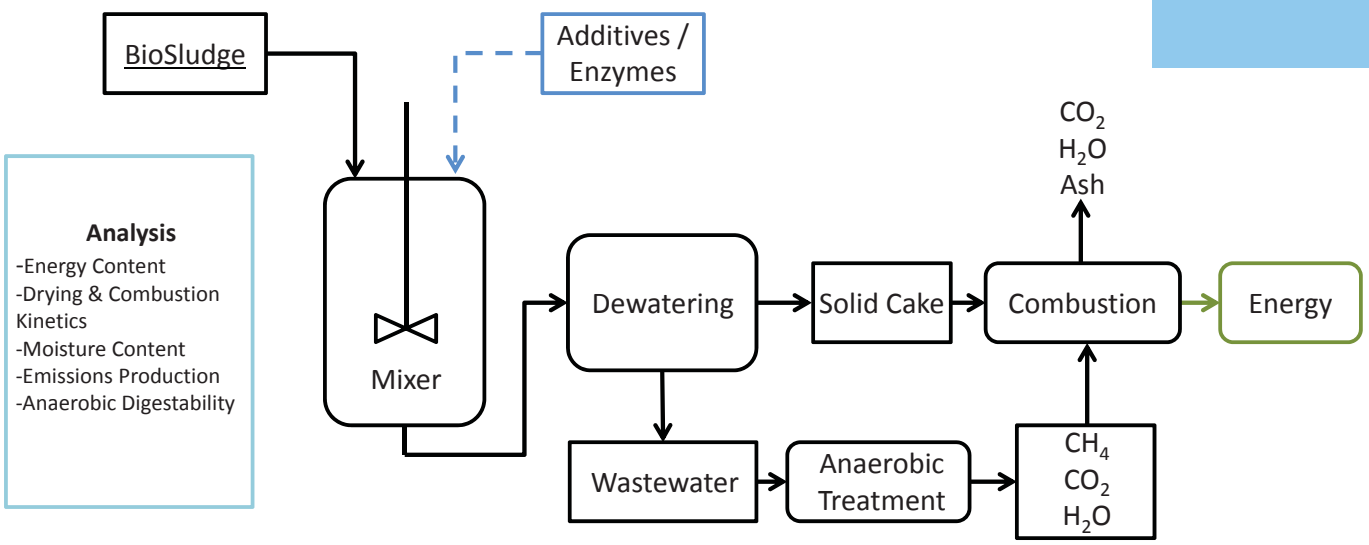


Fig. 1: Schematic representation of testing methodology.

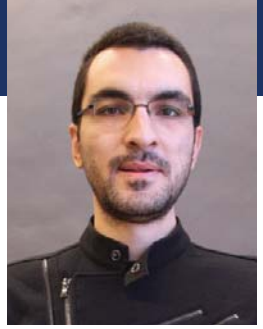
Research Highlights

Anesiadis, N., W. R. Cluett and R. Mahadevan (2011). "Model-driven design based on sensitivity analysis for a synthetic biology application." *Comp. Aided Chem. Eng.* (29):1446-1450

Anesiadis, N. *et al.* (2011). Enhancing bioprocess productivity through dynamic control of gene expression (invited speaker). *Recent Advances in Fermentation Technology (RAFT IX)*, Marco Island, FL

Anesiadis, N. *et al.* (2011). Dynamic metabolic engineering for lactate production. *American Institute of Chemical Engineers (AIChE) Annual Meeting*, Minneapolis, MN

Anesiadis, N., W. R. Cluett and R. Mahadevan (2008). "Dynamic metabolic engineering for increasing bioprocess productivity." *Met. Eng.* 10(5):255-266



Nikolaos Anesiadis
Ph.D. Student

M.A.Sc., 2007, University of Toronto
B.A.Sc., 2005, Aristotle University of Thessaloniki

Supervisor: Radhakrishnan Mahadevan
Co-Supervisor: William R. Cluett

Dynamic control of gene expression for improved bioprocess productivity

The rising worldwide demand for petroleum and derivatives and the depletion of fossil fuels is driving research towards using microorganisms to convert renewable feedstock into fuels and chemicals. Genetic engineering and process optimization have been applied extensively in the past, however, many processes are still not economically viable or cannot compete with existing methods. A common approach is to increase the yield of the desired chemical by eliminating non-desired byproducts. However, this sometimes comes at the expense of impaired growth rate and productivity. In my project we focus on increasing the productivity of processes where the microorganism is growing poorly. Our strategy involves the dynamic expression of genes contributing to growth to allow for an initial high growth rate phase.

The strategy utilizes two elements from the emerging synthetic biology discipline: (a) the natural quorum-sensing mechanism that bacteria use to sense their own population, and (b) the artificial genetic toggle switch that manipulates gene expression in an on-off manner.

The application of this strategy has two components: (a) a mathematical model for the initial design, prediction, analysis and optimization of the process, and (b) the experimental implementation to show the proof of concept for the production of lactate and succinate.

Mathematical modelling is driving hypothesis testing and optimization of the initial experimental design to estimate the optimal parameters and conditions (Fig. 1). Experiments are then updated from the model in an iterative process. Experimental results of the initial design show increased productivity of lactate and decrease of the batch time of the process is possible (Fig. 2).

The ultimate goal of this project is to develop a tool that can be used in addition to genetic engineering, in cases where modifications cause growth impairment to the designed strain.

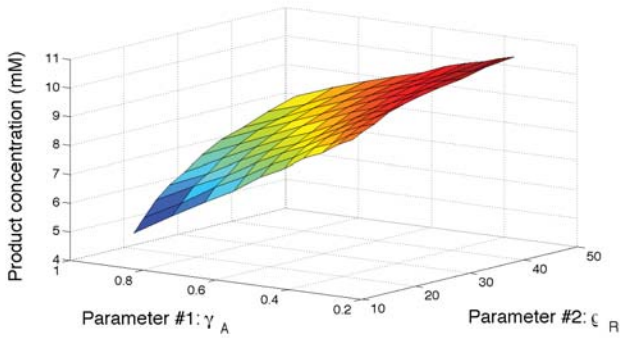


Fig. 1: Product concentration as a function of the toggle switch parameters.

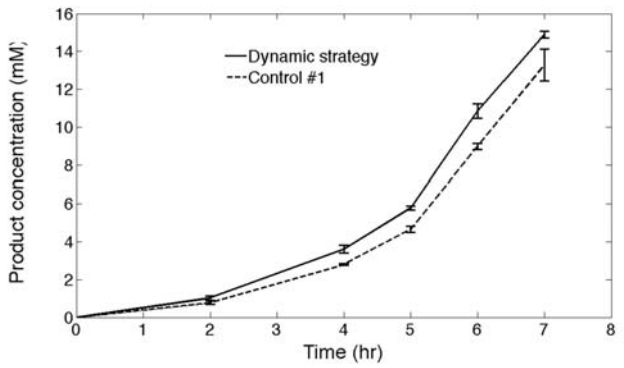


Fig. 2: Product concentration profile (comparison of the dynamic strategy to the control experiment).

Yaldah Azimi
Ph.D. Student

M.Sc., 2008, University of Toronto
B.A.Sc., 2006, Sharif University of Technology

Supervisor: Ramin Farnood
Co-Supervisor: Grant Allen

Biofloc structure and composition in relation to UV light disinfection of secondary treated effluents

Ultraviolet (UV) disinfection is a well established, environmentally friendly technology for disinfection of secondary wastewater effluents. However, its effectiveness decreases in the presence of suspended microbial flocs formed in the activated sludge process. The decrease in the UV disinfection efficiency at high UV doses could be detected in a typical UV dose-response curve. As illustrated in Fig. 1, the presence of microbial flocs leads to a UV dose where the survival ratio of microorganisms exhibits a near plateau or tailing region.

My project's focus is to understand the effect of physical, chemical and microbial structure of microbial flocs on UV disinfection of municipal wastewaters.

Based on flocs having a double layer structure composed of a dense core material surrounded by a loose outer shell, we hypothesized that the dense core contributes to a larger extent to tailing in UV disinfection. By using hydrodynamic shearing the dense cores were extracted from the flocs and their UV disinfection kinetics were compared to that of flocs. The results indicated that cores are harder to disinfect (Fig. 1).

In addition, we are collaborating with the National Water Research Institute (NWRI) in Burlington, where I am running pilot studies of activated sludge secondary treatment reactors under various conditions and comparing the sludge flocs UV disinfection, composition, and mechanical strength. An example of reactors from which samples are taken from is shown in Fig. 2 (biological nutrient removal system UCT type vs. conventional aerobic activated sludge system).

The outcome of this project will provide the basis to develop better treatment processes for water reuse applications and discharges.

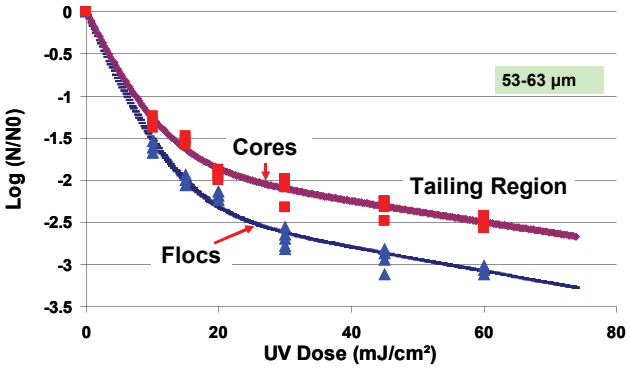


Fig. 1: UV dose response curves showing the tailing phenomenon, as well as a higher tailing level for cores showing that they are harder to disinfect.

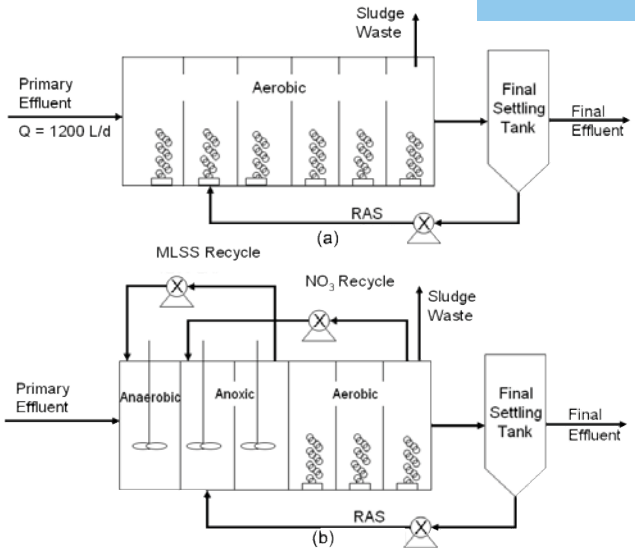


Fig. 2: Process schematics of a conventional fully aerobic activated sludge system (a) and a biological nutrient removal system (BNR-UCT) (b) , which in addition to organics also removes nitrogen and phosphorus.

Research Highlights

Azimi. Y. *et al.* (2011). Tailing phenomenon and the effect of secondary treatment process conditions on UV disinfection. *84th Annual Water Environment Federation Technical Exhibition and Conference (WEFTEC)*, Los Angeles, CA

Azimi. Y. *et al.* (2011). Tailing in ultraviolet disinfection of wastewater. *WEF Disinfection 2011*, Cincinnati, OH

Azimi. Y. *et al.* (2011). Cause of tailing in UV disinfection and the effect of sludge retention time and process type. *The 64th Canadian Water Resources Association (CWRA) National Conference*, St. John's, NL

Azimi. Y. *et al.* (2011). "Surface roughening by wetting of coated papers at meso- and micro-scale". *Appita Journal* 64(5):428-435

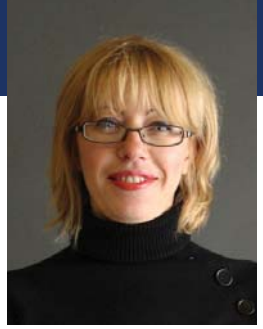
Research Highlights

Beloglazova N.*et al.* (2011). "Structure and activity of the Cas3 HD nuclease MJ0384, an effector enzyme of the CRISPR interference". *EMBO J.* 30(22):4616-4627

Babu M., Beloglazova N. *et al.* (2011). "A dual function of the CRISPR-Cas system in bacterial antivirus immunity and DNA repair". *Mol. Microbiol.* 79(2):484-502

Beloglazova N. *et al.* (2008). "A novel family of sequence-specific endoribonucleases associated with the clustered regularly interspaced short palindromic repeats". *J. Biol. Chem.* 283(29):20361-71

Beloglazova N. *et al.* (2011). Cas1,Cas2, Cas3...: structure and activity of the core CRISPR nuclease (poster). *International Conference on Structural Genomics*, Toronto, ON



Natalia Beloglazova
Postdoctoral Fellow

Ph.D., 2003, Institute of Chemical Biology & Fundamental Medicine, Novosibirsk, Russia
M.Sc., 1994, Novosibirsk State University, Novosibirsk, Russia

Supervisor: Alexander Yakunin

Molecular mechanisms of the CRISPR-based anti-viral defence system

Most prokaryotic genomes contain structures known as Clustered Regularly Interspaced Short Palindromic Repeats (CRISPRs) and, associated with them, large groups of uncharacterized proteins (CAS proteins). CRISPRs and CAS proteins represent an adaptive and heritable RNA-mediated defence system which targets invading phages and exogenous nucleic acids in three steps: (1) adaptation via integration of viral or plasmid DNA-derived spacers into the CRISPR locus, (2) expression of short guide CRISPR RNAs (crRNA) consisting of unique single repeat-spacer units, and (3) interference with the invading cognate foreign genomes (Fig. 1).

To date, the CRISPR system comprises several thousand uncharacterized proteins found in most bacteria, including many pathogens. Most Cas proteins have been predicted to have nuclease activity, but the molecular mechanisms of their activity remain unclear.

My research has been directed towards understanding the molecular mechanisms of the bacterial defence system. I am particularly interested in studying CRISPR-associated nuclease proteins from diverse organisms participating in different stages of CRISPR interference. Currently I am focused on biochemical characterization and structural analysis of main Cas proteins with the aim of elucidating their role in the CRISPR mechanism.

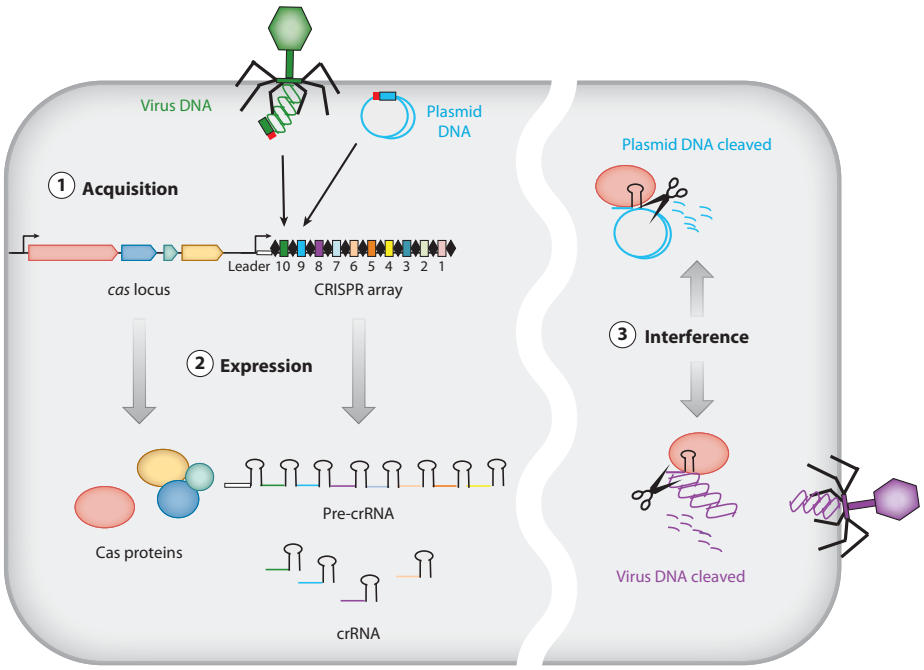


Fig. 1: Features of the CRISPR-Cas adaptive immune system (from Bhaya et.al., 2011).

Sofia Bonilla
Research Assistant

M.Sc., 2010, University of New South Wales
B.A.Sc., 2008, Universidad De Los Andes

Supervisor: Grant Allen

Research Highlights

Bonilla, S. (2010). Isolation, Identification and characterization of hydrocarbon degrading bacteria from seawater. M.A.Sc. thesis, *Faculty of Science*, University of New South Wales

Enhancing sludge dewatering through enzyme conditioning

The management of wastewater sludge is a major cost in wastewater treatment that increases with the volume processed. Therefore, there is considerable value in maximizing dewatering to reduce the volume of sludge for disposal or other uses. In the pulp and paper industry, disposal practices of wastewater sludge are mainly in landfill and/or incineration. Landfills are becoming scarcer and more expensive due to environmental concerns, and incineration process efficiency is affected by the high moisture content of sludge. **In either scenario, industry will benefit if sludge dewatering is improved.**

Enzymes have been reported to improve dewaterability of sludge. However, it is not fully understood how enzymes change floc structure in sludge affecting dewatering characteristics. Therefore, there is great value in understanding floc changes during enzymatic treatment and their relation to dewatering in order to design and optimize a conditioning system to increase dewaterability of wastewater activated sludge in the pulp and paper industry. There are also significant opportunities to look for new enzymes that can efficiently dewater wastewater sludges.

Various enzymes with different activities will be screened for dewatering potential: glycosyl hydrolases, esterases, proteases, phosphatases, nucleases, oxidases. **The goal of this project is to design a novel enzyme conditioning treatment to enhance secondary sludge dewatering in pulp and paper mills.**

Using Capillary Suction Time (CST) as a measure of dewaterability, we have found that a treatment with lysozyme improves dewaterability of sludge by approximately 40% (Fig. 1). This is explained by the reduction of surface area and increase in floc size, limiting water attachment to solids (Fig. 2).

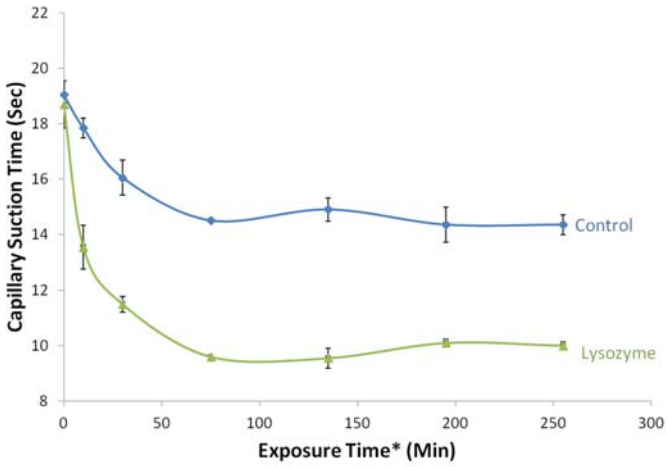


*Fig. 1: Lysozyme treatment effect on sludge dewaterability. *Incubation at 37°C and 75 rpm.*

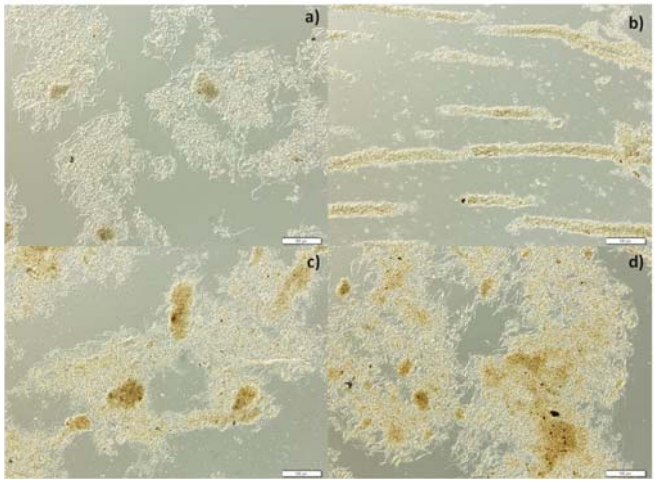


Fig. 2: Microscope images of floc changes using lysozyme treatment in sludge. a) 0 min. b) 35 min. c) 75 min. d) 135 min. Scale bar 100 µm.

Research Highlights

Bourdakos, N. and R. Mahadevan (2010). Defined consortia for the degradation of complex wastes in microbial fuel cells (poster). *CsChE Ontario Quebec Biotechnology Meeting,* Montreal, QC

Bourdakos N. and R. Mahadevan (2011). Defined consortia for the degradation of complex wastes in microbial fuel cells (poster). *3rd International Microbial Fuel Cell Meeting*, Leeuwarden, Netherlands

Nicholas Bourdakos
M.A.Sc. Student

B.A.Sc., 2009, University of Toronto

Supervisor: Radhakrishnan Mahadevan

Reducing limitations from microbial fuel cells using a defined culture

One of the most important concerns that we face as a society is finding affordable and environmentally sound sources for energy. Another serious concern is the remediation and degradation of the wastes that we produce. This project's aim is to address both of these concerns (on a small scale) using microbial fuel cells (MFCs). **In particular, the goal of this project is to develop a co-culture MFC that is capable of removing some of the limitations faced by MFC technology.**

MFCs function in the same way as traditional fuel cells, in that they oxidize some fuel source on an anode electrode and reduce an electron acceptor on the cathode. The electrons from the anode travel to the cathode through an external circuit, where power can be harvested. In the case of MFCs, microbes drive the anodic process, typically consuming acetate as a substrate (Fig. 1). The most promising microbe for generating power on MFC anodes is *Geobacter sulfurreducens*, whose preferred substrate is acetate.

Acetate however is already easily remediated in wastewater, and is not always found in conventional wastewater streams. **In order to expand the possible uses of MFCs for power production, it is critical that they be able to degrade a wide variety of substrates.** With this in mind, we are currently investigating the effect of adding *E. coli* to the MFCs in order to break down more complex substrates, such as glucose or glycerol, into substrates that can be converted to power by *Geobacter*. The *E. coli* has the added benefit of scavenging oxygen from the cell, which is toxic to *G. sulfurreducens*, and this process allows for the removal of some of the more expensive MFC components. The MFC system used in this project can be seen in Fig. 2.

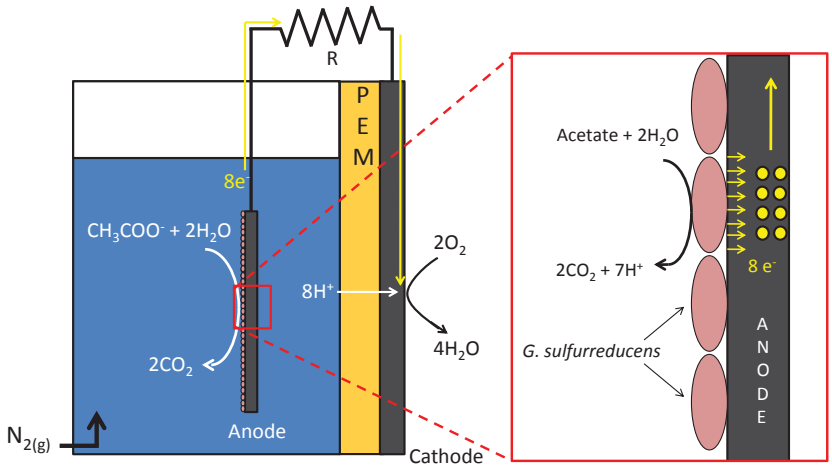


Fig. 1: Schematic of an air cathode MFC using acetate as substrate.

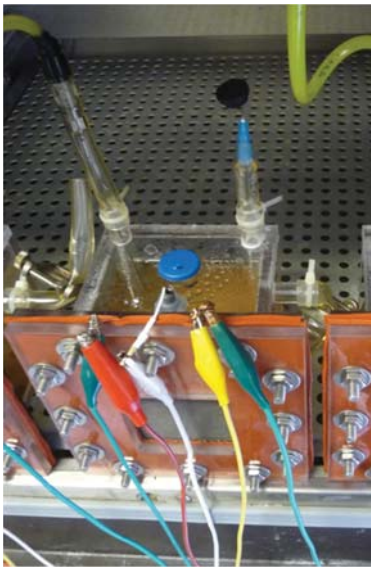
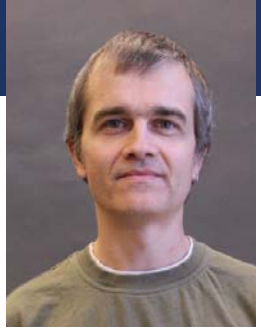


Fig. 2: MFCs containing defined co-culture of G. sulfurreducens and E. coli.



Greg Brown
Technician

H.B.Sc., 1986, University of Western Ontario

Supervisor: Alexander Yakunin

Enzyme discovery, profiling and characterization

Our group has developed a set of general enzyme screens for the functional annotation of purified unknown proteins. Purified proteins are assayed against general chromogenic substrates individually or in pools using 96-well plates and spectrophotometry. We assay for several broad enzymatic activities: phosphatases, carboxylesterases, dehydrogenases, oxidases, proteases and phosphodiesterases.

For proteins with enzymatic activity identified in the general screen, we have a set of secondary screens with which we can determine an enzyme's substrate profile and possibly identifiy the enzyme's specific or natural substrate. For example, we have a phosphatase screen composed of 95 naturally occurring phosphorylated metabolites (Fig. 1). Activities identified from substrate pools can be deconvoluted to identify the specific substrate utilized by the enzyme.

Once optimal *in vitro* substrates have been identified for a particular enzyme, we do a biochemical workup; determining optimal pH, temperature, divalent cation requirements and other cofactors. With these parameters we can determine the enzyme's kinetic parameters such as maximal rate of reaction (V_{max}), catalytic constant (k_{cat}) and substrate affinity (K_m) (Fig. 2).

Site-directed mutagenesis (SDM) is used to map the active site of an unknown enzyme. By comparing the amino acid sequence of a putative enzyme to amino acid sequences of known enzymes with similar activities, we choose conserved amino acids and mutate them to Ala or any other amino acid. This is done by changing individual nucleotides (SDM) in the gene encoding the protein. If a particular mutation inactivates the enzyme's activity, that amino acid is part of the enzyme's active site. For the catalytically active mutant proteins, the analysis of their kinetic parameters can help to determine if these residues contribute to substrate binding.

Research Highlights

Brown, G. *et al.* (2008). "Functional and Structural Characterization of Four Glutaminases from *Escherichia coli* and *Bacillus subtilis*." *Biochemistry* 47(21):5724-35

Brown, G. *et al.* (2009). "Structural and Biochemical Characterization of the Type-II Fructose-1, 6-bisphosphataes GplX from *Escherichia coli*." *Journal of Biological Chemistry* 284(6):3784-92

Babu, M. *et al.*(2011). "A Dual Function of the CRISPR-Cas System in Bacterial Antivirus Immunity and DNA Repair." *Molecular Microbiology* 79(2):484-502

Kuznetsova. E. *et al.* (2006). "Genome-wide Analysis of Substrate Specificities of the *Escherichia coli* Haloacid Dehalogenase-like Phosphatase Family." *Journal of Biological Chemistry* 281(47):36149-61

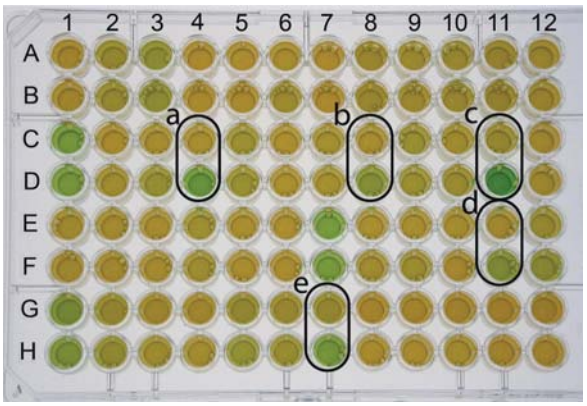


Fig. 1: Assay for natural phosphatase activity. Each well contains one of 95 naturally occurring phosphorylated metabolites. A putative phoshphatase, identified in the general screen, is added to each well. If it cleaves the phosphate group from the substrate, the solution will turn green identifying it as a potential cellular substrate of the enzyme.

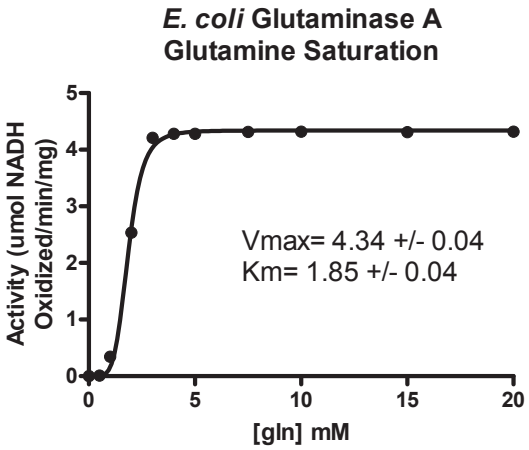


Fig. 2: Substrate saturation curve for E.coli Glutaminase A. Glutaminase cleaves an amino group from the amino acid glutamine and is important for cellular nitrogen metabolism.

Research Highlights

Brunetti, J.A. and A. P. McGuigan (2011). Shear-induced morphological response of human umbilical vein endothelial cells in microchannels (poster). *TERMIS North America 2011*, Houston, TX

Jonathan Brunetti
M.A.Sc. Student

B.Sc. Eng., 2010, Queen's University

Supervisor: Alison McGuigan

Shear-induced morphological response of human endothelial cells in microchannels

Engineered tissues, which are designed to replace or improve the function of damaged organs and tissues, can reduce the need for donors. A major obstacle to the development of engineered tissue is the difficulty of establishing a functional vascular network to deliver oxygen and nutrients. Endothelial cells form a cell monolayer that lines the inner walls of blood vessels, which coordinate into organized structures during development. **Understanding endothelial cell organization will improve vascularisation of engineered tissue.**

Endothelial cell organization in a monolayer is strongly influenced by mechanical forces such as shear stress. Shear stresses cause changes in cell morphology, contributing to changes in cell shape and function. In addition, cell interaction is involved in the response of the cell monolayer. Morphological response to force across a cell monolayer often results in a coordinated cell response, which is likely a result of biological signaling mechanisms. **We aim to investigate the organization of endothelial cell monolayers under flow.** We will examine the role of signalling proteins in the cell mechanobiological response, that is, the reorganization due to interaction with neighbouring cells and the applied shear stress.

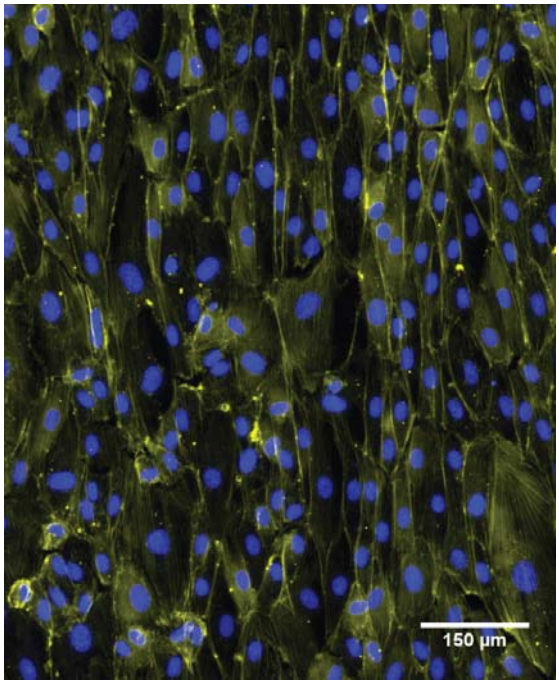


Fig. 1: Fluorescent staining showing HUVEC oriented perpendicular to flow after exposure to shear stress for 48 hrs. Scale bar 150 um; direction of flow from left to right; blue: nuclei, yellow: f-actin.

Resmi Capron
Postdoctoral Fellow

Ph.D., 2003, Nat'l Research Center for Plant Biotech., Indian Agricultural Research Institute
M.Sc., 1997, Govind Vallabh Pant University for Agriculture and Technology
B.Sc., 1994, Kerala Agricultural University

Supervisor: Emma Master

Engineering plant fiber using carbohydrate active enzymes of microbial origin

The plant cell wall is a heterogeneous, complex and highly dynamic structure predominantly composed of diverse polysaccharides. Structural polysaccharides form a crosslinked macromolecular network determining cell shape and wall tensile properties, crucial components of fiber quality. Understanding of the formation and modification of the cell wall is a fundamental step toward the creation of plants with desirable cell wall compositions. **Furthermore, it would allow for more efficient utilization of these plants for innovative industrial applications such as biofuels and biomaterials.** *In planta* expression of polysaccharide modifying enzymes represents a promising strategy to examine the contributions of these polysaccharides to cell wall structure and function. More recently, expression of microbial hydrolases has been proposed as a viable approach for improving bioconversion of plant biomass.

To that end, an experimental system for routine, systematic creation of *A. thaliana* transgenic plants expressing different cell wall hydrolases and esterases has been established in our lab. A collection of transformed plants with modified cell wall compositions is currently being characterized. Initial analysis of this set of plants demonstrated that microbial enzymes expressed and localized to the apoplast can modify plant cell wall composition without deleterious effects, thus providing a toolset to investigate how cell wall structure can be manipulated toward industrial applications.

In particular, our screen has successfully identified transgenic plants with significant differences in cell wall digestibility. I am now interested in exploring the compositional and structural changes in these lines. For instance, I am applying light and confocal microscopy, combined with well established staining techniques, to understand how changes in cell wall polysaccharides affect fiber cell morphology as well as spacing and orientation of cellulose macrofibrils. I am also working closely with others in BioZone to develop ToF SIMS for spatial and compositional analysis of transgenic *Arabidopsis* stem.

Research Highlights

Radhamony R.N., Prasad, A.N., and R. Srinivasan (2005). T-DNA insertional mutagenesis in *Arabidopsis*: a tool for functional genomics. *Electronic Journal of Biotechnology* 8(1):82-106

Radhamony, R.N. and S.M. Theg (2006). Evidence for an ER to Golgi to chloroplast protein transport pathway. *Trends in Cell Biology* 16(8):385-387

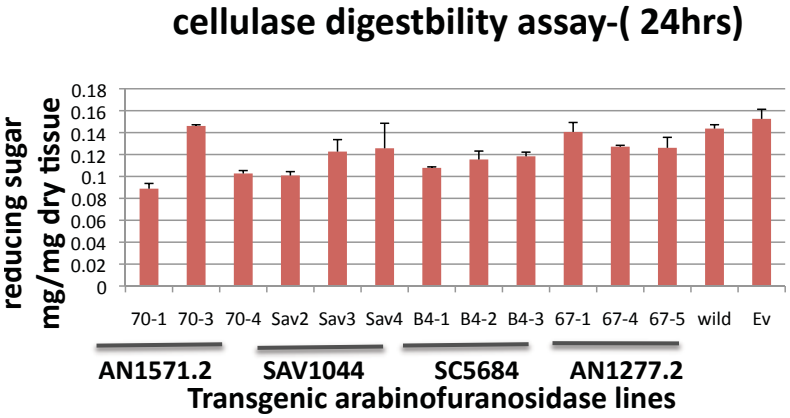


Fig. 1: Transgenic lines expressing arabinofuranosidases from different microbial sources shows changes in sugar release after 24 hrs of cellulase treatment. AN- Aspergillus niger; SC- Streptomyces coelicolor; SA- Streptomyces avermitilis.

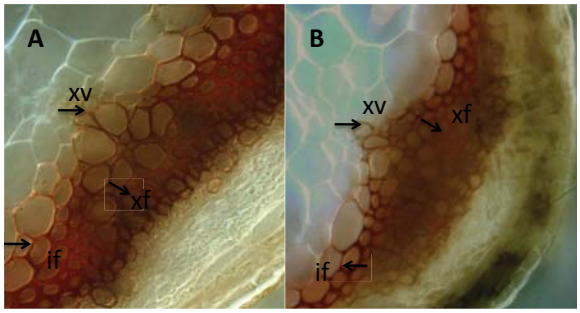


Fig. 2: Maule staining reveals variations in fiber cell organization and lignin distribution in stem sections of (A) wild type and (B) transgenic line expressing arabinofuranosidase gene from Streptomyces avermitilis. Red stain represents S lignin and yellow/brown stain is G lignin. xv-xylem vessels: xf-xylem fiber: if- interfascicular fiber.

Cautha, S.C. and R. Mahadevan (2011). Model based design of *Saccharomyces cerevisiae* for improved L-tyrosine production. *61st CSCHE*, London, ON

Cautha, S.C. and R. Mahadevan (2011). *In silico* design of *Saccharomyces cerevisiae* for improved amino acid production. *SIM Annual Meeting and Exhibition*, New Orleans, LA

Dubey, S., Cautha, S.C., Sambaraju, S., Arya, V. and V.S. Chakravarthy (2009). "A phase dynamic model of systematic error in simple copying tasks". *Biological Cybernetics* 101(3):201-213

Sarat Chandra Cautha
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B.Tech., 2009, Indian Institute of Technology Madras

Supervisor:
Radhakrishnan Mahadevan

Model-driven design of *Saccharomyces cerevisiae* for improved L-tyrosine production

Tyrosine or 4-hydroxyphenylalanine is an aromatic amino acid with several industrial applications. It is used as a dietary supplement and is an important precursor for the production of many high-value chemicals like L-dopa, a drug used to treat Parkinson's disease.

As a Master's student, my research focuses on designing a tyrosine producing strain of *Saccharomyces cerevisiae.* Microbial production of tyrosine provides an eco-friendly alternative to the conventional chemical synthesis. Traditional approaches to develop microbial strains for tyrosine overproduction have relied on amplifying only the enzymatic steps within the shikimate pathway. However, in order to obtain maximum possible yield of tyrosine, it is necessary to engineer the genetic network such that most of the carbon consumed is utilized for tyrosine production.

For this project, we designed a tyrosine-producing yeast strain using computational algorithms. We used a bi-level optimization framework that suggested the necessary genetic manipulations required for maximizing the production of tyrosine while assuming that the strain is homeostatic.

The mutant strain that we designed using the algorithm contained genetic modifications that couple the production of tyrosine to cell growth. In other words, the genetic network of the mutant is altered such that tyrosine becomes an obligatory by-product of growth, which is not the case in wild-type yeast. The predicted strategies seemed to focus mainly on i) altering the central carbon metabolism to increase the intracellular pools of the two main precursors to the shikimate pathway, erythrose-4-phosphate and phosphoenolpyruvate, and ii) minimizing the production of competing aromatic amino acids, tryptophan and phenylalanine.

My current focus is to incorporate kinetic parameters into the model framework and select the best possible targets out of all the suggested genetic manipulations for experimental validation. **The final goal of this project is to be able to design a strain of *S.cerevisiae* that can produce the maximum possible yield of tyrosine with minimum possible genetic modifications.**

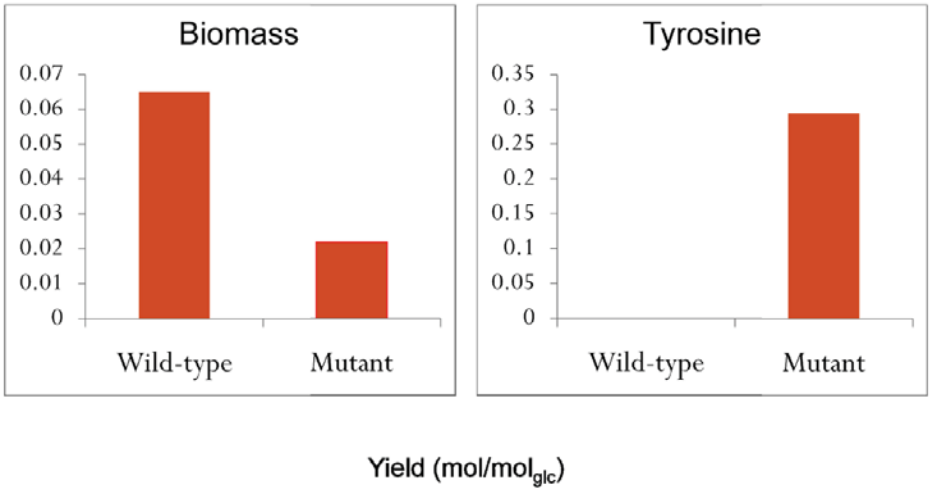

Fig. 1: Model predicted tyrosine and biomass yields for wild-type and mutant strains.

Johanna Chan
M.A.Sc. Student

B.A.Sc., 2010, University of Waterloo

Supervisor: Levente Diosady
Co-Supervisor: Edgar J. Acosta

Microalgae milking with microemulsions

Escalating oil prices and global warming issues have increased interest in using algae as an alternative to petroleum based fuels. Traditionally, sources such as soybeans and corn oil are used to produce biodiesels. However, microalgae have a greater lipid content and the potential to provide more energy per area of crop land. **As a carbon neutral and renewable source, microalgae would be a beneficial fuel alternative.** Extracted lipids can be used for biofuels or nutraceuticals.

Currently, the main methods used to extract oil from microalgae require sacrificing the algae. A major bottleneck in the production of lipids from microalgae is the regeneration and utilization of biomass. Ideally, the biomass is preserved and continuously reused to extract lipids, also known as, 'microalgae milking'. **The objective of my project is to develop a lipid extraction method using microemulsions**, to avoid cell death.

A microemulsion consists of a surfactant, an oil component, an aqueous component, and possibly linkers. To achieve thermodynamic stability, four types of microemulsions can form: type I (water in oil), type II (oil in water), type III (bicontinuous, three-phase), and type IV (bicontinuous, single-phase). Three of these types are shown in Fig. 1.

This project has two areas of focus: lipid yield and biocompatibility. First, a formulated microemulsion was used to extract lipids from the algae. The lipid yield from this method was compared to the yield obtained by traditional extraction techniques. Preliminary results show that Type I microemulsions can achieve similar or better lipid yields than hexane or ethyl caprate. As well, the carotenoids extracted from the microalgae can be quantified using UV-visible spectroscopy. In the second phase, the biocompatibility between the algae and microemulsion will be tested by quantifying cell death after microemulsion exposure.

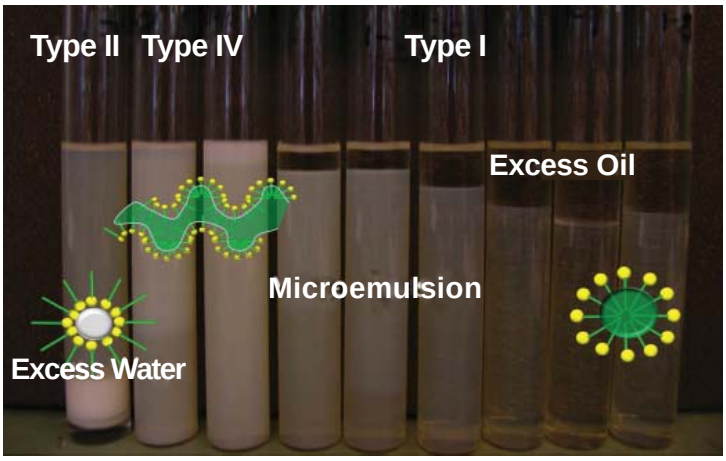

Fig. 1: Microemulsion phase scan. As the ratio of the surfactant to hydrophilic linker increases, the solubility of the micromeulsion changes.

Research Highlights

Chan, J., Chu, C., Diosady, L.L., and E.J. Acosta (2011). Microalgae milking with microemulsions (speaker). *61st Canadian Chemical Engineering Conference*, London, ON

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Amow, G., A. Parisien, and J. Chan. (2008) In investigation of the physical properties of the $La_4Ni_{3-x}B_xO_{10-\delta}$ system (B=Cu, Fe) for solid oxide fuel cell cathodes. *8th European Solid Oxide Fuel Cell Forum,* Lucerne, Switzerland

Research Highlights

Diosady L.L., Lei, X., and B.-K. Chen. US Patent 8,048,463 (2011). "Production of high quality protein isolated from oil seeds"

Diosady L.L. Lei, X., and B.-K. Chen. US Patent 6,905,713 (2005). "Production of high quality protein isolates from defatted meals of *Brassica* seeds"

Bih-King Chen
Research Associate

M.A.Sc, 1989, University of Toronto

Supervisor: Levente Diosady

Process development for protein isolation from oil seeds

Development of processes for oil seeds has been one of our group's major research trends. Currently, we are focusing on protein isolation and oil extraction from different varieties of mustard seed. **The driving force behind this work is that, despite being a popular food ingredient and condiment, the nutritional value of mustard has not been fully exploited as yet.** Furthermore, mustard may also play a substantial role in sustainable energy generation.

To produce high quality protein isolate, a series of well-designed unit operations is required, namely oil extraction, protein dissolution, chemical treatment, centrifugation, membrane filtration (ultrafiltration and diafiltration), isoelectric precipitation, and drying. Even on the bench scale, each individual step must be optimized and carefully monitored to ensure the desired quality of the final product.

Once a bench-scale experimental process has been finalized, scale-up tests are required to obtain engineering parameters that allow

- determination of the technical and economic feasibility of the process,
- selection of appropriate production-scale equipment,
- optimization of unit operations,
- quality assurance and yield consistency of the final products.

We have done pilot-plant tests in several facilities in Canada and the U.S. The products are excellent binders for meat products. Flavour and texture are indistinguishable between meat products prepared with mustard protein isolate and those made with a standard soy protein isolate currently used by the industry.

Our process is readily scalable with no spoilage of intermediate products, which demonstrates the process' stability. Moreover, **the yield consistency and the quality of the final products proved the technical feasibility of the process.** We are now exploring aqueous extraction that recovers the mustard oil as biodiesel, while producing high-valued protein products for the food industry.

Fig. 1: Membrane filtration units used for our pilot-plant experiments at The Food Protein R&D Center, Texas A&M University, with 300-gal reaction tanks in the background.

Fig. 2: Jacketed stainless steel reaction tanks with 300-gal capacity, also used as holding tanks.

Hong Cui
Technician

M.Sc, 2002, Capital University of Medical Sciences
B.A.Sc, 1996, Capital Normal University

Supervisor: Alexei Savchenko

Large scale protein purification and crystallization

Protein structure plays an important role because the function of a protein is closely tied to its 3-dimensional structure. Our research aims to provide high-quality structural models for fungal enzyme in industrial applications and pathogen proteins in pharmaceutical application.

High quality of protein is essential for protein crystallization

Our technological aims are to express, purify and crystallize proteins on a scale required for structural studies. Large-scale protein expression starts from transformation of recombinant constructs into high-efficiency expression bacterial strains. The Lex Parallel Bioreactor System is used to grow up to 40 bacterial cultures in 1 L format in parallel. Various media and temperatures are tested to optimize growing conditions. The proteins are purified through affinity chromatography by using Ni-NTA beads at either native or denatured conditions. Additional gel filtration columns or ion exchange columns are applied if the protein purity is not high enough. The purity is the most fundamental prerequisite for successful protein crystallization

Crystal hunting — Crystallization initial screen set up

After purification, each concentrated protein is subjected to one or two crystallization screens to search for crystals. The Mosquito crystallization robot is a convenient tool for setting up these screens. Protein crystallization occurs when the concentration of protein in solution is greater than its limit of solubility and the protein is therefore in a supersaturated state. However, even when pure soluble protein is available, producing high-quality crystals remains a major bottleneck in structure determination. *In situ* proteolysis protocols are applied if the protein does not produce crystals or produce poor quality crystals. The proteases employed modify protein molecules in real time and promote protein crystallization. The method is straightforward and effective. Our objective is to produce high-quality proteins and apply *in situ* proteolysis methods to obtain well-diffracting crystals for solving structures.

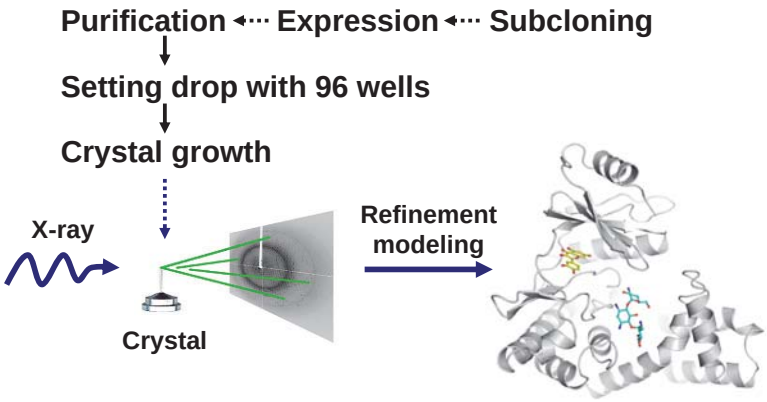

Fig. 1: Determination of Protein Crystal Structure.

Research Highlights

Clasquin, M.F. *et al.* (2011). "Riboneogenesis in yeast." *Cell* 145(6):969-980

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Kuznetsova, E. *et al.* (2010). "Structure and activity of the metal-independent fructose 1,6-bisphosphatase YK23 from *Saccharomyces cerevisiae*". *Journal of Biological Chemistry* 285:21049-21059

Nisa Dar
Bioinformatician

M.Sc. (Bioinformatics), 2008, York University
M.Sc. (Biochemistry), 1999, Punjab University, Lahore Pakistan

Supervisor: Radhakrishnan Mahadevan
Co-Supervisor: Elizabeth Edwards

Identification of functionally important residues of carboxylase enzymes

Carboxylase enzymes catalyze the addition of carbon dioxide (CO_2) to another molecule to create an additional –COOH group. These enzymes are involved in a variety of biological processes e.g., respiration, metabolism, and biosynthesis of amino acids. Carboxylase enzymes can be divided into many subfamilies depending on their structure, cofactors, substrates, and sequence. **My current project focuses on predicting the specificity determining amino acid residues (SDRs) of each carboxylase subfamily.** Results from this project will be used in the following two projects:

1. Identification of benzene carboxylases from the metagenomic sequence pool of benzene degrading cultures.
2. Engineering carboxylase enzymes with desired substrate specificities.

We started with exploring the sequence and functional diversity of carboxylase enzymes by building the sequence similarity networks, followed by manual clustering based on protein names. We identified 19 distinct groups of carboxylases by using BLASTp with e-value ≤ e-136 as a similarity threshold. For instance, Fig. 1 shows a cluster of 14 puruvate carboxylase enzymes. We have also predicted the SDRs of aromatic carboxylases.

Currently, we are clustering the carboxylases into subfamilies based on structure, cofactors, substrate and sequence, collectively. Subsequently, we will perform statistical analysis to find the SDRs of each carboxylase subfamily. The results obtained from these analyses will be released in the form of a web-based database, along with the kinetic parameters of each enzyme, as provided by the BRENDA database (http://www.brenda-enzymes.org/).

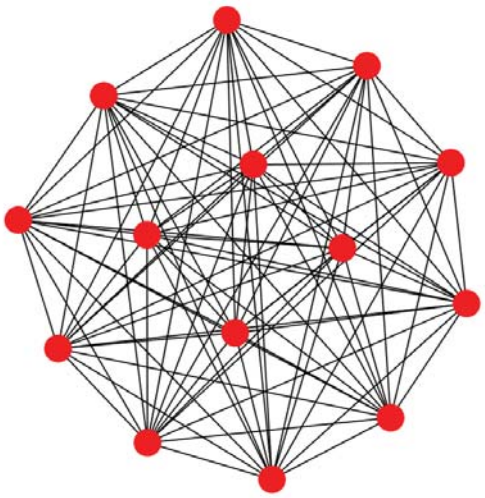

Fig. 1: Sequence similarity cluster of pyruvate carboxylase enzymes (network nodes) connected by similarity (network edges).

Research Highlights

Dar, N., Edwards, E., and R. Mahadevan (2011). Sequence similarity networks reveal conservation and diversity of carboxylase enzymes (poster). *2nd Annual BEEM Meeting,* Toronto, ON

Gfeller, D., Butty, F., Wierzbicka, M., Verschueren, E., Vanhee, P., Huang, H.M., Ernst, A., Dar, N., Stagljar, I., Serrano, L., Sidhu, S.S., Bader, G.D., and P.M. Kim (2011). "The multiple-specificity landscape of modular peptide recognition domains." *Molecular Systems Biology* 7:484

Foxe, J.P., Dar, N., Zheng, H., Nordborg, M., Gaut, B.S., and S.I. Wright. (2008). "Selection on amino acid substitutions in *Arabidopsis*." *Molecular Biology and Evolution* 25(7):1375-1383.

Cheryl Devine
Ph.D. Student

M.A.Sc., 2004, University of Toronto
B.Sc. (Eng.), 2002, Queen's University

Supervisor: Elizabeth Edwards

Characterization of benzene-degrading methanogenic consortia

Benzene is a carcinogenic groundwater contaminant that can be cleaned up *in situ* via bioremediation. While benzene is quickly mineralized aerobically, contaminated aquifers often contain large anaerobic zones where biodegradation occurs much less readily. Under these conditions little is known about the mechanisms of degradation and the organisms involved. **My goal is to identify key benzene-degrading microbes, genes and enzymes in methanogenic enrichment cultures.**

An important characteristic of the benzene-degrading methanogenic cultures is that they are syntrophic. In this type of culture, fermenting organisms gain energy from reactions that are only thermodynamically favorable if the products are kept at very low concentrations, while organisms that achieve this by metabolizing the products depend on the fermenters for food (Fig. 1). As a result of these relationships, we have been unable to isolate a single benzene-degrading organism, and we have used whole community approaches to assign function in these cultures.

Through cloning studies and quantitative PCR analyses, we identified two or more *Desulfuromonadales* species as abundant members of several enrichment cultures. These organisms likely play an important role in benzene degradation, and experiments have been carried out to optimize the growth conditions for these organisms in the presence of benzene.

We used a combined proteomic and metagenomic approach to identify proteins that are expressed in the cultures during benzene degradation. We successfully identified several enzymes associated with the downstream benzoate degradation pathway, along with several novel proteins of unknown function. Some of these may be the enzymes responsible for the initiating benzene degradation. Comparative metagenomic analyses suggest that the mechanism of attack or enzymes involved may be slightly different in these cultures compared to two other benzene-degrading enrichment cultures.

Ultimately, identifying key genes and organisms associated with anaerobic benzene biodegradation will help us to develop biomarkers to monitor benzene bioremediation at contaminated sites.

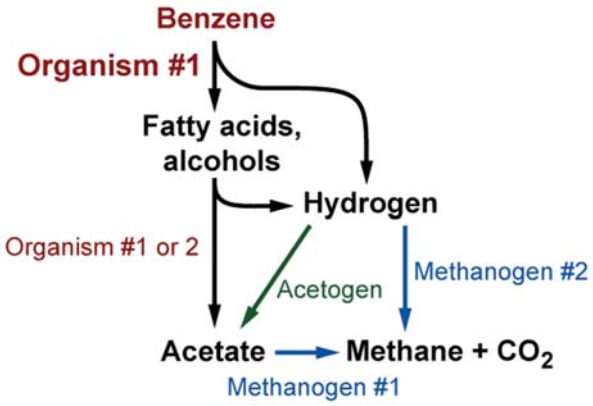

Fig. 1: Schematic showing proposed relationships between organisms in the benzene-degrading methanogenic cultures.

Research Highlights

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Dumitrache, A. *et al.* (2011). Understanding the carbon mass balance and carbon dioxide production profiles of *Clostridium thermocellum* 27405 biofilms (poster). *Genome Canada AGM*, Winnipeg, MN

Alexandru Dumitrache
Ph.D. Student

M.Sc., 2008, Ryerson University
B.Sc., 2006, Ryerson University

Supervisor: Gideon Wolfaardt
Co-Supervisor: Grant Allen

Understanding biofilms of anaerobic thermophilic cellulolytic bacteria: a study towards the advancement of consolidated bioprocessing strategies

In recent efforts towards the emergence of a greener economy, plant biomass is being considered a sustainable source for liquid fuels and biomaterials production, as lignocellulosic material remains a cost-attractive and relatively abundant source of feedstock. A cost-reducing strategy, defined as consolidated bioprocessing (CBP) has been proposed where the conversion of lignocellulose occurs in a single step process via the actions of cellulolytic microorganisms.

The goal of my project is to **characterize biofilms of cellulolytic organisms which posses the desired functions of substrate utilization and product formation that are important towards advancing the CBP technology.**

While traditional lignocellulose conversion studies focus primarily on suspended microbial cell populations, recent evidence suggests that adherent cell populations (biofilms) are central elements in these bioprocesses. Cellulose degrading biofilms differ substantially from those typically studied on inert interfaces, therefore the objective of this project is to provide a detailed description of "transient" anaerobic biofilms developing on solid cellulosic substrates, while also assessing the ability of these microbes to minimize the loss of nutrients extracted from the cellulose interface to the bulk aqueous phase, and to compare their contribution towards substrate hydrolysis and metabolic activity against the free-floating cells that coexist in a typical culture.

We developed a continuous-flow system for *in-situ* detection of cellulose colonization with a strong focus on high resolution confocal laser scanning microscopy (Fig. 1) and qualitatively assayed metabolic activities and behavior of cellulolytic biofilms by monitoring carbon dioxide production profiles and determining the carbon mass balance of cellulose conversion.

My work provides a fresh insight into the cellulolytic bacterium association with cellulose and demonstrates the utility of flow cell technology coupled with fluorescence imaging and and real-time carbon dioxide tracing in studying anaerobic cellulolytic biofilms and consortia.

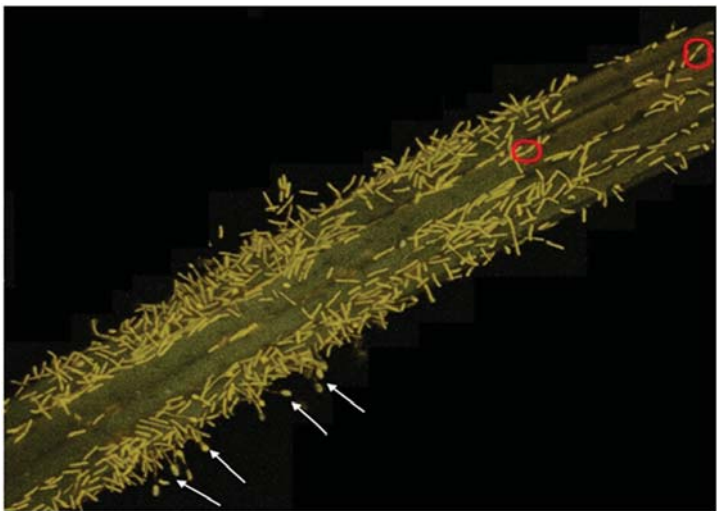
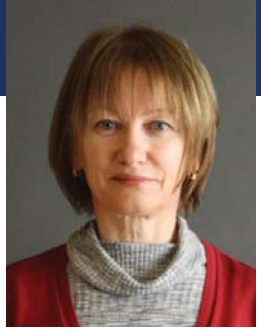


Fig. 1: Confocal laser scanning micrograph of Clostridium thermocellum biofilms on cellulose showing characteristic spores (arrows) and dividing cells (circles).



Elena Evdokimova
Technician

M.Sc., 1983, Moscow Veterinary Academy

Supervisor: Alexei Savchenko

Expression, purification and crystallization of proteins for determination of three-dimensional structure by X-ray crystallography

An important use of three-dimensional structural information of proteins is to uncover clues to a protein's function that are not detectable from sequence analysis, or to get a detailed understanding of its functional mechanism, if it is already known.

Using my extensive experience in the area, I apply new techniques and developments to modify routine analyses for greater efficiencey and effectiveness.

My targets of my interest include potential industrial enzymes, effector proteins that involved in the development of infection and proteins responsible for antibiotic resistance in bacteria.

The extensive use of antibiotics in the treatment of serious bacterial infections has resulted in the emergence of bacterial strains resistant to these antimicrobial drugs. We study two classes of antibiotics and their modifying enzymes: aminoglycosides (kanamycin, erythromycin, lincomycin, etc.) and glycopeptides (vancomycin). Aminoglycoside-modifying enzymes are represented by three families: acetyltransferases, nucleotidyltransferases and phosphotransferases (APHs) (Figs. 1 and 2). In our study we have used inhibitors for APHs, provided by our collaborators. They were selected from functional screening and all were desirable ligands to crystallize proteins to provide direction for further development of antibiotic adjuvants. I have contributed to the structure determination of several APHs (apo-structures as well as substrate and inhibitor contained structures).

Research Highlights

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Taylor P.L. et al. (2008). "Structure and function of sedoheptulose-7-phosphate isomerase, a critical enzyme for lipopolysaccharide biosynthesis and a target for antibiotic adjuvants". *J. Biol. Chem*. 283(5):2835-45.

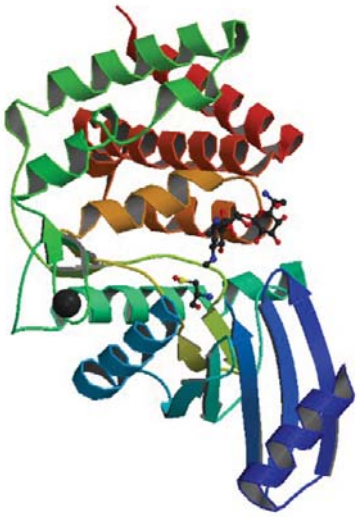

Fig. 1: 3D structure of aminoglycoside-modifying enzyme APH(4)-Ia, responsible for resistance to the atypical amynoglycoside antibiotic hygromycin B, in complex with Hyg B.

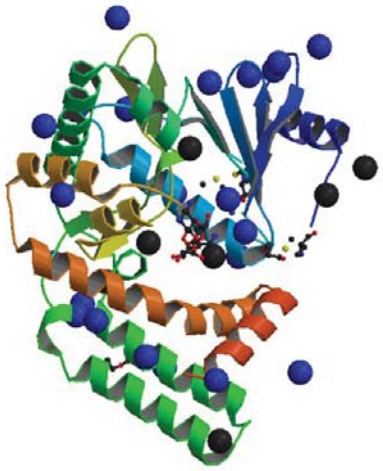

Fig. 2: Crystal structure of aminoglycoside phosphotransferase APH(2")-Id in complex with Kanamycin.

Research Highlights

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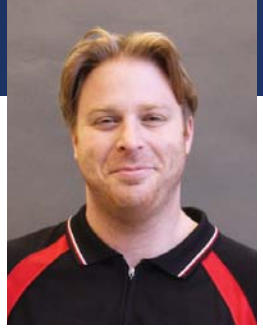


Robert Flick
Technician

M.Sc., 2008, University of Toronto
B.Sc., 2005, McMaster University

Supervisor: Alexander Yakunin

Biochemical characterization of novel enzymes for industrial applications

A large proportion (up to 50%) of predicted genes in sequenced genomes encodes proteins of unknown biochemical function. These proteins represent missing links in various biochemical pathways with the potential for commercial and industrial applications.

I am interested in the characterization of these proteins to identify their function and to further expand our understanding of biochemical pathways.

Using generalized screens designed for testing broad substrate specificity, we have been able to identify the sub-class of enzyme (esterase, phosphatase, nuclease, dehydrogenase, etc.) to which specific proteins belong. Following detection of an activity in the general screens, we further characterize the enzymes using substrate specific assays that employ colorimetric, HPLC, PAGE, and/or Mass Spectrometry-based techniques.

For proteins exhibiting nucleotide hydrolase activity (particularly phosphorylated nucleosides), in addition to using a colorimetric enzyme-coupled assay I have optimized a HPLC-based method with the UV detection. This method allows us to not only detect the presence of phosphohydrolase activity, but also to identify the specific product(s) produced in these reactions by coupling both detection and separation of the reagent(s) and product(s) into one assay (Fig. 1).

In order to bridge the gap between substrate specificity observed *in vitro* and *in vivo*, I have been working on the development of a screen to identify changes in the intracellular metabolome resulting from recombinant protein expression. This method employs extraction of the total intracellular metabolome from a cellular culture, followed by LC/MS separation and detection of metabolites present, providing not only a direct identification of substrate specificity *in vivo* but also a novel assay for substrates and products not detectable by traditional means.

My work is centered on expanding and optimizing the methods of detection for novel enzymatic activities.

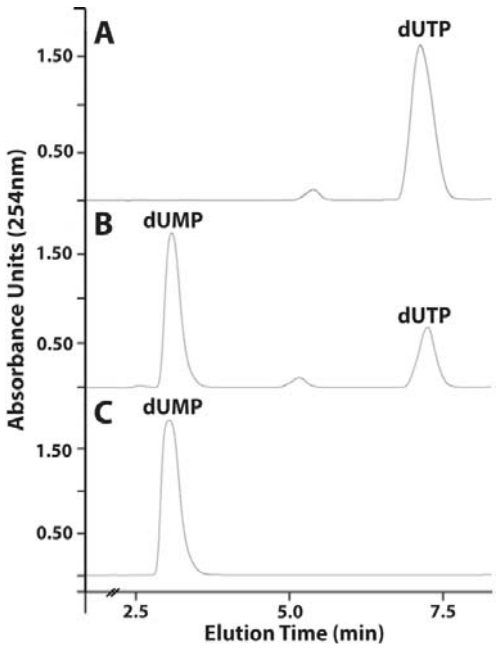
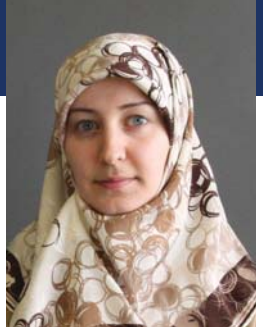


Fig. 1: Assay for dUTPase activity by HPLC at 254nm using a C18 column. (A) Substrate, (B) substrate plus enzyme and (C) product.



Maryam Foumani
Ph.D. Student

M.Sc., 2007, University of Toronto
B.Sc., 2005, University of Tehran

Supervisor: Emma Master

Engineering and production of glucooligosaccharide oxidases for site-directed activation of cellulosic substrates

Oxidation of oligo- and poly-saccharides can alter the rheology and reactivity of corresponding polymers, and facilitate subsequent addition of aliphatic molecules that lead to water-repelling surfaces. Chemical oxidation of cellulose is known to compromise the degree of polymerization and/or crystallinity of the substrates. Accordingly, **the aim of my Ph.D. thesis is to engineer oxidase enzymes that would enable selective oxidation of specific hydroxyl groups on highly functionalized carbohydrates** without arduous protection/deprotection steps, and that would function in mild reaction conditions to overcome losses in the degree of polymerization and crystallinity of cellulosic substrates.

While sugar oxidases have been well described, only three flavin-containing oligosaccharide oxidases have been sequenced to date. One is the oligosaccharide oxidase from *Acremonium strictum* (GOOX), which was targeted for a structure-functional analysis to reveal key amino acids that contribute to the substrate specificity of the enzyme.

A sequence comparison revealed two aromatic amino acids residues close to the enzyme active site (Fig. 1) that were different among the enzyme's homologues that have different substrate selectivity. Replacing these residues with amino acids present in other homologues using site-directed mutagenesis showed enhanced substrate specificity as compared to the wild-type enzyme. For instance, the k_{cat} of enzymes with either Y300A or Y300N substitutions was nearly 2-fold higher on all monomeric sugars tested as well as cello-oligomers and xylo-oligomers. And the W351F substitution improved the catalytic efficiency (k_{cat}/K_m) of the enzyme on galactose. Still, in most cases, the binding affinity of mutant enzymes decreased on sugar substrates, suggesting that these residues participate in stacking interactions with oligomeric substrates.

Having identified residues that contribute to substrate specificity, the current aim of this project is to evaluate the potential of using carbohydrate-binding modules (CBMs) to further increase GOOX activity on polymeric substrates. To date, six chimeras of GOOX and CBMs have been generated, and are being recombinantly expressed for biochemical characterization.

Accordingly, the aim of my Ph.D. thesis is to engineer oxidase enzymes that would enable selective oxidation of specific hydroxyl groups on highly functionalized carbohydrates without arduous protection/deprotection steps. I anticipate that the mild reaction conditions required for oxidase activity will also minimize losses in the degree of polymerization and crystallinity during cellulose derivatization.

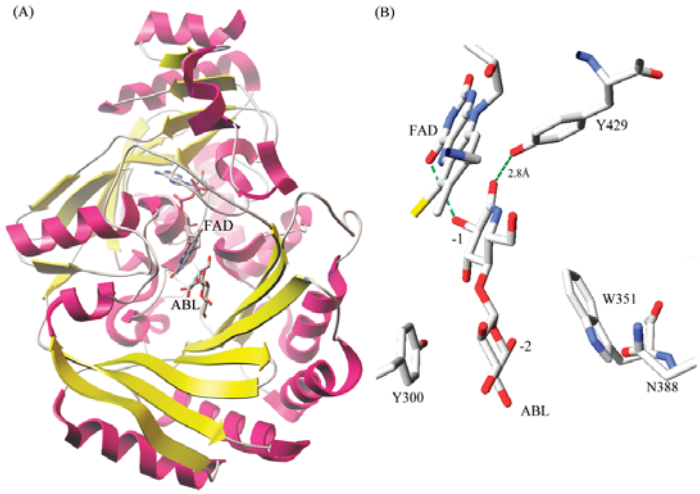


Fig. 1: A) The structural model of GOOX with the active site containing the substrate analogue (ABL) and FAD cofactor. B) The location of key residues, Y300 and W351, that was predicted and experimentally proven to play role in substrate specificity of the enzyme.

Research Highlights

Foumani M. *et al.* (2011). "Altered substrate specificity of the gluco-oligosaccharide oxidase from *Acremonium strictum*". *Biotechnology and Bioengineering* 108(10):2261-2269

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Foumani M, and E.R. Master (2009). Site-directed Mutagenesis for the Production of Oligosaccharide Oxidases with Altered Sugar Specificity (poster). *11th Annual Ontario-Quebec Biotechnology Meeting*, Waterloo, ON

Patent: Foumani, M. *et al.* (2010). Oligosaccharide oxidases derived from *Acremonium strictum*, and uses thereof.

Research Highlights

Gandier, J.A. and E.R. Master (2011). An investigation of the self-assembling properties of class I hydrophobin proteins (poster). *13th CSChe Ontario-Quebec Biotechnology Meeting*, Kingston, ON

2011 recipient of the Canada Vanier Graduate Scholarship

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Gandier J.A. (2009). The encapsulation of *Escherichia coli* KO11 for improved lignocellulosic ethanol production. B.Sc. thesis, *Dept. of Biochemistry, Microbiology and Immunology*, University of Ottawa

Julie-Anne Gandier
Ph.D. Student

B.Sc. Biochemistry (Hons.), 2010, University of Ottawa
B.A.Sc. (Chem. Eng.), 2010, University of Ottawa

Supervisor: Emma Master

Molecular determinants of self-assembly by class I hydrophobins for use in the design of novel enzyme complexes

As the world turns towards greener chemical processes, we face the challenge of developing catalysts, which while being effective, must also be non-toxic and biodegradable. Much work has been invested in engineering the active and/or binding site of an enzyme to develop biocatalysts suited to industry. Often, a cocktail of these enzymes is necessary to achieve a desired product.

Accordingly, the aim of my doctoral work is to lay the foundations for a novel method in which enzyme cocktails would self-assemble in solution to form catalytic "production lines" that could be scaled and rearranged, depending on the number and type of enzyme activities required for a given application. In such an arrangement, the distance an intermediate must travel from one enzyme to the next is significantly reduced, and so the overall reaction rate can be increased, in the case of a series of diffusion-limited enzyme catalyzed reactions.

To achieve this, I am engineering industrially relevant enzymes to contain a class I hydrophobin that can drive the assembly of synergistic activities in solution (Fig. 1). Hydrophobins are small structural proteins that are characterised by their ability to self-assemble at interfaces. The family is subdivided in two classes, I and II. While class II assemblies can be disassociated using alcohol-detergent mixtures, class I hydrophobins are unique in their ability to form highly stable supramolecular structures that are disassembled by strong acids. Class I assemblies are highly resistant to heat, pressure and shear, conditions that characterize many industrial processes; they can also adopt amyloid-like structures similar to those observed in neurodegenerative diseases.

By applying biochemical techniques and molecular dynamics predictions to evaluate class I hydrophobins and corresponding fusion proteins, I will shed light on fundamental aspects of protein self-assembly that can be harnessed to improve the performance of enzymes increasingly used in industrial bioprocesses.

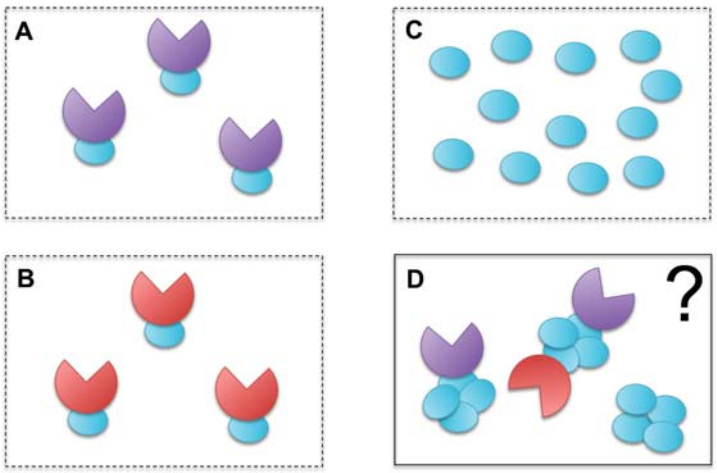

Fig. 1: Industrially relevant enzymes engineered to contain a class I hydrophobin (A,B) will be mixed with wild type hydrophobins (C) at varying ratios to study how these different proteins interact (D)

Srinath Garg
Ph.D. Student

M.A.Sc, 2009, University of Toronto
B.Tech, 2006, Anna University

Supervisor: Radhakrishnan Mahadevan
Co-Supervisor: V.G. Papangelakis

Bioleaching of pyrrhotite tailings for Ni extraction

The extractive metallurgy of nickel, especially from iron sulfide minerals, focuses on using a combination of pyrometallurgical (smelting) and hydrometallurgical techniques. Typically, these ores are concentrated initially through either froth-floatation/magnetic separation and then the concentrates are subjected to hydrometallurgical/pyrometallurgical processing techniques. Processing low grade ores usually leads to the rejection of material termed as mining waste, which may contain valuable metals such as Ni. Thus, if these mining wastes are discarded as is, they indeed represent a loss in the production capacity of Ni and also represent an environmental liability. Furthermore, certain concentrates may be recalcitrant to conventional processing technologies, thereby incurring a high smelter cost. All of these factors have pushed the industry and researchers alike to look for newer and cleaner technologies to treat these recalcitrant ores and concentrates. In this regard, bioleaching or biologically mediated leaching represents an upcoming technology that utilizes the catalytic ability of certain acidophilic iron and sulfur oxidizers to oxidize iron/sulfur and hence solubilize base metals such as Ni in solution (Fig. 1). For example, bacterially-assisted heap leaching of low-grade copper sulfide and nickel sulfide ores is a developing technology that has been applied successfully to the extraction of copper from secondary sulfide minerals such as chalcocite at a number of operations worldwide.

In this regard, my research program focuses on using indigenous and defined cultures of iron and sulphur oxidizing bacteria, including mesophiles and thermophiles, to catalyze the oxidation of an iron sulphide mineral, namely pyrrhotite, that is obtained as a reject from the beneficiation process in Vale's facility in Sudbury, Ontario. Initial studies have focused on evaluating the catalytic influence of two different indigenous iron oxidizers sampled from a cobalt mining site and an acid mine drainage site in Sudbury on the oxidation of iron in solution . In addition, we have completed a proof of concept study on pyrrhotite tailings using the indigenous culture that showed faster kinetics of iron oxidation. Future studies will focus on identifying the factors responsible for limiting the kinetics of pyrrhotite bio-oxidation and optimizing these factors with the goal of making the bioleaching process of pyrrhotite economically attractive.

Research Highlights

Garg, S. *et al.* (2010). Thermodynamic analysis of regulation in metabolic networks using constraint-based modeling. *BMC Research Notes* 3:125

Fang, Y. *et al.* (2010). Direct coupling of a genome scale microbial in silico model and a groundwater reactive transport model. *Journal of Contaminant Hydrology* 122(1-4):96-103

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Garg, S. *et al.* (2011). Comparing the catalytic influence of two indigenous iron oxidizing microbial cultures. *Conference of Metallurgists,* Montreal, QC

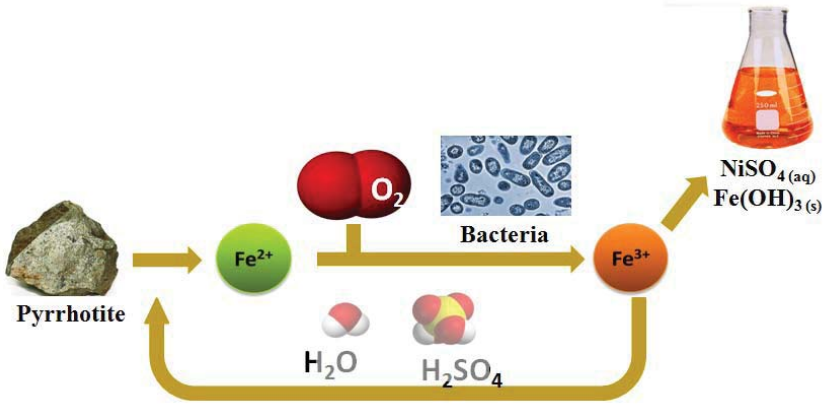

Fig. 1: A general mechanistic overview of the pyrrhotite bio-oxidation mechanism

Research Highlights

Gawand, P. and R. Mahadevan. (2011). Higher order gene deletion mutants give insights into latent pathway activation. *AIChE Annual Meeting*, Minneapolis, MN

Gawand, P., Hyland, P., and R. Mahadevan (2010). Metabolic engineering strategies for substrate co-utilization in *Escherichia coli*. *Metabolic Engineering VIII,* Jeju Island, South Korea

Gawand, P. (2007). Fermentative production of macrolide antibiotic. M.Tech. (Bioprocess Technology) thesis, *Food Engineering and Technology Department,* Institute of Chemical Technology

Pratish Gawand
Ph.D. Student

M.Tech, 2007, Institute of Chemical Technology, Mumbai
B. Tech, 2005, Institute of Chemical Technology, Mumbai

Supervisor: Radhakrishnan Mahadevan

Metabolic engineering for substrate co-utilization

Environmental concerns and the need to reduce our dependence on petroleum are the drivers behind research into sustainable production of biofuels. Use of lignocellulosic biomass as a feedstock for biofuels production is of particular interest, as it does not compete with food production, but it presents numerous technical challenges at various stages of the process. The current project addresses major challenges in the fermentation stage of biofuel production from lignocellulosic feedstock.

Specifically, the project aims to engineer microorganisms for efficient co-utilization of glucose and xylose. Glucose and xylose are the major sugars present in the hydrolysate obtained from lignocellulosic biomass, but fermentation of this mixture is an inefficient process. Use of industrial microorganisms that can efficiently utilize glucose and xylose simultaneously can reduce the fermentation batch times, leading to increased process efficiency.

Rational metabolic engineering of industrial microorganisms has been previously used to construct strains with desired growth characteristics. With the availability of metabolic models, computational design of metabolic engineering strategies is now possible, which can lead to superior and non-intuitive strains (Fig. 1). We developed a novel bilevel optimization algorithm – SIMUP – that could use metabolic network models to predict engineering strategies to force co-utilization of glucose and xylose. By applying the model predicted strategies to *Escherichia coli*, we obtained mutants that could co-utilize glucose and xylose, whereas the wild type strain showed sequential utilization (Fig. 2). The algorithm predicted non-intuitive strategies that could not have been rationally designed. These were experimentally verified, thereby establishing the predictive capability of the SIMUP algorithm.

Current studies are focused on the application of the SIMUP algorithm to other relevant microorganisms, and additional engineering of the previously constructed mutants to improve productivity of important target compounds. **Ultimately, the project is expected to result in superior microorganisms capable of producing biofuels from lignocellulosic biomass with high productivity.**

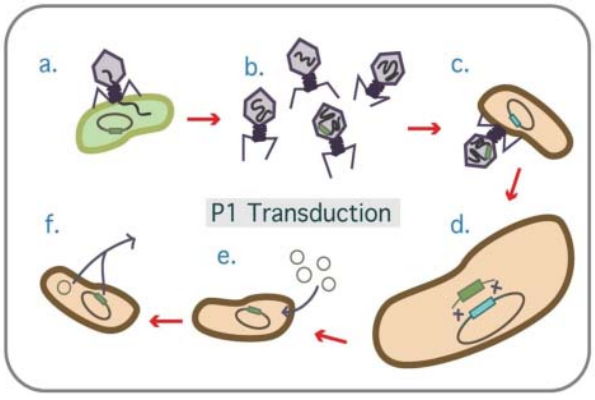

Fig. 1: Construction of model predicted mutants. The mutants are constructed using generalized techniques such P1 transduction.

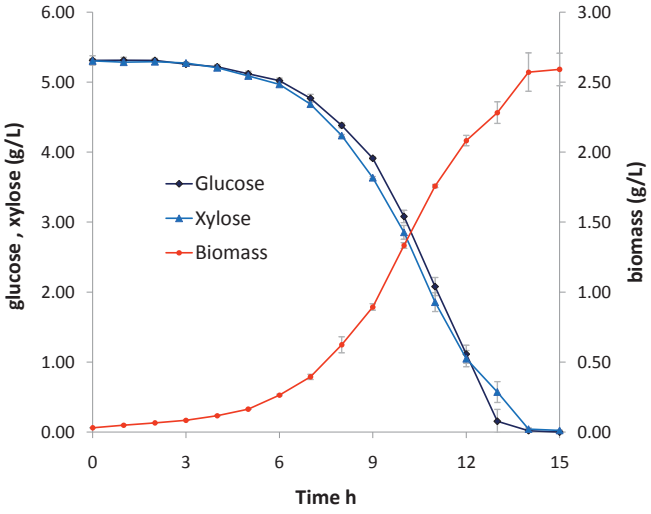

Fig. 2: Batch cultivation results of one of the mutants. The mutant clearly shows co-utilization of glucose and xylose.

Scott Genin
M.A.Sc. Student

B.A.Sc., 2011, Queen's University

Supervisor: Grant Allen

Development of biofilm based photobioreactors to maximize the production of algae

My proposed research involves the design of photobioreactors for the cultivation of algae in biofilms for the production of renewable products. The concept of using agricultural crops for the production of biofuels is well known and shows potential of reducing dependence on fossil fuels. Issues have been raised regarding mass production and competing use of crops as a food source vs. a fuel source. Due to these problems, there has been increased advocacy for the cultivation of microalgae for the production of biodiesel because microalgae are fast growing, have high lipid content, use less land for growth and one can use land that is not useful for food. In spite of all these benefits, microalgae as a source of biofuel is still not economically viable due to the high costs associated with processing and dewatering the resulting light limited, dilute algal suspensions.

Growing algae as a biofilm has several potential advantages over growing algae in suspension. An algae biofilm can be mechanically removed from a surface and has a significantly higher biomass concentration per volume than a suspended culture, and therefore has the potential to dramatically reduce processing costs. However, algal biofilms present unique challenges from a reactor design perspective, as delivery of nutrients and light is complicated by the immobility of the film.

I plan to construct and modify glass surfaces and shapes to enhance light scattering using various techniques such as sandblasting or laser etching. These modified glass surfaces will be placed in photobioreactors operating at different conditions to determine optimum operating parameters. Algae growth will be measured using various techniques such as dry weight measurements and light absorption. Once the technical feasibility of this approach has been demonstrated, I will assess the effect of key parameters in order to optimize algal production at larger scales.

Fig. 1: Photobioreactor.

Research Highlights

Genin, S.N., Hesp, S. A.M., Kodrat, I., and H.F. Shurvell (2011) Modification of asphalt cements with used engine oil residues (presentation). *Peterson Asphalt Research Conference*, Laramine, WY

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Research Highlights

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Roya Gitiafroz
Ph.D. Student

M.A.Sc., 2006, University of Toronto
B.Sc., 1999, Tehran Polytechnic University of Technology

Supervisor: Elizabeth Edwards
Co-Supervisor: Lutgarde Raskin

Microorganisms and metabolic pathways involved in anaerobic benzene biodegradation under nitrate-reducing conditions

The monoaromatic hydrocarbons benzene, toluene, ethylbenzene and the xylenes are major sources of groundwater contamination. Benzene in particular is of great concern due to its toxicity and relatively high water solubility. Anaerobic mineralization of benzene by indigenous microorganisms has attracted attention as a potentially efficient and inexpensive means of remediation of polluted sites. While anaerobic degradation of benzene has been extensively studied in the past, little is known about microorganisms and metabolic pathways involved in this process. **The overall aim of my research is to identify microorganisms and metabolic steps involved in benzene mineralization in nitrate-reducing benzene-degrading cultures.**

We have been maintaining nitrate-reducing benzene-degrading enrichment cultures for over 16 years. To attribute functional roles to the community members, and in particular to identify the organism responsible for the initial attack on benzene, we quantified the change in abundance of the dominant microbial phylotypes during the course of benzene degradation. The results indicated that mineralization of benzene in our enrichment cultures was mediated by syntrophic association between *Peptococcaceae*, which were responsible for the initial attack on and fermentation of benzene, hydrogen-scavenging *Chlorobi*, and nitrate-reducing *Azoarcus* and *Dechloromonas* that oxidize the hydrogen and low molecular weight metabolites of benzene oxidation (Fig. 1). *Anammox* recycle nitrite to nitrate and help stabilize the enrichment cultures by reducing the inhibitory effect of accumulating nitrite.

We employed a meta-transcriptomic approach to attempt to identify genes for enzymes involved in benzene metabolism. We identified carboxylase genes that were specifically transcribed in the presence of benzene. These genes may encode for subunits of a recently proposed putative carboxylase enzyme that is involved in the initial attack on the benzene ring. These results provided compelling evidence for carboxylation as the initial reaction in benzene degradation.

Identification of the microbes and genes involved in the anaerobic degradation of benzene will be useful for creating molecular tools to assess and monitor bioremediation at contaminated sites.

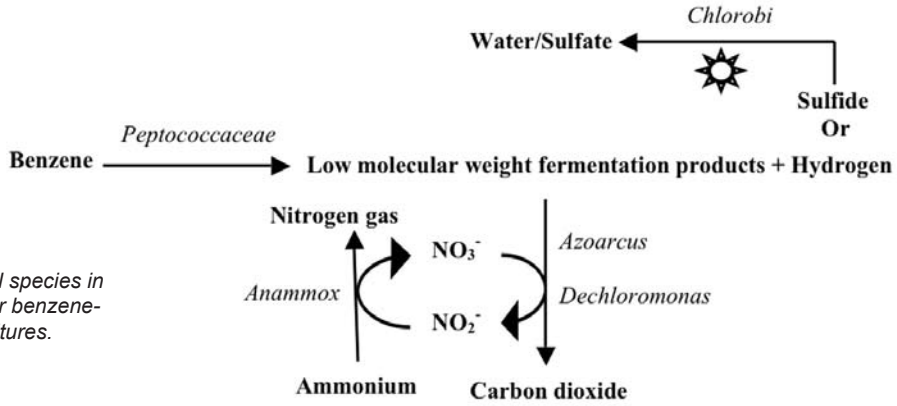


Fig. 1: Proposed role of different bacterial species in the flow of carbon and electrons within our benzene-degrading nitrate-reducing enrichment cultures.

Robyn Goacher
Postdoctoral Fellow

Ph.D., 2009, University at Buffalo
B.S., 2005, Principia College

Supervisor: Elizabeth Edwards
Co-Supervisor: Emma Master

Development of ToF-SIMS for detecting enzyme activity on solid substrates: lignocellulose degradation

Enzymatic conversion of plant biomass comprised mainly of polysaccharides and lignin to fermentable sugars is a key step in the production of renewable fuels and chemicals. The identification and characterization of new carbohydrate-active enzymes is inhibited by our limited ability to rapidly assay the activity of such enzymes on realistic lignocellulosic substrates.

In this project, **we aim to utilize Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS) for the direct measurement of enzyme activity on solid lignocellulosic materials such as wood.** The direct analysis of solid substrates is a feature of ToF-SIMS that obviates the need for soluble substrate analogs or coupled assays to rapidly screen for industrially relevant, lignocellulose-active enzymes.

This work began by identifying ions distinguishing polysaccharides from lignin in ToF-SIMS spectra of wood. Red pine (*Pinus resinosa*) and de-lignified holocellulose and cellulose fractions of red pine were analyzed by ToF-SIMS and the main sources of variation among the wood constituents were elucidated by Principal Component Analysis (PCA). Many ions characteristic of lignin and polysaccharides were identified, which could be used for more rapid characterization of solid wood samples. PCA of high-resolution ToF-SIMS images of solid pine cross-sections also distinguished the lignin-rich middle lamella from the polysaccharide-rich layers in the cell walls, confirming the ion assignments.

Proof-of-principle assays showed ToF-SIMS as capable of providing qualitative screening for enzyme activity on complex solid substrates. Extracted aspen and spruce fibers were immersed in commercial cellulase and laccase enzymes, using water/buffer and denatured enzymes for controls. PCA clearly distinguished cellulase tests from controls through the loss of polysaccharide peaks and relative enrichment of lignin peaks (Fig. 1). Active laccase (with mediator) was indicated by a relative decrease in G- and S-lignin peak intensities and increase in generic aromatic peaks, resulting from the cleavage of hydroxyl and methoxy groups from lignin benzoid units. **Future work will explore the ability of ToF-SIMS to identify activity of recombinantly expressed, putative CAZymes on lignocellulosic feedstocks.**

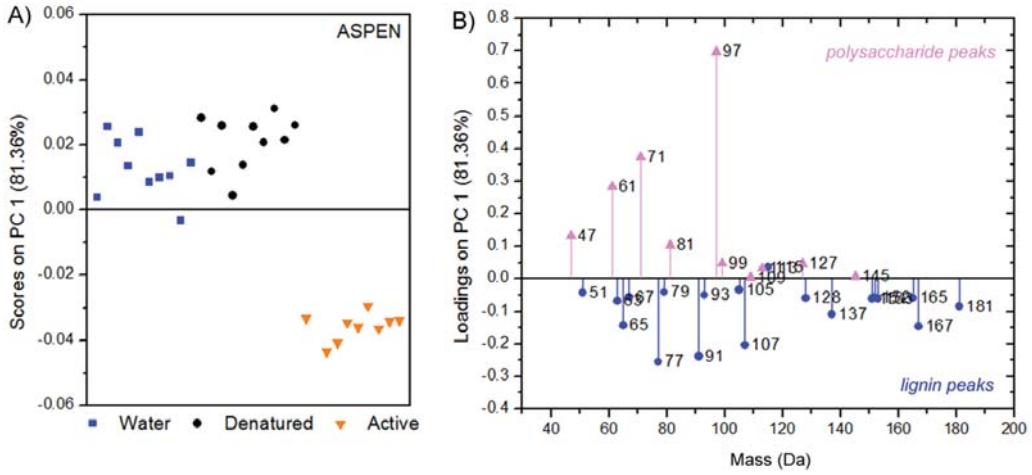


Fig. 1: Scores (A) and loadings (B) for PCA of ToF-SIMS cellulase assay. Aspen treated with active cellulase is distinct from controls by a relative lack of polysaccharide peaks and enrichment of lignin peaks.

Research Highlights

Goacher, R. E. *et al.* 2011). First use of ToF-SIMS for screening assays: enzymes active on wood. *18th International Conference on Secondary Ion Mass Spectrometry,* Riva del Garda, Italy

Goacher, R.E. *et al.* (2011). Distinguishing wood biopolymers by ToF-SIMS (invited speaker). *23rd Annual Workshop on Secondary Ion Mass Spectrometry,* Baltimore, MD

Goacher, R.E. *et al.* (2011). "Expanding the library of secondary ions that distinguish lignin and polysaccharides in ToF-SIMS analysis of wood." *Anal. Chem.* 83(3): 804-812

Mahajan, S. *et al.* (2011). "Mode of coniferous decay by the white rot fungus *Phanerochaete carnosa* as elucidated by FTIR and ToF-SIMS." (accepted) *Appl. Microbiol. Biot.*

Christopher Gowen
Postdoctoral Fellow

Ph.D., 2011, Virginia Commonwealth University
M.S., 2008, Virginia Commonwealth University
B.S., 2006, Clemson University

Supervisor: Radhakrishnan Mahadevan

Systems and synthetic biology for malonyl-CoA production in *Saccharomyces cerevisiae*

Plants exhibit a huge amount of diversity in their native biochemical pathways, producing an enormous range of valuable chemicals with pharmaceutical, nutritional, and industrial importance. Polyketides are one class of these phytochemicals, which themselves cover a wide range of applications. Modern synthetic biology techniques are rapidly being applied to search known and novel plant sources for biochemical pathways that produce interesting polyketides, so that these pathways can be ported to microorganisms such as yeast. Efficient production of these compounds in yeast would significantly reduce their cost and therefore greatly increase availability of important medicines and nutritional supplements. However, efficient microbial production of polyketides will require an optimized and balanced metabolism.

The goal of this project is to apply systems and synthetic biology techniques to engineer *Saccharomyces cerevisiae* metabolism for over-production of malonyl-CoA, a common precursor to polyketides. An optimal strain of *S. cerevisiae* will serve as a platform organism that can host a range of exogenous downstream pathways leading to reliable and efficient polyketide production.

To accomplish this task, we are applying a variety of systems and synthetic biology approaches for modeling metabolism and implementing strain design strategies. The genome-scale model of *S. cerevisiae* metabolism incorporates over 900 genes and over 1400 reactions, and it can be used to predict the impact of genetic manipulations on the metabolic phenotype. A small portion of the model is displayed in Fig. 1. We first combine this model with algorithms based on optimal control theory to systematically discover manipulations that will lead to improved malonyl-CoA production. Future work will also utilize a combination of genetic engineering and synthetic biology techniques to implement genetic controls predicted by the computational models. **Ultimately, we anticipate that a malonyl-CoA overproducing strain will serve as a host platform for several pathways ported from plants for the economical production of important polyketides.**

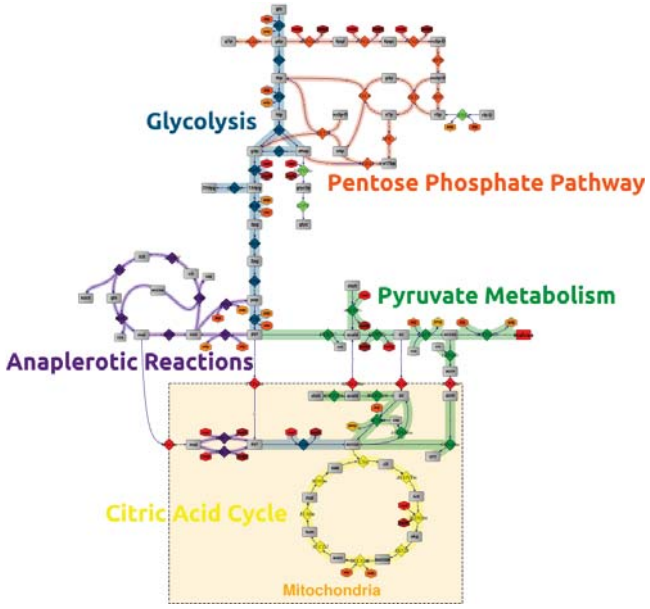


Fig. 1: Schematic representation of model reactions in yeast central metabolism. Drawing was made using Omix software (www.13cflux.net).

Research Highlights

Gowen, C.M. and R. Mahadevan (2012). Model-guided engineering of *Saccharomyces cerevisiae* for over-production of malonyl-CoA." (in preparation)

Gowen, C.M. and R. Mahadevan (2012) "A methodology for including alternative substrates in the computational strain design search space." (in preparation)

Gowen, C.M. and S.S. Fong (2011) "Applications of systems biology towards microbial fuel production." *Trends in Microbiology* 19(10):516-524

Gowen, C.M. and S.S. Fong (2010). "Genome-scale metabolic model integrated with RNAseq data to identify metabolic states of *Clostridium thermocellum*." *Biotechnology Journal* 5(7):759-767

Mahbod Hajighasemi
Ph.D. Student

M.Sc. (Microbiology), 2007,
University of Tehran, Iran
B.A.Sc. (Cell. Mol. Biol.), 2004,
Azad University of Tehran, Iran

Supervisor: Elizabeth Edwards
Co-Supervisor: Alexander Yakunin

Metagenome screening for bioplastic depolymerase activities

Polylactic acid (PLA), polyhydroxybutyrate (PHB) and their co-polymers are the most attractive alternatives for oil-derived conventional plastics due to their ideal physicochemical properties. However, there are some challenges remaining as we transition to these greener plastics, such as higher production costs, slower production cycles and the absence of effective recycling procedures.

For my doctoral thesis, **I am interested in bioplastic degradation using novel enzymes that are capable of cradle-to-cradle degradation of PLA and PHB bioplastics.** Moreover, by analyzing the degradation products and intermediates, we will take serious steps toward the development of recycling systems.

Using the richness of our metagenome libraries, we have access to numerous gene fragments that are isolated directly from different environments and used to make recombinant *E. coli* cells. These recombinant bacteria are cultured on double layer assay plates. The bottom layer supports the selective growth of host *E. coli* and overexpression of cloned gene fragments. The top layer however contains only a bioplastic emulsion agar as the indicator substrate. This primary screening is performed in a high throughput manner, assaying more than 2300 clones on a single plate (Fig. 1). Positive clones capable of degrading bioplastics produce transparent halos around their colonies.

The positive clones are accordingly selected for subcloning, overexpression and purification of responsible bioplastic depolymerases. The enzymatic activity of purified proteins is verified using bioplastic substrates directly, both in emulsion and solid-state reactions. The resulting products of enzymatic degradation are recovered and analyzed subsequently.

Among different groups of enzymes, esterases, lipases and proteases from the alpha/beta hydrolase fold superfamily include the most probable groups of enzymes to harbour bioplastic depolymerase activities. **By screening with bioplastics of various molecular-weight and enantiomeric compositions, we expect to find novel enzymes capable of degrading a diversity of bioplastics at different stages.**

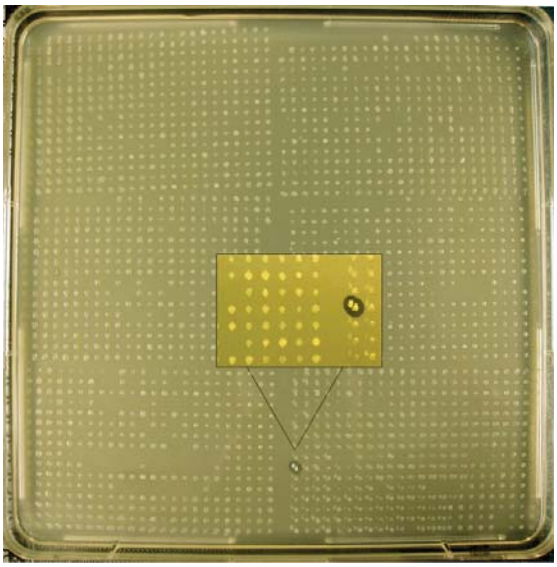


Fig. 1: Functional screening of metagenomic libraries for PLA depolymerase activity. Magnified rectangle shows a positive clone which has produced a clear halo.

Research Highlights

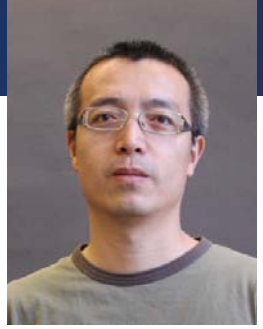
Hajighasemi, M., Amoozegar, M.A., Hamedi, J., Asad, S. and A. Ventosa (2011). "Azo dye decolorization by halophilic and halotolerant microorganisms." *Annals of Microbiology,* 61(2):217-230

Hajighasemi, M., Karbalaei-Heidari, H.R., Amoozegar, M.A., Ziaee, A.A. and A. Ventosa (2009). "Production, optimization and purification of novel extracellular protease from the moderately halophilic bacterium *Halobacillus karajensis*." *Journal of Industrial Microbiology and Biotechnology* 36(1):21-27

Research Highlights

Han, J., Hendzel, M.J., and J. Allalunis-Turner (2011). "Notch signaling as a therapeutic target for breast cancer treatment?" *Breast Cancer Research* 13(3):210

Han, J., Ma, I., Hendzel, M.J., and J. Allalunis-Turner (2011). "The cytotoxicity of .γ-secretase inhibitor I to breast cancer cells is mediated by proteasome inhibition, not by γ-secretase inhibition". *Breast Cancer Research* 11(4):R57



Jianxun Han
Postdoctoral Fellow

Ph.D., 2010, University of Alberta
M.Med., 1999, Fourth Military Medical University, China
B.Med., 1995, Fourth Military Medical University, China

Supervisor: Alison McGuigan

Elucidation of the molecular mechanisms underlying tip/stalk cell specification during angiogenesis

Angiogenesis, the formation of new blood vessels to meet increased demand for nutrition and/or oxygen supply, is essential for the growth of cancers and, therefore, angiogenesis inhibition holds great therapeutic promise in battling cancer. However, currently available approaches to block angiogenesis have failed to demonstrate significant therapeutic benefits. Thus, more research to reveal fundamental mechanisms underlying the process is urgently needed.

Endothelial cells are specified into tip cells and stalk cells at the beginning of angiogenesis: tip cells instruct the direction, and stalk cells via their proliferation mediate the growth of new sprouts. However, the molecular mechanisms underlying tip/stalk cell specification is yet to be fully understood. Most studies on tip/stalk cell specification use an *in vivo* mouse retina angiogenesis model. Although this is a very valuable system, it is time-consuming and difficult to manipulate. **We will use engineering tools, such as micropatterning and microfabrication, to create an *in vitro* system that can recapitulate the tip/stalk cell specification.**

Conceptually, a well-defined pattern of human endothelial cells that mimics the new sprout from the existing blood vessels will be exposed to a variety of combinations of growth factors that are applied uniformly or in gradient, which resembles the *in vivo* situation. We will validate the system by examining the characteristics of tip cells and stalk cells. **This system will help to study some basic questions regarding angiogenesis regulation and help to develop novel strategies to modulate angiogenesis for therapeutic benefits.**

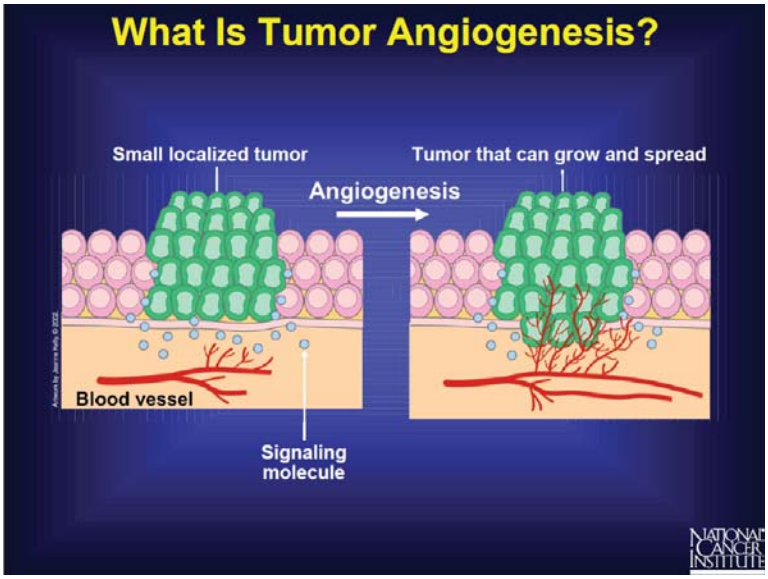


Fig. 1: Schematic diagram of tumor angiogenesis. (Source: National Cancer Institute)

Benjamin Hijar
M.Sc. Student

B.A.Sc. (Chem. Eng.), 2001, Instituto Tecnologico y de Estudios Superiores de Occidente, Guadalajara, Mexico

Supervisor: Levente Diosady

Production of protein isolates from yellow mustard flour by aqueous extraction and membrane processing

Aqueous extraction of several oleaginous materials, such as coconut, palm, peanut, rapeseed and sunflower, has been studied in order to find an organic-solvent free alternative for the extraction of oil and protein from oil seeds, due to increasing organic solvent prices and health and environmental concerns. The main disadvantage of the process is lower oil yields, ranging from 60-90% in comparison to the 99%+ typical of hexane extraction.

In our food engineering group, several research projects have focused on the aqueous extraction of oil seeds, in order to obtain high extraction yields for both oil and protein. The goal is a unified aqueous process that would give an optimum yield of oil and protein for their subsequent processing for food in the form of protein products and for fuel from the oil. **The project aims to find an optimized process for the production of high quality protein isolates with low levels of antinutritional factors and low oil content starting with the aqueous extraction of dehulled yellow mustard flour.**

Based on previous work, the conditions for extraction in the proposed study are as follows: pH 11, 4:1 water to seed ratio, 3 minutes blending time, room temperature, 30 minutes extraction time, and 3 stages. The proposed processing procedure is illustrated in Fig. 1.

Current studies are focused on the application and optimization of an ultrafiltration and diafiltration process on the yellow mustard flour protein extract, prior to isoelectric precipitation, purification and residual protein recovery, this will be followed by the determination of the food functionality of the protein products produced. **Ultimately, the project is expected to result in protein isolates with at least 90% purity, low oil content and with the required food functionality for their application in the food industry.**

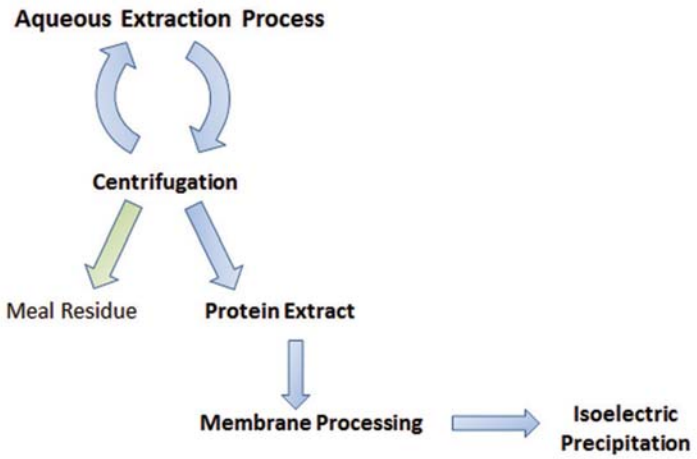


Fig. 1: General process for aqueous extraction and membrane processing for dehulled yellow mustard flour.

Research Highlights

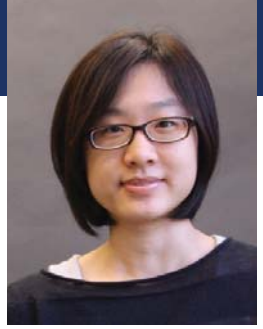
Co-developed a method for the manufacture of highly stable pigment powders and assessed its performance in layer egg yolk pigmentation for Kemin Agrifoods.

Co-developed a method for the manufacture of propylene glycol free liquid pigment for Kemin Agrifoods.

Research Highlights

Ho, H.C., Devine, C., Hug, L., Edwards, E.A., and R. Mahadevan (2011). A functional analysis of the metagenome of an anaerobic benzene-degrading community (poster). *13th CSChE Ontario-Quebec Biotechnology Meeting,* Kingston, ON

Ho, H. C., C. Devine, R. Beiko and R. Mahadevan (2011). Phylogenetic and functional analysis on an anaerobic benzene-degrading community (speaker). *61st Canadian Chemical Engineering Conference,* London, ON



Cleo Ho
M.A.Sc. Student

B.A.Sc., 2010, University of British Columbia

Supervisor: Radhakrishnan Mahadevan

Unravelling the microbial interactions in a benzene-degrading community using genome-scale models

Hydrocarbon discharges from anthropogenic activities often lead to benzene contamination in soil and groundwater systems. With its toxicity and carcinogenicity, benzene poses threats to human health because it is thermodynamically stable and able to persist in the environment. Although benzene can be metabolized by local microorganisms and transformed into non-toxic substances, such a degradation process is not well-characterized under anaerobic conditions, and benzene is broken down more slowly than it is accumulated.

To accelerate anaerobic benzene degradation, I study the metabolic interactions between the bacterial and archaeal species in a benzene-degrading culture using genome-scale models. Computational models facilitate the focused generation of testable hypotheses, and this capability is particularly crucial in the study of anaerobic benzene degradation because the number of experiments that can be performed is often limited by the slow growth of cells.

Genome-scale models are constructed based on DNA sequences. I assembled the DNA sequences representing the metagenome of the benzene-degrading community, and the assembled sequences were then classified into either the bacterial or archaeal domains by my collaborators. Subsequently, a metabolic network was reconstructed for each domain to represent the biochemical reactions that may be employed by the microbes. I will develop a novel approach to combine these separate networks and to capture the complex interactions between archaea and bacteria. In turn, this community model will allow us to accurately predict microbial activities, including metabolite exchange and benzene degradation.

Because these organisms cannot grow in isolation and must rely on each other syntrophically, it is essential to understand the microbial relationships, in order to identify substrates required to promote growth and benzene degradation. **With the knowledge gained from this study, we can improve our cultivation techniques and ultimately harness the metabolic capabilities of the culture to remediate benzene at polluted sites.**

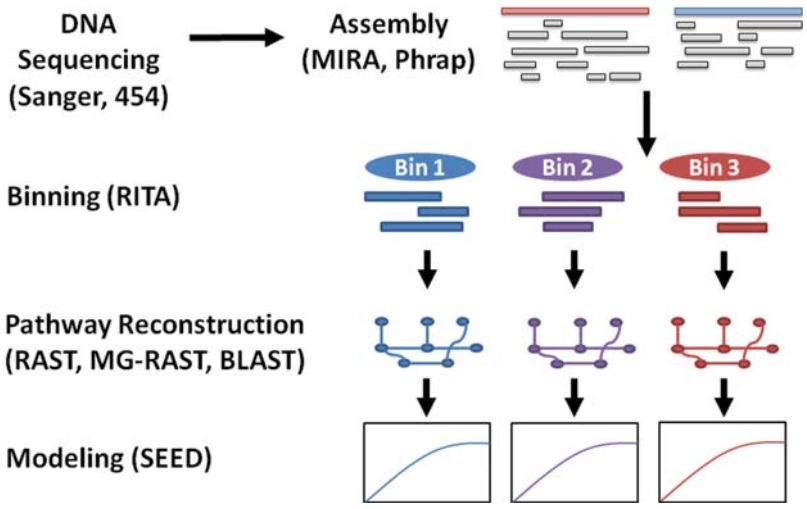


Fig. 1: A pipeline used to construct organism-specific models from metagenomic sequences.

Laura Hug
Ph.D. Student

M.Sc., 2007, Dalhousie University
B.Sc., 2005, University of Guelph

Supervisor: Elizabeth Edwards

A metagenome-based examination of dechlorinating enrichment cultures: *Dehalococcoides* and the role of the non-dechlorinating organisms

Bioremediation of chlorinated solvents to a non-toxic end product can be achieved with *Dehalococcoides* sp., through reductive dehalogenation of the chlorinated organics. *Dehalococcoides* sp. are typically maintained in enrichment cultures containing multiple microorganisms, and which typically exhibit better growth and dechlorination rates than *Dehalococcoides* isolates.

For my doctoral thesis, **I am interested in the nature of the relationships between the *Dehalococcoides* and the non-dechlorinating organisms in enrichment cultures.**

Using comparative metagenomics, we identified differences and similarities in taxonomy and functional gene complements between three *Dehalococcoides*-containing enrichment cultures. From this, we were able to identify pivotal supporting organisms involved in maintaining dechlorination activity through provision of nutrients and other factors to the *Dehalococcoides*.

We designed a *Dehalococcoides* pan-genus microarray using available sequenced genomes as well as a draft genome generated from an in-house metagenome sequence. This array serves as a common platform across collaborating laboratories, facilitating comparison of results obtained from different enrichment cultures.

Using an enrichment culture containing three phylogenetically distinct dechlorinating organisms, we examined the interactions of niche-specific organisms that are alternately competing and collaborating for access to their required electron acceptors. This was accomplished through single-cell sorting on a laser directed microfluidics platform with subsequent genome sequencing. The use of this cutting-edge technique allowed generation of partial genomes from novel dechlorinating organisms without the need for isolation, typically a lengthy and difficult process for obligate dechlorinating anaerobes. From the partial genomesequences, we identified novel reductive dehalogenase genes, as well as evidence of lateral gene transfer between the three dechlorinating organisms.

My work has focused on the use of genomic and metagenomic datasets, which serve as metabolic blueprints, **to identify areas where bioremediation can be optimized through manipulation of the naturally occurring microbial communities.**

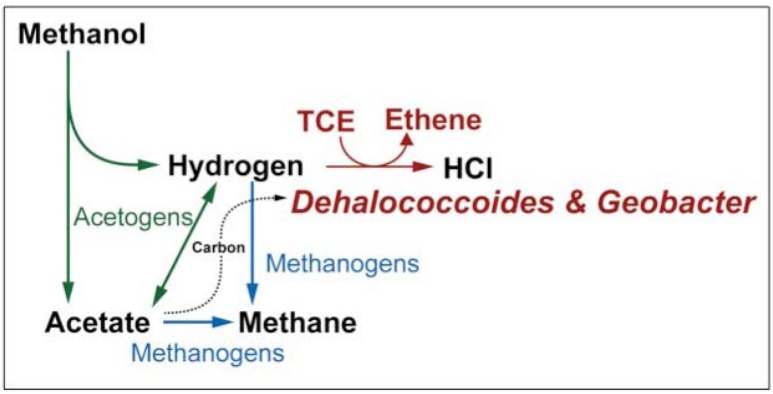


Fig. 1: Schematic representation of the organisms within the Dehalococcoides-containing KB-1 enrichment culture and their roles in electron donor utilization and dechlorination.

Research Highlights

Hug L.A. *et al.* (2011). "Design and verification of a pan-genome microarray oligonucleotide probe set for *Dehalococcoides* spp." *Appl. Environ. Microbiol.* 77(15):5361-5369

McMurdie, P.J., Hug, L.A., *et al.* (2011). "Site-specific mobilization of vinyl chloride respiration islands by a mechanism common in *Dehalococcoides*." *BMC Genomics* 12(1):287-301

Hug, L.A., Stechmann, A., and A.J. Roger (2009). "Phylogenetic distributions and histories of proteins involved in anaerobic pyruvate metabolism in eukaryotes." *Mol. Biol. and Evol.* 27(2):311-324

Hampl, V., Hug, L., *et al.* (2009). "Phylogenomic analyses support the monophyly of Excavata and resolve relationships among eukaryotic "supergroups." *PNAS U.S.A.* 106(10):3859-3864

Research Highlights

Two invention disclosures relating to technologies for the biosynthesis of target chemicals.

Gawand P., Hyland, P., and R. Mahadevan (2010). Metabolic engineering strategies for substrate co-utilization in *Escherichia coli*." *Metabolic Engineering for Green Chemicals*, Jeju Island, Korea.

Patrick Hyland
Ph.D. Student

B.A.Sc., 2010, University of Toronto

Supervisor: Radhakrishnan Mahadevan

Metabolic engineering for production of value-added chemicals: adipic acid

Recent global awareness of the limitations of petrochemical feedstock has underscored the need for sustainable and renewable synthesis of fuels and chemicals. Metabolic engineering offers a viable alternative to such processes, harnessing microbial metabolism to synthesize such chemicals from sugars. Adipic acid, a chemical used for the production of Nylon(6,6), is a commodity chemical produced on the order of 2.2 billion pounds per annum from petroleum.

My doctoral research is specifically focused on the development and implementation of pathways for the biosynthesis of adipic acid, a non-natural chemical intermediate, in the yeast *Saccharomyces cerevisiae*.

Using publicly available enzyme databases, we identified candidates for novel enzyme activity required for completion of biosynthetic pathways. Through our collaboration with the Structural Proteomics in Toronto laboratory, we demonstrated the activity of candidate enzymes on pathway intermediates.

Following the identification of the best enzyme candidates for required activity, they are expressed in a host strain of *S. cerevisiae* that overproduces pathway precursors. Rational strain design, employing computational algorithms to elucidate gene deletion targets that will enable a microbe to overproduce a metabolic intermediate, has previously been used to overproduce other valuable chemicals in microbes. In addition to gene deletions, it has been shown that moderate expression of target genes provides optimal production. We employed a novel computational algorithm, developed by our laboratory, to design strains that include gene deletions and finely tuned gene expression to overproduce pathway precursors.

My current studies are focused on the optimization of a base strain of *S. cerevisiae*, investigation of the robustness of the strain to genetic perturbations and quantification of the metabolic shift incurred during growth in the presence of weak acids. **This research is expected to establish a pipeline for the development of strains that overproduce valuable compounds to be used as drop in substitutes for petrochemicals.**

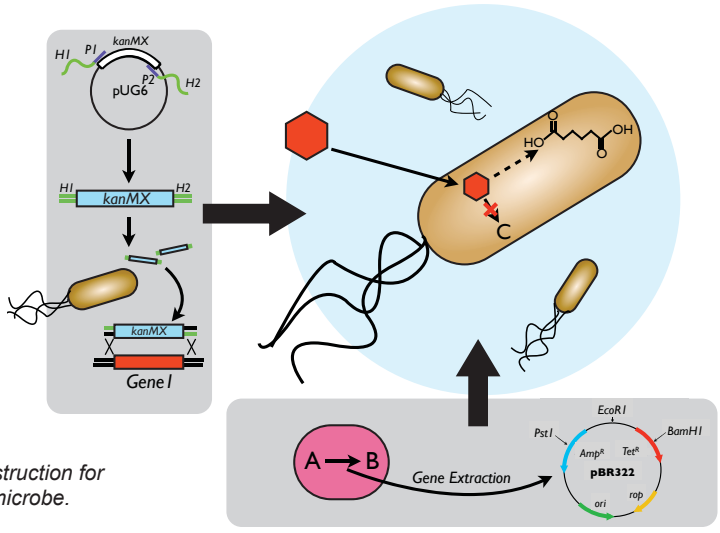


Fig. 1: Workflow of strain construction for an adipic acid overproducing microbe.

M. Ahsanul Islam
Ph.D. Student

M.Sc., 2005, Imperial College of Science, Technology and Medicine, London
B.Sc.Eng., 2003, Bangladesh University of Engineering and Technology, Dhaka

Supervisor: Radhakrishnan Mahadevan
Co-Supervisor: Elizabeth A. Edwards

A system-wide investigation of metabolism of a dechlorinating microbial community

Halogenated organics, including known human carcinogens trichloroethene and vinyl chloride, as well as chlorobenzenes and polychlorinated biphenyls, are found in soil and groundwater as a result of their widespread industrial and agricultural use as pesticides, herbicides, flame-retardents, solvents and degreasing agents. Due to their toxicity and persistence, they contaminate soil, sediments, and ground water aquifers, thus posing a serious threat to human health and to the environment. However, biological degradation of halogenated organic compounds can be achieved by a group of tiny and strictly anaerobic bacteria called *Dehalococcoides*. These specialized bacteria harness energy for growth from the dehalogenation reaction — a process termed organohalide respiration. To better apply this novel and natural bioremediation process, we need to have a detailed understanding of the unusual metabolism of *Dehalococcoides*, and the microbial community they are intricately linked to.

Thus, **the goal of my doctoral research project is to develop a fundamental understanding of the metabolism of *Dehalococcoides* and associated community members using novel tools from genomics, bioinformatics, systems biology, and environmental microbiology.**

Specifically, I am developing computer models of metabolism for *Dehalococcoides* and associated microorganisms using the information from available whole genome-sequences, microbial physiology and biochemistry, as well as the knowledge from experiments with our own dechlorinating community, KB-1.

First, I developed a pan-genome-scale computer model of *Dehalococcoides* metabolism (Fig. 1). The model revealed several limitations in the metabolism of *Dehalococcoides*; for example, an inefficient use of energy from the reductive dechlorination reactions, and a clear lack of information about molecules and enzymes involved in energy flow in the cell.

Future work includes construction and integration of genome-scale metabolic models for other microbes with that of *Dehalococcoides* for better understanding interspecies interactions in subsurface environments.

This knowledge will, ultimately, help accelerate the detoxification of chlorinated solvent contaminated sites around the world.

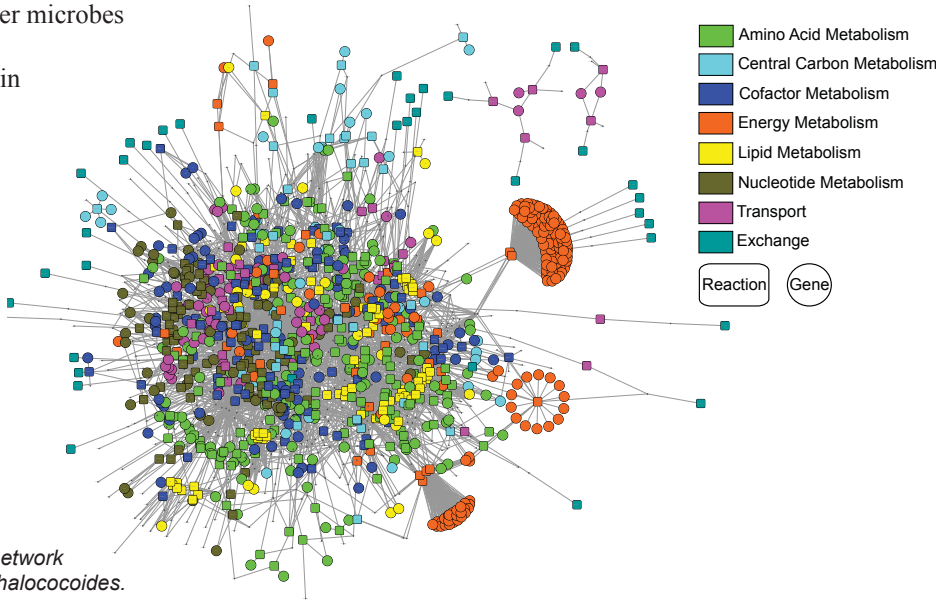


Fig. 1: Pan-genome-scale reconstructed biochemical network and metabolic model for Dehalococoides.

Research Highlights

Islam, M. A. *et al.* (2011). A systems-level study of the transcriptional changes of *Dehalococcoides* metabolism (poster). *Gordon Research Conference on Cellular Systems Biology*, Davidson College, Davidson, NC

Islam, M. A. *et al.* (2010). Characterizing the metabolism of *Dehalococcoides* with a constraint-based model. *PLoS Comp. Biol.* 6(8):e1000887

Islam, M. A. *et al.* (2010). Pan-genome-scale modeling of *Dehalococcoides* metabolism (speaker). *12th Annual Ontario-Quebec CSChE Biotechnology Meeting,* Montreal, QC

Islam, M. A. and R. Kumar (2006). An oxygen transport model of human bone marrow for haemolytic sickle cell anaemia. *Journal of Chemical Engineering* IEB-BD Vol. ChE 24(1):7-13

Research Highlights

Javaherian S., Paz, A.C., and A.P. McGuigan (2011). "Tools for micropatterning epithelial cells into microcolonies on transwell filter substrates". *Lab on a Chip* 11(20):3440-8

Javaherian S., O'Donnel, K.A., and A. P. McGuigan (2011) "A fast and accessible methodology for micropatterning cells on standard culture substrates using Parafilm™ inserts". *PLoS ONE* 6(6) e20909

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Javaherian S. *et al.* (2009) Selection of aptamers to a protein target in cell lysate and their application to protein purification from cell lysate. *Nucleic Acid Research* 37:e62

Sahar Javaherian
Ph.D. Student

M.Sc., 2009, York University
B.Sc., 2007, York University

Supervisor: Alison McGuigan

Patterning gene expression in *in vitro* engineered tissues

Gene expression patterns play a central role in patterning tissues of a developing embryo, where they specify body and organ axes and impose distinct functions on equipotent cells. **The purpose of this project is to develop an approach for patterning gene expression *in vitro* in engineered tissues comprised of genetically identical cells.**

We propose that this goal can be reached by using a drug-inducible gene expression system and patterning the delivery of the inducing agent. We are also modeling our gene expression patterning approach to understand the boundary conditions that cellular rearrangements within the tissue (such as cell migration) impose on the feasibility of obtaining a gene expression pattern using a gene expression system with given dynamics of induction.

Our gene expression patterning technology is expected to be instrumental in developing a more realistic *in vitro* model system of developmental processes as well as introducing better functionality to tissues engineered *in vitro*.

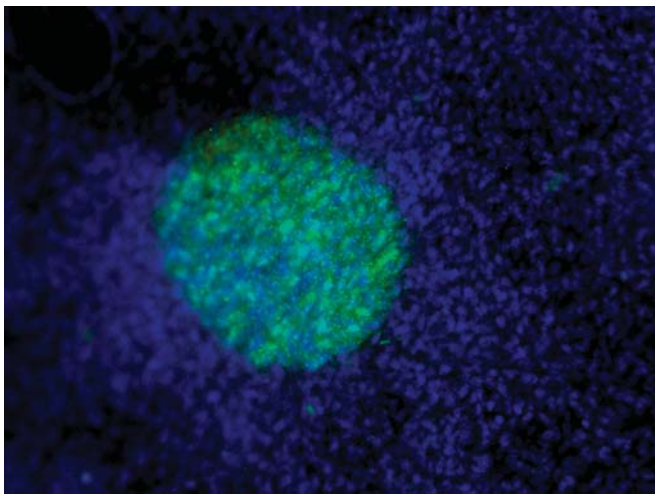


Fig. 1: Controlled delivery of a drug (CFDA) to a cellular monolayer. Green - CFDA; Blue - DAPI.

Ph.D., 2007, University of Toronto
M.Sc.F.E., 1999, University of New Brunswick
B.Sc.F.E., 1996,
University of Belgrade

Dragica Jeremic Nikolic
Research Associate

Supervisor: Emma Master

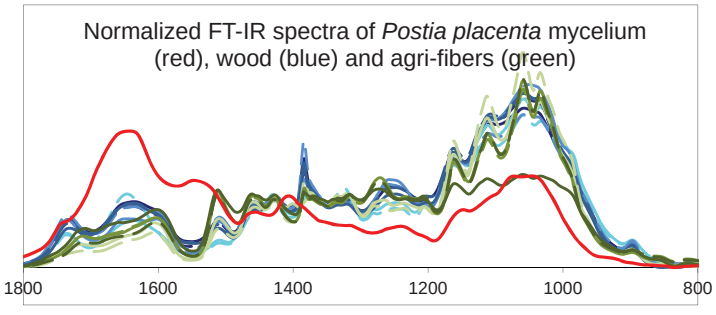
Proteomic analysis of wood degrading fungi and development of techniques for detection of penetration and adsorption to lignocellulose substrate

This project involves proteomic analysis of enzymes secreted by the brown-rot basidiomycete *Postia placenta* while transforming various lignocellulosic substrates: agricultural crops, and coniferous and deciduous wood species relevant for Canadian industry. The goal of the study is to:

1. identify enzymes that are most efficient at transforming particular lignocellulosic feedstocks,
2. compare enzyme profiles excreted in the presence of various substrate compositions
3. correlate the enzymatic profile to chemical changes introduced to the substrate, and
4. identify feasibility of proteomics to solely identify enzymes of importance for biotechnology uses.

Besides heterogeneous chemistry of the substrate, the main challenges in bioconversion of lignocellulose arise from our limited understanding of accessibility of enzymes within dense lignocellulose cell walls and parameters that determine adsorption of enzymes to the substrate. It is clear that our limited appreciation of enzyme penetration and catalysis dynamics is partly due to the constraints of the techniques employed previously. The current study, therefore, aims to identify suitable techniques for examination of penetration and adsorption of enzymes to substrate.

In particular, we are working on developing methods for determining these parameters using Quartz Crystal Microbalance with Dissipation Monitoring (QCM-D), Time-of-flight Secondary Ion Mass Spectrometry (TOF-SIMS) and Scanning Transmission X-ray Microscopy (STXM). **We anticipate that these methods will enhance our fundamental understanding of enzymatic activity on lignocellulosic substrates and enable tailored applications of enzymes for processing different lignocellulose resources.**



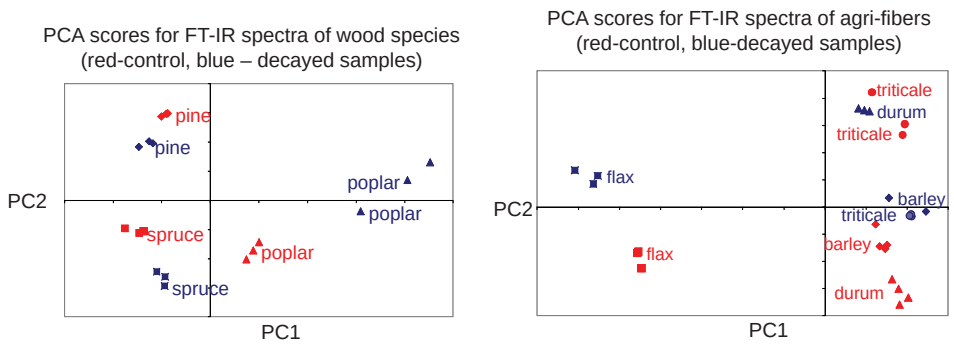


Fig. 1: FT-IR chemical profile of wood and agri-fiber samples before and after Postia placenta degradation as analyzed by Principal Component Analysis

Research Highlights

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Goacher, R., Jeremic, D., and E. R. Master (2011). "Expanding the library of secondary ions that distinguish lignin and polysaccharides in time-of-flight secondary ion mass spectrometry analysis of Wood." *Anal. Chem.* 83:804-812

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Jeremic, D., Quijano-Solis, C., and P. Cooper (2009). "Diffusion rate of polyethylene glycols into cell walls of red pine following vacuum impregnation." *Cellulose* 16:339-348

Research Highlights

Aitken, R.A., Al-Awadi, N.A.,Dawson, G., El-Dusouqi, O.M.E., Farrell. D.M.M., Kaul K., and A. Kumar (2005). "Synthesis, thermal reactivity and kinetics of substituted [(benzoyl)(phenylcarbamoyl) methylene] triphenylphosphoranes and their thiocarbamoyl analogues". T*etrahedron* 61:129-135

Ph.D., 1986, University of Poona, Maharashtra, India
M.Sc., 1981, Department of Chemistry, University of Poona, India
B.Sc. Hons., 1979, Fergusson College, University of Poona, India

Kamini Kaul
Research Assistant

Supervisor: Radhakrishnan Mahadevan

Characterisation of metabolic turnover using isotopic labeling

^{13}C based metabolic flux analysis (MFA) is an exclusive approach to experimentally quantify the integrated responses of metabolic networks to environmental and genetic alterations.

Metabolic conversion of isotopically labeled substrates generates molecules with distinct labeling patterns, i.e. isotope isomers (isotopomers), that can be detected by mass spectrometry. Isotope measurements provide many additional independent constraints for metabolic flux analysis. At metabolic and isotopic steady state the isotopomer composition of metabolic intermediates is determined by the metabolic flux distribution and isotopic labeling. **The obtained isotope-based metabolic flux distribution is of key importance in validating and refining the metabolic network models as well as in assessing the metabolic engineering strategies.**

My work involves derivatizing an amino acid standard as well as microbial biomass hydrolysates and performing GC/MS analysis. We were able to define a GC method for chromatographic separation of different amino acids in the derivatized amino acid standard. The 20 amino acids in the mixture were then identified by MS, through a fragmentation pattern defined by the cleavage of 5 main fragments of the tert-butyl silylated amino acid derivatives. The same GC method was applied to the derivatised microbial biomass hydrolysates and the main fragments of all the 20 amino acids were identified by the MS for the sample as well.

After optimization of the protocol, **we can characterize the metabolic state of the cell by quantifying the flux through the various pathways in the cell. Such insights will be valuable for engineering metabolites for chemicals or fuels production.**

In addition to this project, my research expertise is in the area of synthesis and characterization of organics, pharmaceuticals, carbohydrates, heterocycles and other biological compounds. Another area of speciality is in the field of organic environmental pollutants.

Marina Kudritska
Technician

M.Sc, 1995, Odessa National University, Odessa, Ukraine

Supervisor: Alexei Savchenko

Optimization of protein purification and crystallization conditions

I have a background in biology with specialization in hydrobiology, but my current research interest focuses on bacterial protein purification and crystallization. **Obtaining high purity protein extracts is crucial for the production of high quality crystals, and well-diffracting crystals are a key factor for resolving the three-dimensional structure of proteins.** The crystal structure provides information for understanding protein interactions and functions (Fig. 1).

Together with other members of Dr. Savchenko's group, I have participated in many projects mainly focused on obtaining structural information of proteins of important bacterial pathogens to facilitate development of new antibacterial drugs.

Protein structure determination involves a series of steps with two bottlenecks in the procedure: protein purification and crystallization. My work includes the following steps of the procedure: bacteria transformation, culture growing, testing expression of recombinant proteins, protein purification, setting up initial crystallization screening trials and checking the quality of crystals using diffractometers.

The process of protein crystallization is an empirical, complex and multiparametric process. In close cooperation with other members of Dr. Savchenko's group I focus on finding favourable crystallization conditions by setting up initial crystallization screening trials with variable protein concentrations, different buffer compositions, pH, variable temperatures or additives.

Research Highlights

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Chruszcz, M. *et al.* (2008). Function-biased choice of additives for optimization of protein crystallization – the case of the putative thioesterase PA5185 from Pseudomonas aeruginosa PAO1. *Crystal Growth & Design* 8(11):4054–4061

Beloglazova, N. *et al.* (2008). A novel family of sequence-specific endoribonucleases associated with the clustered regularly interspaced short palindromic repeats. *Journal of Biological Chemistry* 283(29):20361–20371.

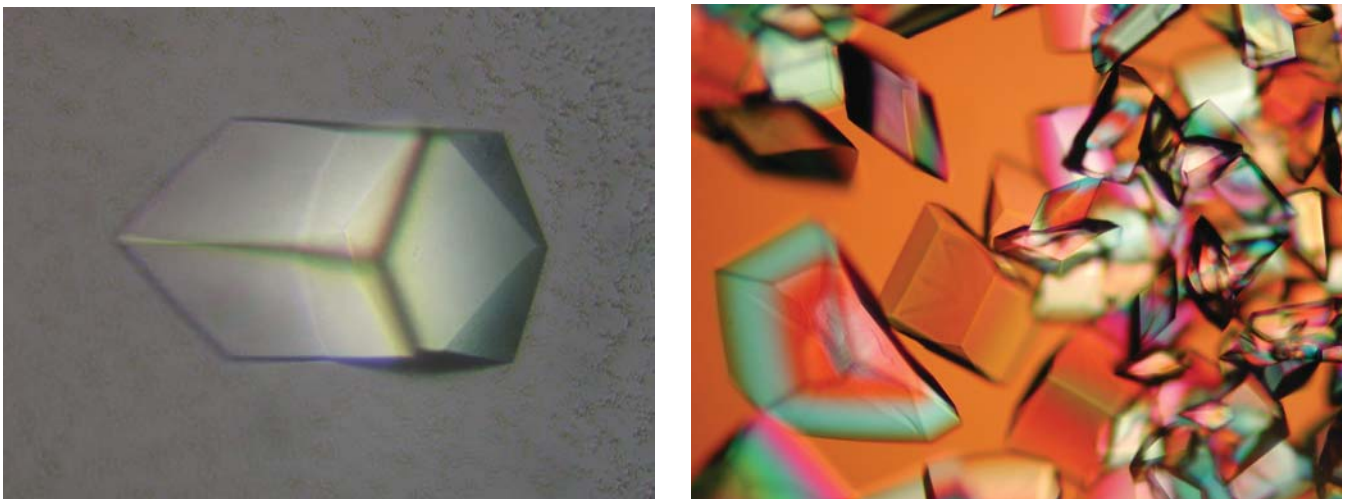

Fig. 1: Crystals from proteins of unknown function from Thermoplasma acidophilum and Archaeoglobus fulgidus.

Research Highlights

Kwan, L., and L. Diosady (2011). Integrated platform for delivery of nutraceuticals: triple fortification of salt (poster). *13th CSChE Ontario-Quebec Biotechnology Conference*, Kingston, ON

Kwan, L., and L. Diosady (2011). Integrated platform for delivery of nutraceuticals: triple fortification of salt with vitamin A, iron, and iodine (poster). *4th International Delivery of Functionality in Complex Food Systems*, Guelph, ON

Lana Kwan
M.A.Sc. Student

B.A.Sc., 2009, University of Guelph

Supervisor: Levente Diosady

Triple fortification of salt with vitamin A, iron, and iodine using a self-emulsifying drug delivery system

Micronutrient deficiencies in the developing world are prevalent, but food fortification programs can increase the intake of multiple micronutrients simultaneously in a cheap and sustainable manner. There are three micronutrients that are particularly important: vitamin A, iron, and iodine.

My research objectives are (1) to test the concepts of delivering fat soluble bioactive compounds in systems that can survive the harsh acidic conditions of the stomach, (2) to deliver the fat-soluble bioactive compound through a O/W microemulsion in order to enhance absorption of the compound of interest, and (3) to look into the chemical interaction between the fat-soluble bioactive compound in the microemulsion with the additional compounds in the surrounding system. **My work focuses on creating self-emulsifying drug delivery systems that form microemulsions on contact with intestinal fluid in order to optimize vitamin A delivery.**

We designed self-emulsifying drug delivery systems, or SEDDS, containing the primary micronutrient, vitamin A, which was then encapsulated with a protective coating through the use of a spray dryer (Fig. 1). The main idea behind the SEDDS is that it can form fine oil-in-water (O/W) microemulsions in an aqueous phase. We then triple fortified salt with encapsulated vitamin A, encapsulated iron, and a spray solution of iodine. Salt was chosen as the food vehicle because it is universally consumed. Fortified samples were stored under typical conditions and the stability of the micronutrients was followed over a period of several months.

The use of self-emulsifying drug delivery systems to contain the bioactive compound has never been thoroughly investigated and its application to food is unique. **In the near future, this technology will enable the accurate releasing and absorption of compounds of interest in targeted areas of the digestive system.**

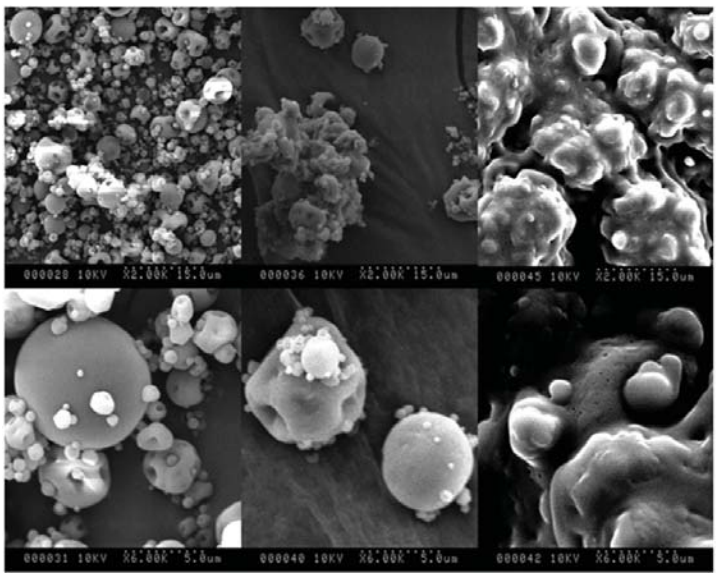

Fig. 1: SEM images of three capsules with varying ratios of enteric coating to micronutrient. Each sample is shown at 2000x (top row) and 6000x (bottom row) magnification.

Michael Lacourt
Research Assistant

M.A.Sc., 2011, University of Toronto
B.A.Sc., 2009, University of Toronto

Supervisor: Elizabeth Edwards
Co-Supervisors: Emma Master, Honghi Tran

Biogas from woody biomass

Lignocellulosic biomass is difficult to degrade due to the crystalline structure of cellulose, the irregular and complex molecular structure of lignin, and the presence of anti-microbial extractives. Due to this complexity, a mixed microbial community is required for anaerobic biotransformation, for example to produce methane.

My Master's thesis focused on the biogas production rate, yield, and methane fraction present in the gas produced from a variety of lignocellulosic substrates by mixed microbial communities derived from moose rumen fluid, beaver droppings, and anaerobic digestor granules. Additional studies included a microbial community analysis of the enriched communities compared to the initial inoculum, and the design and commissioning of a lab-scale continuous anaerobic reactor.

The substrates tested included cellulose, pine needles, lignosulphonate, tannic acid, or poplar hydrolysate. Tannic acid delayed biogas production compared to cellulose enrichments, both by reducing initial rates (up to 50% in beaver dropping cultures) and increasing lag times (up to 50 days in moose rumen cultures). Subsequently, to test the robustness of the microbial communities enriched on these different feedstocks, the enrichments were amended with a recalcitrant pulp and paper wastewater feed (post extraction wash, PEW). Enrichment on pine needles or poplar hydrolysate promoted bioconversion of PEW to methane, suggesting that components in pine needles and poplar hydrolysate are also found in PEW.

Next steps for this project include continued enrichment and scale-up to continuous reactor conditions to better understand the performance of cultures on an industrial scale, and the identification of potentially useful enzymes within the enriched communities via metagenome screens.

Research Highlights

Lacourt, W.M. (2011). Enrichment of methanogenic microcosms on recalcitrant lignocellulosic biomass. M.A.Sc. thesis, *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto

Lacourt, W.M. *et al.* (2011). Enrichment of methanogenic microcosms on recalcitrant lignocellulosic biomass (poster). *2nd Annual Bioproducts and Enzymes from Environmental Metagenomes Meeting*, Toronto, ON

Lacourt, W.M. *et al.* (2011). Biogas from woody biomass (conference presentation). *Ontario-Quebec Biotechnology Meeting*, Kingston, ON

Lacourt, W.M. *et al.* (2010). Biogas from woody biomass (conference presentation). *Pulp and Paper Technical Association of Canada Annual Meeting,* Montreal, QC

Fig. 1: Microcosm bottles.

Fig. 2: Moose.

Research Highlights

Li, Y.X., and E.A. Edwards (2010). Searching for microbes in deep hypersaline anoxic basins (poster). *CSChE Ontario-Quebec Biotechnology Meeting*, Kingston, ON

Li, Y.X. (2011). Adapting the dechlorinating enrichment culture KB-1 to dechlorinate acidic contaminated sites (speaker). *61st Canadian Chemical Engineering Conference*, London, ON

Jine Jine (Yi Xuan) Li
M.A.Sc. Student

B.A.Sc., 2010,
University of British Columbia

Supervisor: Elizabeth Edwards

Adaptation of a dechlorinating culture to acidic environments

Bioremediation of chlorinated ethenes and many other chlorinated compounds is optimal at neutral pH with dechlorination slow or incomplete at pH below 6. Contaminated sites with pH below 6 are common and given that reductive dechlorination and fermentation of commonly used electron donors are both acid generating processes, bioremediation has the potential to decrease the pH to well below 6.

In recent years, modifying aquifer pH using buffering agents such as sodium bicarbonate and various commercial formulations has become increasingly common. Aquifer pH modification has had varying degrees of success depending on application method, site geology and geochemistry but is generally considered challenging and effective alternatives would be beneficial. **The objective of this work is to assess/develop bioaugmentation culture KB-1®, containing *Dehalococcoides* and *Geobacter* spp. that can completely dechlorinate chlorinated volatile organic compounds at pH levels near 5.0.**

Microcosms were constructed with anaerobic media to evaluate the dechlorinating ability of the culture at different pH levels ranging from 5.5 to 7.0. Methanol and hydrogen were used as electron donors with VC as the electron acceptor. The VC amended microcosms specifically target Dehalococcoides. Microcosms were sampled over time and dechlorination rates compared between the different pH levels. Chlorinated ethenes and dissolved hydrocarbon gases were measured by GC/FID analysis.

Dechlorination rates decreased with decreasing pH, but complete dechlorination was observed below pH 6.0 (Fig. 1). The low pH tolerant formulation of KB-1® is being evaluated for dechlorination ability at various pH levels with periodic examination of the microbial community using molecular biological tools to monitor population shifts. Site geologic materials and groundwater from an acidic aquifer (pH ~ 5.5) are also being used to evaluate the performance of the low pH KB-1® formulation under site conditions.

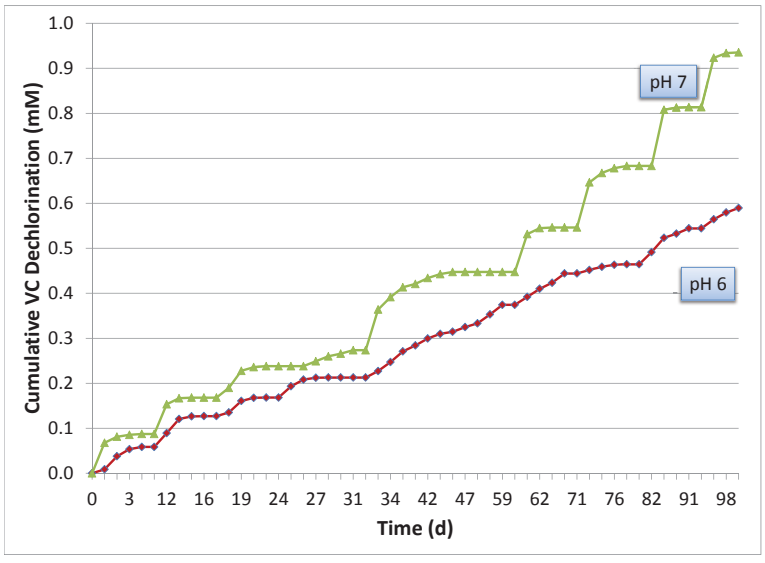

Fig. 1: Comparison of dechlorination rates of VC at pH 7 and pH 6 in a methanol-amended cultures.

The results of these experiments will have implications for field application. **It is believed that bioaugmentation cultures acclimated to lower pH could reduce the need for adjusting aquifer pH with buffering agents during bioremediation.**

Xiaoming Liang
Postdoctoral Fellow

Ph.D., 2009, University of Oklahoma
M. Eng., 2004, Tongji University, China
B.A. Eng., 1997, University of Jinan, China

Supervisor: Elizabeth Edwards

Sequential dechlorination of chlorobenzenes via monochlorobenzene to benzene coupled to benzene transformation to methane and carbon dioxide

My research interests focus on the exploration of fundamental biogeochemical processes of hazardous materials in natural and engineered systems. My long-term goals are to (1) unravel the mechanisms governing the transformation of contaminants in subsurface environments, (2) develop cost-effective wastewater treatment methods for both legacy and emerging contaminants, and (3) comprehend the environmental impacts of different groundwater remediation technologies and wastewater treatment options over an entire life cycle.

These goals involve a variety of topics across the fields of environmental chemistry, microbiology, geochemistry, and environmental sustainability. My research employs interdisciplinary approaches integrating surface analysis tools, molecular biology techniques, and photochemistry with stable environmental isotopes (e.g., C, H, Cl, and N isotopes), when appropriate.

I am currently investigating the anaerobic mineralization of chlorinated benzenes, in particular monochlorobenzene (MCB) in methanogenic microcosms. Chlorinated benzenes and MCB are widespread groundwater contaminants found at many industrial sites. Complete dechlorination of chlorinated benzenes results in problematic accumulation of benzene, which is more toxic than the parent compounds. In this work, we developed cultures containing microbes capable of MCB dechlorination and benzene degradation (Fig. 1). The mineralization of MCB via benzene as an intermediate led to non-toxic degradation products, CO_2 and methane. Moreover, our results suggested that benzene derived electrons fueled MCB dechlorination, removing the need to provide exogenous electron donors. Some ongoing experiments include exploring the microbial community and potential enzymes (dehalogenases) responsible for MCB mineralization, using quantitative polymerase chain reaction (q-PCR), next generation gene sequencing (e.g., 454 pyrotag sequencing) and protein analysis techniques (e.g., blue-native polyacrylamide gel electrophoresis (BN-PAGE)). **Our findings have promising implications for sustainable bioremediation of monochlorobenzene- and benzene-contaminated sites.**

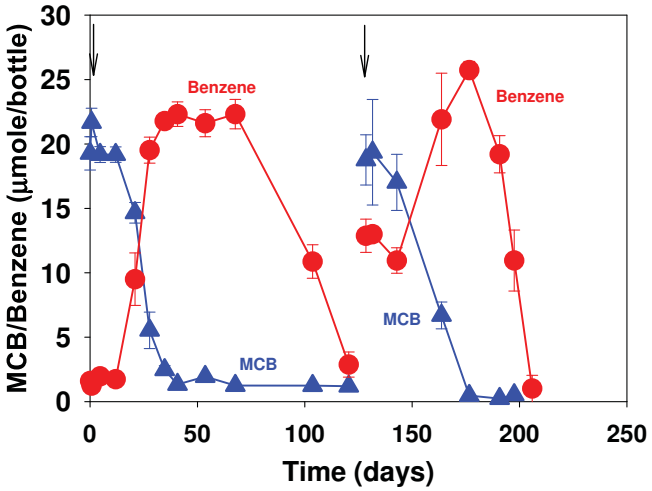

Fig. 1: Microbial degradation of MCB and benzene in microcosms. Arrows indicate amendments of 0.5 mM MCB and 0.1g/L yeast extract (Day 0) and 0.5 mM MCB and 0.25 mM benzene (Day 125).

Research Highlights

Liang, X. *et al.* (2011). Pathway-dependent isotope fractionation during aerobic and anaerobic degradation of monochlorobenzene and 1, 2, 4-trichlorobenzene. *Environ. Sci. Technol.* 45:8321-8327

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Liang, X. and E.C. Butler (2010). Effects of natural organic matter model compounds on the transformation of carbon tetrachloride by chloride green rust. *Water Res.* 44:2125-2132

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Research Highlights

Lomheim, L. (2002). Anaerobic biodegradation of high concentrations of perchloroethene (PCE) and 1,2-dichloroethane (1,2-DCA). M.A.Sc. thesis, *Dept. of Civil Engineering*, University of Toronto

Lomheim, L. (1997). Hydrochemistry in subtill groundwater magazines at Laag Jaeren. M.Sc thesis, *Faculty of Applied Earth Sciences and Petroleum Engineering*, Norwegian University of Science and Technology

Line Lomheim
Technician

M.A.Sc., 2002, University of Toronto
M.Sc., 1997, Norwegian University of Science and Technology

Supervisor: Elizabeth Edwards

Microcosm studies to assess *in situ* biodegradation rates

As a laboratory technician I work on various research projects and provide assistance with equipment and procedures to students and laboratory staff. Some of the techniques that I teach are gas chromatography, preparation of standards, calibration of analytical instruments, accuracy of measurements, use of syringes, techniques involved in microbial growth and maintenance of anaerobic microorganisms, and the creation of anaerobic microcosms. I also provide support to collaborating laboratories.

With a background in environmental technology and contaminant hydrogeology, and work experience as an environmental consultant, I have taken on projects with industrial partners. With our collaborator, Geosyntec Consultants, **I am conducting treatability studies to assess bioremediation options for chlorinated hydrocarbons at contaminated sites.** In these studies we create microcosms using soil and groundwater from the contaminated site (Fig. 1). We assess intrinsic biodegradation in the groundwater, evaluate ways to improve degradation efficiency through various amendments, and evaluate whether bioaugmentation with microbial consortia is needed.

In a new project, **I am working in collaboration with researchers at Université des Antilles et de la Guyane Guadeloupe, French West Indies, to investigate the potential for bioremediation of herbicides and pesticides.** Many banana plantations in Guadeloupe are severely impacted by chlordecone and lindane (Fig. 2). Previous studies have shown that these compounds resist aerobic biological treatment, but their chemistry suggests that anaerobic reductive dechlorination may be possible. We constructed microcosms from materials from 6 sites on or near banana plantations. Results show that under anaerobic conditions, lindane is reductively dechlorinated to chlorobenzene and benzene. I am currently working on establishing analytical methods for lindane, chlordecone and their potential degradation products.

Collaborations with other universities and industy partners involve the use of chemical reduction, such as zero valent iron, in combination with biodegradation as remediation technologies.

Fig. 1: Anaerobic microcosms created from soil and groundwater from a contaminated site

Fig. 2: A banana plantation in Guadeloupe, where many areas are impacted by pesticides.

Camila Londono
M.A.Sc. Student

B.A.Sc, 2008, University of Toronto

Supervisor: Alison McGuigan

The role of the planar cell polarity signaling pathway on coordination of collective cell migration

Cell migration is important to both the developmental and adult phases of an organism's life. In some cases, cells migrate independently of one another (e.g., in immune response). In many other cases, however, cells move as a group, and must remain tightly connected. This process, known as collective cell migration, requires that the intracellular changes involved in individual cell migration be coordinated among the whole migrating group.

My project focuses on **whether the Planar Cell Polarity (PCP) signalling pathway, known to cause coordination in whole tissues, is part of the mechanism of coordination of collective cell migration.** Specifically, we are modifying components of the PCP signalling pathway and analyzing the effect of these modifications on single and group cell migration using high-throughput microscopy.

Understanding the mechanism of coordination of collective cell migration is important because of how pervasive collective cell migration is in healthy and disease states. **Increased knowledge about collective cell migration will give us insight into morphogenic processes, which could strongly impact cancer research and tissue engineering.**

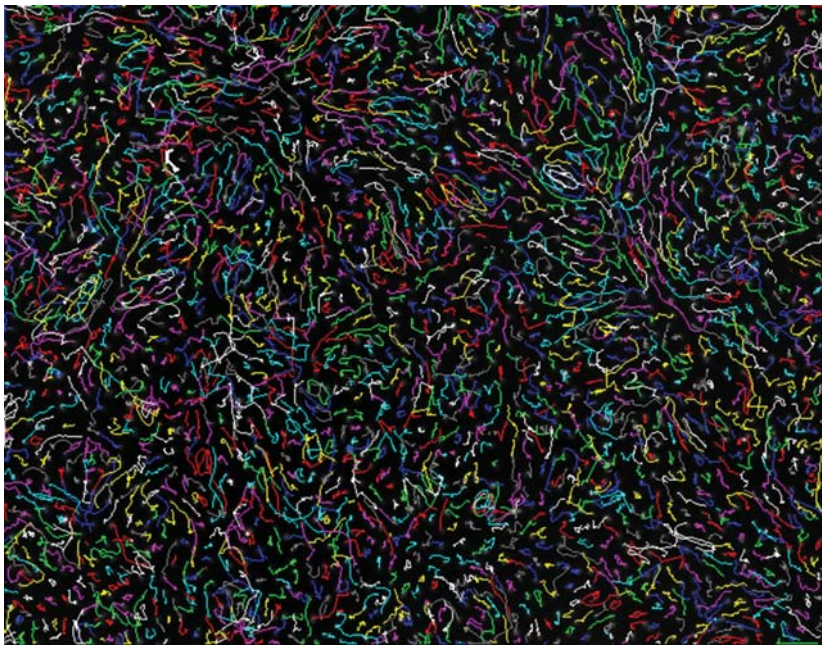


Fig. 1: Tracks of cells migrating within an intact sheet. Track colours are randomly assigned.

Research Highlights

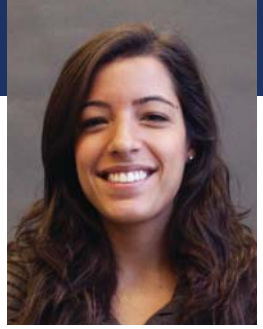
Londono, C. and A.P. McGuigan (2011). The role of planar cell polarity signaling in collective endothelial cell migration (poster). *Gordon Research Conference on Gradient Sensing and Directed Cell Migration*, Les Diablerets, Switzerland

Research Highlights

M.J.L Gines, Loureiro, M.J., and F.J. Williams (2009). “Effect of phosphorous on the hardness temperature resistance of nanostructured Ni-W electrodeposited coatings”, *Plating and Surface Finishing*

G. Sacripante, Loureiro, M.J., Vong, C., Veregin, R., Hawkins, M., and E. Zwartz (2009). “Curable toner compositions and processes”, US & European Patents: 20090550-US-NP & 100175193.1-1217

C. Zamecnik, Loureiro, M.J., Postnikoff, C., Kong, Y., and A. Penlidis (2011). Synthesis and morphology of poly(N-isopropylacrylamide) nanocomposites with emulsion templated nanoporous structure. B.A.Sc. thesis, *Dept. of Nanotechnology Engineering*, University of Waterloo



Maria Jimena Loureiro
M.A.Sc. Student

B.A.Sc. (Nanotech. Eng.), 2011, University of Waterloo

Supervisor: Alison McGuigan

Engineering a device to characterize collective cell migration in the presence of growth factor gradients for tissue engineering and regenerative medicine applications

Cells *in vivo* respond to chemotactic cues in the environment. In fact, it is the resulting migration of cells as a *cohesive group* that underlies embryonic morphogenesis, wound repair and cancer tumour development and invasion. There are well-known techniques that allow an understanding of chemotaxis (directional movement of cells as a response to chemical stimuli) in solitary cell migration. But there is a need for developing a more relevant yet simple method for the characterization of chemotaxis in collective cell migration.

The primary goal of my project is to engineer and scale up a device for the high-throughput analysis of the effect of chemical gradients on collective cell migration *in vitro*. Using microfabrication and engineering tools, we will build a system to expose groups of cells to a gradient of growth factor and analyze their response. The optimized system will be designed to be compatible with high throughput fluorescence microscopy. Scaling up the technique to perform such tests in tissue culture plates will allow for screening of tens of experimental conditions within hours.

Engineering tools to gain mechanistic understanding of the underlying concepts of collective cell migration will lead to the development of novel strategies to enhance or suppress collective cell movement in a controlled manner. **The resulting engineered platform will help understand the basis of cell migration *in vitro*, but it will also significantly contribute to advances in tissue engineering, invasive disease research, and regenerative medicine.**

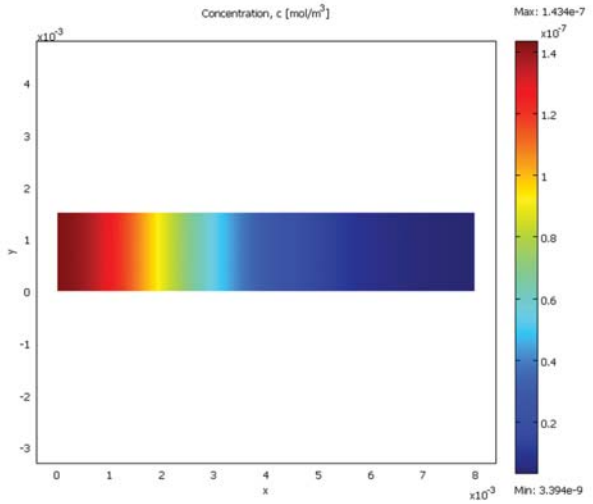


Fig. 1: Modelling diffusion of growth factor gradient to which cells will be exposed.

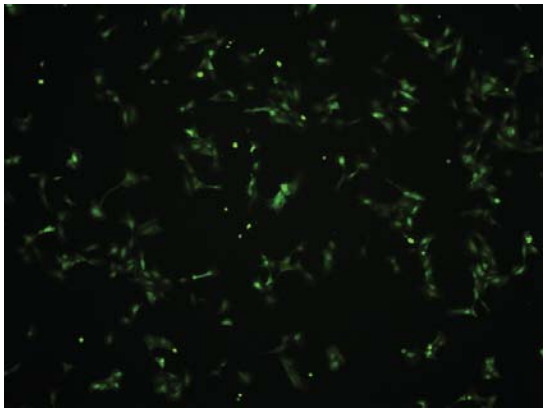


Fig. 2: HUVEC cells migrating as single cells or small clusters.

Fei Luo
Ph.D. Student

M.Sc., 2009, Nanyang Technological University, Singapore
B.Eng., 2008, Nanjing University of Technology, China

Supervisor: Elizabeth Edwards

Elucidating the initial activating mechanism of anaerobic benzene biodegradation

Benzene is a prevalent contaminant in the environment as a result of inadvertent leaks or spills of petroleum products. The presence of benzene in groundwater is of particular concern because it is a known human carcinogen and, therefore, its concentration in drinking water is regulated to a few parts per million. Groundwater impacted by petroleum hydrocarbon contamination typically becomes anaerobic. Therefore my overall goal is to understand the fate of benzene in anaerobic groundwater environments. Although anaerobic benzene biodegradation has been studied for more than 20 years, the initial reactions in this process are still not well understood.

For my doctoral studies, **I am interested in identifying the anaerobic activating mechanism of the stable unsubstituted benzene ring.**

To facilitate this study, we conducted medium experiments to improve cell growth in the enrichment cultures. We developed a medium preparation procedure that accelerates cell growth and associated benzene degradation. This method has increased the efficiency of the the generation of larger volumes of culture.

We also used comparative metatranscriptomics to provide clues to the metabolic pathway. Sequences from RNA samples extracted from the culture amended with benzene and on benzoate were sequenced with Illumina technology. We assembled the sequences into larger fragments and annotated the coding regions to identify genes that are up-regulated when the culture is amended with benzene. In this way, we were able to identify some genes that are involved in activating the benzene ring. Based on these results, I will design enzyme assays to provide activity-driven evidence for the proposed reaction.

The difficulty in isolating pure strains of anaerobic benzene-degraders suggests strong and complex interspecies interactions in these cultures (Fig. 1). Therefore, **I would also like to identify the microbial interactions within the microbial community that enable benzene detoxification.**

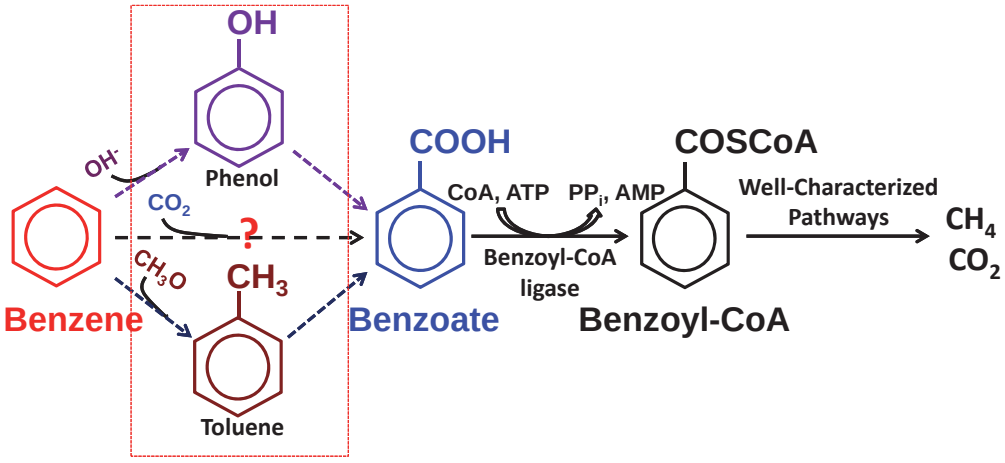


Fig. 1: Anaerobic benzene metabolic pathway and the hypothesized activating reactions (shown in dashed box).

Research Highlights

Luo F., Devine C.E., Gitiafroz R., Heidorn C., Gong Y. and Edwards, E. A. (2011). Anaerobic benzene biodegradation in enriched cultures. *13th CSChE Ontario Quebec Biotechnology Meeting*, Kingston, ON

Xu, Y., Luo, F., Pal, A., Gin, Y., Reinhard, M. (2011) "Occurrence of emerging organic contaminants in a tropical urban catchment in Singapore." *Chemosphere* 83(7):963-969

Research Highlights

Zhao, J. *et al.* (2011). Detailed modeling of subsurface microbes and interaction between microbial and hydro-geochemical process for sustained uranium removal (poster). *Subsurface Biogeochemical Research (SBR) Contractor-Grantee Workshop*, Washington, DC

Zhuang, K. *et al.* (2011). Dynamic Metabolic Modeling of communities and the design of effective uranium bioremediation strategies (poster). *111th American Society of Microbiology Conference,* New Orleans, LA

Zhuang, K., Ma, E., and R. Mahadevan (2011). Microbial community modeling and the design of effective uranium bioremediation strategies (presentation). *61st Canadian Chemical Engineering Conference*, London, ON

Eugene Ma
M.A.Sc. Student

B.A.Sc., 2010, University of Toronto

Supervisor: Radhakrishnan Mahadevan

A computational investigation of the metabolism of uranium-reducing microbes: *Anaeromyxobacter* and its role in uranium bioremediation

In recent years, *in situ* bioremediation of uranium has become a notable and novel process for addressing uranium (VI) groundwater contamination. By stimulating microbial metabolism in these environments, the results of field-scale experiments have shown that the successful reduction of uranium levels can be accomplished (Fig. 1). However, extensive repetitions of these experiments are often costly and time-consuming. My graduate project aims to alleviate these constraints via *in silico* computational analysis. **By using mathematical models, I am interested in understanding the complex microbial metabolic interactions and their influence on uranium bioremediation.**

In particular, my project focuses on understanding the microbial metabolism of *Anaeromyxobacter dehalogenans*, a bacterial strain that is both an effective metal reducer and an able dechlorinator. Although extensive laboratory studies have been conducted on this microbe, its unique metabolism is still poorly understood, and its contribution to uranium bioremediation in large field-scale scenarios has yet to be identified.

The approach of using constraint-based genome-scale models has proven to be effective in characterizing and representing the complex physiology of microorganisms in the natural environment. Built using genome sequences and available physical data, it is possible to reconstruct metabolic networks of various microbes and predict intracellular fluxes during growth. Based on this rationale, we are currently building a detailed genome-scale model for *Anaeromyxobacter dehalogenans*, using the Model SEED resource developed by Dr. Chris Henry. In addition, we are investigating the uranium-reducing kinetics of this microbe.

By utilizing and manipulating these mathematical models, it is possible to examine and predict the microbial behaviour of microorganisms in a community setting. **This work will enable us to optimize and design effective long-term engineering strategies for uranium bioremediation.**

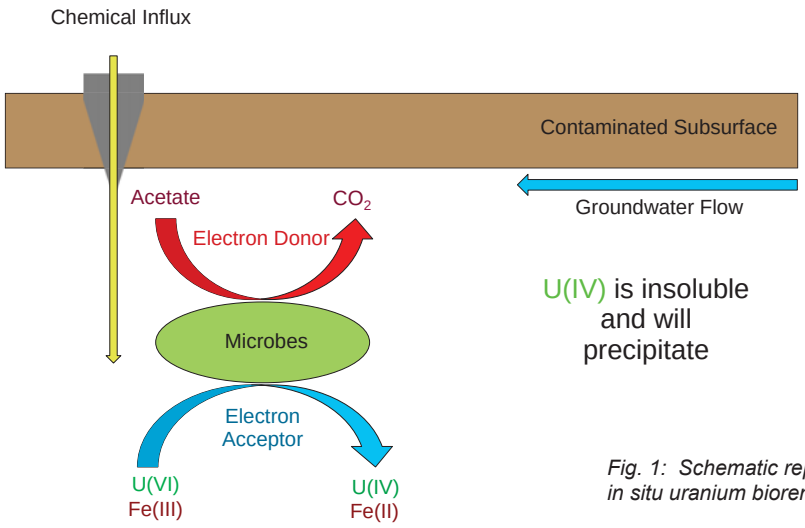


Fig. 1: Schematic representation of in situ uranium bioremediation.

Jacqueline MacDonald
Ph.D. Student

M.Biotech., 2006, University of Toronto
B.Sc., 2004, University of Western Ontario

Supervisor: Emma Master

Genetic adaptations of a softwood-degrading fungus

In Canada, softwood is a large renewable resource that could contribute to the production of sustainable fuels, biopolymers, and other chemicals. To create such products, wood needs to be separated into various molecular components that can each be made into different commodities. This separation can be achieved using enzymes from white-rot fungi — a group of organisms with the ability to degrade all major components of wood, as well as the bonds that hold these components together. While most white-rot fungi grow preferentially on hardwood, *Phanerochaete carnosa* is a white-rot fungus that has been found growing almost exclusively on softwood. **My goal is to uncover the genetic adaptations that promote softwood utilization by *P. carnosa*, which could be exploited for industrial bioprocessing of softwood.**

We have sequenced the *P. carnosa* genome to identify differences in gene content compared to the hardwood-degrading white-rot fungus, *Phanerochaete chrysosporium*. We used transcriptome sequencing to detect the expression levels of all *P. carnosa* genes during growth on four types of wood compared to a control substrate. Finally, I used quantitative real-time PCR to follow the temporal expression of specific genes related to wood degradation during growth of *P. carnosa* on four wood types.

Of particular interest is the discovery that *P. carnosa* differs from *P. chrysosporium* in the number and expression levels of two genes used to make enzymes - LiP and MnP - that modify the wood component lignin. *P. chrysosporium* has more genes for LiP and these are typically more highly expressed than its genes for MnP. In contrast, *P. carnosa* has more genes for MnP with higher expression levels than LiP. This distinction may allow *P. carnosa* to readily degrade the type of lignin found in softwood. Current experiments aim to detect variations in the efficiencies of lignin degradation by these enzymes.

Research Highlights

MacDonald, J., and E.R. Master (2011). "Time-dependent profiles of transcripts encoding lignocellulose-modifying enzymes of the white rot fungus *Phanerochaete carnosa* on multiple wood substrates". *Appl. Environ. Microbiol.* (submitted)

MacDonald, J. *et al.* (2011) "Transcriptomic responses of the softwood-degrading white-rot fungus *Phanerochaete carnosa* during growth on coniferous and deciduous wood". *Appl. Environ. Microbiol.* 77(10):3211-3218

Koehler, A. *et al.* (2009) "Molecular evolution of SPARC: absence of the acidic module and expression in the endoderm of the starlet sea anemone, *Nematostella vectensis*". *Dev. Genes Evol.* 219:509-521

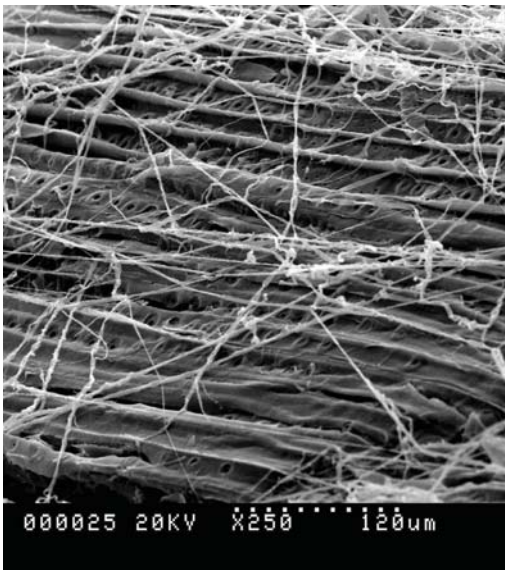


Fig. 1: Scanning electron micrograph of P. carnosa growing on Balsam fir (softwood).

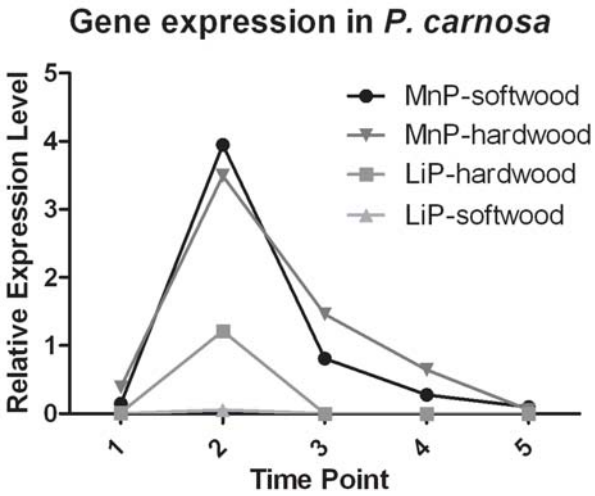


Fig. 2: Expression levels of genes for MnP and LiP in P. carnosa during growth on softwood and hardwood.

Sonam Mahajan
Postdoctoral Fellow

Ph.D., 2011, University of Toronto
M.A.Sc., 2006, University of Toronto
B.Tech., 2004, Indian Institute of Technology, IIT Delhi

Supervisor: Grant Allen

Developing molecular tools for analyzing algal biofilms

Algal biomass is a promising source of renewable fuel, bioproducts and specialty nutraceuticals. **Microalgae offer high growth rates, significant oil content, low nutrient requirements and the ability to grow on non-arable land including polluted water.** While several research initiatives are underway to harness the potential of algal biomass, process costs for the most part remain prohibitive, primarily due to the low concentration of algal biomass. Cultivating and maintaining algae as biofilms enables easy separation of algal biomass from the broth, and could potentially lead to increased cell density, and allow for growth and separation to take place in a single reactor.

Scaling-up and replication of bench top experiments require microbial fingerprints of the biofilms. Most investigations on succession studies in biofilms have focused on microscopic methods for identification of algal species and molecular methods only for bacteria. Microscopic methods are tedious, and discount the variance in morphology due to age and culture conditions of algae. The fragility of algae poses additional limitations. The purpose of this research is to **develop molecular tools to determine the microbial complexity of algal biofilms cultivated on treated wastewater.** Extracted DNA will be amplified and then sequenced to determine the microbial species present. An insight into the species composing the biofilm will allow systematic manipulation of physical and biochemical environment for optimal cultivation and end-product properties like lipid content and biomass concentration.

Fig. 1: Biofilm grown on a glass slide.

Research Highlights

Mahajan, S. (2011). The inaugural analysis of the white-rot fungus, *Phanerochaete carnosa*, through proteomic methods and compositional analysis of decayed wood fibre. Ph.D. Thesis, *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto

Mahajan, S., Jeremic, D., Goacher, R., and E. Master (2011). "Mode of coniferous wood decay by the white rot fungus *Phanerochaete carnosa* as elucidated by FTIR and ToF-SIMS". *Appl. Microbiol. Biotechnol.* (Accepted)

Mahajan, S. and E. Master (2010). "Proteomic characterization of lignocellulose-degrading enzymes secreted by *Phanerochaete carnosa* grown on spruce and microcrystalline cellulose. *Appl. Microbiol. Biotechnol.* 86(6):1903-1914

Elisa McGee
M.A.Sc. Student

B.A.Sc., 2010, University of Toronto

Supervisor: Levente Diosady

Triple fortification of salt with iodine, iron, and folic acid

In developing countries micronutrient (vitamin and mineral) deficiencies are common. People in these countries often have a diet that consists predominantly of micronutrient-poor food. Micronutrient deficiencies have been tied to devastating impacts on human health, national growth, and national productivity. The missing micronutrients can be provided by supplementation, dietary diversification or food fortification. Food fortification refers to the addition of micronutrients to broadly consumed foods. It is the most effective method of alleviating micronutrient deficiencies, as a large population may receive the nutrients on a regular basis with no active participation or dietary changes.

My research group, the Food Engineering Group, has developed methods for the fortification of both salt and rice. Double fortified salt that contains both iodine and iron is now distributed in developing countries. **The objective of my project is to develop appropriate fortification technologies for the incorporation of folic acid into salt fortified with iodine and iron.** Folic acid is a synthetic form of vitamin B9 (folate) that is often used in food fortification and supplements because of its augmented stability and bioavailability. Vitamin B9 deficiency is a leading cause of birth defects, and been linked to heart disease, poor growth and anaemia.

In this project I will fortify salt with iodine, iron, and folic acid using several technologies: development of folate solutions that may be sprayed onto the salt (Fig. 1); preparation of folic acid premixes by spray drying encapsulation, and extrusion agglomeration with surface coating. I will monitor the stability of the micronutrients for several months under conditions typical of developing countries. **At the conclusion of this project the most appropriate technologies for the triple fortification of salt with iron, iodine, and folic acid will be identified.**

Research Highlights

McGee, E. (2010). Triple fortification of salt: Folic acid stability testing using HPLC. B.A.Sc. thesis, *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto

McGee, E. and L. L. Diosady (2011). Triple fortification of salt with iron, iodine, and folic acid (poster). *13th CSChE Ontario-Quebec Biotechnology Meeting*, Kingston, ON

Fig. 1: Figure 1: Nutrient Solutions for Spray Application (1% Folic Acid, 2% Folic Acid, 3% Folic Acid)

Research Highlights

Meyer, T., Lei, Y.D., and F. Wania (2006)."Measuring the release of organic contaminants from melting snow under controlled conditions." *Environmental Science and Technology* 40:3320-3326

Meyer, T., De Silva, A., Spencer, C., and F. Wania (2011). "Transport of perfluorinated carboxylates and sulfonates during snowmelt within an urban watershed." *Environmental Science and Technology* 45:8113-8119

Torsten Meyer
Research Associate

Ph.D., 2008, University of Toronto
Dipl. Ing., 2002, Technische Universität Berlin

Supervisor: Elizabeth Edwards

Anaerobic digestion and lignocellulosic biomass biotransformation

My research interests comprise a wide range of areas that are related to water resource science and engineering.

In my position within BioZone I am interested in the fields of anaerobic digestion of pulp and paper mill wastewater streams and lignocellulosic biomass biotransformation. Currently, I am involved in a project to study the cause of degranulation of sludge granules in anaerobic reactors.

Certain in-mill wastewater streams contain high concentrations of compounds, such as resin acids and fatty acids (RFAs), that adversely affect the anaerobic digestion process (Fig. 1). The impact of those substances includes diminished biogas production and disintegration of anaerobic sludge granules. We designed, constructed, and tested a laboratory-scale anaerobic reactor setup (Fig. 2). We will feed those reactors with post extraction washer (PEW) effluent from Tembec's pulp mill in Temiscaming, where PEW effluent is largely excluded from anaerobic treatment because of its detrimental impact on process performance. This study aims to assess and minimize the impact of PEW effluent and other problematic in-mill streams on anaerobic sludge granulation and digester performance. In order to gain an understanding of the underlying processes, we will closely monitor the microbial community, the RFA composition, and the physical properties of the granules within the reactors using pyrotag sequencing, liquid chromatography-mass spectrometry, and granule analysis methods. Process optimization will further be supported by simulation studies using the software GPS-X.

An overarching goal of this project is to convert as much waste organic matter from pulp and paper mill streams as possible into utilizable biogas.

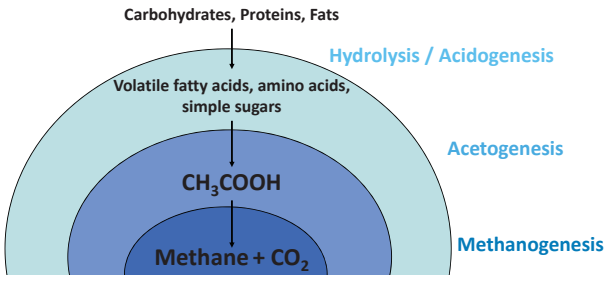


Fig. 1: Simplified schematic describing the transformation of complex organic matter to utilizable methane

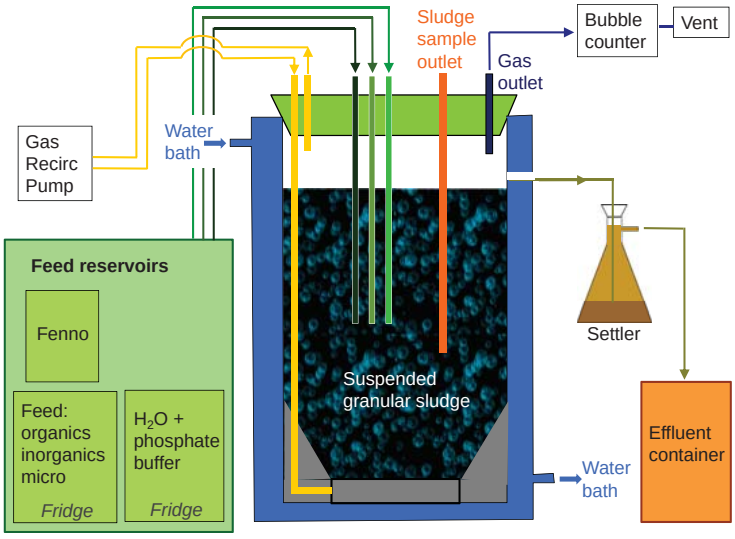


Fig. 2: Laboratory reactor setup

Olivia Molenda
Ph.D. Student

B.Sc., 2011, York University

Supervisor: Elizabeth Edwards

Deducing the role of reductive dehalogenase enzymes in mixed microbial cultures used for bioremediation of chlorinated aliphatic hydrocarbons

Most chlorinated aliphatic hydrocarbons (CAHs) are highly toxic. Some are even known human carcinogens. CAHs are being produced worldwide at an estimated rate of 3 x 10^6 ton $year^{-1}$. Production is primarily due to the manufacturing of PVC, aerosol, propellants and refrigerants, or for use as solvents for degreasing and dry-cleaning. Problems with handling and storage have lead to the accumulation of these persistent toxic chemicals in various environments including groundwater. Decontamination can be achieved through bioremediation – using bacteria to reduce toxic compounds to their non-lethal constituents and, in the case of CAHs, through reductive dechlorination.

Two enriched mixed microbial cultures known as KB-1 and WBC-2 have been developed in Professor Edwards lab. KB-1 has been successfully used for commercial bioremediation of groundwater at over 200 sites. Recent research efforts have focused on classifying which bacteria are present in the cultures and what role each bacterium plays in the dechlorination process (Fig. 1). **My research will attempt to further classify the cultures by identifying the genes encoding for reductive dehalogenase enzymes (RDases) and connecting them to dechlorinating functions.**

The study of RDases in *Dehalococcoides* (Dhc) has shown the presence of novel RDases as well as evidence of horizontal gene transfer between organisms within KB-1 and WBC-2. For example, *Dehalogenimonas eccentricus*, an organism in the WBC-2 culture has been identified as the main dechlorinator of trans-1,2-dichloroethene, a reduce chlorinated compound. This is the first time a non-*Dehalococcoides* organism has been found to dechlorinate reduced compounds; understanding where this organism gained this ability will help us enhance the dechlorinating properties of both KB-1 and WBC-2 cultures. **Complete understanding of dechlorinating mechanisms is essential to optimizing bioremediation of CAHs – persistent, toxic compounds which are continuously being produced worldwide.**

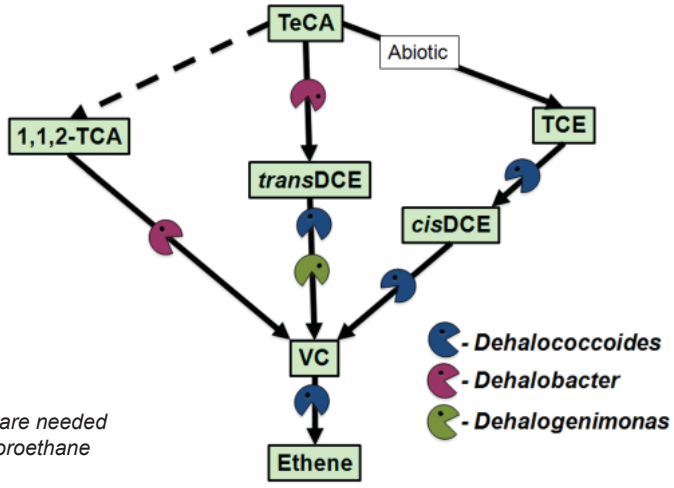


Fig. 1: Three different dechlorinating bacteria are needed for complete transformation of 1,1,2,2-tetrachloroethane (TeCA) in the WBC-2 culture.

Research Highlights

Jones, B., Molenda, O., Hayward,C., D'Aguiar, M., Miller, N., Cottenie, K., and L. Rye (2011). "Patterns of tree diversity in response to logging in Algonquin Provincial Park". *SURG.* 4:56-62

Molenda, O., Reid, A., and C. Lorite (2011) "The alpine cushion plant *Silene acaulis* as a foundation species: a plant and bug's eye view to facilitation and microclimate". *Oikos* (Under revision ID=o20159)

Research Highlights

Mottiar, Y. and E. R. Master (2011). Engineering galactose oxidase to enable wood carbohydrate derivatisation (poster). *Gordon Research Conference on Cellulosomes, Cellulases & Other Carbohydrate Modifying Enzymes*, Easton, MA

Mottiar, Y. and I. Altosaar (2011). "Iodine sequestration by amylose to combat iodine deficiency disorders." *Trends Food Sci. Technol.* 22(6):335-340

Yaseen Mottiar
M.Sc. Student

B.A.Sc., 2008, University of Ottawa
B.Sc., 2007, University of Ottawa

Supervisor: Emma Master

Enzymes and feedstocks for sustainable biomass utilisation

The sustainable forest bioproducts industry in Canada is potentially worth up to 100 billion dollars annually. But lignin in recalcitrant woody feedstocks reduces processing efficiency and inhibits the downstream fermentation that produces biofuel. Amid concerns about the long-term economic feasibility of biofuels production, there is also a drive to develop high-value products from wood fibre that would economise future biorefineries.

In light of these opportunities, **the aim of my research is to study natural low-lignin woody species which have relevance in feedstock engineering and to improve the usefulness of carbohydrate oxidase enzymes** for the derivatisation of plant-derived carbohydrates.

Eastern leatherwood (*Dirca palustris* L.) is a native understory shrub found uncommonly in rich soils across eastern North America (Fig. 1). I have found that xylem tissues of this species have remarkably low levels of lignin in comparison to two other reference species. The lignin is also comparatively rich in syringyl-type units and is more easily hydrolysed. Besides noteworthy wood chemistry, leatherwood also displays a number of secondary adaptations that have co-evolved with hypolignification.

Carbohydrate oxidases, such as galactose oxidase and glucooligosaccharide oxidase, are unique biocatalysts capable of converting hydroxyl groups of cellulose and hemicellulose to carbonyl and carboxylic acids. These resulting reactive handles can be targeted for further derivatisation to produce a wide array of high-value bioproducts. I have worked at improving the substrate range of these enzymes through mutagenesis and domain swapping. Galactose oxidase is particularly appealing because of its carbohydrate-binding module and surface-exposed active site which accepts bulky hemicellulosic substrates (Fig. 2).

Through my work, **I have sought to improve the efficiency and economics of sustainable biomass utilisation.** I have promoted leatherwood as a model species for the study of hypolignification in woody plants. In addition, I have produced a range of carbohydrate oxidase variants that may be useful in the production of high-value bioproducts from plant-derived carbohydrates.

Fig. 1: Eastern leatherwood is a model hypolignified woody species that could inspire efforts to engineer low-lignin plants.

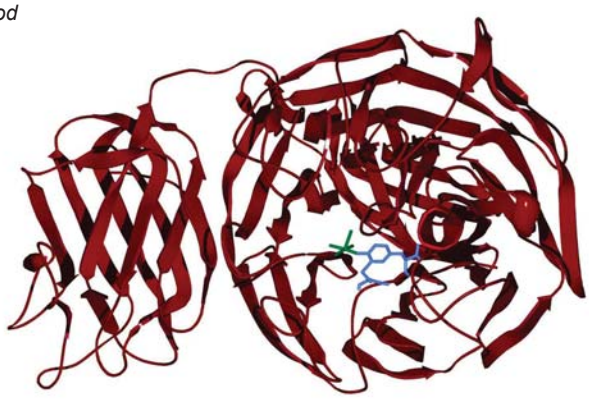


Fig. 2: Galactose oxidase is a carbohydrate oxidase which can be used to derivatise plant-derived polysaccharides (PDB: 1GOF).

Ph.D., 2011, University College Cork
M.Sc.Eng., 2007, Chalmers University of Technology
B.Sc. (Hons.), 2004, University of the Punjab

Abdul-Sattar Nizami
Postdoctoral Fellow

Supervisor: Bradley Saville
Co-Supervisor: Heather MacLean

Life cycle analysis of biofuels and bioproducts

Biofuels were used for energy before the industrial revolution, including wood for heat, while ethanol was used for early automobiles, and the diesel engine was designed to run on plant oils. Later, large scale economic development occurred with the expanded use of coal, oil and natural gas. As a consequence, greenhouse gas (GHG) emissions have increased significantly, affecting all human societies living on Earth. Biofuels and bioenergy have re-emerged, and along with bioproducts, can help to mitigate the GHG emissions and other air pollutants. However there is a need to make biofuels and bioproducts economically profitable, socially acceptable and environmental friendly.

Commercialization of biofuels is expected in the near future due to improved process technologies and added-value products. Thus, **to ascertain optimal biofuel strategies, it is necessary to take into account environmental impacts of bioproducts (by-products) from cradle to grave.** Life cycle assessment (LCA) techniques allow detailed analysis of various bioproducts along with the main biofuels. Fig. 1 provides an example of bioproducts created as a result of biomethane production. At the same time, if not developed strategically, these processes can lead to incorrect and inappropriate actions on the part of industry and policy makers, and affect people's perception of bioenergy.

I have recently joined BioZone researchers as a postdoctoral fellow, working with Prof. Brad Saville and Prof. Heather MacLean to evaluate environmental, economic and social aspects of biofuels and bioproducts in an integrated sustainability assessment. I have a Ph.D. in green grass: developing grass for sustainable gaseous biofuel. I have published 14 peer review journal papers (total impact factor of 54.5), 8 international conference papers, 4 book chapters and a further 2 invited lectures; my work has been cited over 73 times in peer review press (H factor of 5). For more details, see http://individual.utoronto.ca/Nizami/

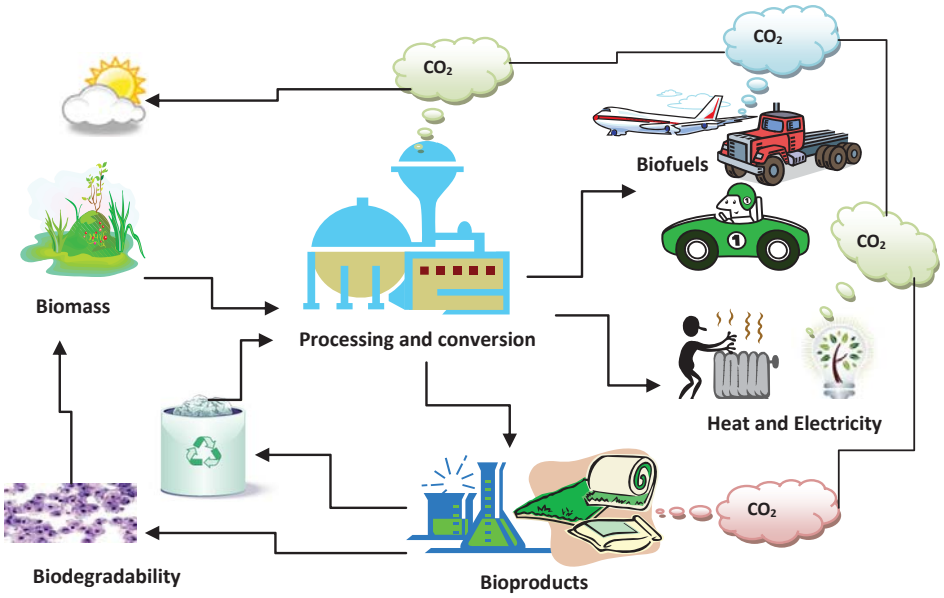


Fig. 1: Biorefining: the cycle of biomass to bioenergy and bioproducts

Research Highlights

Nizami, A.S., Orozcoc, A., Groom, E., Dieterich, B. and J.D. Murphy (2011). How much gas can we get from grass? *Applied Energy* 92:783-790

Nizami, A.S. and J.D. Murphy (2011). Optimizing the operation of a two-phase anaerobic digestion system digesting grass silage. *Environ. Sci. Technol.,* 45 (17):7561 -7569

Nizami, A.S., Singh, A. and J.D. Murphy (2011). Design, commissioning, and start-up of a sequentially fed leach bed reactor complete with an upflow anaerobic sludge blanket digesting grass silage. *Energy and Fuels* 25 (2):823-834

Nizami, A.S., Thamsiriroj, T., Singh, A. and J.D. Murphy (2010). The role of leaching and hydrolysis in a two phase grass digestion system. *Energy and Fuels* 24(8):4549 -4559

Mehdi Nouraei
M.A.Sc. Student

M.Sc., 1996, Shiraz University
B.Sc., 1993, Isfahan University of Technology

Supervisor: Levente Diosady

Developing a microencapsulated self-microemulsifying delivery system to enhance the bioavailability of lipophilic nutraceuticals

Food fortification is a viable strategy for delivering nutraceuticals and micronutrients for the prevention and cure of micronutrient deficiency diseases. However, the development of novel fortified functional foods faces technological barriers such as stability, solubility and bioavailability. Many of bioactive compounds and nutraceuticals have low bioavailability due to inherently poor water-solubility or instability during processing, storage and consumption.

We have developed a delivery system for lipophilic nutraceuticals by integrating two delivery approaches: fortified self-microemulsifying delivery system (S(M)EDS) to address the solubility and bioavailability issues, and microencapsulation technology for improved stability and protection against harsh environmental and biological conditions prior to digestion. We developed a pre-concentrate composed of an oil (ethyl caprate), a surfactant (lecithin), and lipophilic/hydrophilic linkers in appropriate ratios, fortified with the target ingredient (ß-carotene as a model bioactive) (Fig.1). Upon dilution with intestinal fluid and mild agitation, this structured vehicle spontaneously generates a microemulsion with nano-sized droplets and delivers the dissolved active molecule directly to the intestine wall.

Next, I examined the functionality of three coating materials for microencapsulating the S(M)EDS. A shellac-based formulation, made from food grade and FDA-approved materials, was selected as the suitable enteric coating agent and was spray dried to coat the structured vehicle. This film-forming and pH-responsive formulation protects the fortified S(M)EDS in the acidic conditions of the stomach and releases its content in an emulsified state into the intestines (Fig. 2). **By developing proper formulations and optimizing the process conditions, this project is expected to create a novel delivery system that can be incorporated into food products as a fortifying agent and improve the bioavailability of water-insoluble micronutrients.**

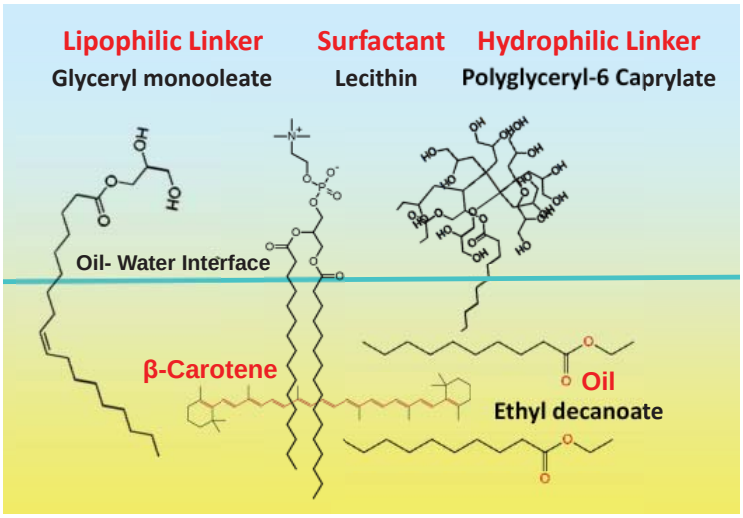


Fig. 1: The composition and structure of SMEDS upon dilution.

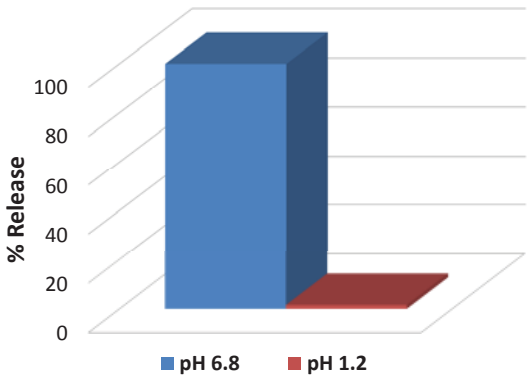
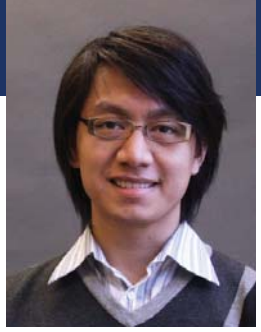


Fig. 2: Release profile of enteric coated spray dried microcapsules in pH 1.2 and pH 6.8



Jon Albert Obnamia
M.A.Sc. Student

B.A.Sc., 2011, University of Toronto

Supervisor: Bradley Saville

Kinetics-based enzymatic hydrolysis model for the optimization of hydrolysis process parameters and prediction of sugar concentration profiles

Research into the production of sustainable biofuels from lignocellulosic biomass has focused on the three key areas of pre-treatment, enzymatic hydrolysis, and fermentation. In particular, the area of enzymatic hydrolysis has seen developments in enzyme engineering and process optimization and scale-up.

In my Master's research thesis, I am exploring the kinetics-based mathematical model that characterizes the lignocellulose hydrolysis process, as it relates to process optimization and scale-up.

The kinetics-based model is a mathematical tool with implications for optimizing process variables and for predicting results and performance of reaction and reactor system configurations (Fig. 1). Process variables such as substrate (biomass) load and enzyme dose are optimized such that maximum amounts of substrate are hydrolyzed into fermentable sugars, and minimum amounts of enzymes are utilized. Performance is gauged on hydrolysis rates and the yields of fermentable sugars, in reference to the maximum sugars in the biomass.

The ultimate goal in lignocellulose hydrolysis is to attain high levels of fermentable sugars like glucose and xylose for conversion to biofuel or other valuable co-products. With an accurate and versatile model for the process, optimum variables would be readily determined and results for various reaction schemes would be predicted.

In validating the model, experiments would be performed to determine whether or not the model is capable of simulating various conditions. Of particular interest is to study the flexibility of the model in terms of its ability to accommodate different substrates (and substrate pre-treatment methods) and enzyme cocktails, while predicting reasonable results.

There are also possibilities to be discovered in conjunction with system wide models currently using yield-based lignocellulose hydrolysis models. **The kinetics or rate-based models can be developed to provide more insightful information and allow greater flexibility in systems modeling, thus enabling more accurate technical and economic decisions to be made.**

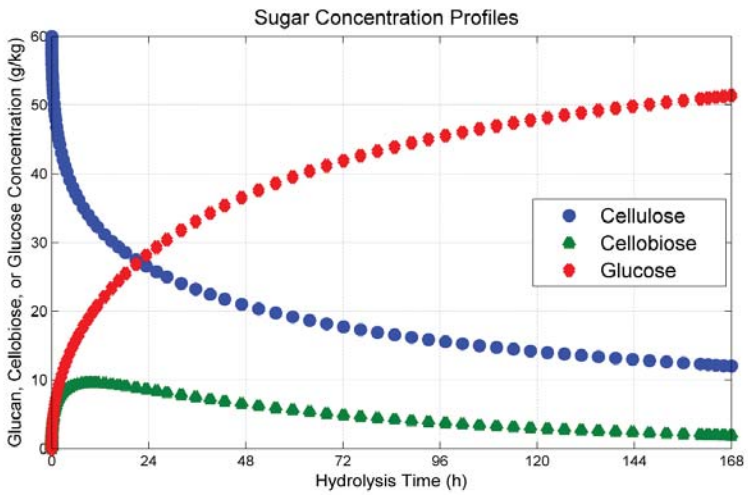


Fig. 1: Model predictions of the hydrolysis profiles of lignocellulose sugars in a batch reactor setup.

Research Highlights

Obnamia, J.A. (2011). A study on the effects of enzyme dose and substrate load on the enzymatic hydrolysis of steam-pretreated poplar wood. B.A.Sc. Thesis, *Dept. of Chemical Engineering and Applied Chemistry,* University of Toronto

Obnamia, J.A. (2011). Substrate load and enzyme dose study on the enzymatic hydrolysis of steam-pretreated poplar wood (poster). *2nd NSERC Bioconversion Network Annual General Meeting,* Toronto, ON

Research Highlights

Petkowski, J.J., Chruszcz, M., Zimmerman, M.D., Zheng, H., Skarina, T., Onoprienko, O., Cymborowski, M.T., Koclega, K.D., Savchenko, A., Edwards, A., and W. Minor (2007). "Crystal structures of TM0549 and NE1324--two orthologs of *E. coli* AHAS isozyme III small regulatory subunit." *Protein Science* 16(7):1360-1367

Delvaux, D., Murty, M.R.V.S., Gabelica, V., Lakaye, B., Lunin, V.V., Skarina, T., Onopriyenko, O., Kohn, G., Wins, P., De Pauw, E. and L. Bettendorff (2011). "A specific inorganic triphosphatase from *Nitrosomonas europaea*: structure and catalytic mechanism." *J. Biol. Chem.* 286(39):34023-34035

B.A.Sc, 2005, Université du Québec à Montréal
M.Sc, 1998, Académie d'État des Technologies d'Alimentation d'Odessa, Ukraine

Olena Onopriyenko
Technician

Supervisor: Alexei Savchenko

Optimization of protein purification and crystallization for obtaining X-ray crystal structures

Together with colleagues from Dr. Savchenko's group **I participate in projects leading to the development of new antibacterial drugs, by obtaining structural information of proteins of important bacterial pathogens.**

Because of the high degree of hydration, typically reaching 40 to 60% water by volume of crystal, crystalline proteins maintain their native conformation and function. The three-dimensional crystal structures of proteins can provide a basis for understanding protein function and biological activity and, therefore, the crystal structure can be employed as a starting point for designing drugs specifically interacting with target proteins.

Determination of crystal structure by X-ray crystallography is a complex multistep process that involves cloning and expression of selected target proteins, protein purification, crystallization screening and crystal optimization, followed by data collection and structure determination. The success of crystallization experiments strongly depends on the purity of proteins and the conditions chosen for protein crystallization. With my background in biology and a degree in analytical chemistry earned at Université du Quebec à Montreal, I focus on optimizing protein purification using Ni-Affinity Chromatography and Fast Protein Liquid Chromatography (FPLC). Although I specialize in FPLC, I also have the skills required for cloning, growing bacterial cultures, testing expression of proteins, setting up initial crystallization screening trials and checking the quality of crystals using diffractometer.

My work in Dr. Savchenko's group is centered on obtaining high purity proteins and finding crystallization conditions for different proteins.

Fig. 1: Crystals from proteins from Thermoplasma acidophilum.

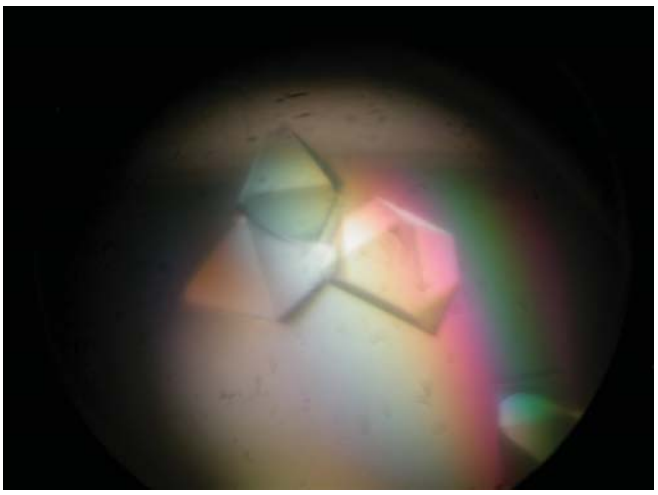


Fig. 2: Crystals from proteins from Pseudomonas aeruginosa.

A. Vikram Pandit
Ph.D. Student

B.A.Sc., 2009, University of Toronto

Supervisor: Radhakrishnan Mahadevan

Microbial electrosynthesis for metabolic engineering of biochemicals

In response to economic and environmental considerations, there has been increased interest in the development of commercial bioprocesses that produce biofuels and specialty chemicals. Conventional petrochemical routes are environmentally unfriendly. Furthermore, as the cost of petrochemical feedstocks becomes increasingly expensive, alternative processes or feedstocks are eventually expected to replace or at least supplement them. Bioprocesses have the capacity to manufacture bulk and fine chemicals and fuels at significant scale, and offer the advantage of being cost competitive and sustainable. Metabolic engineering of microorganisms is important for establishing economically viable bioprocesses.

A critical concern in metabolic engineering is the need to balance the demand and supply of redox intermediates such as NADH. Bioelectrochemical techniques offer a novel and promising method to alleviate redox imbalances during the synthesis of biochemicals and biofuels. We have developed a method to characterize the role of bioelectrosynthesis in chemical production using the genome-scale metabolic model of *E. coli*. **The lessons learned from these methods are now being applied to *in vivo* to engineer strains of *E. coli* that are able to use electrochemical power to improve yields of biochemicals and increase productivity.** My project looks at improving yield of the model compound succinic acid.

In depth analysis of these techniques will help to (1) provide insight in understanding necessary mutations to upregulate certain pathways; (2) explore the possibility of strain evolution on an electrode; (3) develop processes that are able to use carbon dioxide as the main carbon source; (4) establish bioprocesses that consider bioelectrosynthesis dynamically during batch operation leading to greater process productivity. With the knowledge gained in this study, overall bioprocesses will see economic benefits from greater product yield, and underlying changes observed in the *E. coli* regulatory and metabolic network may provide insights on how to improve traditional metabolic engineering strategies.

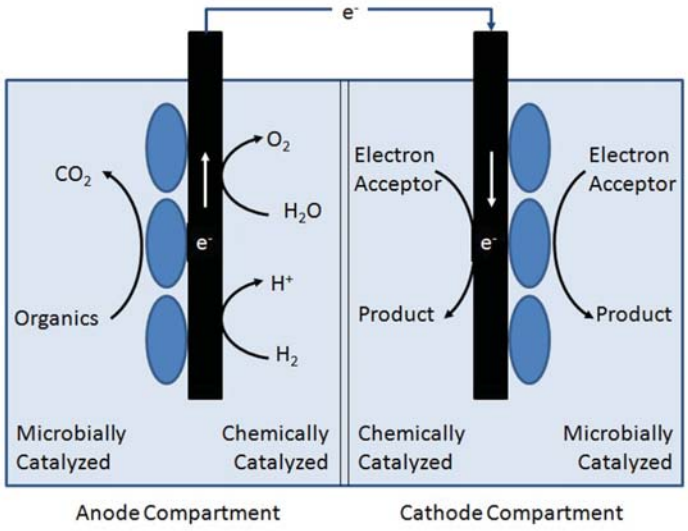


Fig. 1: A typical BES consists of two compartments separated by an ion exchange membrane that separates the oxidation reaction from the reduction reaction.

Research Highlights

Pandit, A.V. and R. Mahadevan (2011). "*In silico* characterization of microbial electrosynthesis for metabolic engineering of biochemicals." *Microb. Cell. Fact.* 10:76

Research Highlights

Pérez-de-Mora, A. *et al.* (2010). Anaerobic bioremediation of volatile organic compounds in a fractured bedrock system: insights from a bioaugmentation trial in Canada (poster). *13th Intl Symp.on Microb. Ecol.*, Seattle, WA

Lebrón, C. *et al.* (2010). Standardized procedures for nucleic acid-based tools for microbial monitoring (poster). *Partners in Environ. Technol. Technical Symp. & Workshop,* Washington, DC

Mundle, S.O.C. *et al.* (2011). Using carbon isotope fractionation to identify ethylene degradation at contaminated field sites (invited speaker). *Intl Symp.on Bioremed. and Sust. Environ. Tech.*, Reno, NV

Peréz-de-Mora, A. *et al.* (2011). Enhanced *in situ* bioremediation of chloroethenes in fractured bedrock: A combined approach to assess remediation and microbial activity (invited speaker). *OMICS Group 2nd World Congress,* Philadelphia, PA

Ph.D., 2006, Institute of Natural Resources and Agrobiology and University of Sevilla, Spain
M.Sc., 2001, University of Sevilla, Spain

Alfredo Pérez de Mora
Postdoctoral Fellow

Supervisor: Elizabeth Edwards

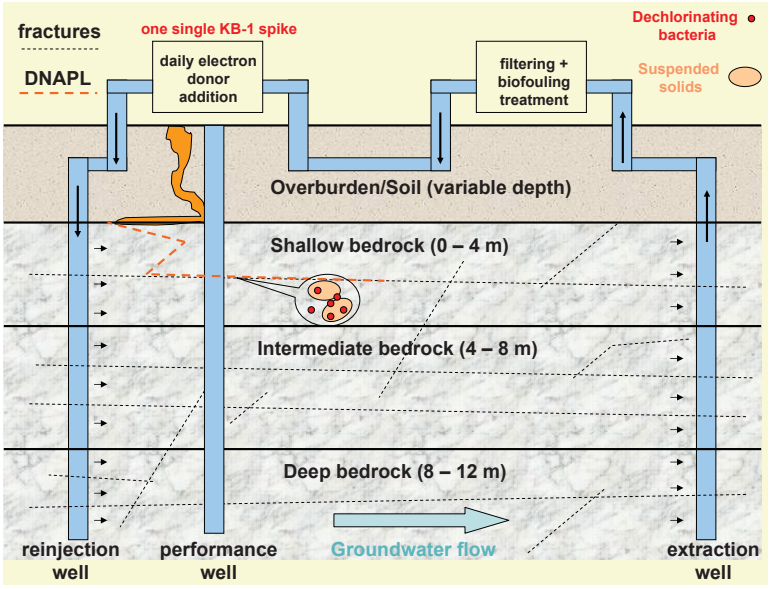
Unraveling key biotic-abiotic factors for sustainable biotransformation of chlorinated compounds in groundwater

Chlorinated solvents are priority pollutants frequently found in groundwater. Anaerobic biotransformation of these compounds to non-toxic products such as ethene via reductive dechlorination is exclusively carried out by microorganisms living in consortia rather than acting as individual species. In such consortia many different species work together akin to a multicellular life form.

This project aims to provide new insights into the biogeochemical interactions affecting chlorinated solvent bioremediation in subsurface anaerobic environments. Specifically our research focuses on three main aspects:

- contaminant metabolism and interspecies interactions in mixed microbial communities using metagenome sequence and metabolomic profiling
- identification of biomarkers through molecular screens and isotopic fractionation signatures to monitor biodegradative mechanisms and the key players involved in these processes
- abiotic-biotic interactions and physiological influences on microbial activity in the subsurface at contaminated sites.

Research involves both field and laboratory studies. In collaboration with Geosyntec Consultants Inc. (Guelph, Canada) we conducted a field trial at a fractured bedrock site where enhanced reductive dechlorination of chlorinated ethenes was achieved with biostimulation of indigenous microbial populations and later bioaugmentation using the mixed microbial consortium KB-1®. Special emphasis was placed on studying population dynamics of organisms relevant to reductive dechlorination including fermenters, acetogens, methanogens and dechlorinators. In addition, the utilization of reductive dehalogenase genes from *Dehalococcoides* (main dechlorinator at the site) as biomarkers to distinguish between native and non-native *Dehalococcoides* was examined.



With collaborators in Germany (Dr. Philippe Schmitt-Kopplin, Helmholtz Centre Munich) we are also investigating species interactions within mixed microbial consortia capable of biotransformation of various chlorinated compounds under anaerobic conditions via non-targeted metabolomics using advanced mass spectrometric tools. **This multi-faceted approach can be used to accelerate the discovery and characterization of novel microbial consortia that transform these problematic pollutants.**

This research is supported by the European Commission through a Marie Curie Outgoing International Fellowship (Project AnDeMic).

Fig. 1: Vertical profile of the groundwater recirculating system used for enhancing reductive dechlorination at a fractured bedrock contaminated site.

Ana Popovic
Technician

M.Sc., 2008, University of Toronto
B.Sc., 2005, University of Toronto

Supervisor: Alexander Yakunin

Screening environmental metagenomes for enzyme activities

One of the objectives of the project *BEEM: Bioproducts and Enzymes from Environmental Metagenomes* is to develop new and better strategies for bioremediation of industrial waste sites and engineer new bioprocesses; thus **we have undertaken the task of identifying new industrial enzymes present in natural microbial communities at contaminated sites and in specialized extreme environments.** Since less than 1% of environmental microbes can be cultured in the laboratory, we have turned to functional metagenomics to tap this wealth of genes.

Currently, we have over 20 different metagenome DNA libraries made in-house with our BEEM project colleagues and obtained from our European collaborators at MAMBA and Genoscope. These libraries sample genomes from microbial communities all over the world, from deep-sea hypersaline basins in the Mediterranean, to oil and heavy metal contaminated regions in northern Europe, even cellulose-digesting herbivores here in Canada.

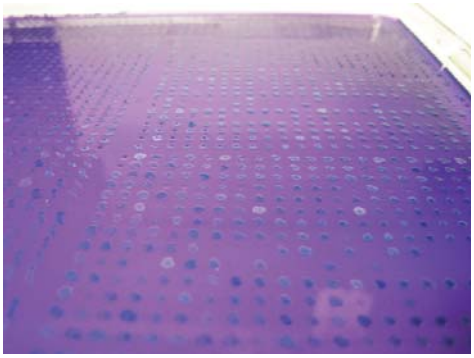


Fig. 1: Fosmid library screen for dehalogenase activity.

The focus of my work is to screen these metagenome DNA libraries expressed in *E. coli* cells for enzyme activity (Fig. 1), and ultimately to identify the genes responsible (Fig. 2). At the moment we are screening the libraries for esterase, lipase, glycosyl hydrolase, protease and dehalogenase acitivities, although additional enzyme screens are under development in the lab.

To date, we have screened over two million clones, found over 800 enzyme activity hits, and cloned over 150 target genes. Protein purification and biochemical characterization of these enzymes are in progress.

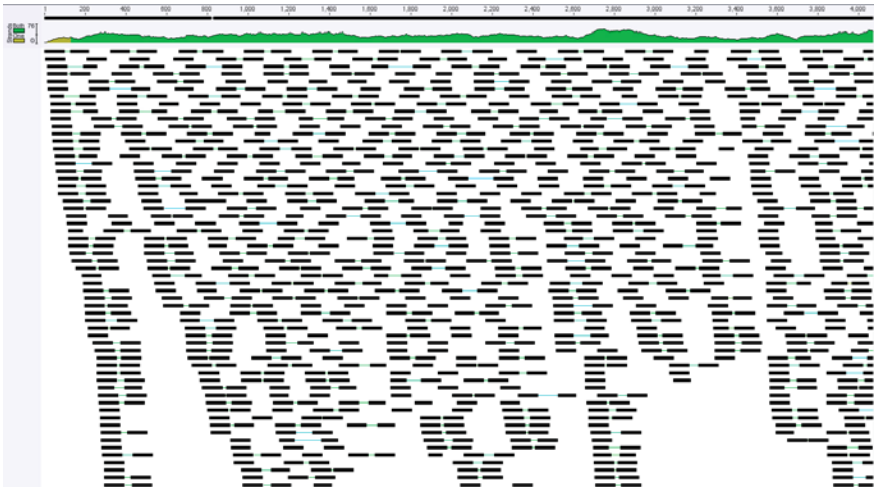


Fig. 2: Next Generation sequence assembly of a fosmid for an isolated positive clone. (Graphic generated in Geneious Pro)

Research Highlights

Popovic, A., *et al.* (2011). Screening of environmental metagenomes for enzyme activities (speaker). *2nd Annual BEEM Meeting*, Toronto, ON

Tchigvintsev, A., Lemak, S., Brown, G., Yakunin, A., and T. Hai (2010). Screening of environmental metagenomes for enzyme activities (poster). *1st Annual BEEM Meeting*, Toronto, ON

Popovic A. (2008). Characterization of a virally encoded chaperone involved in λ DNA packaging. M.Sc thesis. *Dept. of Biochemistry*, University of Toronto

Popovic, A., Maxwell, K.L., and A.R. Davidson (2007). Role of gpFI in bacteriophage lambda DNA packaging (invited speaker). *American Society for Microbiology General Meeting*, Toronto, ON

Research Highlights

Pourbafrani, M., McKechnie, J., Maclean, H. and B. Saville (2010). Life cycle assessment of a citrus waste biorefinery (speaker). *2nd Annual BEEM Research Meeting*, Toronto, ON

Pourbafrani, M., McKechnie, J., Maclean, H. and B. Saville. "Greenhouse gas impacts of biofuel and limonene production from citrus waste". (in prep)

Pourbafrani, M., Forgacs, G., Horvath, S.I., Niklasson, C. and M.J. Taherzadeh (2010). "Production of biofuel, limonene and pectin from citrus wastes". *Bioresour. Technol.* 101:4246-4250.

Lohrasbi, M., Pourbafrani, M., Niklasson, C. and M.J. Taherzadeh (2010). Process design and economic analysis of a citrus waste biorefinery with biofuels and limonene as products. *Bioresour. Technol.*101:7382-7388.

Ph.D. (Chem. Eng.), 2010, Chalmers University of Technology
M.Sc., 2005, Sharif University of Technology

Mohammad Pourbafrani
Postdoctoral Fellow

Supervisor: Bradley Saville
Co-Supervisor: Heather L. Maclean

Life cycle assessment and cost analysis of biofuel

Many studies are currently conducted on the development and optimization of biofuel production from various biomasses such as woody biomass, agricultural and municipal wastes. Biofuel could replace petroleum fuels such as natural gas, diesel, jet fuel and gasoline. This replacement reduces dependency on oil and gas and also helps the environment through reduction of greenhouse gas emissions (GHG).

However, there are issues concerning the economic and environmental aspects of biofuel processes. Each biofuel process needs to be studied in detail, the cost of biofuel production has to be calculated and the effect of various parameters such as biomass cost and plant capacity on production cost must be evaluated. Furthermore, the environmental aspect of biofuel process should be studied through a life cycle assessment of the process.

My research focus is the economic and environmental evaluation of biofuel production from biomass. These evaluations are carried out through detailed process modeling based on pilot and experimental data. The process modeling helps us to calculate the energy requirements of a process, product distributions and yields, and the size of process equipment. Later, the modeling results are used in economic and life cycle analyses.

At this stage, we have studied the GHG emission from biomethane and bioethanol production from citrus wastes and results showed that significant reductions in GHG emissions are achieved through electricity generation from biomethane and use of bioethanol as light-duty vehicle fuel. **We will work on the economic analysis of bioethanol production from hybrid poplar, which has already proven environmental benefits in replacing gasoline.** The environmental analysis of biojet production employing various pathways will also be investigated.

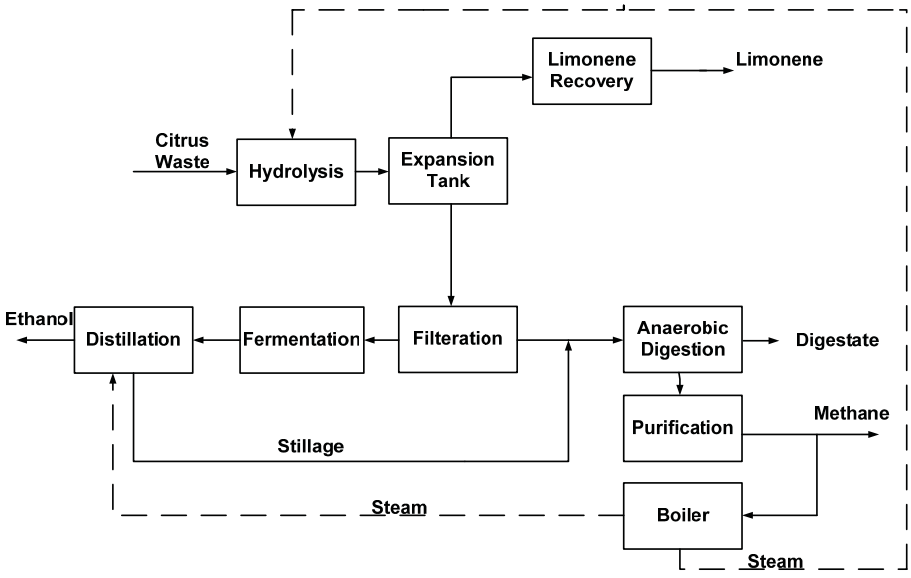


Fig. 1: Block Flow Diagram of Citrus Waste Biorefinery.

Fahimeh Salimi
Ph.D. Student

M.Sc., 2005, Sharif University of Technology
B.Sc., 2002, Sharif University of Technology

Supervisor: Radhakrishnan Mahadevan

Clostridial co-culture for cellulosic biobutanol production

Increasing world energy demands and environmental concerns due to the fossil fuel consumptions motivate considerable efforts toward the development of sustainable and renewable energy resources such as biofuels. Biobutanol has recently been introduced as a biofuel and direct substitute of gasoline. An alternative method for the production of biobutanol from cellulosic biomass in a consolidated bioprocessing approach is the use of mesophilic clostridial co-culture.

It has been demonstrated that the rate of cellulose utilization in the co-culture of *Clostridium acetobutylicum* and *Clostridium cellulolyticum* is improved compared to the mono-culture of *C. cellulolyticum*, suggesting the presence of syntrophy between these two species (Fig.1). However, the phenotypic and metabolic behaviours of this co-culture are not well understood. **Developing methods for the analysis of metabolism in this co-culture and developing a genome-scale model of metabolism in this clostridial co-culture is the focus of my study.**

We applied thermodynamics based metabolic flux analysis, to obtain thermodynamically feasible flux distributions, along with metabolomics data analysis to study the metabolism of *C. acetobutylicum*. Furthermore, the genome-scale model of *C.cellulolyticum* metabolism was developed. This model has been validated against experimental data, and is able to predict the metabolism of *C.cellulolyticum* on cellulose and cellobiose.

Moreover, this model of *C. cellulolyticum* metabolism has been integrated with a *C. acetobutylicum* model to develop a genome-scale model of the clostridial co-culture metabolism. This co-culture model was used to analyze the integrated physiology of this co-culture. Further investigation of the mechanism behind *C. cellulolyticum* growth arrest at high cellulose concentrations is required for improving the model predictions as well as predictions of the co-culture physiology. **The overall goal of this project is to applying this model to design strategies for further optimization of this co-culture for improved cellulose degradation and biobutanol production.**

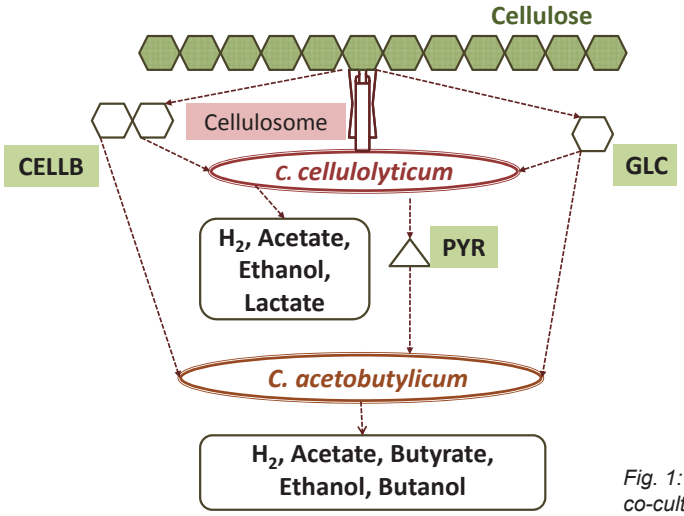


Fig. 1: A simplified scheme of the clostridial co-culture fermentation.

Research Highlights

Salimi, F. *et al.* (2010). "Genome-scale metabolic modeling of a clostridial co-culture for consolidated bioprocessing". *Biotechnology Journal* 5:726 -738

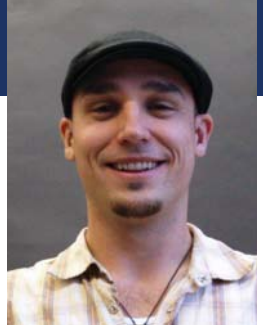
Salimi, F. *et al.* (2010). Understanding *Clostridium acetobutylicum* ATCC 824 metabolism using genome-scale thermodynamics and metabolomics-based modeling. *Proceedings of the Computer Applications in Biotechnology*, Leuven, Belgium

Salimi, F. *et al.* (2010). Genome-scale characterization of metabolic potential of solventogenic and cellulolytic *Clostridia* (poster). *110th American Society of Microbiology Annual General Meeting*, San Diego, CA

Research Highlights

Schnurr, P.J., Irving, T., Espie, G. and D.G. Allen (2011). The development of mixed algal biofilms for biofilm based bioreactors (presentation). *61st Canadian Chemical Engineering Conference*, London, ON.

Chandok, G., Razzak, S. and P.J. Schnurr (2010). Investigation of lipid and biodiesel production from *Chlorella vulgaris* (UTEX 2714) microalgae cultured in photo bioreactors (presentation). *First Canada – U.S. Symposium on Microalgae for Bioenergy and Other Applications*, Sarnia, ON



Peter Schnurr
M.A.Sc. Student

B.A.Sc., 2010, University of Western Ontario
Applied Science Diploma, 2007, Fanshawe College

Supervisor: Grant Allen

The production of biofuels & biomaterials from the growth of algae cultures grown as a biofilm

The production of biofuels and biochemicals from the growth of algae cultures has great promise due to their rapid growth rates and high lipid (oil) concentrations in comparison to conventional crops. However, current algal growth systems are not economical, in part because of the high cost of harvesting and de-watering the dilute suspension of algal cells. To circumvent much of these costs we are attempting to grow the algae as a highly concentrated, immobilized biofilm (Fig. 1).

For my thesis I am investigating the factors that affect the development of algal biofilms in order to develop a system that will allow for the rapid growth of thick biofilms. In addition, I am investigating the potential to manipulate these biofilms to significantly increase the lipid concentration of the film.

There has been very little research conducted on generating algal biofilms for biofuel production. My first objective is to investigate the importance of species selection by screening several different algal species and mixed communities (consortia) to determine their ability to rapidly grow as a biofilm. After identifying the most promising species/consortia, I will investigate the effect of physical/chemical conditions (e.g. carbon dioxide, light intensity) on biofilm growth in order to determine the optimal conditions for rapid biofilm development.

In order to test the ability to manipulate biofilms for greater lipid concentrations, the algae biofilms will be starved of the key nutrients nitrogen and silicon. Although previous research on planktonic algae has shown that nutrient starvation of these key nutrients can double lipid concentrations (up to 80% lipids by dry weight), there have not been any studies to determine if similar media manipulation will affect algae grown as a biofilm.

Preliminary studies have shown that we can effectively grow a thick algal biofilm (Fig. 2). **We expect that the results of this research can yield valuable information for scale-up to pilot scale biofilm photobioreactors.**

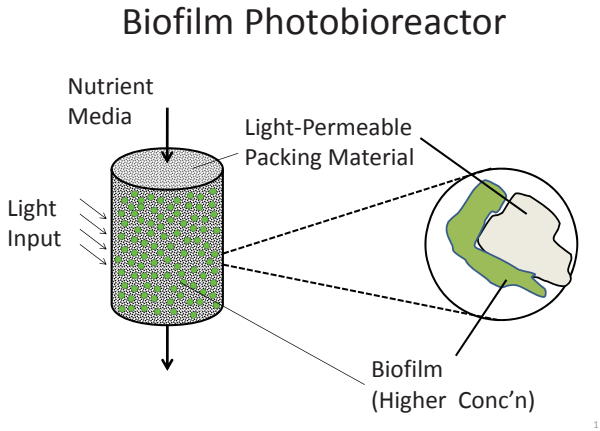


Fig. 1: Representation of an algal biofilm photobioreactor.

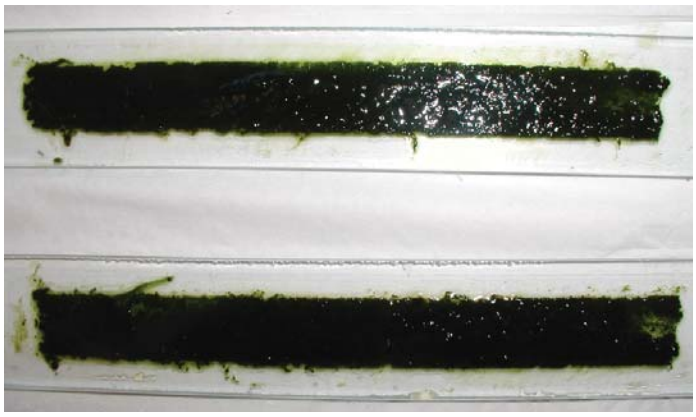


Fig. 2: Culture of a thick algal biofilm grown on horizontal substrate.

Tim Shen
M.A.Sc. Student

B.A.Sc., 2009, University of Toronto

Supervisor: Bradley Saville
Co-Supervisor: Heather MacLean

Life cycle analysis of multiple co-product systems

Concern regarding the sustainability of petroleum has motivated the worldwide development of 2nd generation lignocellulosic ethanol. With "bioethanol" on the verge of commercialization, life cycle analysis has become an integral tool in evaluating the impact of large scale implementation strategies. As only a portion of lignocellulose is convertible to ethanol, interest exists in transforming leftover residues into value-added co-products, which may prove key in enabling bioethanol to reach economic parity with petroleum fuels. However, given the diverse composition of lignocellulose, a large number of co-product options are possible, with variable environmental benefits and consequences.

Thus, the objective of this project is to evaluate a range of ethanol production life cycles employing leading conversion technologies, considering promising co-product strategies. Specific synergies may exist between combinations of certain lignocellulosic feedstock types, conversion technologies and co-product strategies. Greater understanding in regards to how and which co-products are beneficial from a life cycle perspective will aid parties at all levels of lignocellulosic ethanol development to better direct future commercialization, policy and research.

With the aid of computer simulation and life cycle data from both the public and private sector, "cradle-to-grave" life cycle models were constructed for future ethanol pathways. The models were able to predict the energetic and environmental impacts of each pathway in relation to the volume of ethanol produced and combusted in a vehicle at the end of its life cycle. Several pathway combinations were identified as optimal from a life cycle perspective, based on a number of life cycle performance metrics. Additionally, environmental benefits and penalties for each co-product strategy on the ethanol life cycle were also quantified, to indicate the effectiveness of producing certain co-products. **Ultimately this work will identify key tradeoffs in bioethanol co-product production and their synergies with existing and future ethanol conversion technologies.**

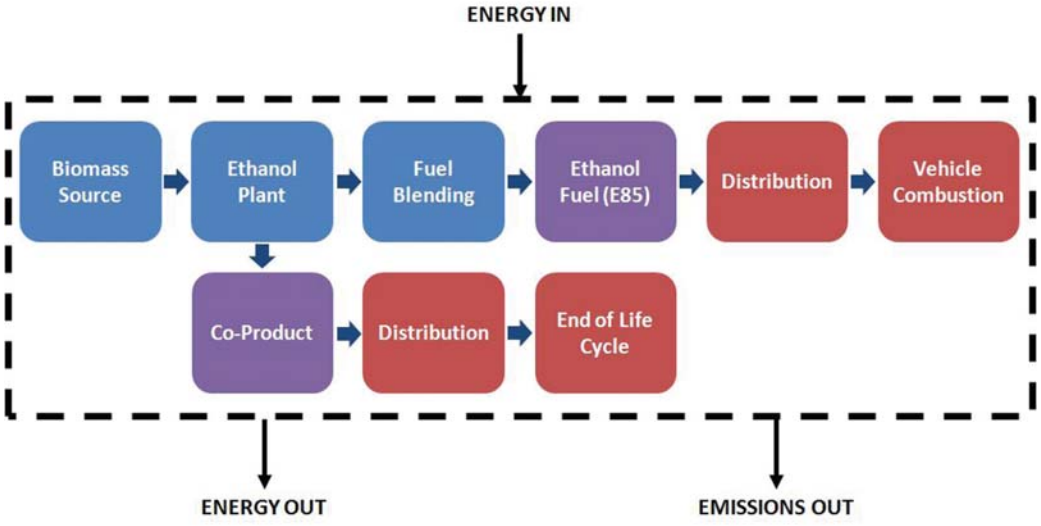


Fig. 1: Generalized block representation of cradle-to-grave lignocellulosic ethanol life cycle pathway with co-product production.

Research Highlights

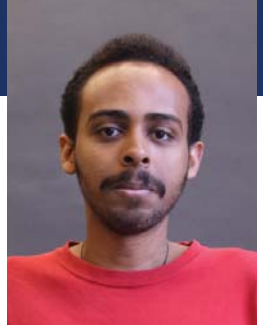
Shen, T., Saville, B.A., and H.L. MacLean (2011). Energetic and environmental impacts of co-products on lignocellulosic ethanol (poster). *2nd Annual BEEM Research Meeting*, Toronto, ON

Shen, T., Saville, B.A., and H. L. MacLean (2010). Life cycle assessment of lignocellulosic co-product systems (speaker). *1st Annual BEEM Research Meeting,* Toronto, ON

Research Highlights

Sherif, M. and E. R. Master (2011). Synthetic derivatization of plant phenolics using esterase and lipase enzymes (poster). *NSERC Bioconversion Network 2nd Annual General Meeting*, Toronto, ON

Two months research experience at NRCan, Canadian Forest Service, Great Lakes Forestry Centre



Mohammed Sherif
Ph.D. Student

B.Sc., 2008, University of Toronto

Supervisor: Emma Master

Synthetic derivatization of plant phenolics using enzymes

Plants synthesize a wide variety of phytochemicals, including phenolic compounds that have notable antioxidant or pesticidal activity. The nutraceutical and biocidal value of certain of these phytochemicals could be enhanced by conjugating them to hydrophobic or lignocellulosic substrates. T**he objective of my project is to identify enzymes and optimal reaction conditions that catalyze the addition of aliphatic molecules or carbohydrates to plant phenolics.** Enzymes are desirable catalysts due to their specificity and ability to catalyze under relatively mild conditions. Two potential enzymatic routes to derivatization of plant phenolics are with the use of: (1) esterases/lipases enzymes and (2) laccase enzymes.

In the case of esterases/lipases, a transesterification-type reaction can be used to attach the phenolic to a carbon-chain molecule, making the phenolic more fat soluble. Such reactions need to be carried out in a non-aqueous solvent to avoid an undesired side-reaction with water. Non-aqueous solvents are known to have detrimental effects on enzymes, therefore, one challenge is identifying such a solvent that minimizes inhibition of enzyme activity. With a commercial lipase, we identified a general trend of solvent compatibility, whereby the most water-immiscible solvent supported highest lipase activity for a phenolic reaction, which was in agreement with previously observed general trends. Transesterification by a thermostable and solvent stable aryl esterase from an archael species and a bacterial species is now being evaluated in terms of substrate selectivity, product yields, and influence of solvent conditions.

Laccases are multi-copper oxidases that produce a reactive free radical form of the phenolic molecule, which can then be linked to carbon-chains or other phenolic molecules. Most laccases that have been characterized to date were isolated from fungi. We characterized a bacterially derived laccase and more than 10 corresponding mutants, and showed that it has oxidative activity on a broad range of phenolics, including the natural compounds quercetin and resveratrol that have known antioxidant properties.

With the above two enzyme types, we aim to harness and enhance the bioactivity of phenolics that are abundant in plant biomass sources, thereby utilizing what are currently considered waste streams in many lignocellulosic processing industries.

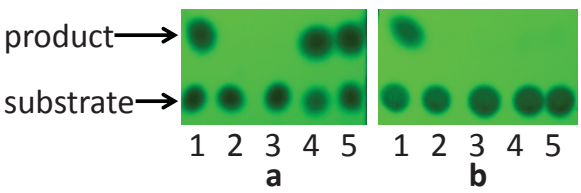


Fig. 1: Thin-layer chromatograms from lipase-catalyzed reaction samples. Reactions from: (a) water-immiscible solvent, (b) water-miscible solvent. Lane 1: standard; lane 2, 3: reactions without enzyme; lane 4, 5: reactions with enzyme.

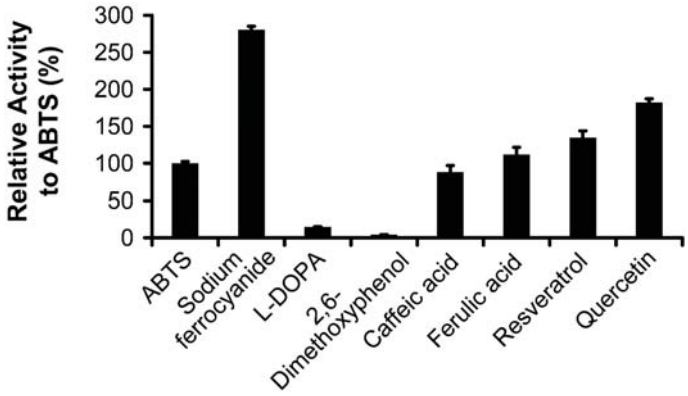


Fig. 2: Oxidase activity of laccase on some phenolic and non-phenolic substrates.

Vyacheslav Shuvalov
M.A.Sc. Student

B.Sc., 2008, Tallinn University of Technology

Supervisor: Levente Diosady
Co-Supervisor: Olev Trass

Aspects of protein and phenolic compound extraction from canola

Phenolic compounds are secondary plant metabolites with potentially useful antioxidant properties that are present in many plant species. Sinapine and sinapic acid are the main phenolic compounds found in rapeseed, which may have value as neutraceuticals.

The aqueous process used to extract proteins from rapeseed generates large amounts of wastewater. Most of the phenolic compounds present in rapeseed are left in the wastewater after protein precipitation. Extraction and concentration of those components is a way to retrieve high value compounds and at the same time reduce the amount of wastewater generated by protein purification process.

For my Master's thesis, **I am interested in developing a process for extraction and concentration of phenolic compounds such as sinapic acid and sinapine from rapeseed.**

Using methods like microfiltration, nanofiltration and reverse osmosis (Fig. 1) it is possible to isolate and concentrate sinapic acid from wastewater generated by aqueous extraction. Hydrolysis of sinapine to sinapic acid and application of membrane technologies are a possibility for process development for the recovery of sinapic acid as a separate product from waste streams generated by protein isolate production.

My work is focused on **identifying the parameters of hydrolysis of sinapine, microfiltration, nanofiltration and reverse osmosis processes applied to wastewater generated by aqueous protein extraction to yield concentrated sinapic acid with good overall recovery rates based on initial sinapic acid content of rapeseed.**

Fig. 1: Membrane processing equipment used for filtration and reverse osmosis.

Research Highlights

Singer, A.U. *et al.* (2011). "Structural analysis of HopPmaL reveals the presence of a second adaptor domain common to the HopAB family of *Pseudomonas syringae* type III effectors". *Biochemistry* 55(1):1-3

Wu, B., Skarina, T., Yee, A., Jobin, M.C., Dileo, R., Semesi, A., Fares, C., Lemak, A., Coombes, B.K., Arrowsmith, C.H., Singer, A.U., and A. Savchenko (2010). "NleG Type 3 effectors from enterohaemorrhagic *Escherichia coli* are U-Box E3 ubiquitin ligases". *PLOS Pathog.* 6(6):1-17

Singer, A.U., Rohde, J.R., Lam, R., Skarina, T., Kagan, O., Dileo, R., Chirgadze, N.Y., Cuff, M.E., Joachimiak, A., Tyers, M., Sansonetti, P.J., Parsot, C., and A. Savchenko A. (2008). "Structure of the *Sigella* T3SS effector IpaH defines a new class of E3 ubiquitin ligases". *Nat Struct Mol Biol.* 15(12):1293-301

Alex Singer
Research Associate

Ph.D., 1998, University of Toronto
M.Sc., 1991, University of Toronto
B.Sc., 1986, University of Guelph

Supervisor: Alexei Savchenko

Structure and function of type III effectors

Type III effectors are virulence factors secreted by pathogenic gram-negative bacteria; these effectors in turn weaken the host immune system and help the pathogen colonize the host. The function of many of these type III effectors often cannot be predicted, as they are often divergent from other proteins in sequence and protein structure databases. **My work involves structure determination of these effectors, in whole or in part, from both from plant and animal pathogens,** using both X-ray crystallography or heteronuclear NMR. We then use the resulting information to predict the role of these proteins in pathogenicity.

One particular set of effectors we have studied extensively are those that catalyze the addition of long poly-ubiquitin chains onto specific proteins (called E3 ligases), often targeting them for destruction by the proteosome. We have demonstrated E3 ligase activity for the NleG family of type III effectors from *E. coli* (Fig. 1), and solved the structure of a C-terminal fragment by NMR. Its structure contained a U-box, a common E3 ligase fold. However, bacterial pathogens can also develop novel folds for this function, as the ubiquitination system is not a staple of prokaryotic metabolism. Thus we found a novel fold for the E3 ligase domain of the IpaH family of effectors from *Shigella* sp. by X-ray crystallography (Fig. 2), and will soon also report the structure of another novel E3 ligase, this time from an effector found in the plant pathogen *Xanthomonas*. **Bacterial virulence factors often create novel folds to perform functions unique to eukaryotic metabolism, and we expect many more such structures in the future.**

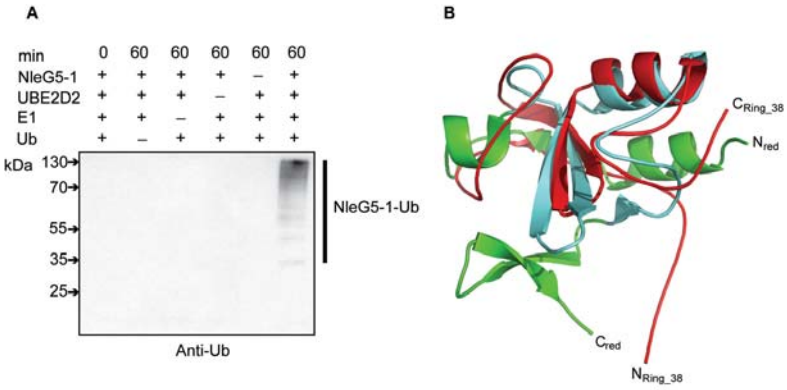


Fig. 1: NleG proteins are E3 ligases. A. Immunoblot analysis with anti-ubiquitin antibodies of reactions performed in the presence of ATP and in the presence or the absence of ubiquitin, E1, UBE2D2 E2 and NleG5-1[1-203]. B. Superimposition of the NleG C-terminal domain (reduced NleG2-3[90-191]) with RING finger 38 protein (PDB 1X4J, red). The region of the NleG C-terminal domain which follows the fold of the U-box/RING finger is coloured cyan, the regions outside this fold are colored green (Source: Wu et al, 2010).

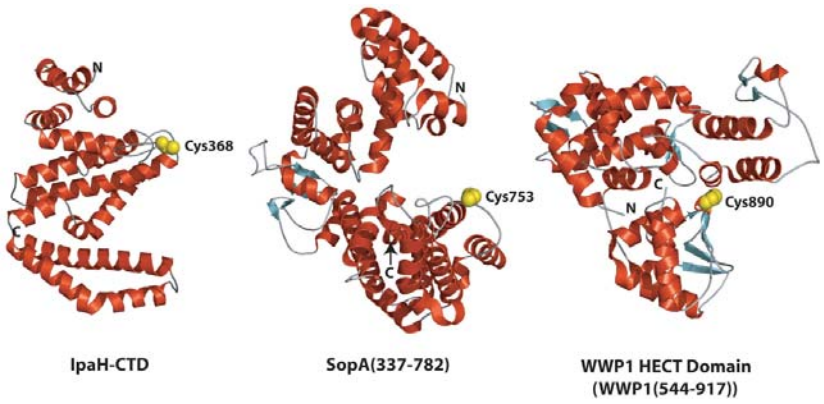


Fig. 2: Structures of different classes of E3 ligases which require a catalytic cysteine for activity. Helices are shown in red, strands in cyan and loops in gray,and positions of the N and C termini of each protein are labeled. The WWP1 HECT domain (PDB 1ND7) is used to represent the HECT domain fold. The catalytic cysteine is shown as a space-filling model and colored yellow (Source: Singer et. al., 2008).

Sayeh Sinichi
Ph.D. Student

B.A.Sc., 1999, Azad University

Supervisor: Levente Diosady

Production of isopropyl ester from mustard oil

Fossil fuels represent a significant source of worldwide energy consumption. The search for alternative sources of energy is a necessity. **Vegetable oils which have an energetic content close to fossil fuels are an example of a renewable and potentially inexhaustible source of energy.** An annual production of almost 160,000 tonnes of mustard (2008), which accounts for over 50% of worldwide exports of all mustard, makes Canada the world's largest exporter of mustard seed.

Relative to the recommended intakes for proper nutrition, the proteins in oilseeds such as mustard are very well balanced with low allerginicity.

Mustard seed oil contains erucic acid which is known to contribute to certain heart conditions such as myocardial lipidosis. As a result, in Europe and North America mustard oil is banned for human consumption. It is also known that the presence of erucic acid in mustard seed oil is also responsible for its high lubricity, a positive property of biofuels. **As a result mustard seed is a good candidate for production of biodiesel.**

Therefore, the objectives of my research are:

a. To extract oil from de-hulled yellow mustard flour using isopropyl alcohol that has been proven as potentially a good replacement for hexane by using the direct solvent extraction method. Oil extraction at ambient temperature would maintain the high quality of the proteins in mustard flour.
b. The miscella (IPA+Oil) would then be dried, its free fatty acid and impurity content reduced prior to transesterification.
c. I will investigate the production of isopropyl as well as methyl esters from the miscella for use as a biodiesel fuel.
d. As water forms azeotropes with IPA the recovery and reuse of the solvent and the reaction products will be studied.

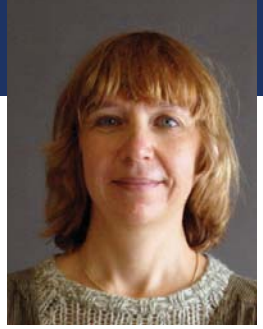
Research Highlights

Sinichi, S. (1999). Designing a laboratory scale rotary dryer. B.Sc. thesis, *Dept. of Chemical Engineering,* Azad University, Iran

Research Highlights

Singer, A.,Rohge, J., Lam, R., Skarina, T., Kagan, O., Dileo, R., Chirgadze, N., Cuff, M., Joachimiak, A., Tyers, M., Sansonetti, P., Parsot, C. and A. Savchenko (2008). "Structure of the *Shigella* T3SS effector IpaH defines a new class of E3 ubiquitin ligases." *Nature Structural & Molecular Biology,* 15(12):1293-1301

Babu, M., Beloglazova, N., Flick, R., Graham, C., Skarina, T., Nocek, B., Gagarinova, A., Pogoutse, O., Brown, G., Binkowski, A., Phanse, S., Joachimiak, A., Koonin, E., Savchenko, A., Emili, A., Greenblatt, J., Edwards, A. and A. Yakunin (2011). "A dual function of the CRISPR-Cas system in bacterial antivirus immunity and DNA repair." *Molecular Microbiology,* 79(2):484-502



Tatiana Skarina
Technician

M.Sc., 1983, Novosibirsk State University, Russia

Supervisor: Alexei Savchenko

Optimization of protein crystallization

Protein crystallization is a key step in obtaining high quality 3D structures using X-ray crystallography. Finding the unique set of buffers and salt solutions, which would be suitable to crystallize a number of different proteins, is a challenging task for crystallographers. **Since starting my work in Professor Savchenko's group, I have been focused on the improvement of the protein crystallization process by trying to develop more effective crystallization screens.** Our database of crystallization conditions is based on more then 700 crystallized not structurally characterized targets from all three kingdoms of life (Bacteria, Archaea and Eukarya). Structures of 450 of them have been solved and deposited in the Protein Databank. The final result of automatic data processing led to the creation of new, more universal crystallization screens.

Among 200 deposited structures I have worked on is the structure of E3 ubiquitin ligase IpaH1.4, the T3SS effector protein from important human pathogen *Shigella*, which is responsible for bacillary dysentery. The Type 3 Secretion System (T3SS), one of the best-studied weapons in the bacterial molecular arsenal, is used by various pathogenic bacteria to inject effector proteins into host cells and affect host functions in favor of bacterial internalization into the cells. The number of confirmed and potential effectors secreted by T3SS has mounted to over a hundred protein families. The structures and specific functions of most of these proteins remain unknown. The structure of the IpaH C-terminal domain, determined to 2.65Å resolution, represents an all-helical fold bearing no resemblance with previously defined E3 ubiquitin ligases. Results from ubiquitination assays demonstrated that the IpaH C-terminal domain carries the catalytic activity for ubiquitin transfer (Fig. 1).

My current studies are focused on the use of protein crystallization techniques and functional assays (ubiquitination, protein-protein interaction) to help characterize different effector proteins.

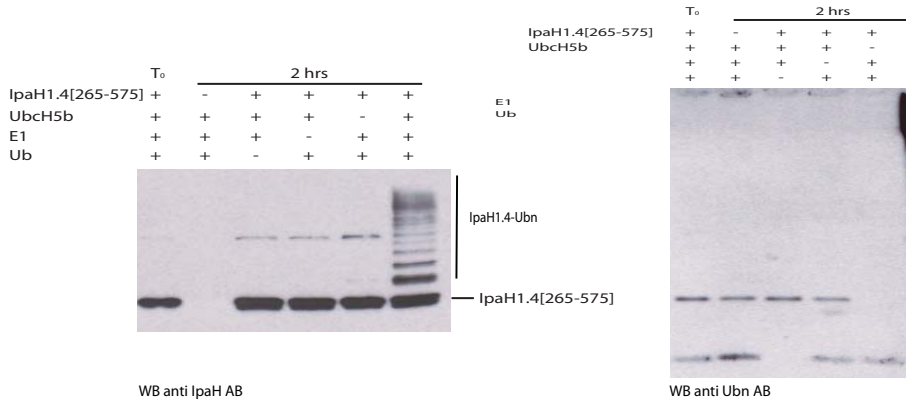


Fig. 2: Western blot analysis using anti-IpaH or anti-ubiquitin antibodies in the presence of ATP, ubiquitin, E1, UbcH5b and IpaH1.4[265-575]. The reactions were performed at 25ºC for 2 hours (except for T_o representing the degree of ubiquitination at t=0 minute).

John Soleas
M.Sc. Student

B.M.Sc, 2010, The University of Western Ontario

Supervisor: Alison McGuigan
Co-Supervisor: Thomas Waddell

Engineering organized tracheal epithelium using nanogroove surface topography

There is currently no accepted method for rebuilding extended segments (>6cm) of injured trachea. Early attempts at engineering tracheal grafts led to fatal airway obstruction in animal models and suggested that the development of organized epithelium in the graft is important for clinical success. The standard method to construct epithelium *in vitro* is through transwell filter technology to create an air-liquid-interface (ALI) culture system. While this method creates apical-basal polarized epithelium, the resulting tissue is not organized in a planar orientation.

Growing cells on grooved topography is a well-known strategy to align cell morphology and organization in artificial tissues. However, the substrates currently used to align cells on nanogrooved topography are not appropriate for ALI culture, which requires nutrient diffusion from the basal compartment.

Our challenge is to create a system that controls both apical-basal and planar polarity while allowing for differentiation and maturation of tracheal epithelial cells. We propose a unique, interdisciplinary project combining biological and engineering expertise to approach the problem of generating mature epithelium in ALI culture by imprinting gelatin hydrogels with nanogroove surface topography.

We characterize the influence of substrate topography on the organization of various epithelial cell types on gelatin nanogrooves (Fig. 1) that allows maintenance of ALI culture while exposing the cells to nanotopographic cues.

Using nanogroove topography we hope to create better organized tracheal epithelium that has coordinated cilia beating leading to improved *in vitro* models of respiratory epithelium and increased clinical success.

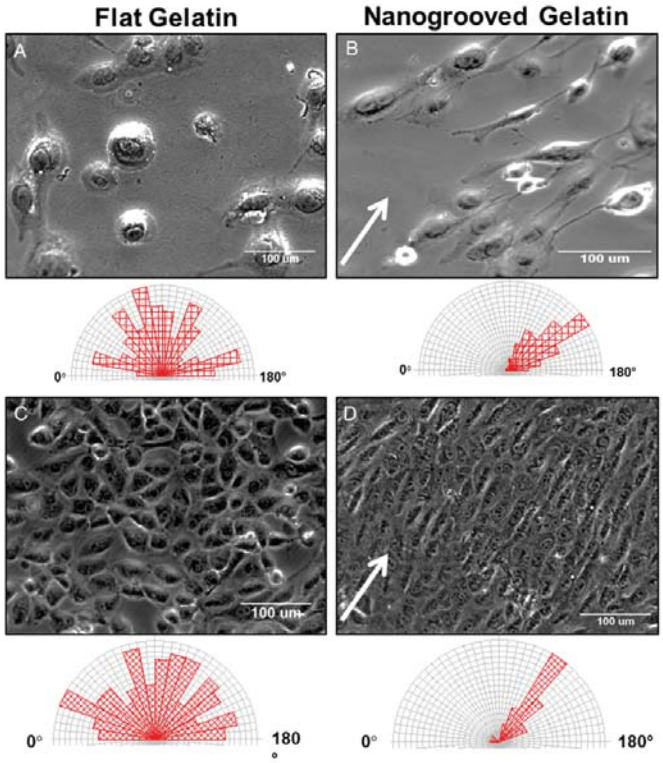


Fig. 1: Phase contrast micrographs of various epithelial cell lines. Bronchial epithelial cell line BEAS2B (A and B) grown at sparse seeding density and retinal epithelial cell line ARPE19 (C and D) grown at confluent seeding density. On flat gelatin (A and C) random whole-cell orientation is present as indicated by the corresponding angular histograms. On nanogrooved gelatin hydrogels (B and D) whole-cell orientation parallel to the nanogrooves occurs, as indicated by the angular histograms.

Research Highlights

Soleas, J.P. *et al.* (2011). "Engineering airway epithelium." *J. Biomed. Biotechnol.* (accepted)

Haykal, S., Soleas, J.P. *et al.* (2011). "Evaluation of the structural integrity and extracellular matrix components of tracheal allografts following cyclical decellularization techniques: Comparison of three protocols." *Tissue Eng. Pt. C-Meth.* (submitted)

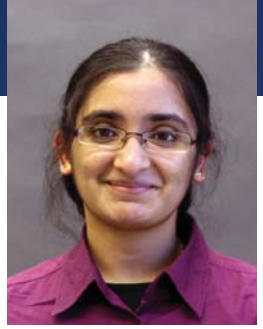
Soleas, J.P. *et al.* (2011). Engineering organized epithelium using nanogroove topography (invited speaker). *Tissue Eng. & Regen. Med. Intl. Soc. - North American Annual Conference and Exposition*, Houston, TX

Soleas, J.P. *et al.* (2011). Engineering osrganized tracheal epithelium using nanogroove topography (invited speaker). *4th Annual Regenerative Medicine Symposium*, Toronto, ON

Research Highlights

Sridharan, S. (2010). Variable angle transmission Fourier transform infrared spectroscopy of peptide membrane interactions. B.A.Sc. thesis, *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto

Sridharan, S. and C.M. Yip (2009). Variable angle transmission Fourier transform infrared spectroscopy of peptide membrane interactions (presentation). *8th World Congress of Chemical Engineering*, Montreal, QC



Supraja Sridharan
M.Eng. Student

B.A.Sc, 2010, University of Toronto

Supervisor: Levente Diosady

Transesterification of yellow Canadian mustard oil using a mixed alcohol system

The increasing demand for energy coupled with changing government policies has resulted in technological developments and improvements in renewable energy technologies. In particular, biodiesel has been praised due to its potential for lower emissions of SO_2, hydrocarbons and smoke, its successful commercialisation, as well as the tax exemptions across several European countries. Biodiesel production (transesterification) has been conducted using vegetable oils, such as soybean and canola, and alcohols, such as methanol, ethanol, and isopropanol. In particular, methyl esters have been praised for their significantly lower viscosity.

Work by the Diosady Food Engineering Group with dehulled yellow Canadian mustard flour has shown mustard seed to be a contender and a contributor in the production of biodiesel. In particular, we have examined the production of biodiesel from isopropanol. Biodiesel created from isopropanol is advantageous, as the resulting esters demonstrate superior cold-flow properties. However, the resulting ester yield is significantly lower than that generated from methanol.

My research approach begins with the extraction of oil from dehulled yellow Canadian mustard flour using isopropyl alcohol, with an oil extraction yield greater than 90%. Following extraction, a mixed alcohol system of methanol and isopropanol as a co-solvent and reagent (respectively), and sodium hydroxide catalyst is used to convert the resulting mustard oil into biodiesel. **The focus of my Master's thesis work is on determining the appropriate ratio of methanol, isopropanol and mustard oil that will maximise the ester quantity in the resulting biodiesel.** The idea behind using both methanol and isopropanol is to satisfy the ultimate goal of creating a biodiesel of high yield and high quality.

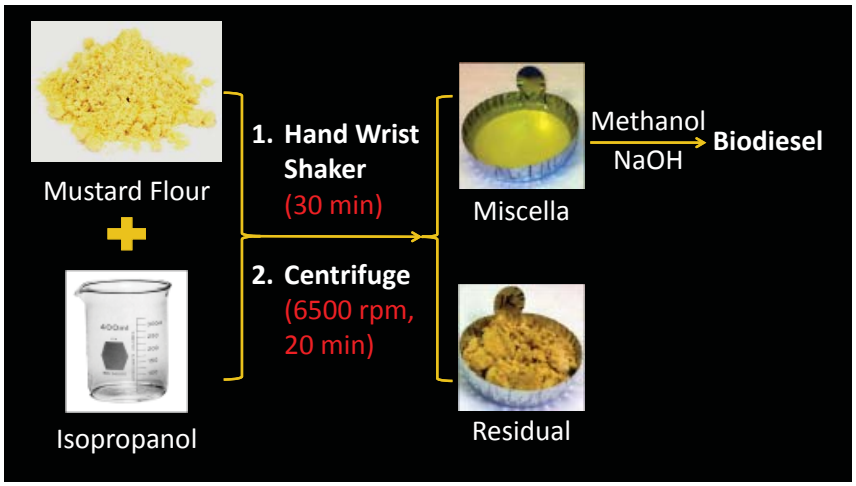


Fig. 1: Integrated oil extraction and transesterification from dehulled Canadian yellow mustard.

Peter J. Stogios
Postdoctoral Fellow

Ph.D., 2008, University of Toronto
Hon. B. Sc., 2001, University of Toronto

Supervisor: Alexei Savchenko

Structural and functional analysis into the mechanism and inhibition of enzymes conferring antibiotic resistance

The discoveries of antibiotics as treatments against bacterial diseases revolutionized medicine in the early 20th century. However, the effectiveness of nearly every class of antibiotic has been compromised due to the evolution of resistance, leading to disease outbreaks. Drug-resistant bacteria have evolved a variety of resistance mechanisms; the most clinically relevant is modification of the drugs, which alleviates their bactericidal effects by reducing their affinity for their cellular target.

The focus of my research is to study antibiotic resistance with the goal of developing small molecule therapeutics as adjuvants to restore the efficacy of obsolete antibiotics. We gain molecular insight into the activity of enzymes involved in antibiotic modification by determining the 3D structures of their complexes with antibiotic substrates/inhibitors through x-ray crystallography, a method that provides atomic-resolution details.

We study two classes of antibiotics and their modifying enzymes: aminoglycosides and glycopeptides (vancomycin), which have been rendered ineffective by chemical modification. Three families of enzymes, including acetyltransferases, nucleotidyltransferases and phosphotransferases (Fig. 1) add different small functional groups to aminoglycosides. Vancomycin modification is mediated by a series of enzymes that work in unison, including peptidases, dehydrogenases, racemases and ligases.

Together with other members of Professor Savchenko's group and the Center for Structural Genomics of Infectious Diseases (www.csgid.org), I have contributed to determination of the 3D structures of several aminoglycoside and vancomycin-modifying enzymes, now publically available at the Protein Databank (www.rcsb.org/pdb). These structures have been pivotal in rationalizing small molecule inhibitor data (Fig. 2). Using the structures as guides, we are embarking on efforts to modify these lead compounds to improve specificity and potency.

Ultimately, we expect that the 3D structures determined of these enzymes will be used by the scientific community for understanding their functional mechanisms and will be foundational in developing antibiotic adjuvant therapies.

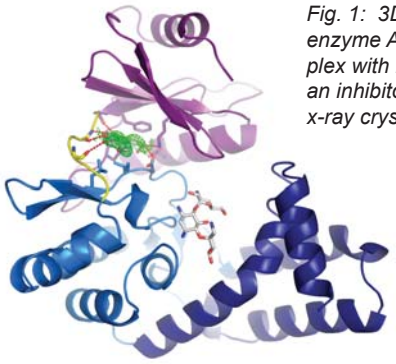


Fig. 1: 3D structure of aminoglycoside-modifying enzyme APH(2")-IVa (purple/blue cartoon), in complex with its substrate kanamycin (grey sticks) and an inhibitor, quercetin (green mesh). Determined by x-ray crystallography.

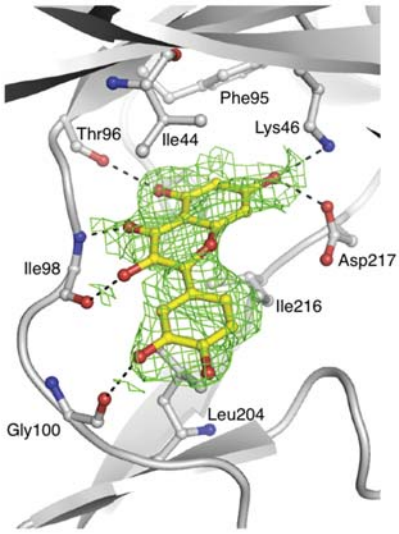


Fig. 2: Flavonoid inhibitor quercetin (yellow sticks, green mesh) bound to the active site of APH(2")-IVa (grey sticks, amino acids labeled). Structure reveals intermolecular interactions that correlates with structure-activity-relationship data (Source: Shakya et al 2011).

Research Highlights

Shakya, T., Stogios, P.J. *et al.* (2011). "A small molecule discrimination map of the antibiotic resistance kinome." *Chem. Biol.* (in press)

Stogios, P.J. *et al.* (2011). "Structure and function of APH(4)-Ia, a hygromycin B resistance enzyme." *J. Biol. Chem.* 286(3):1966-75. JBC Paper of the Week

Stogios, P.J. *et al.* (2011). Resisting resistance: Structural genomics and drug design against antibiotic resistance (poster). *51st Interscience Conference on Antimicrobial Agents and Chemotherapy,* Chicago, IL

Stogios, P.J. *et al.* (2011). Resisting resistance: Structural genomics and drug design against antibiotic resistance (poster). *International Conference on Structural Genomics*, Toronto, ON

Research Highlights

Sun, T. T. W. and B. A. Saville (2011). Alkaline pretreatment of softwood pine as a method for increasing enzyme access to cellulose and hydrolysis yield (poster). *The Eighth Annual World Congress on Industrial Biotechnology & Bioprocessing,* Toronto, ON

Sun, T. T. W. (2011). Increasing softwood pine enzyme hydrolysis efficiency: a scaled-up approach (presentation). *Second Annual NSERC Bioconversion Network AGM*, Toronto, ON

Tim Sun
M.A.Sc. Student

B.A.Sc., 2010, University of Toronto

Supervisor: Bradley Saville

Scale-up optimization of bioreactors to produce reducible sugars from softwood pine

The second generation lignocellulose-derived biofuel has come to be regarded as a feasible alternative to the depleting conventional petroleum-based fuels. Canada's abundant softwood lumber stands are an ideal source as the pulp and paper industry diminishes. In addition, it is essential to dispose of pine-beetle ravaged trees. **Our objective is to investigate the efficacy of softwood as a suitable feedstock and its enzyme hydrolysis scale-up performance, to cost-effectively produce biomass-derived sugars.**

The main challenge of using softwood as a feedstock is the low hydrolysis yield when compared to hardwood. It has been proposed that enzyme deactivation, and non-productive binding between the enzymes and the lignin structure is the cause the low yield. We are currently looking at different lignin-removal methods including autohydrolysis (steam explosion) at much higher severity, and alkaline delignification using sodium hydroxide. Once a cost-effective method for delignification is identified, we will scale up the reaction by increasing the volume and substrate load to investigate rheological properties and reactor performance. In addition, the enzyme load will be optimized by comparing the hydrolysis yield when different cocktails are used.

Ultimately, **the results from this project will make it possible to identify the appropriate reactors for the commercialization of the biofuel production process, and lead to economic, sensitivity, and feasibility analyses for evaluating the use of softwood pine as a potential lignocellulosic material for the process.**

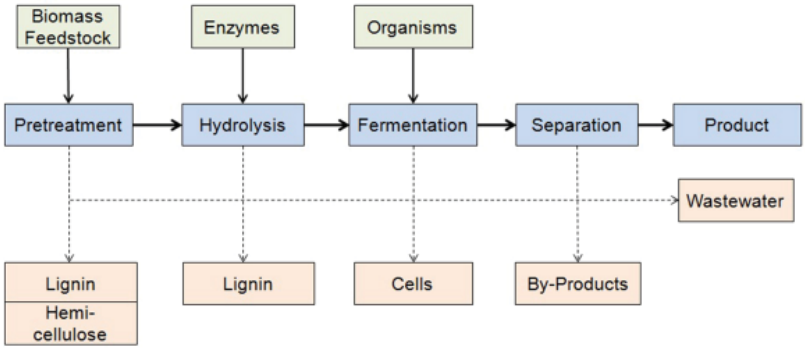


Fig. 1: Generic Process Flow for Biomass Conversion Process - Pretreatment is often necessary to increase the accessibility of cellulose to digestive enzymes. The rate limiting step in the process is the enzyme hydrolysis which can vary between 12 to 16 hrs in semi-continuous or up to 48 hrs in batch process conditions. Downstream processing is required to purify and separate the ethanol produced.

Fig. 2: Reactors in Action - Our 20L Chemglass reactor used for scale-up investigation. Here paper sludge (another biomass feedstock) is converted into monomers. The reaction is observed for 3 days to ensure that generation has plateaued.

Hitoshi Suzuki
Postdoctoral Fellow

Ph.D., 2010, The University of Tokyo
M.Sc., 2007, The University of Tokyo
B.Sc., 2005, The University of Tokyo

Supervisor: Emma Master

Comparative genomics and transcriptomics of white-rot fungi to elucidate the genetic basis of the distinct wood types they colonize

The lignocellulose fraction of plant cell walls is the most abundant renewable carbon source on earth, and is a key resource for substituting petroleum in the production of energy, chemicals and materials. Among the various types of lignocellulosic biomasses, softwood is the predominant land plant biomass in the Northern hemisphere.

The distribution of two white-rot fungi, *Phanerochaete carnosa* and *P. chrysosporium*, in nature suggests that these fungi effectively transform coniferous and hardwood species, respectively, even though their phylogeny indicates that they are genetically very similar. Accordingly, **an underlying hypothesis of the current project is that by comparing subtle differences in the distribution, sequence and expression of genes encoded by these fungi, we can more easily decipher candidate enzymes that promote the conversion of recalcitrant softwood feedstocks.**

Our analysis represents the first description of the *P. carnosa* genome and its comparison to *P. chrysosporium*. The study includes direct comparison of the growth of these fungi on model and lignocellulosic carbon sources, including heartwood and sapwood samples from a variety of coniferous and hardwood species. The conversion of these carbon sources was evaluated in the context of predicted metabolic models and differences in the genes encoded by the respective fungi. Our results to date indicate that the *P. carnosa* genome is enriched with genes that encode P450 monooxygenases that can participate in extractives degradation, and manganese peroxidases involved in lignin degradation. The comparative genomic analysis also revealed that the most divergent glycoside hydrolase families were predicted to encode hemicellulases and glycoprotein degrading enzymes. These differences could be correlated to the utilization of woody feedstocks.

Given the particular recalcitrance of softwood resources and limited analysis of softwood bioconversion at the molecular level, **this work contributes to both practical and fundamental aspects of biomass conversion.**

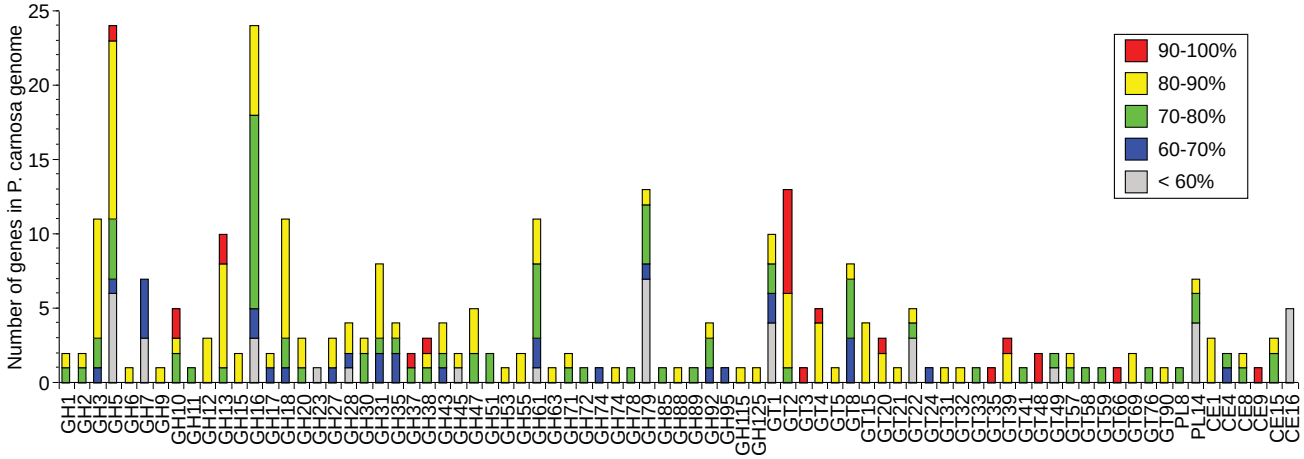


Fig. 1: The distribution of percent identities of carbohydrate-active enzymes between P. carnosa and P. chrysosporium. The sequence identities were evaluated using blastp search. The number of P. carnosa genes are indicated according to the percent identities. The original figure has been submitted for publication.

Research Highlights

Suzuki, H. *et al.* (2009). "Quantitative transcriptional analysis of the genes encoding glycoside hydrolase family 7 cellulase isozymes in the basidiomycete *Phanerochaete chrysosporium*." *FEMS Microbiol. Lett.* 299:159-165.

Suzuki, H. *et al.* (2010). "Cellotriose and cellotetraose as inducers of the genes encoding cellobiohydrolases in the basidiomycete *Phanerochaete chrysosporium*." *Appl. Environ. Microbiol.* 76(18):6164-6170

Suzuki, H. *et al.* (2011). Comparative genomics of the closely related white-rot fungi, *Phanerochaete carnosa* and *P. chrysosporium*, to elucidate the genetic basis of the distinct wood types they colonize (poster). *Gordon Research Conferences on Cellulosomes, Cellulases & Other Carbohydrate Modifying Enzymes,* Easton, MA

Research Highlights

Tabtabaei, S. and L.L. Diosady (2011). "The isolation of yellow mustard oil using water and cyclic ethers." *Journal of the American Oil Chemists' Society*, (in press)

Tabtabaei, S. and L.L. Diosady (2011). Destabilization of the emulsion produced during aqueous extraction of dehulled yellow mustard flour using organic solvents. *AOCS Annual Meeting and Expo*, Cincinnati, OH

Tabtabaei, S. and L.L. Diosady (2011). Factors minimizing emulsion stability during aqueous extraction of dehulled yellow mustard flour. *CSChE*, London, ON

Solmaz Tabtabaei
Ph.D. Student

M.Sc., 2007, University of Tehran
B.A.Sc., 2004, Azad University, Science and Research Branch

Supervisor: Levente Diosady

The production of biodiesel from dehulled yellow mustard flour

"Food or Fuel" is an international controversy about the use of agricultural land to produce biofuels, which results in higher food prices and adversely affects poor people. Therefore, it is essential to find a compromise that satisfies the needs for both food and green transportation fuel. Mustard seeds are sources of oil and protein and potentially could be used to produce both biodiesel and high quality protein products. The well-balanced amino acid profile found within the seeds makes them attractive potential sources of food-grade vegetable protein. In addition, the high levels of erucic acid found in yellow mustard oil could potentially introduce it into the industrial market for biodiesel production.

My program's objective is to recover both protein and biodiesel from dehulled yellow mustard flour.

To achieve this goal, an aqueous extraction process (AEP), in which water is used to extract all of the non-oil materials of the seed and release the oil as a separate phase, was developed for dehulled yellow mustard flour. An oil-rich (stable oil-in-water emulsion) and protein-rich aqueous phases were produced simultaneously with minimal protein damage. The first part of my study focused on the destabilization of the emulsion produced during aqueous oil and protein extraction. Organic solvents including tetrahydrofuran and dioxane totally destabilized the emulsion to form a single-phase oil-solvent-water miscella. After reducing its water content this miscella will be used in the next phase of my research as the medium for a single-phase transmethylation of the oil to produce fatty acid methyl esters. This integrated process will have the advantage to produce biodiesel using less flammable solvents than the conventional hexane extraction.

Finally, I believe that this study will help Canada to balance food and biofuel production and to lower food prices, benefiting many poor people in the world.

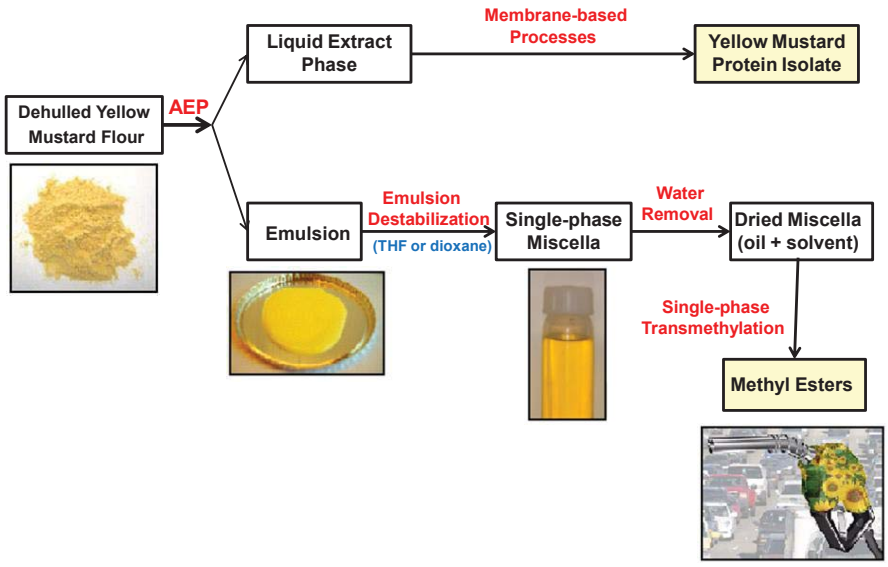


Fig. 1: Schematic diagram for the production of protein and biodiesel from dehulled yellow mustard flour.

Shuiquan Tang
Ph.D. Student

M.Sc., 2009, University of Ottawa
M.Sc., 2006, Tianjin University
B.A.Sc., 2003, Nanjing University of Science and Technology

Supervisor: Elizabeth Edwards

Investigation of *Dehalobacter*-containing cultures that reductively dechlorinate 1,1,1-trichloroethane and chloroform

1,1,1-trichloroethane and chloroform are common contaminants to underground water due to massive applications as organic solvents in industry in the past. Known for their recalcitrance and toxicity, these two compounds inhibit many microbial processes, including the biodegradation of other chlorinated organics. Our previous research established a mixed culture, called ACT-3, which can reductively dechlorinate these two compounds. **My Ph.D. thesis aims to uncover interesting features of this powerful culture, such as the biology of the dominant organism, its potential interactions with other supporting organisms and the mechanism of the enzymes that catalyze the dechlorination reactions.**

First, we sequenced the metagenome of ACT-3 to obtain the genomic blueprint of all the microbes in the culture. Previous studies have shown that *Dehalobacter* is the dominant organism in ACT-3, and is the organism responsible for dechlorinating 1,1,1-TCA and CF. By applying a novel genome assembly strategy, we successfully assembled two complete genomes of this dominant organism from the metagenomic data. One of them was polished as a finished genome (Fig. 1). The annotations of these genomes are unveiling the biology of this important genus. We also successfully isolated two pure cultures of *Dehalobacter* from ACT-3, which will serve as platforms to test hypotheses generated from interpretation of the two *Dehalobacter* genomes.

Reductive dechlorination reactions are catalyzed by reductive dehalogenases (RDases); the genes encoding these proteins are known as *rdhA* genes. Metagenomic sequencing of ACT-3 revealed 21 rdhA homologs belonging to *Dehalobacter*. We identified the function of two RDases using blue native PAGE to partially purify expressed RDases. One of the two RDases, named CfrA, is the first known RDase that can dechlorinate chloroform; it also dechlorinates 1,1,1-TCA (methyl chloroform). Genes encoding such functionally-characterized RDases are used as bio-markers for assessing biodegradation of halogenated organics at contaminated sites.

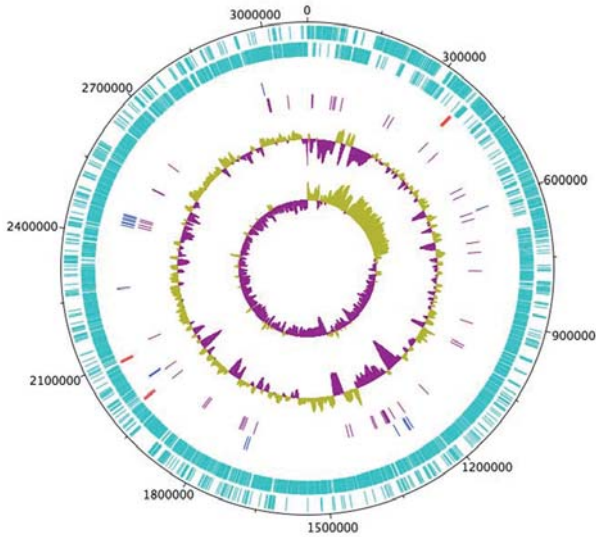


Fig. 1: The circular map of the finished Dehalobacter genome. The annotation circles include (from outside to inside) coding sequences (CDS) on the forward strand, CDS on the reverse strand, 16S ribosomal RNA genes, rdhA genes, repetitive elements (including genes of transposases and reverse transcriptases), GC content, and GC skew.

Research Highlights

S. Tang, Grostern, A., Chan W. and E. A. Edwards (2010). Characterization of *Dehalobacter* cultures that reductively dechlorinate chlorinated ethanes and chloroform. *13th ISME Conference,* Seattle, WA

S. Tang and E. A. Edwards (2010). Identification of *Dehalobacter* reductases that dechlorinate 1,1,1-trichloroethane, chloroform and 1,1-dichloroethane. *111th General Meeting American Society for Microbiology,* New Orleans, LA

S. Tang, H. Lam, L. Boehme and Z. Zhang (2009). "*Pichia pastoris* cultivation for phytase production using crude glycerol from biodiesel production as the sole carbon source". *Biochemical Engineering Journal* 43:157-162

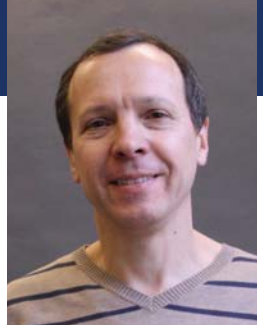
Research Highlights

Tchigvintsev, A. *et al.* (2011). "Structure and activity of the *Saccharomyces cerevisiae* dUTP pyrophosphatase DUT1, an essential housekeeping enzyme". *Biochem. J.* 437:243-253

Tchigvintsev, A. *et al.* (2011). "Biochemical and structural studies of the uncharacterized protein PA0743 from *Pseudomonas aeruginosa* revealed a NAD+-dependent L-serine dehydrogenase". *J. Biol. Chem.* (in press)

Tchigvintsev, A. *et al.* (2010). "Structural insight into the mechanism of c-di-GMP hydrolysis by EAL domain phosphodiesterases". *J. Mol. Biol.* 402 (3):524-38

Tchigvintsev, A. *et al.* (2011). Screening and biochemical characterization of novel esterases from environmental metagenomes and genomes (poster). *2nd Annual BEEM Research Meeting*, Toronto, ON



Anatoli Tchigvintsev
Technician

M.Sc., 1983, Ural State University

Supervisor: Alexander Yakunin

Screening and biochemical characterization of new industrial enzymes from environmental metagenomes and genomes

My work is focused on the screening and biochemical characterization of new enzymes for potential industrial applications with a particular emphasis on novel esterases and cellulases. I have designed and optimized the secondary screens for esterases and cellulases, which we use for substrate profiling of new enzymes.

We have purified over 160 uncharacterized alpha/beta hydrolases from sequenced environmental genomes and metagenomes. General enzymatic screens identified esterase activity in 45 proteins. The 25 most active esterases were selected for substrate profiling against the home-made ester library with 66 different substrates including ethyl, methyl and vinyl esters with different chain length. We have identified three metagenomic esterases with high activity and broad substrate profiles.

Our **substrate library for cellulases includes over 50 chromogenic and natural substrates. Further biochemical characterization of new esterases and cellulases is expected to result in new industrial enzymes for plant biomass degradation and other applications in biocatalysis.**

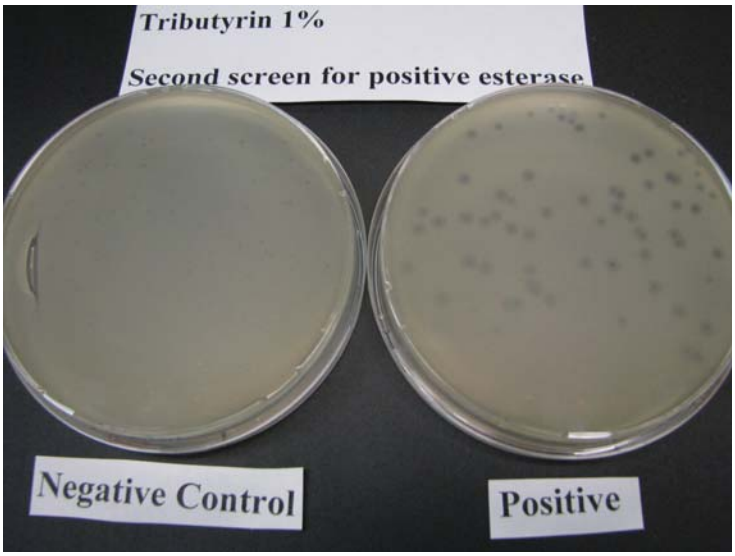


Fig. 1: Enzyme screening.

Christopher Tran
M.Sc. Student

B.A.Sc., 2010, University of Toronto

Supervisor: Elizabeth Edwards

Characterization of hydrolytic dehalogenases

The uses of halogenated hydrocarbons are ubiquitous in modern society. With the chemical stability of the carbon-halide bond, as well as attributes such as increased hydrophobicity and reactivity, halocarbons have established themselves as a key ingredient in many applications from plastics to industrial solvents.

Unfortunately, these same characteristics make these compounds environmentally troublesome. These stable compounds persist within the environment and, frequently, their toxic nature make them priority pollutants.

What is critical to realize is that the carbon-halide bond is central to this problem; breakage of this bond is critical to detoxification. These reactions, termed dehalogenations, are often the rate-limiting steps of halocarbon degradation. The focus of my Master's thesis is on enzymes that can catalyze these reactions. **In particular I am interested in characterizing hydrolytic dehalogenases, which are known to dehalogenate various halocarbon substrates via hydrolysis.** Hydrolytic dehalogenases have a simple, robust reaction mechanism, only using water as a substrate (Fig. 1), and are thus ideal candidates for study under both aerobic and anaerobic conditions.

My research currently involves kinetic characterization of two haloacid dehalogenases (Rsc1362 from *Ralstonia solanacearum*, PA0810, *Pseudomonas aeruginosa*) and two haloalkane dehlogenases (DhlA from *Xanthobacter autotrophicus*, Jann2620 from *Jannschia* sp. CCS1). We have designed a protocol for assessing dehalogenating activity from a variety of substrates, including volatiles organic compounds such as 1,2-dichloroethane.

The pH optima for each enzyme were assessed using ion chromatography to quantify the amount of halide ion produced by the reaction; the general consensus is a pH optimum of 9-10. Kinetic data for the haloacid dehalogenases (with chloroacetate as a substrate) have been measured using isothermal titration calorimetry. Kinetic data for the haloalkane dehalogenases is underway.

My work on the haloacid dehalogenases has been used within a larger project aimed at elucidating the structural differences between enzymes capable of fluoro and chloro dehalogenation. **The kinetic data critical to assess the structure-function relationship of these enzymes. This data will be useful in the engineering of faster, more efficient dehalogenases in the future.**

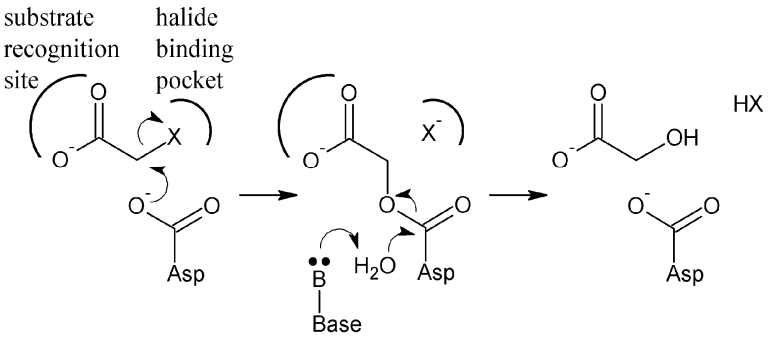


Fig. 1: Reaction mechanism of hydrolytic dehalogenases. An aspartate nucleophile forms an ester intermediate with the substrate via S_N2 reaction, removing the halide ion. A subsequent H_2O molecule cleaves the intermediate through a hydrolysis reaction.

Research Highlights

Cuesta-Seijo, J., Prive, G., and C. Tran (2010). "PagP crystallized from SDS/Consolvent reveals the route for phospholipid access to the hydrocarbon ruler". *Structure* 18(9):1210-1219

Tran, C., Edwards, E.A., and A. Yakunin (2011). Characterization of putative and known hydrolytic dehalogenases (poster). *13th Annual CSChE Ontario-Quebec Biotechnology Meeting,* Queens University, Kingston, ON

Tran, C., Chan, P., Yakunin, A., and E.A. Edwards (2011). Biochemical characterization of haloacetate dehalogenases (poster). *1st Annual BEEM Meeting,* University of Toronto, ON

Research Highlights

Tsai, A. *et al.* (2011). Constitutive expression of a fungal glucuronoyl esterase in *Arabidopsis* reveals altered cell wall composition (invited speaker). *Gordon-Kenan Research Seminar,* Easton, MA

Tsai, A. *et al.* (2011). Constitutive expression of a fungal glucuronoyl esterase in *Arabidopsis* reveals altered cell wall composition (poster). *Gordon Research Conference*, Easton, MA

Tsai, A. *et al.* (2009). Biochemical characterisation of bacterial and fungal hemicellulases and heterologous expression *in planta* (poster). *Gordon Research Conference,* Andover, NH

Tsai, A. *et al.* (2009). Biochemical characterisation of bacterial and fungal hemicellulases and heterologous expression *in planta* (poster). *31st Symposium on Biotechnology for Fuels and Chemicals,* San Francisco, CA

Alex Tsai
Ph.D. Student

B.Sc. (Hons.), 2008, University of Toronto

Supervisor: Emma Master

Isolation, characterisation, and transgenic expression of novel microbial hemicellulases for *in planta* fibre engineering

Plant cell walls are primarily composed of lignocellulose, which mainly comprises cellulose, hemicellulose, and lignin. While these biopolymers can be harnessed for the production of biofuel and biochemicals, cross-linkages and interactions between them contribute to the recalcitrance of lignocellulosic material. Several pretreatment technologies are being applied and further developed to address this challenge; however, they each present their own technical and economic drawbacks.

My project focuses on the development and assessment of transgenic plants that express microbial hemicellulases with potential to reduce fibre recalcitrance. Hemicellulases include enzymes that cleave the linkage between hemicellulose and lignin (i.e. lignin-carbohydrate complexes, or LCCs). LCCs are believed to be particularly important in lignocellulosic recalcitrance.

In collaboration with postdoctoral fellow Dr. Tom Canam, I constructed a library of transgenic *Arabidopsis thaliana* that express one of 25 microbial hemicellulases from either a constitutive or spatially regulated promoter. The hemicellulases targeted in this study were selected based on the likelihood they would effect LCC composition within the plant cell wall. The *Arabidopsis* lines were screened based on their morphology (Fig. 1) and enzyme digestability. More detailed analyses were conducted with a line that expresses a novel glucuronoyl esterase from the wood-degrading fungi *Phanerochaete carnosa* (PcGCE). These analyses included anatomical characterization of plant stem and cell walls by histological staining, electron microscopy, immunolabeling, total sugar and lignin composition, and xylan extractability. Lastly, to investigate the effect of the plant's genetic background on the effect of the transgene, I expressed PcGCE in an *Arabidopsis* mutant with altered lignin composition. The resulting transgenic line displayed similar morphometric and chemical phenotype as the transgenic line constructed in wild type *Arabidopsis*, verifying the robustness of the transgene effect.

My work demonstrates the potential of *in planta* cell wall modification via transgenic expression of microbial hemicellulases. **This strategy can be expanded on through rational enzyme combination, expression regulation, and host plant selection to improve lignocellulosic biomass quality.**

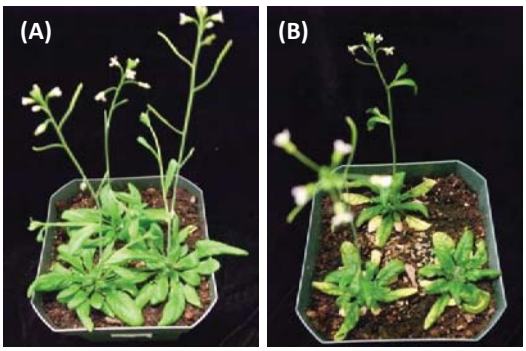


Fig. 1: Transgenic Arabidopsis expressing PcGCE (B) displayed smaller leaf, rosette size , and yellowing of leaf at an earlier developmental stage compared to the wild type plant (A).

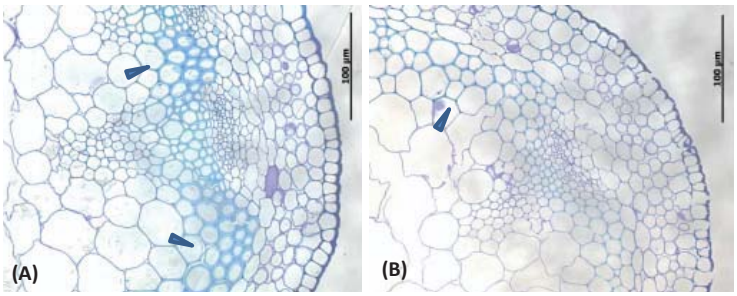
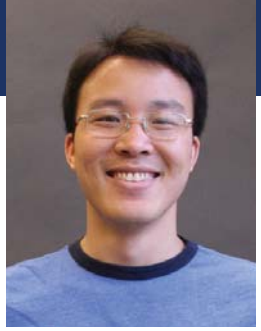


Fig. 2: Toluidine blue staining of Arabidopsis stem cross section. The cell wall thickness of interfascicular fibre (arrow) is halved in the transgenic line expressing PcGCE (B) compared to the wild type plant (A).



Thu Vuong
Postdoctoral Fellow

Ph.D., 2010, Cornell University
M.Biotech., 2004, Flinders University
B.Biotech., 2000, Hanoi University of Science

Supervisor: Emma Master

Engineering of a gluco-oligosaccharide oxidase for hemicellulose derivatisation, and enhancement of lignocellulosic biomass processing by reducing non-productive binding of hemicellulases

In addition to cellulosic biofuels, **the production of high-value biomaterials from plant biomass, including wood and under-utilized agricultural residues is going to be an important part of emerging biorefineries.** It is anticipated that high-value bioproducts can help secure investment in the cellulosic biofuels while diversifying agricultural and forest products. Accordingly, the aim of my project is to harness the specificity of biocatalysts (enzymes) to derivatise plant polysaccharides, particularly hemicelluloses. Specifically, I am engineering an oligosaccharide oxidase with enhanced activity on xylan. Based on computational docking of xylo-oligosaccharides to the active site of gluco-oligosaccharide oxidase (GOOX-VN) from the fungus *Acremonium strictum* (Fig. 1), potential amino acid residues were identified and then mutated for improved enzyme activity. Additionally, a hemicellulose-binding module from a different microorganism was added to *A. strictum* GOOX-VN in order to increase binding affinity of this enzyme on hemicelluloses. These mutant and chimeric enzymes are being purified and characterised using model and industrial hemicellulose samples.

I am also studying the non-productive binding, or binding without hydrolysis, of xylanases to lignin. The goal is to detect and minimise this undesirable binding behaviour, thus **increasing enzyme efficiency on lignocellulosic biomass**. Isothermal calorimetry (ITC) has been being used to measure non-catalytic interactions between lignin and commercial as well as purified xylanases. The enzymes were injected to lignin suspension and the heat that was generated from xylanase-lignin interaction after each injection was measured to calculate binding affinity (Fig. 2). These enzymes will be engineered to minimize their non-productive binding.

Research Highlights

Vuong, T. *et al.* (2011). A biocatalyst for oxidative modification of cello- and xylo-oligosaccharides (invited speaker). *Gordon-Kenan Res. Sem. on Cellulosomes, Cellulases and Other Carbohydrate Modifying Enzymes*, Easton, MA

Foumani, M. *et al.* (2011). "Altered substratespecificity of the gluco-oligosaccharide oxidase from *Acremonium strictum*". *Biotech. Bioeng.* 108(10):2261-2269

Vuong T. and D. B. Wilson (2010). Engineering *Thermobifida fuscacellulases*: catalytic mechanisms and improved activity. In *Protein Engineering: Design, Selection and Applications*, Nova Science Publishers

Vuong T. and D. B. Wilson (2010). "Glycoside hydrolases: Catalyticbase/nucleophile diversity". *Biotech. Bioeng.* 107(2):195-205

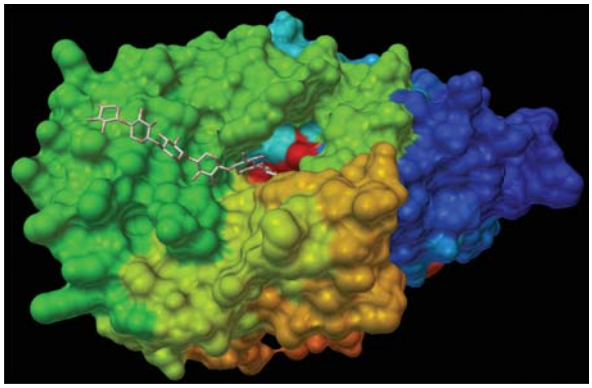


Fig. 1: Computational docking of xylohexaose (ring diagram) into the active site of Acremonium strictum gluco-oligosaccharide oxidase

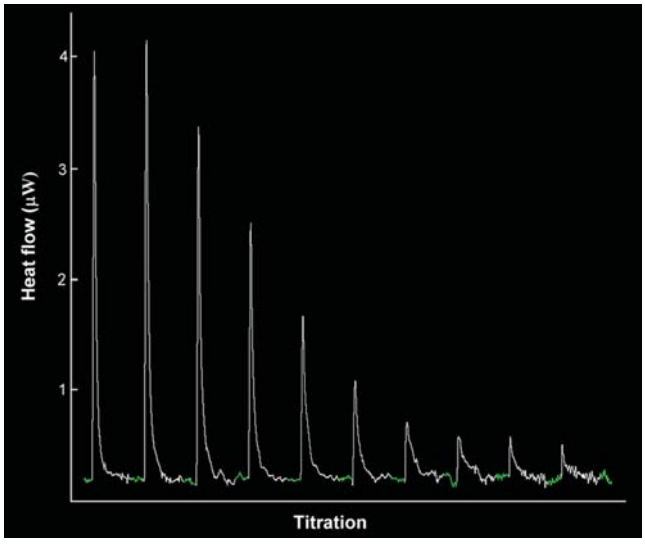


Fig. 2: Heat measurement from each injection of xylanase to lignin suspension

Research Highlights

Wei, K. and E. A. Edwards (2011). Biodegradation of chlorofluorocarbon-113 in anaerobic enrichment cultures (presentation). *13th CSChE Ontario-Quebec Biotechnology Meeting*, Kingston, ON

Han, S.K., Wei, K., Bourne, S., Bohnet, S., English, C., and L.Y. Stein (2010). Nitrifying communities in a sub-Arctic diamond mine tailings pond. *International Society of Microbial Ecology (ISME)*, Seattle, WA

Kai Wei
M.A.Sc. Student

B.A.Sc, 2010, University of Alberta

Supervisor: Elizabeth Edwards

Characterization of the inhibitory effect of different chlorinated compounds on reductive dechlorination

Chlorinated solvents are common contaminants in groundwater. Among all of the technologies to clean up contaminated sites, bioremediation is a cost-effective approach to remove chlorinated contaminants from groundwater. However, when multiple chlorinated compounds are present at contaminated sites, reductive dechlorination may be inhibited. For example, it is well documented that chloroform inhibits many microbial processes in subsurface environments.

For my Master's project, **I am investigating the inhibitory effects caused by the presence of multiple co-contaminant on detoxifying reductive dechlorination reactions.**

Chlorofluorocarbons (CFCs) often exist as co-contaminants with chlorinated solvents in groundwater due to improper disposal of refrigerators and air conditioners. 1,1,2-trichloro-1,2,2-trifluoroethane (CFC-113) is one such CFC that can inhibit reductive dechlorination of certain chlorinated ethenes. My plan was to determine whether certain bacteria can degrade CFC-113 to alleviate its inhibitory effect on reductive dechlorination. Using a combination of whole cell assays and cell-free extract assays, I determined that CFC-113 dechlorination was catalyzed by vitamin B12 supplied in the growth medium, but was not a result of specific enzyme activity.

Chlorinated ethenes and chlorinated ethanes are also found together as co-contaminants at polluted sites. Chloroform strongly inhibits the reductive dechlorination of trichloroethene, dichloroethene and vinyl chloride. Inversely, I found that trichloroethene, dichloroethene and vinyl chloride can also greatly inhibit the reductive dechlorination of chloroform. Currently I am characterizing the cross-inhibition kinetic parameters of chlorinated ethenes and chlorinated ethanes.

Overall, my project is focusing on the inhibitory effect on reductive dechlorination when multiple contaminants are present. This research has important implications for bioremediation strategies at contaminated sites. For example, the degree of inhibition is a strong fuction of the concentration of the inhibiting compound. **My research will guide practioners to optimize bioremediation performance at sites impacted with multiple interfering contaminants.**

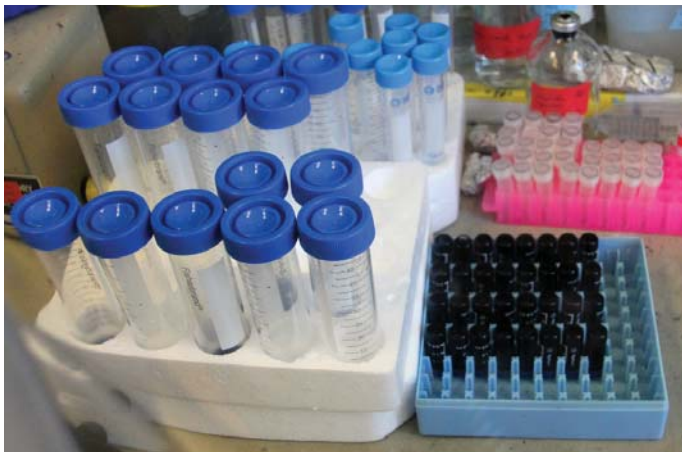


Fig. 1: Dechlorination assays for samples with multiple chlorinated contaminants, conducted in an anaerobic chamber.

Xiaohui (Linda) Xu
Technician

M. Sc., 1988, Northeast Normal University
B.A. Sc., 1985, Northeast Normal University

Supervisor: Alexei Savchenko

Protein crystallization — the key for protein crystallography

The only technique that allows direct visualization of protein structures at the atomic or near-atomic level is X-ray diffraction analysis as applied to single crystals of pure proteins. Obtaining well-diffracting crystals remains the main bottleneck in protein crystallography. My colleague, Hong Cui, and I work as a team to focus on protein expression, purification and crystallization. We express and purify hundreds of proteins each year and deliver dozens of structures quality crystals to the Advanced Photon Source. In 2007, I participated in Dr. A. Edwards' effort to develop a significant rescue technique called `*in situ* proteolysis' which doubled the success rate in protein crystallization and structure determination. As of now, more than 200 structures from my crystals have been deposited to the Protein Databank.

The challenge: protein complex crystallization

Macromolecular interaction is essential, necessary and unavoidable in a living organism. We could gain a more fundamental understanding of protein interactions and their resulting functions from protein-protein or protein-ligand complex structures. The first step in this challenging process is to get well-diffracting crystals containing subject ligands. Fig.1 shows the structure of a complex crystal obtained by the crystal-ligand soaking technique. The '*in situ* proteolysis' method was employed to obtain a crystal of the human nuclear hormone receptor REV-ERB in complex with Heme (Fig.2).

My team currently is focusing on the following 2 areas:

First, express, purify and crystallize enzymes identified from a variety of fungi that are potentially useful in industrial applications, including production of pulp and paper, ethanol and bio-diesel for energy, human food and animal feed, and a host of other uses. Second, express, purify and crystallize the effector proteins including the complexes identified from plant and human pathogens that cause infectious diseases.

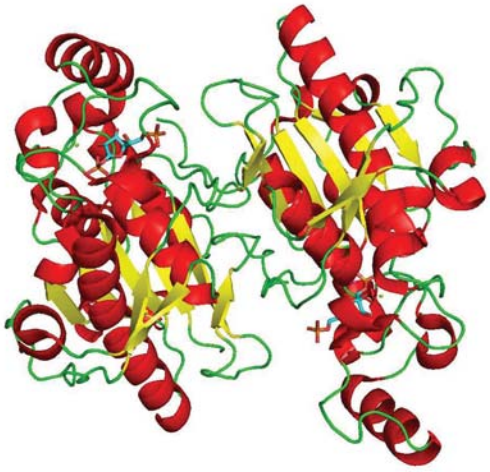


Fig. 1: Structure of the H13A mutant of Ykr043C in complex with sedoheptulose-1,7-bisphosphate(PDB ID: 3OI7).

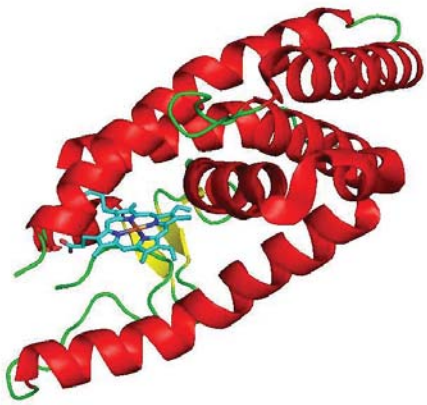


Fig. 2: Structure of human nuclear hormone receptor REV-ERB beta in complex with Heme(PDB ID: 3CQV).

Research Highlights

Clasquin, M.F. *et al.* (2011). "Riboneogenesis in yeast." *Cell* 145(6):969-980

Tchigvintsev, A., Xu. X. *et al.* (2010). "Structural insight into the mechanism of c-di-GMP hydrolysis by EAL domain phosphodiesterases." *J. of Mol. Biol.* 402:524-538

Pardee, K.I., Xu, X., Reinking, J., Schuetz, A., Dong, A., Liu, S., Zhang, R., Tiefenbach, J., Lajoie, G., Plotnikov, A.N., Botchkarev, A., Krause, H.M., and A. Edwards (2009). "The structural basis of gas-responsive transcription by the human nuclear hormone receptor REV-ERBbeta". *PLoS Biology* 7:e43

Dong, A., Xu, X. and A.M. Edwards (2007). "*In situ* proteolysis for protein crystallization and structure determination." *Nature Methods* 4(12):1019-1021

Research Highlights

Yang, L., Cluett, W.R., and R. Mahadevan (2011). "EMILiO: a fast algorithm for genome-scale strain design." *Metab. Eng.* 13:272-281

Garg, S., Yang, L., and R. Mahadevan (2010). "Thermodynamic analysis of regulation in metabolic networks using constraint-based modeling." *BMC Res. Notes* 3:125

Yang, L., Cluett, W.R., and R. Mahadevan (2011). EMILiO: a faster algorithm for genome-scale strain design (invited speaker). *SIM,* New Orleans, LA

Yang, L., Mahadevan, R., and W. R. Cluett (2010). Designing experiments from noisy metabolomics data to refine constraint-based models (Awarded best presentation in session). *ACC*, Baltimore, MD

Laurence Yang
Ph.D. Student

M.A.Sc., 2008, University of Toronto
B.A.Sc., 2006, University of Toronto

Supervisor: Radhakrishnan Mahadevan
Co-Supervisor: William R. Cluett

Computational algorithms to design and understand cell metabolism

The need for sustainable alternatives to petroleum-based chemicals and fuels is widely recognized. One avenue for achieving this goal is the microbial conversion of renewable feedstocks to products. Microbes possess a diverse portfolio of substrates and products, and their use of enzymes ensures great specificity of chemical conversions, potentially reducing purification costs. On the other hand, microbial metabolism is a vast and complex system whose operating principles are still largely unknown. Therefore, **a major challenge is to develop tools to accurately predict metabolic behavior, and to predictably re-design metabolism for engineering purposes.**

To predict metabolic behavior at the genome-scale, constraint-based modeling (CBM) has proven to be effective. This computational approach uses genome annotations to reconstruct a mathematical representation of the metabolic network and predicts network-wide reaction flux distributions.

Our research has two goals: (i) improve the precision of CBM predictions by using high-throughput data, and (ii) develop efficient algorithms to design metabolism for maximum biochemical production. To address the first, we developed a computational algorithm that uses a noisy metabolomics data set to predict which metabolites need to be measured more precisely, in order to reduce the variance of model predictions. Second, we developed a nonlinear optimization-based strain design algorithm, called EMILiO, that rapidly identifies genetic manipulation targets (knockout, inhibition and activation) to maximize biochemical production. EMILiO identifies strain designs with several orders of magnitude speed-up over alternative algorithms.

As our understanding of cell metabolism deepens, our capacity to predictably engineer them will also increase. **Our ongoing goal is to develop cutting-edge tools to aid experimentalists and modelers alike in their quest to accelerate understanding, while making the best use of what we already know to develop efficient microbial strains.**

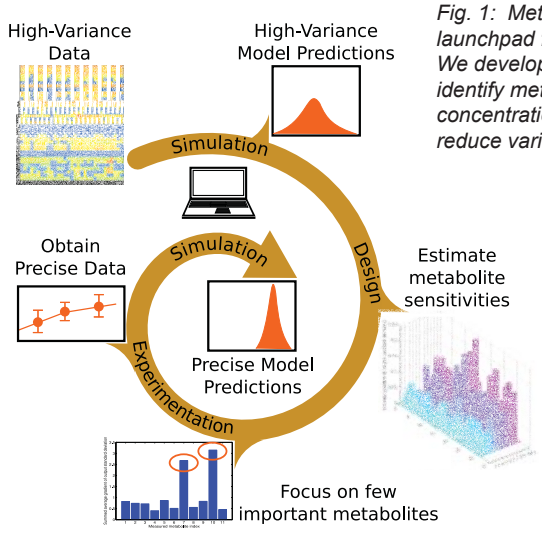


Fig. 1: Metabolomics data serving as the launchpad for iterative modelrefinement. We developed a computational algorithm to identify metabolites needing more precise concentration measurements in order to reduce variability of model predictions.

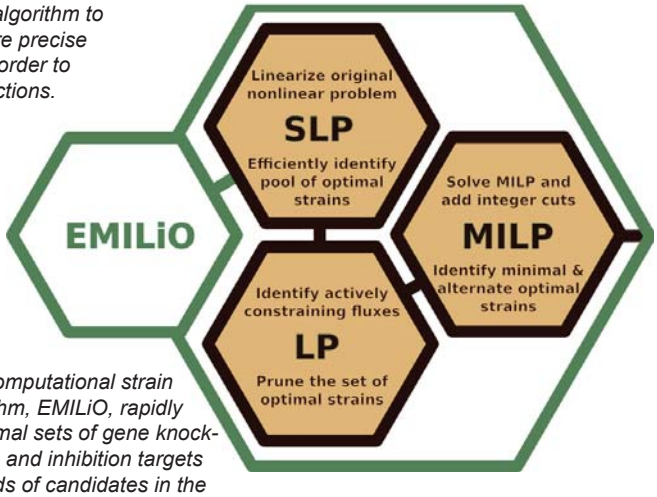


Fig. 2: The computational strain design algorithm, EMILiO, rapidly identifies optimal sets of gene knockout, activation and inhibition targets from thousands of candidates in the metabolic network.

Minqing Ivy Yang
Ph.D. Student

M.A.Sc., 2008, University of Toronto
B.A.Sc., 2005, University of Toronto

Supervisor: Grant Allen
Co-Supervisor: Elizabeth Edwards

Granulation in the anaerobic treatment of pulp mill wastewaters

Anaerobic treatment has been increasingly applied in the pulp and paper industry, where wastewaters contain high concentrations of organic compounds that can be converted into CH_4. In certain high rate reactors, such as internal circulation (IC) reactors, methanogenic archaea and bacteria form granular aggregates (Fig. 1). Granular sludge settles faster than dispersed sludge, so microorganisms in granules are less likely to be washed out. Maintaining granulation and avoiding degranulation is the key to retaining microorganisms in many high rate anaerobic reactors.

The main objectives of this project are to understand the cause of degranulation in the full scale IC reactors in the pulp mill Tembec Temiscaming, and to propose strategies to enhance granulation. Particle size distribution analysis and granule strength tests were developed to examine the physical properties of granules. Molecular methods, such as PCR and denaturing gradient gel electrophoresis (DGGE), were adopted to study the microbial community. These methods were applied to compare the granules collected from four continuous reactors treating different concentrations of sulphite pulp washer effluent (PEW) that was suspected to cause degranulation.

It was found that the sludge from the reactors treating PEW was weaker and smaller than the sludge from the PEW-free reactor, so the negative impact of PEW on granulation was confirmed. The PCR-DGGE results showed that the bacterial community in the sludge treating PEW was different than that in the sludge from the PEW-free reactor.

Further analysis will be performed to link the differences in the granule properties to the constituents in PEW to identify the problematic compounds. Two continuous reactors will also be set up in the lab to study the effect of long-term exposure of PEW on granules (Fig. 2). At the end of the study, **strategies for granulation enhancement will be proposed.**

Research Highlights

Yang, M.I. *et al.* (2010). "Anaerobic treatability and biogas production potential of selected in-mill streams." *Water Sci. Technol.* 62(10):2427-2434.

Yang, M.I., Edwards, E.A. and D.G. Allen (2010). Granulation in the anaerobic treatment of pulp mill effluents (poster). *12th IWA Specialist Conf. on Anaerobic Digestion,* Guadalajara, Mexico

Yang, M.I., Edwards, E.A. and D.G. Allen (2010). The effect of pulp mill effluents on the microbial properties of anaerobic granules. *13th Inter'l Symp. for Microbial Ecol.,* Seattle, WA

Yang, M.I., Edwards, E.A. and D.G. Allen (2009). Anaerobic treatment of pulp mill effluents: Treatability of selected streams and granulation. *9th IWA Symposium on Forest Industry Wastewaters*, Fredericton, NB

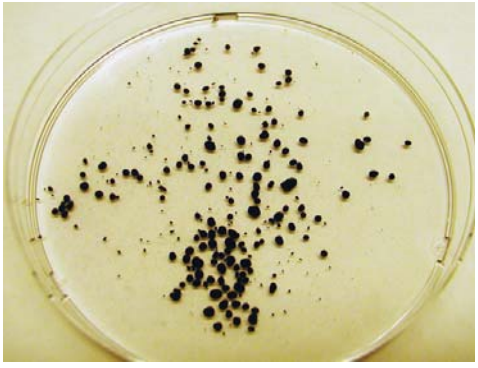


Fig. 1: Granules in petri dish.

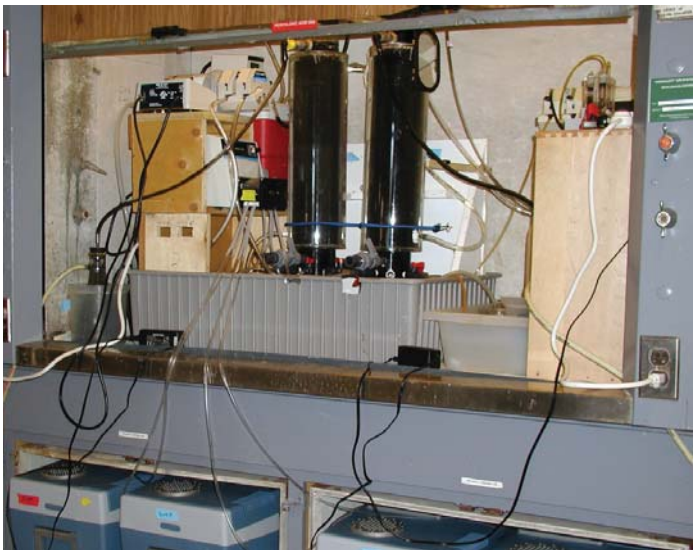


Fig. 2: Set-up for lab-scale upflow anaerobic digesters.

M.Sc. (Biochemistry), 2002,
McMaster University
B.A.Sc. in Biochemistry, 1999,
San Francisco State University

Veronica Yim
Technician

Supervisor: Alexei Savchenko

High throughput cloning and test protein expression

DNA constructs are typically created by ligating two different DNA fragments that have been digested with restriction enzymes containing complementary restriction sites. Cloning options are often limited by lack of available unique sites in the vector and gene of interest. In contrast, In-Fusion is an enabling technology that can join any two pieces of DNA that contain 15bp of shared identity at their ends, without the need for any restriction enzyme digestion. The 15bp overlap can be included in the primers used to PCR-amplify a segment of DNA.

Our research group is part of Midwest Centre for Structural Genomics (MCSG) with affiliations with several centers in the US. **Our lab's main focus is the study of proteins involved in bacterial pathogenesis.** We have already solved structures and characterized proteins produced by important pathogens such as *Bacillus anthracis*, *Bacillus subtilis*, and *Shigella flexneri* and *Escherichia coli*.

We work with effectors, bacterial proteins injected into the host cells by the type III, IV and VI secretion systems. These proteins are to be obtained in pure form to be used in protein crystallization and functional assays using recombinant expression technology. My main duties are to perform high throughput cloning of these effectors in recombinant expression vector and to test for their expression and solubility in *E.coli* expression system.

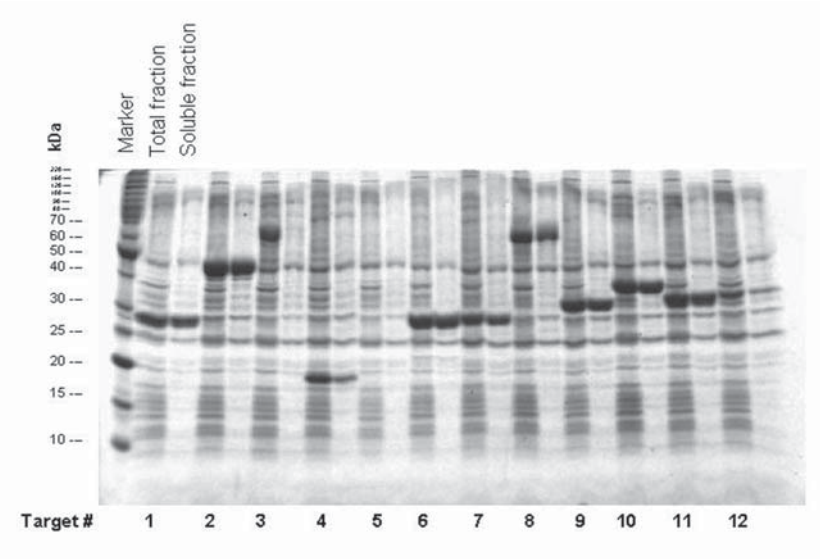


Fig. 1: Gel analysis of 12 recombinant proteins expressed in E. coli from high throughput cloning.

Jiao Zhao
Research Associate

Ph.D., 1998, East China University of Science and Technology (ECUST)
M. Eng., 1994, ECUST
B. Eng., 1991, ECUST

Supervisor: Radhakrishnan Mahadevan

A multi-scale investigation of subsurface bioremediation

In situ bioremediation is defined as the use of microorganisms (either single strains or microbial consortia) to remove pollutants at the contaminated sites. The challenge, however, is how to achieve a sustainable outcome for *in situ* bioremediation, which is synergistically affected by biological, geochemical and hydrological processes at different space-time scales. A multi-scale investigation of subsurface bioremediation can provide insightful analysis and predictions for long-term bioremediation processes.

My research focuses on the development of a multi-scale mechanistic model of *in situ* bioremediation, and the subsequent use of simulations to assess sustainable strategies prior to experimental trials. This computational approach requires a detailed understanding of the microbial activity in the subsurface and its interaction with the geochemical and hydrological processes involved in bioremediation. We have successfully developed a multi-scale mechanistic model for *Geobacter*-mediated uranium bioremediation (Fig. 1), and performed global sensitivity analysis to identify the main sources of prediction uncertainty caused by synergistic effects of biological, geochemical, and hydrological processes, thereby improving the structure and parameterization of the comprehensive model. Based on this progress, we are currently working on coupling the optimal control theory to field-scale models in order to achieve a sustainable uranium bioremediation by controlling the chemical flux (e.g., electron donor and/or acceptor) amendments.

Since bioremediation efforts may lead to long-term changes in microbial communities, which in turn affect the sustainability of bioremediation processes, the evolutionary basis for the development of microbial consortia is one of my other research interests. We intend to computationally process the genetic material recovered directly from environmental samples (metagenomes) and then develop the associated genome-scale models for the microbial community. To refine and validate the genome-scale metabolic models under environmental conditions, we will apply isotope-based metabolic flux analysis to the microbial consortia.

Research Highlights

Zhao, J., Scheibe, T.D., and R. Mahadevan (2011). "Model-based analysis of the role of biological, hydrological and geochemical factors affecting uranium bioremediation." *Biotechnol. Bioeng.* 108(7):537-1548

Zhao, J., Scheibe, T.D., Fang, Y., Lovley, D.R., and R. Mahadevan (2010). "Modelling and sensitivity analysis of electron capacitance for *Geobacter* in sediment environments." *J. Contam. Hydrol.* 112(1-4):30-44

Zhao J. *et al.* (2008) "Extraction of elementary rate constants from global network analysis of *E. coli* central metabolism." *BMC Syst. Biol.* 2(1):41-59

Zhao J., Baba, T., Mori, H. and K. Shimizu (2004). "Effect of zwf gene knockout on the metabolism of *Escherichia coli* grown on glucose or acetate." *Metab. Eng.* 6:164-174

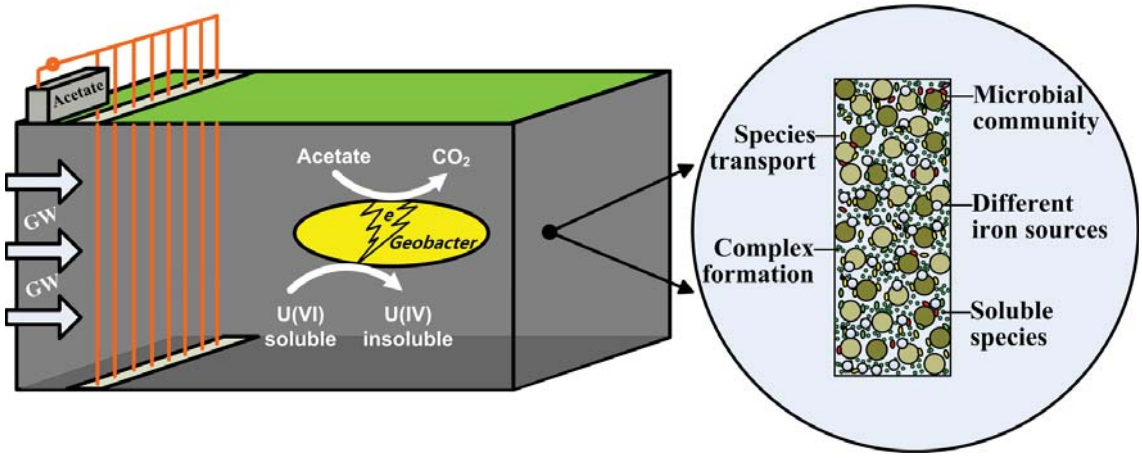


Fig. 1: Multi-scale mechanistic model for Geobacter-mediated uranium bioremediation stimulated using acetate as an electron donor. The mechanistic model takes into account synergistic effects of biological, geochemical, and hydrological processes on uranium reduction as well as microbial community evolution.

Research Highlights

Zheng, H. (2010). Mechanistic, inhibitory, and mutagenic studies of inositol dehydrogenase from *Bacillus subtilis*. Ph.D. thesis, *Dept. of Chemistry*, University of Saskatchewan

van Straaten, K.E., Zheng, H., Palmer, D.R.J., and D.A.R. Sanders (2010). "Structural investigation of myo-Inositol dehydrogenase from *Bacillus subtilis*: Implications for catalytic mechanism and inositol dehydrogenase subfamily classification." *Biochemical Journal*, 432:237-247

Hongyan Zheng
Postdoctoral Fellow

Ph.D., 2010, University of Saskatchewan
M.Sc., 2004, Lanzhou University
B.A.Sc., 2001, Lanzhou University

Supervisor: Radhakrishnan Mahadevan
Co-Supervisor: Alexander Yakunin

Enzymatic production of difunctional alkane

Growing concerns over environment and energy have drawn attention to developing biocatalytic routes to attain useful products from renewable feedstocks. Chemical synthesis needs more energy for controlling reaction conditions, and may require harsh conditions (such as high pressure), and usually produces byproducts. Alternatively, enzymes offer substantial advantages over chemical catalysts in that they are derived from renewable resources, are biodegradable, and work under relatively mild conditions of temperature, pressure, and pH. Additionally, they are capable of high reaction rates and tend to offer exquisite selectivity in both reactant and product stereochemistry.

Production of non-natural compounds in a microbial system has two major challenges: the establishment of a new biosynthetic pathway and the engineering of the host to direct flux to product pathway. An example of a successful metabolic engineering project is the production of 1,3-propanediol (PDO) in *E. coli* developed by Genencor and DuPont, which has led to a commercial process.

My research interests revolve around identification of novel enzyme function and the mechanism by which the enzyme catalyzes the reaction. A direct application of the information obtained is to engineer enzymes for industrially significant substrates, as well as the development of biosynthetic pathways to synthesize compounds that are not naturally produced by living cells. The methodology of the approach comprises several different disciplines, including organic chemistry, molecular biology, and enzymology.

My work has focused on enzyme screening, function characterization, and protein engineering of dehydrogenases, ammonia lyases, and mutases, to produce difunctional alkanes.

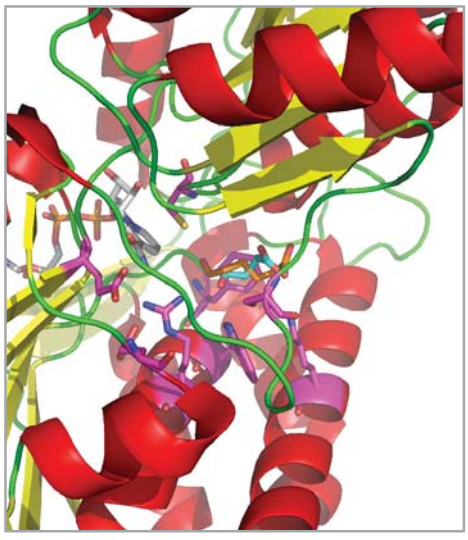


Fig. 1: The active site of succinate-semialdehyde dehydrogenase from Salmonella typhimurium LT2.

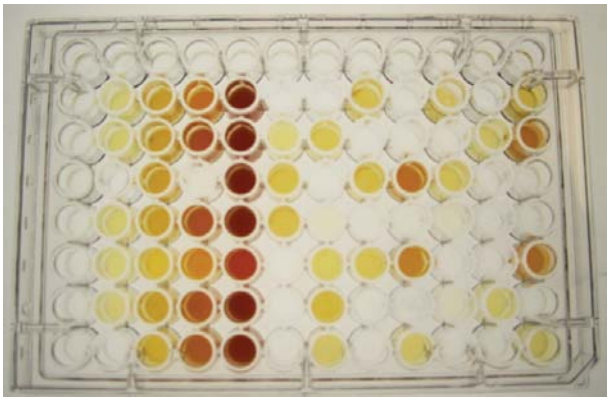


Fig. 2: Screening of ammonia lyases with Nessler's reagent.

Wendy Han Zhou
M.A.Sc. Student

B.A.Sc., 2011, University of Toronto

Supervisor: Elizabeth Edwards
Co-Supervisor: Vladimiros G. Papangelakis

Bioleaching of an ultramafic Ni deposit using anaerobic nitrate reducing bacteria

Worldwide depletion of high-grade nickel deposits has driven the metallurgical industry to look into low-grade resources that were previously uneconomical to exploit. Low-grade resources include waste materials such as pyrrhotite tailings and unexcavated natural deposits with 0.3 to 1.0 wt% Ni. In the latter, Ni exists in the form of nickel sulphide mineral (pentlandite) along with magnesium silicate minerals such as lizardite and magnetite ($Mg_3Si_2O_5(OH)_4$, $FeO{\bullet}Fe_2O_3$).

My master thesis focuses on **how to selectively dissolve Ni from an ultramafic low-grade nickel sulphide deposit by minimizing co-dissolution of gangue elements, such as Mg, Si, and Fe, hence increasing extraction selectivity, by employing the catalytic effect of iron/sulfur oxidizing anaerobes,** specifically *Thiobacillus denitrificans.*

The high magnesium and silicon content in ultramafic ores makes traditional chemical extraction processes unfeasible due to rapid smelter wear, high Ni losses, high reagent consumption, and increased health and environmental concerns related to dust generation. In addition, acidic chemical processes usually require large amounts of neutralizing agents and downstream waste management processes. A promising hydrometallurgical route that makes use of aqueous chemistry involves selective Ni dissolution under near neutral pH environment by using iron and sulfur oxidizing anaerobes such as *T. denitrificans* in the presence of nitrate salts.

T. denitrificans is a widespread, chemolithoautotrophic bacterium with the ability to couple denitrification to sulfur and Fe(II) oxidation (Fig. 1). As Ni exists as a solid solution in FeS minerals, the capability of catalyzing Fe(II) oxidation, and consequently dissolving FeS minerals attracted us to look into *T. denitrificans'* potential for selective Ni extraction from ultramafic ores at near-neutral pH.

My current work is to determine the ability of the bacterium to dissolve pentlandite (NiS) minerals at neutral pH, **with the goal to determine if *T. denitrificans* or similar nitrate-reducing bacteria can be effective for bioleaching of low-grade nickeliferous ultramafic deposits.**

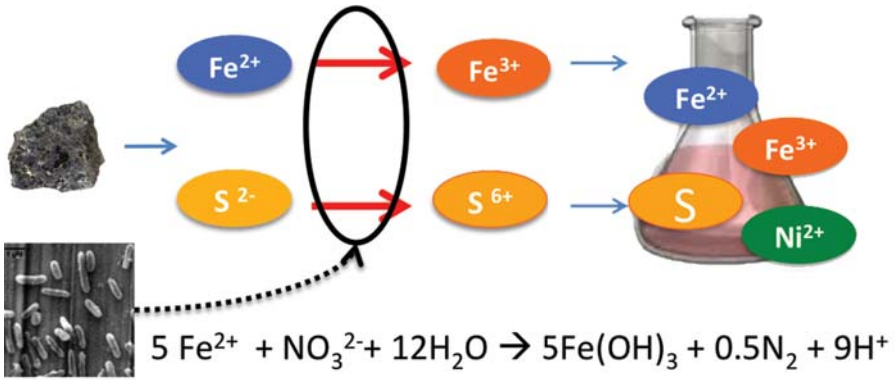


Fig. 1: Reactions Catalyzed by Thiobacillus denitrificans.

Research Highlights

Zhou, W.H. and V.G. Papangelakis (2011). Bacteria catalyzed leaching of Ni from pyrrhotite tailings under different temperatures (poster). *50th Annual Conference of Metallurgists*, Montreal, QC

Zhou, W.H. (2011). Bioleaching of Ni from pyrrhotite tailings. B.A.Sc. thesis, *Dept. of Chemical Engineering and Applied Chemistry,* University of Toronto

Research Highlights

Zhuang, K., Vemuri G. and R. Mahadevan. (2011). "Economics of membrane occupancy and respiro-fermentation." *Nature Molecular Systems Biology* 7(500)

Barlett, M., Zhuang, K., Mahadevan, R. and D. Lovley (2011). "Integrative analysis of the interactions between *Geobacter* spp. and sulfate-reducing bacteria during uranium bioremediation." *Biogeosciences Discussion* 8:11337-11357

Kai Zhuang, Ma, E., and R. Mahadevan (2011). "Model-based design and optimization of an effective uranium bioremediation strategy". (in preparation)

Kai Zhuang
Ph.D. Student

M.A.Sc., 2009, University of Toronto
B.A.Sc., 2006, University of Toronto

Supervisor: Radhakrishnan Mahadevan

Integrative analysis of the interactions between *Geobacter* spp. and sulfate-reducing bacteria during uranium bioremediation

Acetate addition to the subsurface is a promising strategy for uranium bioremediation, however, it remains to be optimized because of a poor understanding of microbial interactions during bioremediation. In previous field trials in which acetate was added to the subsurface, there were two distinct phases: an initial phase in which acetate-oxidizing, U(VI)-reducing *Geobacter* predominated and U(VI) was effectively reduced and a second phase in which acetate-oxidizing sulfate reducing bacteria (SRB) predominated and U(VI) reduction was poor.

We investigated the interaction of *Geobacter* and SRB using both experimental and computational modeling techniques. In sediment incubations, *Geobacter* grew quickly but then declined in numbers as the microbial reducible Fe(III) was depleted, whereas the SRB grew more slowly and reached dominance after 30-40 days. Modeling predicted a similar outcome (Fig. 1). Additional modeling in which the relative initial percentages of the *Geobacter* and SRB were varied indicated that there was little to no competitive interaction between *Geobacter* and SRB when acetate was abundant. Further simulations suggested that the addition of Fe(III) would revive the *Geobacter*, but have little to no effect on the SRB. This result was confirmed experimentally.

The results demonstrate that it is possible to predict the impact of amendments on important components of the subsurface microbial community during groundwater bioremediation. **Our finding that Fe(III) availability, rather than competition with SRB, is the key factor limiting the activity of *Geobacter* during *in situ* uranium bioremediation will aid in the design of improved uranium bioremediation strategies.** This work is currently underway in our lab.

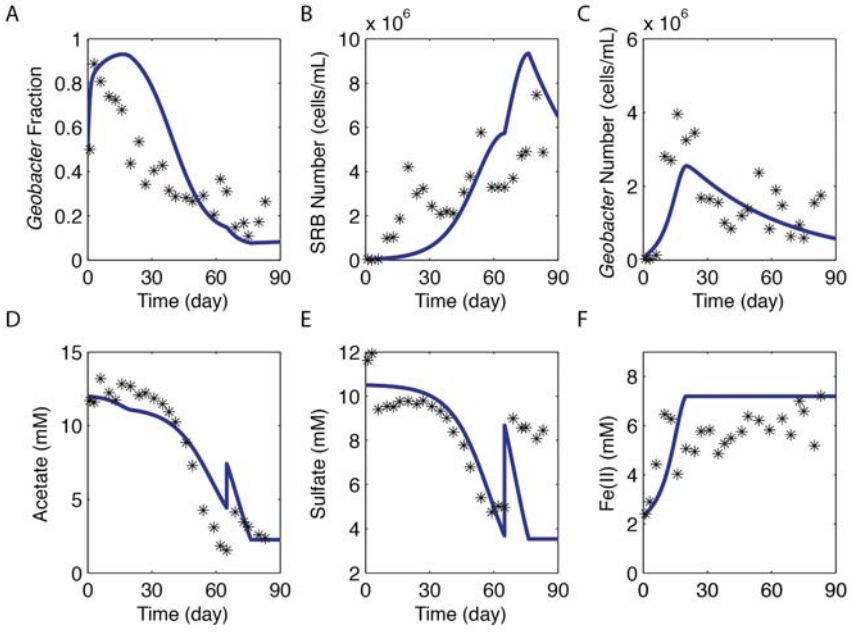


*Fig. 1: In silico predictions of trends in the fraction of Geobacter in the community (A), number of SRB (B), number of Geobacter (C), acetate concentration (D), sulfate concentration (E), and Fe(II) concentration (F). Lines are the in silico predictions; * are the experimental values.*

Anna Zila
Research Assistant

M.Eng., 2011, University of Toronto
B.A.Sc., 2009, University of Toronto

Supervisor: Elizabeth Edwards

Population dynamics and biomarkers of KB-1® genera in a bioaugmentation field trial

KB-1® is a stable microbial consortium capable of complete reductive dechlorination of perchloroethene (PCE) through trichloroethene (TCE), cis-dichloroethene (cDCE) and vinyl chloride (VC) to non-toxic ethene (Fig. 1). My Master's project focused on a field bioaugmentation trial using KB-1® in a TCE-contaminated fractured bedrock site. Fractured bedrock yields complex GW flow patterns, and can be a very challenging medium for bioremediation. Furthermore, the contribution of organisms other than dechlorinators (both native and introduced) to dechlorination is rarely tracked in field-scale studies. Thus, **my study aimed to prove that reductive dechlorination was effective at a site having low groundwater temperatures and fractured bedrock.** It also provided novel information on the population dynamics of dominant KB-1® genera prior to and after biostimulation with ethanol and bioaugmentation with KB-1®.

The microbial community dynamics in groundwater from various locations, including performance monitoring and extraction wells within the treatment area as well as background wells, were studied prior to and after inoculation. Six bacterial genera (*Acetobacterium, Bacteroidetes, Dehalococcoides, Geobacter, Pelobacter,* and *Synergistetes*) and one archaeal genus (*Methanomethylovorans*) were quantified using real-time polymerase chain reaction (qPCR) targeting respective 16S rRNA genes over five sampling events. The study showed that all species increased after bioaugmentation except for *Acetobacterium* which declined in the first two sampling events, and then attained pre-bioaugmentation numbers in the final sampling event. The concentrations of TCE, cDCE and VC confirmed that bioaugmentation was responsible for an increase in dechlorination rates in the intermediate bedrock level, and that bioaugmentation was effective (Fig. 2).

Since the completion of my Master's project, I have been screening a suite of genes coding for reductive dehalogenase enzymes present in *Dehalococcoides* to be used as biomarkers when bioaugmenting with KB-1® in the field. The biomarkers can potentially be used to differentiate native and non-native species of *Dehalococcoides*, thereby attributing enhanced dechlorination to bioaugmentation.

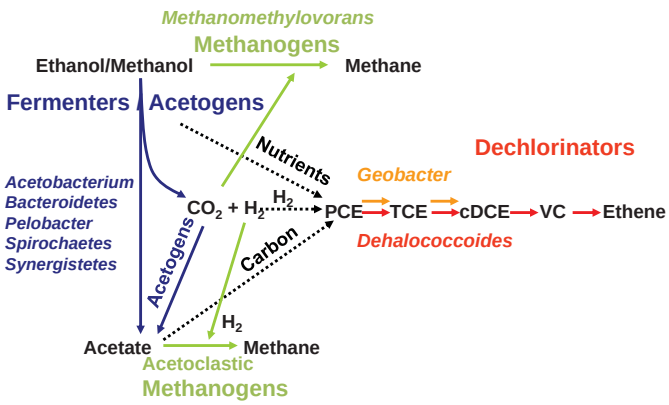


Fig. 1: Substrate utilization pathway of dominant KB-1 organisms whose population dynamics were tracked in a bioaugmentation field trial.

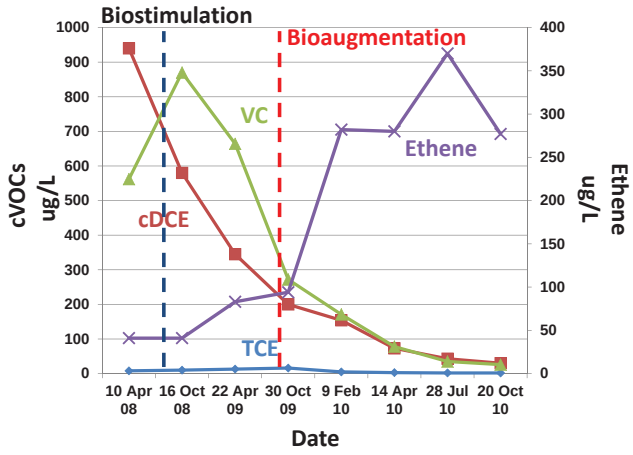


Fig. 2: Chlorinated ethene concentrations over time, with a noticeable increase in ethene concentration only after bioaugmentation.

Research Highlights

Zila, A. (2011). A Molecular study of field bioaugmentation using the KB-1® mixed microbial consortium: the application of real-time PCR in analyzing population dynamics. M.Eng. thesis, *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto

Zila, A. *et al.* (2011). Population dynamics of a mixed microbial bioaugmentation culture (KB-1®) during a bioremediation field trial at a trichloroethene-contaminated fractured bedrock site (poster). *Ontario Quebec Biotechnology Meeting*, Kingston, ON

Alumni Updates

BioZone alumni are involved in exciting endeavours around the world. We are very pleased to be able to include updates about their current activities.

Thomas Canam, *Postdoctoral Fellow, 2010*

My research focuses on using plants for bioenergy and bio-product applications. One aspect of my research involves using biotechnology to tailor plant traits for specific applications, such as bioethanol production.

I am also interested in using bacteria and fungi as pretreatment agents of agricultural residues destined for biomass-to-bioenergy processes, such as gasification. At Eastern Illinois University, my research will complement the numerous green energy initiatives on campus, including the Renewable Energy Center (http://www.eiu.edu/fpm/erec.php) and the Center for Clean Energy Research and Education (http://castle.eiu.edu/energy/).

I am now an Assistant Professor at the Department of Biological Sciences at Eastern Illinois University (Charleston, IL).

Work Contact: Department of Biological Sciences, Eastern Illinois University, 600 Lincoln Avenue, Charleston, IL 61920-3099, (217) 581-6608

Grant Frahm, *M.A.Sc., 2008*

I currently live in Ottawa, Ontario, and work in the Biologics and Genetic Therapies Directorate at Health Canada. Specifically, our laboratory conducts research in the areas of nanotechnology and protein structure.

Claudio F. Gonzalez, *Postdoctoral Fellow, 2007*

I am now an Assistant Professor of the Microbiology and Cell Science Department at the University of Florida. I try to understand the biochemistry of and biological importance of hydrolytic enzymes of microbial origin. I am studying microbial enzymes involved in the release and modification of food bioactive phytophenols. The enzymes isolated from mammal's gut release bioactive food components that can have a positive impact on human health. Since several host signaling pathways are triggered by the action of the released bioactive components, the correct selection of the necessary enzymatic activities is a promising alternative to an adequate and natural stimulation the host immunological system. My research group recently demonstrated a positive correlation between the bio-activation of phytophenols and the prevention of chronic autoimmune disease in animal models.

Web: http://microcell.ufl.edu/personnel/faculty/gonzalez1.shtml

Chris Goode, *Ph.D., 2010*

Currently I work as Senior Program Advisor at the Ontario Ministry of the Environment. I am developing new regulations for a range of industry sectors as part of the Ministry's effort to modernize our environmental approvals process. On the home front, my wife Laura and I are expecting our second child in January 2012 so life is busy.

Work Contact: (416) 325-7421, christopher.goode@ontario.ca

Ariel Grostern, *Ph.D., 2009*

For the last two year I have been in California, doing a post-doc at UC Berkeley in the lab of Lisa Alvarez-Cohen. I am studying the genetic and biochemical basis of 1,4-dioxane metabolism in the actinobacterium *Pseudonocardia dioxanivorans* strain CB1190. It is certainly nice to work with an aerobe, and an isolate to boot! Although I miss the people in Toronto, I don't miss the cold or my Toronto biker-rage. Everyone should live in the San Francisco area at some point in their life – it is multicultural and liberal like Toronto, but with ten times nicer geography!

Work Contact: ariel.grostern@berkeley.edu

Jen Guthrie, *M.Sc., 2006*

In 2006 I accepted a research technician position with the Ontario Ministry of Health, which has since been incorporated into the new government agency known as Public Health Ontario (PHO). Here I have worked in several areas of research, surveillance and diagnostic test development for infectious diseases such as MRSA, Bordetella pertussis, and tuberculosis. In 2009 I was part of the group working on pandemic H1N1 (swine flu) and was able to design the first diagnostic test available in Ontario for this strain. Currently, alongside various research projects I also coordinate the efforts between the laboratory and Public Health Units with respect to strain typing of tuberculosis which helps to monitor for outbreaks, as well as track and trace cases in Ontario. More than a dozen publications and international conference poster presentations have illustrated my contributions to infectious disease research in the last several years.

Alex Hayes, *Ph.D., 2010*

I am currently working as a Research Associate at the National Research Council Canada - Institute for Biological Sciences where I am studying enzymes involved in regulating bioactive lipids.

Work Contact: alexander.hayes@nrc-cnrc.gc.ca

Tyler Irving, *M.A.Sc., 2010*

I like to describe myself as Canada's only chemistry journalist. I am the News Editor for ACCN, the Canadian Chemical News, published by the Chemical Institute of Canada. My job involves researching and writing about people, events, discoveries and government policy as they relate to the chemical profession. It is rewarding work and makes excellent use of my background in both chemical engineering and education. I spend my spare time writing and blogging about Canadian science in general, as well as playing the Great Highland Bagpipe.

Work Contact: ACCN, Chemical Institute of Canada, 130 Slater St., Suite 550, Ottawa, ON K1P 6E2, (647) 378-2883, tirving@chemist.ca

Marie-Claude Jobin, *Postdoctoral Fellow, 2010*

In December 2010, I moved to Gatineau/Ottawa. Since then, I gave birth to my second daughter and stayed at home to take care of her. I will start a part-time teaching position at the University of Ottawa in January and I am currently looking for a research/management position.

Work Contact: mjobi3@uottawa.ca

Ekaterina Kuznetsova, *Ph.D., 2009*

I have received my PhD degree from the Department of Medical Biophysics in 2009. Currently, I work in the Structural Genomics Consortium in the Molecular Biophysics group led by Dr. Masoud Vedadi. Our current focus is on epigenetic proteins, particularly histone modifying enzymes. The goal is to characterize their activities and screen for small molecule inhibitors.

Work Contact: MaRS Centre, South tower, 101 College St., 7th Floor, Room 715, Toronto ON M5G 1L7, (416) 946-0502, kate.kuznetsova@utoronto.ca

Yao Olive Li, *Ph.D., 2009*

After completing my Ph. D. study under the supervision of Professor L. L. Diosady in 2009, I had stayed for about one year to continue my post-doctoral research. During this period of time I had independently taught a session of Food Engineering at U of T in spring 2010. The following summer I moved my family to Nashville, TN, due to the acceptance of a research assistant professor position at Tennessee State University. A year later in July 2011, I was hired by Cal Poly Pomona as a tenure-track assistant professor and once again I relocated my family to the beautiful southern California. Currently my family has settled in and been enjoying the nice weather and numerous entertaining sites/activities here. The small town we are residing now is close to the county of Ontario, CA – what a coincidence: "Ontario, CA", so we are just missing something called "Toronto" here. By all means I welcome everyone to come down visiting me here whenever there is a chance for you.

Work Contact: Department of Human Nutrition & Food Science, California State Polytechnic University, Pomona, CA, USA, (909) 869-3021, yaoli@csupomona.edu

Daniel Liao, *M.Eng., 2011*

I graduated from Prof. Saville's lab with an M.Eng. in June 2011. I am now a research manager with Mascoma Canada, a company that specializes in the development of next-generation biofuels and chemicals from renewable sources, where his expertise is in bioprocess engineering. I became a professional engineer (P.Eng.) in September 2011.

Work Contact: Mascoma Canada Inc., 2121 Argentia Rd. Suite 401, Mississauga, ON L5N 2X4, (905) 821-4000 ext. 416, dliao@mascoma.com

Marie Manchester, *M.A.Sc., 2011*

I started working in the spring as a community engagement coordinator for Queenston Mining, a gold exploration company in the Kirkland Lake area. So far this work has been an amazing opportunity to learn about the mining industry as well as the rich history of the area. The role of community engagement coordinator is to act as a liaison between the community and the company and to help educate the public about proposed projects and to gather comments and concerns from the public to the proposed development. This has involved coordinating community meetings and site visits with Aboriginal people with traditional territory in the area, as well as public meetings and class visits. The role has also allowed some diversification into the environmental field and health and safety. Thus other activities have included bimonthly field sampling duties for the surface water and groundwater sampling baseline data gathering campaign and serving on the joint health and safety committee.

Eve Moore, *M.A.Sc., 2009*

After graduating from Prof. Edwards' Lab in 2009 with a Masters in Chemical and Environmental Engineering, I spent 6 months bush camping along the Eastern Coast of Africa.

In 2010, I helped start HandyMetrics Corporation - a medical software services and technologies company focused on helping fight the spread of hospital acquired infections. The company has expanded quickly and now provides services to over 64% of the academic hospitals in Ontario and over 80 hospital sites across Canada. HandyMetrics is currently planning expansion into the US and international markets. I do a number of things at the company, but primarily fulfill my role as Director of Operations.

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Nalina Nadarajah, *Research Associate, 2011*

Currently I am a professor at the School of Engineering Technology and Applied Science (SETAS) at Centennial College (Morningside campus). I teach courses in Environmental Microbiology, Microbial Genetics and I also supervise students on their final year microbiology projects. I am also involved in microbiology-related applied research carried out at Centennial College.

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David Sanscartier, *Postdoctoral Fellow, 2011*

I completed part of my NSERC postdoctoral fellowship at the University of Toronto working with Heather MacLean and Brad Saville from Nov 2009 to January 2011. Myresearch at Biozone involved a life cycle assessment (LCA) and a techno-economic analysis of electricity generation through anaerobic digestion of household organic waste within the Ontario renewable energy policy environment. The findings of this project have recently been published in Environmental Science and Technology. I am now completing the second portion of this fellowship at the University of Saskatchewan, Saskatoon, SK, where my partner holds a tenure-track position in the Department of Art and Art History, and I am working as a researcher at the Saskatchewan Research Council. I enjoy inter-disciplinary research examining a range of environmental issues, and continue to be involved in LCA research in collaboration with U of T and other institutions.

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Alison Waller, *Ph.D., 2009*

Since finishing my PhD in the Edwards lab I have taken up a postdoctoral fellowship at the European Molecular Biology Laboratory (EMBL) in Heidelberg Germany funded through an NSERC PDF scholarship.

I am working in the group of Peer Bork with a focus on bioinformatics. My various projects include: analyzing a metagenome from a sample taken from the arctic ocean in the winter to understand metabolism of a new archaeal species, analyzing metagenomes from the human gut to infer genetic variation, creating a computer program to simulate metagenomes and using it to determine differences between different sequencing methods, using pathway and genome neighbour information to identify candidate sequences for Orphan Enzymes (enzymes currently lacking a cognate sequence). My husband Sasha is also doing his postdoctoral fellowship at EMBL, and we had our third child while we have been here. Although we are enjoying our time here we are excited to return to Canada and I think that my new expertise in bioinformatics will be a great asset to help analyze the systems-level data that is currently being generated.

Nicholas Wood, *M.A.Sc., 2008*

I am currently working at Hatch Ltd. in the renewable power business unit. During my short time here I've been able to research novel energy storage concepts and have spent a lot of time involved in performing due diligence on solar projects being built in Ontario. I've also been involved in the design and energy production modeling of solar systems.

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Divya Yadava, *M.A.Sc., 2008*

After graduating with a Masters in 2008, I ventured into the field of risk consulting. However, after realizing that my true passion is in the area of food, I founded a food blog, which was followed by the launch of a digital quarterly food magazine, Flavour Fiesta. I am currently working on developing a line of healthy snack products and hope to launch mybusiness in the near future.

Work Contact: divya@flavourfiesta.com

Yi Zhang, *Ph.D., 2008*

I joined the Existing Substances Risk Assessment Bureau (ESRAB) of Health Canada in Nov. 2008, as an evaluator to assess risks to human health associated with exposure to existing chemicals in use in Canada. ESRAB plays a key role in the creation and implementation of Canada's Chemicals Management Plan. My husband Jing, our two boys (5-year and 1-year) and I are living happily in Ottawa.

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Publications

The following publications were published during the September 2010 - August 2011 reporting period.

Refereed articles

Babu, M., Beloglazova, N., Flick, R., Graham, C., Skarina, T., Nocek, B., Gagarinova, A., Pogoutse, O., Brown, G., Binkowski, A., Phanse, S., Joachimiak, A., Koonin, E.V., Savchenko, A., Emili, A., Greenblatt, J., Edwards, A.M., and A.F. Yakunin (2010). **A dual function of the CRISPR-Cas system in bacterial antivirus immunity and DNA repair.** *Molecular Microbiology*, 79(2):484-502.

Bester, E., Kroukamp, O., Hausner, M., Edwards, E.A., and G.M. Wolfaardt (2011). **Biofilm form and function: Carbon availability affects biofilm architecture, metabolic activity and planktonic cell yield.** *Journal of Applied Microbiology*, 110(2):387-398.

Chan, P.W.Y., Yakunin, A.F., Edwards, E.A., and E.F. Pai (2011). **Mapping the reaction coordinates of enzymatic defluorination.** *Journal of the American Chemical Society*, 133(19):7461-7468.

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Deshpande, C.N., Harrop, S.J., Boucher, Y., Hassan, K.A., Di Leo, R., Xu, X.H., Cui, H., Savchenko, A., Chang, C.S., Labbate, M., Paulsen, I.T., Stokes, H.W., Curmi, P.M.G., and B.C. Mabbutt (2011). **Crystal structure of an integron gene cassette-associated protein from *Vibrio cholerae* identifies a cationic drug-binding module.** *PLoS ONE*, 6(3):11.

Di Risio, S., Hu, C.S., Saville, B.A., Liao, D., and J. Lortie (2011). **Large-scale, high-solids enzymatic hydrolysis of steam-exploded poplar.** *Biofuels, Bioproducts and Biorefining*, 5(6):609-620.

Eckert, T. , Brunner, B., Edwards, E.A., and U. G. Wortmann. (2011). **Microbially mediated re-oxidation of sulfide during dissimilatory sulfate reduction by *Desulfobacter latus*.** *Geochimica et Cosmochimica Acta,* 75(12):3469-3485.

Eshaghi, A., Patel, S.N., Sarabia, A., Higgins, R.R., Savchenko, A., Stojios, P.J., Li, Y., Bastien, N., Alexander, D.C., Low, D.E., and J.B. Gubbay (2011). **Multidrug-resistant pandemic (H1N1) 2009 infection in immunocompetent child.** *Emerging Infectious Diseases,* 17(8):1472-1474.

Fang, Y., Scheibe, T.D., Mahadevan, R., Garg, S., Long, P.E., and D.R. Lovley (2011). **Direct coupling of a genome-scale microbial *in silico* model and a groundwater reactive transport model.** *Journal of Contaminant Hydrology*, 122(1-4):96-103.

Filippova, E., Chruszcz, M., Cymborowski, M., Gu, J., Savchenko, A., Edwards, A., and W. Minor. **Crystal structure of a putative transcriptional regulator SCO0520 from *Streptomyces coelicolor A3(2)* reveals an unusual dimer among TetR family proteins.** *Journal of Structural and Functional Genomics*, 12(3):149-157.

Foumani, M., Vuong, T.V., and E.R. Master (2011). **Altered substrate specificity of the gluco-oligosaccharide oxidase from *Acremonium strictum*.** *Biotechnology and Bioengineering*, 108(10):2261-2269.

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Irving, T., and D.G. Allen (2011). **Species and material considerations in the formation and development of microalgal biofilms.** *Applied Microbiology and Biotechnology*, 92(2):283-294.

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Li, Y.O., Diosady, L.L., and S. Jankowski (2011). **Folic acid stability in the presence of various formulation components including iron compounds in fortified extruded Ultra Rice® over prolonged storage at 40°c and 60% relative humidity (RH).** *International Journal of Food Science & Technology*, 46(2):379-385.

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MacDonald, J., Doering, M., Canam, T., Gong, Y.C., Guttman, D.S., Campbell, M.M., and E.R. Master (2011). **Transcriptomic responses of the softwood-degrading white-rot fungus *Phanerochaete carnosa* during growth on coniferous and deciduous wood.** *Applied and Environmental Microbiology*, 77(10):3211-3218.

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Osipiuk, J., Xu, X., Cui, H., Savchenko, A., Edwards, A., and Joachimiak, A. (2011). **Crystal structure of secretory protein Hcp3 from *Pseudomonas aeruginosa*.** *Journal of Structural and Functional Genomics*, 12(1):21-26.

Paz, A.C., Javaherian, S., and A.P. McGuigan (2011). **Tools for micropatterning epithelial cells into microcolonies on transwell filter substrates.** *Lab on a Chip*, 11(20):3440-3448.

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Romita, D., Cheng, Y.-L., and L.L. Diosady (2011). **Microencapsulation of ferrous fumarate for the production of salt double fortified with iron and iodine.** *International Journal of Food Engineering*, 7(3):1-14.

Sherwood Lollar, B., Hirschorn, S., Mundle, S.O.C., Grostern, A., Edwards, E.A., and G. Lacrampe-Couloume (2010). **Insights into enzyme kinetics of chloroethane biodegradation using compound specific stable isotopes.** *Environmental Science & Technology*, 44(19):7498-7503.

Stogios, P.J., Shakya, T., Evdokimova, E., Savchenko, A., and G.D. Wright (2011). **Structure and function of APH(4)-Ia, a hygromycin B resistance enzyme**. *Journal of Biological Chemistry*, 286(3):1966-1975.

Syed, M.A., Koyanagi, S., Sharma, E., Jobin, M.-C., Yakunin, A.F., and C.M. Lévesque (2010). **The chromosomal *mazEF* locus of *Streptococcus mutans* encodes a functional Type II toxin-antitoxin addiction system.** *Journal of Bacteriology*, 193(5):1122-1130.

Tchigvintsev, A., Singer, A.U., Flick, R., Petit, P., Brown, G., Evdokimova, E., Savchenko, A., and A.F. Yakunin (2011). **Structure and activity of the *Saccharomyces cerevisiae* dUTP pyrophosphatase DUT1, an essential housekeeping enzyme.** *Biochemical Journal*, 437(2):243-253.

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Yang, L., Cluett, W.R., and R. Mahadevan (2011). **EMILiO: A fast algorithm for genome-scale strain design.** *Metabolic Engineering*, 13(3):272-281.

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Zhao, J., Scheibe, T.D., and R. Mahadevan (2011). **Model-based analysis of the role of biological, hydrological and geochemical factors affecting uranium bioremediation.** *Biotechnology and Bioengineering*, 108(7):1537-1548.

Zhuang, K., Vemuri, G.N., and R. Mahadevan (2011). **Economics of membrane occupancy and respiro-fermentation.** *Molecular Systems Biology*, 7:500.

Book Chapters

Saville, B.A. **Pretreatment Options.** In *Plant Biomass Conversion,* E. E. Hood, P. Nelson and R. Powell (Eds.), pp. 376: Wiley (2011).

Theses

Garcia Becerra, F.Y. (2010). **Production of detergents and adhesives from municipal wastewater activated sludge.** Ph.D., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Botes, N. (2010). **Analyzing the effect of hypoxia on metabolism in the human brain: a mathematical model-based approach.** M.Eng., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Brei, E. (2011). **Bacterial adhesin proteins associated with microbial flocs and EPS in the activated sludge.** Ph.D., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Catalfo, L. (2010). **Detection of soluble metabolites produced by microbial enrichments that anaerobically degrade recalcitrant cellulosic biomass.** M.Eng., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

D`Costa, C. (2010). **Design of a bench-scale photobioreactor for assessment fo the effect of carbon dioxide on growth of *Scenedesmus obliquus*.** M.Eng., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Irving, T.E. (2010). **Factors influencing the formation and development of microalgal biofilms.** M.A.Sc., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Jayasinghe, N. (2011). **Metabolic modeling of spatial heterogeneity of biofilms in microbial fuel cells.** M.A.Sc., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Jung, T. (2011). **Production of biodiesel using mustard seed oil and isopropyl alcohol.** M.A.Sc., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Lacourt, M. (2011). **Enrichment of methanogenic microcosms on recalcitrant lignocellulosic biomass.** M.A.Sc., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Mahajan, S. (2011). **The inaugural analysis of the white-rot fungus, *Phanerochaete carnosa*, through proteomic methods and compositional analysis of decayed wood fibre.** Ph.D., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Palynchuk, K. (2010). **Fortified rice premix formulations for the alleviation of micronutrient deficiencies: Stabilization of Vitamin A in the presence of iron.** M.A.Sc., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Paz Mejia, A.C. (2011). **Generation of planar cell polarity (PCP) *in vitro* for epithelium tissue engineering.** M.Sc., *Dept. of Chemical Engineering and Applied Chemistry* and *Institute of Biomaterials and Biomedical Engineering*, University of Toronto.

Perruzza, A.L. (2010). **Exploring pretreatment methods and enzymatic hydrolysis of oat hulls.** M.A.Sc., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Romita, D. (2011). **Spray drying based technologies for the double fortification of salt with iron and iodine.** M.A.Sc., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Waung, D.W. (2010). **Optimizing enzymatic preparations of mechanical pulp through the characterization of new laccases and non-productive interactions between enzymes and lignin.** M.A.Sc., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Zila, A. (2011). **A molecular study of field bioaugmentation using the KB-1® mixed microbial consortium: The application of real-time PCR in analyzing population dynamics.** M.Eng., *Dept. of Chemical Engineering and Applied Chemistry*, University of Toronto.

Conference presentations and invited talks

Allen, D.G. **Bioprocess engineering to treat wastewater and air emissions and convert biosolids into value-added products.** *SCION*. Rotorua, New Zealand. Feb. 23, 2011

Allen, D.G. **Harnessing biology for treating reduced sulphur and fixing carbon dioxide from air emissions.** *Massey University.* Palmerston North, New Zealand. Feb. 24, 2011

Allen,D.G. **Bioenergy from pulp and paper wastewater treatment.** *APPITA Bioresources Forum*. Rotorua, New Zealand. Apr. 12, 2011

Allen, D.G. **Utilizing biofilm bioreactors to treat reduced sulphur and fix carbon dioxide.** *University of Canterbury*. Christchurch, New Zealand. May 25, 2011

Allen, D.G., Gapes, D., Jack, M., Lei, R. and T. Stuthridge. **Opportunities for environmental technologies for current and future biorefineries.** *SCION*. Rotorua, New Zealand. June 7, 2011

Allen, D.G., Gapes, D., Jack, M., Lei, R. and T. Stuthridge. **Opportunities for environmental technologies for current pulp mills and future biorefineries.** *Appita Environmental Strategy and Discussion Forum*. Norske Skog Tasman Training Centre, Kawerau, New Zealand. June 10, 2011

Allen, D.G., M.I. Yang and E. Edwards. **Challenges and opportunities in the anaerobic conversion of wastewater into energy in the pulp and paper industry.** *Technical Association of the Australian and New Zealand Pulp and Paper Industry (APPITA) Technical Meeting,* Rotorua, New Zealand. Mar. 16, 2011

Anesiadis, N., Cluett, W.R., and R. Mahadevan, **Model-driven design based on sensitivity analysis for a synthetic biology application.** *21st European Symposium on Computer Aided Process Engineering.* Chalikidiki, Greece. May 29 - June 1, 2011

Chan, C.H., Tang, S., Eckert, T., Lacrampe-Couloume, G., Edwards, E.A., and B. Sherwood Lollar. **Compound specific isotope analysis on biotic degradation of chloroform and implication for monitoring anthropogenic-sourced trichlorinated carbon compounds.** *Battelle International Symposium on Bioremediation and Sustainable Technologies.* Reno, NV. June 27-30, 2011

C.E. Devine, Gitia-Froz, R., and E.A. Edwards. **Metabolic pathways, genes and enzymes in anaerobic benzene-degrading dultures: From "omics" to application.** *Battelle International Symposium on Bioremediation and Sustainable Technologies.* Reno, NV. June 27-30, 2011

Diosady, L.L. **Canola protein isolation technology.** International Symposium on Canola Processing. Winnipeg, MB, Sept. 2010

Diosady, L.L. **Industrial uses of castor oil.** *Castor Meeting - OMAFRA.* Guelph, ON. Mar. 21, 2011

Diosady, L.L. **Development of salt double fortified with iodine and iron for the prevention and cure of micronutrient deficiency diseases** (FPE28). *ICEF 11.* Athens, Greece. May 22-26, 2011

Diosady, L.L. **Simultaneous production of food protein isolates and a biodiesel from mustard seed** (FPE49), *ICEF 11.* Athens, Greece. May 22-26, 2011

Diosady, L.L. **The role of chemical engineering in eliminating micronutrient deficiency diseases.** *Institute of Food Technologists Annual Meeting.* New Orleans, LA, USA. June 11-14, 2011

Edwards, E.A. **The benefits of microbial genomics to the environment.** *Ontario Genomics Institute.* Toronto, ON. Mar. 29, 2011

Edwards, E.A. **Metagenomics and chlorinated solvents.** *Environmental Metagenomics – the Ontario Landscape*. Ontario Genomics Institute, Toronto, ON. Apr. 8, 2011

Edwards, E.A. ***Dehalobacter* unveiled: A key player in the detoxification of chlorinated alkanes at contaminated sites.** *Battelle International Symposium on Bioremediation and Sustainable Technologies.* Reno, NV, USA. June 27-30, 2011

Foumani, M., Vuong, T. and E.R. Master. **A biocatalyst for oxidative modification of cello- and xylooligosaccharides.** *Gordon Research Conference (Gordon-Kenan Research Seminar):Cellulosomes, Cellulases & Other Carbohydrate Modifying Enzymes.* Stonehill College, Easton, MA, USA. July 24-29, 2011.

Goacher, R., Jeremic, D., and E.R. Master. **ToF-SIMS and iterative PCA on complex materials: lignin and polysaccharides in wood.** *7th European Workshop on Secondary Ion Mass Spectrometry.* Münster, Germany. Sept. 19-21, 2010

Goacher, R. **Secondary ion mass spectrometry of complex solid materials.** *Indiana University - Purdue University*. Fort Wayne, IN. Feb. 17, 2011

Goacher, R. **Secondary ion mass spectrometry of complex solid materials: wood degradation and diffusion in semiconductors.** *Duquesne University*. Pittsburgh, PA. Mar. 21, 2011

Goacher, R. E., Jeremic, D., and E. Master. **Distinguishing wood biopolymers by ToF-SIMS.** *23rd Annual Workshop on Secondary Ion Mass Spectrometry.* Baltimore, MD, USA. May 16, 2011

Islam, M. A., E. A. Edwards and R. Mahadevan. **Investigating the metabolism of *Dehalococcoides* and associated community members.** Genome Biology and Bioinformatics Work in Progress Seminar Series, Toronto, ON. May 17, 2011.

Kwan, L and L.L.Diosady. **Integrated platform for delivery of nutraceuticals: Triple fortification of salt with vitamin A, iron, and iodine.** *4th Delivery of Functionality in Complex Food Systems International Symposium.* Guelph, ON. Aug. 21-24, 2011

Lacourt, M., and E.R. Master. **Biogas from woody biomass.** *Pulp and Paper Centre Consortium Meeting.* Toronto, ON. Nov. 12, 2010

Li, Y.O. and L.L. Diosady (organizers). **Symposium on the challenges of food fortification for micronutrient deficiencies.** *Institute of Food Technologists Annual Meeting.* New Orleans, LA, USA. June 11-14, 2011

Liang, X., Howlett, M.R., Nelson, J.L., Grant, G., Dworatzek, S., Lacrampe-Couloume, G., Zinder, Z.H., Edwards. E.A., and B. Sherwood Lollar. **Pathway-dependent isotope fractionation during aerobic and anaerobic degradation of monochlorobenzene and 1,2,4-trichlorobenzene.** *241st American Chemical Society National Meeting.* Anaheim, CA. Mar. 27-31, 2011

Luo, F., Devine, C., Gitiafroz, R., Heidorn, C., Gong, Y., and Edwards, E. **Anaerobic benzene biodegradation in enriched cultures.** *13th CSChE Ontario-Québec Biotechnology Meeting,* Kingston, ON, May 12-13, 2011

MacDonald, J., and E.R. Master. **Quantitative analysis of transcripts that encode lignocellulose-active enzymes and are expressed by *Phanerochaete carnosa* at progressive stages of wood decay.** *26th Fungal Genetics Conference*. Asilomar, CA. Mar. 15-20, 2011

Mahadevan, R. **Constraint-based modeling.** *SRI Pathway Tools Workshop.* Palo Alto, CA. Oct. 27, 2010

Mahadevan, R. **Model-based metabolic engineering.** *University of Pittsburgh.* Pittsburgh, PA. Dec. 1, 2010

Mahadevan, R. **Rational bioprocess design.** *Queens University.* Kingston, ON. Jan. 26, 2011

Mahadevan, R. **Integrated modeling of microbial ecology in subsurface environments.** *PNNL Frontiers in Biological Sciences Seminar.* Richland, WA. May 12, 2011 (Received Outstanding Lecturer Award)

Mahadevan, R. **Model-based analysis and design of metabolism for biofuels and biochemicals.** *Workshop on Complexity and Systems Biology of Microbial Biofuels.* Coventry, UK. June 20-24, 2011

Mahadevan, R. **Dynamic genome-scale modeling of microbial communities.** *Institute for Computing in Science (ICiS) Genomics Driving Modeling in Biology.* Park City, UT. July 23-30, 2011

Manchester, M., Zarek, M., Hug, L., Dworatzek, S., Lorah, M., and E. Edwards. **Characterization of dechlorinating populations in the WBC-2 consortium.** *Battelle International Symposium on Bioremediation and Sustainable Technologies.* Reno, NV. June 27-30, 2011

Master, E.R. **Enzymatic valorization of plant fibre.** *Genecor*. Palo Alto, CA. Mar. 1, 2011

Master, E. **Comparative genomics of the closely related white-rot fungi, *Phanerochaete carnosa* and *P. chrysosporium*, to elucidate the genetic basis of the distinct wood types they colonize.** *JGI User Meeting*. Walnut Creek, CA, USA. Mar. 21-24, 2011

Mundle, S.O.C., Johnson, T., Lacrampe-Couloume, G., Duhamel, M., Perez-de-Mora, A., Edwards, E.A., Kluger, R., Sherwood Lollar, B., Tiedeman, C. R., Revesz, K., Imbrigiotta, T. E., and E. Cox. **Using ^{13}C isotope signatures of ethene as a direct indicator to assess the accumulation of toxic daughter products of trichloroethene.** *Battelle International Symposium on Bioremediation and Sustainable Technologies.* Reno, NV. June 27-30, 2011

Pandit, A.V., and R. Mahadevan. **An *in silico* characterization of microbial electrosynthesis for metabolic engineering of biochemcials.** *111th General Meeting American Society for Microbiology.* New Orleans, LA. May 21-24, 2011

Romita, D. and L.L. Diosady. **Production and optimization of engineered iron sources for the addition of iron to sensitive foods.** *CIFST Annual Meeting.* Winnipeg, MB. May 30-June 1, 2010

Sanscartier, D., MacLean, H., and B.A. Saville. **Environmental and economic analysis of industrial scale anaerobic digestion of household source separated organic waste.** *Growing the Margins Conference*. London, ON. Feb. 28 - Mar. 2, 2011

Sanscartier, D. Saville, B. and H.L. MacLean. **Life-cycle approach to the evaluation of the Ontario biogas feed-in-tariff.** *LCA X.* Portland, OR. Nov. 2-4, 2010

Savchenko, A. **Structural insights into the function of effector proteins – bacterial pathogenic factors orchestrating eukaryotic cell biology.** *4th CSGID Annual Meeting*. Northwestern University, Chicago IL, March 29, 2011

Savchenko, A. **Structural insight into the function of effector proteins – bacterial pathogenic factors orchestrating eukaryotic cell biology.** *International Conference on Structural Genomics*. Toronto, Ontario. May 10-14, 2011

Saville, B.A.. **Advantages and disadvantages of various economic incentive frameworks.** *Biofuels Law and Policy Conference.* Ottawa, ON. June 9-10, 2011

Tabtabaei, S. and L.L. Diosady. **Destabilization of the emulsion produced during aqueous extraction of dehulled yellow mustard flour using organic solvents.** *AOCS Annual Meeting.* Cincinnati, OH, USA. May 1-4, 2011

Tsai, A. and E.R. Master. **Constitutive expression of a fungal glucuronoyl esterase in *Arabidopsis* reveals altered cell wall composition.** *Gordon Research Conference (Gordin-Kenan Research Seminar):Cellulosomes, Cellulases& Other Carbohydrate Modifying Enzymes.* Stonehill College, Easton, MA, USA. July 24-29, 2011.

Wei, K., S. Tang and E. A. Edwards. **Biodegradation of chlorofluorocarbon-113 in anaerobic enrichment cultures.** *13th CSChE Ontario-Québec Biotechnology Meeting,* Kingston, ON. May 12-13, 2011

Yakunin, A. **Application of enzymatic assays for experimental annotation of unknown proteins.** *18th International Microbial Genomes Conference*. University of California, Los Angeles, CA. Sept. 12-16, 2010

Yakunin, A. **Functional annotation of unknown proteins using enzymatic assays.** *Institute for Molecular Enzyme Technology.* University of Düsseldorf, Jülich, Germany. Feb. 10, 2011

Yakunin, A. **Structure and nuclease activity of the Cas3 HD domain protein MJ0384.** *4th CRISPR Symposium.* University of California, Berkeley, CA, USA. July 12-13, 2011

Yang, L., Cluett, W.R., and R. Mahadevan. **Efficient redesign of metabolism for biochemicals.** *IBE 16th Annual Conference*. Atlanta, GA. Mar. 3-5, 2011

Yang, L., and R. Mahadevan. **Rational genome-scale design of metabolism.** *111th General Meeting American Society for Microbiology.* New Orleans, LA. May 21-24, 2011

Zhuang, K., Ma, E., and R. Mahadevan. **Dynamic community modelling of uranium bioremediation.** *AIChE Annual Meeting.* Salt Lake City, UT. Nov. 7-12, 2010

Zhuang, K., Vemuri, G., and R. Mahadevan. **Economics of membrane occupancy and the respiro-fermentation.** *AIChE Annual Meeting.* Salt Lake City, UT. Nov. 7-12, 2010

Conference posters

Azimi, Y., Allen, D.G. and R.R. Farnood. **UV disinfection of wastewater effluents: How to control the tailing level, and improve the efficiency.** *46th Central Canadian Symposium on Water Quality Research.* Canada Centre for Inland Waters, Burlington, ON. Feb. 22-23, 2011

Azimi, Y., Allen, D.G. and R.R. Farnood, R.R. **The effect of microbial flocs size and structure on UV disinfection.** *Water Environment Federation Disinfection 2011 Conference.* Cincinnati, OH, USA. Apr. 10-12, 2011

Balderas-Hernandez, V.E., Usher, J., Baetz, K., Gleddie, S., Johnston, A., Harris, L., and R. Mahadevan. **Ethanol production by a xylose fermenting *Saccharomyces cerevisiae* using wheat straw hydrolysate.** *111th General Meeting American Society for Microbiology.* New Orleans, LA. May 21-24, 2011

Beloglazova, N., Petit, P., Flick, R., Brown, G., Skarina, T., Nocek, B., Kudritska, M., Zimmerman, M.D., Binkowski, A., Chruszcz, M., Wang, S., Osipiuk, J., Minor, W., Joachimiak, A., Savchenko, A., and A.F. Yakunin. **Cas1, Cas2, Cas3…: structure and activity of the core CRISPR nucleases.** *International Conference on Structural Genomics*. Toronto, Ontario. May 10-14, 2011

Bourdakos, N., and R. Mahadevan. **Defined consortia for the degradation of complex wastes in microbial fuel cells.** *3rd International Microbial Fuel Cell Conference.* Leeuwarden, Netherlands. June 6-8, 2011

Chandra, S., and R. Mahadevan. Ensemble **Modeling of *S. cerevisiae*.** *111th General Meeting American Society for Microbiology.* New Orleans, LA. May 21-24, 2011

Cox, E., Austrins, C., Spain, J., Shin, K., Nishino, S., Gossett, J., Giddings, C., Johari, W.L.B.W., Edwards, E.A., Perez de Mora, A., Sherwood Lollar, B., and S.O.C. Mundle. **The truth is out there: unraveling the mystery of the missing cDCE, vinyl chloride and ethene.** *SERDP / ESTCP Partners in Environmental Technology Technical Symposium and Workshop.* Washington, DC. Nov. 30 - Dec. 2, 2010

Hayes, A.C., Liss, S.N. and D.G. Allen. **Effect of methanol addition on the performance and microbiology of biofilters treating dimethyl sulfide.** *2010 Duke-UAM Conference on Biofiltration for Air Pollution Control.* Washington, DC. Oct. 28-29, 2010

Ho, H., Devine, C., Beiko, R., Hug, L., Edwards, E., and R. Mahadevan. **A functional analysis of the metagenome of an anaerobic benzene-degrading community.** *13th CSChE Ontario-Québec Biotechnology Meeting,* Kingston, ON, May 12-13, 2011

Hug, L.A., Rowe, A.R., Parks, D., Beiko, R.G., Richardson, R.E., and E.A. Edwards. **Comparative metagenomics of three *Dehalococcoides*-containing dechlorinating microbial consortia.** *American Society for Microbiology General Meeting.* New Orleans, LA. May 21-24, 2011

Hug, L.A., McMurdie II, P.J., Waller, A.S., Holmes, S., Spormann, A., and E.A. Edwards. **Testing an enrichment culture metagenome binning heuristic with a dechlorinating microbial consortium.** *JGI Users Meeting,* Walnut Creek, CA. Mar. 21-24, 2011

Islam, M.A., Edwards, E., and R. Mahadevan. **New insights into *Dehalococcoides* metabolism from an integrated metabolic transcriptomics study.** *Biochemical and Molecular Engineering XVII.* Seattle, WA. June 26-30, 2011

Islam, M. A., Edwards, E., and R. Mahadevan. **A systems-level study of the transcriptional changes of *Dehalococcoides* metabolism.** *Gordon Research Conference on Cellular Systems Biology.* Davidson College, Davidson, NC. July 24-30, 2011

Javaherian, S., and A.P. McGuigan. ***In vitro* model system of gene expression patterning.** *TERMIS-NA Annual Meeting.* Orlando, FL. Dec. 5-8, 2010

Kwan, L and L.L.Diosady. **Microencapsulation of self emulsifying microemulsions.** *13th CSChE Ontario-Quebec Biotechnology Meeting.* Queen's University, Kingston, ON. May 12-13, 2011

Lebron, C., Major ,D., Dennis, P., Loeffler, F.E., Ritalahti, K., Hatt, J.K., Edwards, E.A., Yeager, C., Ogles, D., and C. Acheson. **Standardized procedures for use of nucleic acid-based tools for microbial monitoring.** *SERDP / ESTCP Partners in Environmental Technology Technical Symposium and Workshop.* Washington, DC. Nov. 30 - Dec. 2, 2010

Li, Y.X., Adler, I., Devine, C., and E. Edwards. **Searching for microbes in deep hypersaline anoxic basins.** *13th CSChE Ontario-Québec Biotechnology Meeting,* Kingston, ON, May 12-13, 2011

Liang, X., Devine, C.E., Nelson, J.L., Sherwood Lollar, B., Zinder, Z.H., and E.A. Edwards. **Anaerobic conversion of monochlorobenzene and benzene to CH_4 and CO_2 in bioaugmented microcosms.** *111th American Society for Microbiology General Meeting.* New Orleans, LA. May 21-24, 2011

Löffler F.E., Ritalahti, K., Edwards E.A., and M. Lee. **Comprehensive MBT approaches for site assessment and bioremediation monitoring at chlorinated solvent sites,** *SERDP / ESTCP Partners in Environmental Technology Technical Symposium and Workshop*, Washington, DC, USA. Nov. 30-Dec. 2, 2010

Lücker, P., Javaherian, S., Axelrod, J., and A.P. McGuigan. **Microfabricated culture systems for understanding and controlling coordinated tissue morphogenesis.** *TERMIS-NA Annual Meeting.* Orlando, FL. Dec. 5-8, 2010

MacDonald, J., and E.R. Master. **Quantitative analysis of transcripts that encode lignocellulose-active enzymes and are expressed by *Phanerochaete carnosa* at progressive stages of wood decay.** 26th Fungal Genetics Conference. Asilomar, California, USA. March 15-20, 2011

MacDonald, J., and E.R. Master. **Expression of lignocellulose-active enzymes by Phanerochaete carnosa at progressive stages of wood decay.** *JGI User Meeting*. Walnut Creek, CA, USA. Mar. 21-24, 2011

McMurdie, P.J., Hug, L., Löffler F.E., Edwards, E.A., and A.M. Spormann. **Patterns of horizontal gene transfer in vinyl chloride respiration of *Dehalococcoides* as revealed by comparative (meta)genomics.** *111th American Society for Microbiology General Meeting.* New Orleans, LA. May 21-24, 2011

Mottiar, Y. and E. Master. **Engineering galactose oxidase to enable wood carbohydrate derivatisation.** *Gordon Research Conference: Cellulosomes, Cellulases & Other Carbohydrate Modifying Enzymes.* Stonehill College, Easton, MA. July 24-29, 2011

Namazi, A., Allen, D.G. and C.Q. Jia. **Microwave-assisted pyrolysis of pulp mill sludge for porous carbon productions.** *60th CSChE Conference.* Saskatoon, SA. Oct. 24-27, 2010

Paz, A.C., and A.P. McGuigan. **Generation of planar cell polarity (PCP) *in vitro* for epithelium tissue engineering.** *TERMIS-NA Annual Meeting.* Orlando, FL. Dec. 5-8, 2010

Perruzza, A., and B.A. Saville. **Factors affecting enzymatic hydrolysis of oat hulls.** *10th Agricultural Biotechnology International Conference.* Saskatoon, SK. Sept. 12-15, 2010

Sangakkara, A, Li, Y.O. and L.L. Diosady. **Improving organoleptic properties of simulated rice fortified with micronutrients.** *nstitute of Food Technologists Annual Meeting.* New Orleans, LA, USA. June 11-14, 2011

Savchenko, A. **Collaborative efforts in antibiotic resistance.** *International Conference on Structural Genomics*. Toronto, Ontario. May 10-14, 2011

Suzuki, H. et al. **Comparative genomics of the closely related white-rot fungi, *Phanerochaetecarnosa* and *P. chrysosporium*, to elucidate the genetic basis of the distinct wood types they colonize.** *Gordon Research Conference: Cellulosomes, Cellulases & Other Carbohydrate Modifying Enzymes.* Stonehill College, Easton, MA, USA. July 24-29, 2011

Tang, S. and E. Edwards. **Identification of *Dehalobacter* reductases that dechlorinate 1,1,1-trichloroethane, chloroform and 1,1-dichloroethane**. *American Society for Microbiology General Meeting*. New Orleans, LA, USA. May 21, 2011

Tran, C., Edwards, E.A., Yakunin, A., Chan, P., and J. Dinglasan-Panlilio. **Isolation of a putative and characterization of known hydrolytic dehalogenases.** *13th CSChE Ontario-Québec Biotechnology Meeting*, Kingston, ON. May 12-13, 2011

Tsai, A., Canam, T., Mellerowicz, E.J., Campbell, M.M., and E.R. Master. **Constitutive expression of a fungal glucuronoyl esterase in *Arabidopsis* reveals altered cell wall composition.** *Gordon Research Conference: Cellulosomes, Cellulases & Other Carbohydrate Modifying Enzymes.* Stonehill College, Easton, MA. July 24-29, 2011

Vuong, T. **A biocatalyst for oxidative modification of cello- and xylooligosaccharides.** *Gordon Research Conference on Cellular Systems Biology*. Davidson, NC. July 24-29, 2011

Yang, L., Cluett, W.R., and R. Mahadevan. **Genome-scale robust strain design.** *Biochemical and Molecular Engineering XVII.* Seattle, WA. June 26-30, 2011

Yang, M.I., Edwards, E.A., and D.G. Allen. **Granulation in the anaerobic treatment of pulp mill effluents.** *The 12th World Congress on Anaerobic Digestion.* Guadalajara, Mexico.

Zhao, J., Zhuang, K., Ma, E., Richter, H., Barlett, M., Fang, Y., Mahadevan, R., Scheibe, T., and D. Lovley. **Detailed modeling of subsurface microbes and interaction between microbial and hydrogeochemical process for sustained uranium removal.** *US DoE-SBR 6th Annual PI Meeting.* Washington, DC. Apr. 26-28, 2011

Zhuang, K., Barlett, M., Ma, E., Lovley, D., and R. Mahadevan. **Modeling community metabolism during uranium bioremediation.** *US DoE-SBR 6th Annual PI Meeting.* Washington, DC. Apr. 26-28, 2011

Zhuang, K., Ma, E., and R. Mahadevan. **Dynamic community modelling of uranium bioremediation.** *111th General Meeting American Society for Microbiology.* New Orleans, LA. May 21-24, 2011

Zhuang, K., Vemuri, G., and R. Mahadevan. **Economics of membrane occupancy and the respiro-fermentation.** *111th General Meeting American Society for Microbiology.* New Orleans, LA. May 21-24, 2011

Awards, Grants & Scholarships

During the 2010–2011 year, BioZone researchers and students were recognized for excellence in research, teaching and communication with scholarships, awards and professional accolades. Several of our professors also received prestigious grants from Canadian and international funders in support of their innovative research programs.

Awards

International

Fellow
American Association for the Advancement of Science
Elizabeth Edwards

Jay Bailey Young Investigator Best Paper Award
Metabolic Engineering Journal
Radhakrishnan Mahadevan

Outstanding Lecturer Award
Pacific Northwest National Laboratory
Radhakrishnan Mahadevan

Project of the Year (Environmental Restoration)
Strategic Environmental Research Development Project
Elizabeth Edwards, Winnie Chan, Melanie Duhamel, Alfredo Perez de Mora (co-performers)

Travel Grant
American Society for Microbiology

Canadian

13th CSChE Ontario-Quebec Biotechnology Meeting Oral Presentation Awards
Canadian Society for Chemical Engineering
(2nd Place and 2 Honourable Mentions)

13th CSChE Ontario-Quebec Biotechnology Meeting Poster Awards
Canadian Society for Chemical Engineering
(2nd Place and 2 Honourable Mentions)

Bill Stolte Silver Student Award
Canadian Water Resources Assocation

Fellow
Canadian Academy of Engineering
Elizabeth Edwards

Class of 1T2 Best Teaching Assistant Award
University of Toronto Dept. of Chemical Engineering and Applied Chemistry

Conference Grant (3)
University of Toronto School of Graduate Studies

Fellow
Engineering Institute of Canada
Grant Allen

Engineering Medal for Research and Development
Professional Engineers of Ontario
Elizabeth Edwards

Student Life Catalyst Award
University of Toronto Dept. of Chemical Engineering and Applied Chemistry

Undergraduate Engineering Research Day Presentation Awards
University of Toronto Faculty of Applied Science and Engineering
(Top Presenter and 2 Runners-Up)

Grants

The following new grants were announced during the 2010 - 2011 reporting period.

International

A toilet that uses mechanical dehydration and smoldering of feces to recover resources and energy
Bill and Melinda Gates Foundation: Reinvent the Toilet Challenge
PI: Yu-Ling Cheng, Centre for Global Engineering
Co-investigators: Levente Diosady, Elizabeth Edwards and others

Enzymatic modification of plant fibres
Tekes Finnish Funding Agency for Technology and Innovation: Finland Distinguished Professor Programme
Emma Master

Canadian

Advanced Tissue Engineering Strategies for Generating Replacement Tracheal Tissues
Natural Sciences and Engineering Research Council of Canada & Canadian Institutes of Health Research: Collaborative Health Research Projects
Alison McGuigan

Bioreaction engineering to maximize mixed microbial systems that convert wastes into value added products
Natural Sciences and Engineering Research Council of Canada: Discovery Grant
Grant Allen

Forest FAB: Applied Genomics for Functionalized Fibre And Biochemicals
Ontario Ministry of Economic Development and Innovation: Ontario Research Fund - Research Excellence Award
Emma Master

High throughput culturing system for evolutionary engineering
Natural Sciences and Engineering Research Council of Canada: Research Tools and Instruments Grant
Radhakrishnan Mahadevan

Multi-scale genome-based modeling of human metabolism for diagnostic and therapeutic applications
Natural Sciences and Engineering Research Council of Canada: Discovery Grant with Accelerator Supplement
Radhakrishnan Mahadevan

Quartz crystal microbalance with dissipation for characterization of new biomaterials from underutilized biomass and activated sludge
Natural Sciences and Engineering Research Council of Canada: Research Tools and Instruments Grant
Emma Master

Rational design of microbial strains for novel biochemical products
Natural Sciences and Engineering Research Council of Canada: Strategic Grant
Radhakrishnan Mahadevan

Reagent Development and Assessment for Biomass Conversion
Natural Sciences and Engineering Research Council of Canada: Strategic Grant
Emma Master

Tool Kit for Quantifying Gene Expression
Natural Sciences and Engineering Research Council of Canada: Research Tools and Instruments Grant
Alison McGuigan

Scholarships

The following scholarships were announced during the 2010 - 2011 reporting period.

Canada Graduate Scholarship (2)
Natural Sciences and Engineering Research Council of Canada

Canada Graduate Scholarship - Michael Smith Foreign Study Supplement
Natural Sciences and Engineering Research Council of Canada

Consejo Nacional de Ciencia y Tecnología (Mexico) Scholarship

Doctoral Completion Award
University of Toronto School of Graduate Studies

Frances Bradfield Graduate Fellowship in Environmental Engineering
University of Toronto Dept. of Chemical Engineering and Applied Chemistry

Graduate Student Endowment Fund Scholarship
University of Toronto

IMS Entrance Award
University of Toronto Institute of Medical Science

Marie Curie Postdoctoral Fellowship
European Commission

Mitacs Elevate Postdoctoral Fellowship

Postgraduate Scholarship
Natural Sciences and Engineering Research Council of Canada

Ontario Graduate Scholarship (2)
Ontario Ministry of Training, Colleges and Universities

Ontario Graduate Scholarship in Science & Technology (5)
Ontario Ministry of Training, Colleges and Universities

Undergraduate Summer Research Award
Natural Sciences and Engineering Research Council of Canada

W. H. Rapson Memorial Award
University of Toronto Dept. of Chemical Engineering and Applied Chemistry

Yoshio Masui Prize in Developmental, Molecular or Cellular Biology
University of Toronto Dept. of Cell and Systems Biology

Events

BioZone students, personnel and PIs organize and participate in a large variety of research-related events as well as social outings. In addition to regular meetings among individual research groups, the events listed below reach across lab boundaries to foster collaboration.

Research & Training

BioZone Big Thinking

To encourage thinking "outside the box", students in BioZone organized a bi-monthly get-together focused around discussion topics of their choosing. Topics have included biofuels and the ethics of creating synthetic life.

In July 2011, a special BEEM Big Thinking event brought together over 90 researchers, students and staff for presentations by PIs to highlight their research interests, followed by a social reception. Participants had the chance to learn about their colleagues' research in an informal, social setting.

BEEM Theme Meetings

BEEM Theme is a monthly series for researchers involved in one of BioZone's biggest projects, *BEEM: Bioproducts and Enzymes from Environmental Metagenomes*. The series features research seminars and open discussions to share the latest results between members of this very interdisciplinary team and to bring questions for discussion.

Seminars presented in 2010-2011 were:

- Ana Popovic, "Screening of environmental metagenomesfor enzyme activities"
- Dragica Jeremic, "Investiation of degradation of lignocellulosic feedstock by *Postia placenta*"
- Mike Lacourt, "Biogas from woody biomass"
- Pierre Petit, "Macromolecular crystallography: A tool for investigating drug and enzyme interactions"
- Robyn Goacher, "Time-of-Flight Secondary Ion Mass Spectrometry (ToF-SIMS)"; Rob Flick, "LC/MS Facilitated Screening of Metabolites"
- Nisa Dar, "Bioinformatics"
- Laura Hug, "Comparative metagenomics of three dechlorinating microbial consortia"
- Tim Shen, "Life cycle assessment"
- Dragica Jeremic, "Research beyond the lab"
- Shuiquan Tang, "Identification of *Dehalobacter* reductases that dechlorinate 1,1,1-TCA, CF and 1,1-DCA"
- Christina Heidorn, "Bioproducts for sustainability: What BEEM can do to raise public support"

1st Annual BEEM Research Meeting

On Nov 18 and 19, 2010, BioZone hosted the 1st Annual BEEM Research Meeting. This event allowed researchers involved in this large, multi-disciplinary project to share recent results and discuss future directions.

Over 70 researchers, students, collaborators and partners from various institutions attended two days of research presentations and lively discussion, as well as a student poster session and dinner. Also in attendance were the members of the BEEM Science Advisory Board, who met with the project's principal investigators to review progress to date.

The meeting's full program can be found on the BEEM website at http://www.beem.utoronto.ca/news/1st-annual-beem-research-meeting-great-success.

Tembec Tour

In May 2011, eight of BioZone's students, postdoctoral fellows and professors travelled to Temiscaming, Quebec, to visit one of our long-time industrial supporters, Tembec Inc. The visit included a guided tour of the pulp and paper mill with special attention paid to the 30 metre high internal circulation anaerobic digesters and the associated biogas production. Prior to the tour, Profs. Emma Master and Elizabeth Edwards gave short presentations about our research at an introductory meeting in Temiscaming. Tembec and the University of Toronto are associated by a long-lasting collaboration.

Outreach

Sanofi-Aventis BioTalent Challenge (SABC)

The SABC is an international high school science competition for projects in biotechnology. Prof. Emma Master was a speaker at this year's regional competition in April 2011 in Toronto and spoke to about 250 high school students and their teachers about "Mighty microbes: Turning trash into cash".

DEEP Summer Academy

BioZone was involved in this summer program for high school students in two ways. Ph.D. student Cheryl Devine hosted a group of grade 9/10 students for a lab demomstration about bioremediation during the DEEP Summer Academy course "World of Water".

BioZone also hosted a "Lunch & Learn" session during which Master's students Michael Lacourt and Anna Zila gave a 30 minute presentation about bioengineering with a focus on bioconversion and bioremediation. The session was attended by about 120 high school students.

Student Volunteers

Master's student Anna Zila spoke with high school girls in March, 2011, about being a woman in engineering at the Youth in Motion Career Learning Day for Young Women in Grades 8-12, held in Toronto.

Graduate students Laura Hug and Peter Schnurr served as volunteer judges at the regional Toronto competition of Canada Wide Science Fair.

Postdoctoral fellow Robyn Goacher served as a volunteer judge at the Toronto regional competition of the Sanofi-Aventis BioTalent Challenge.

Spring Reunion

The FASE's annual Spring Reunion brings alumni back to campus to revisit their alma mater. Graduate students Mike Lacourt, Fei Luo, Victor Balderas, Ana Paz, and Director Elizabeth Edwards and Communications Manager Christina Heidorn lead tours of lab facilities, highlighting research into bioremediation and biotransformation for about 20 alumni.

Guinness World Record

In May 2011, members of Prof. Levente Diosady's food engineering research group helped Dairy Queen set a new Guinness World Record for the world's largest ice cream cake in Toronto. Weighing in at a hefty 10,130.35 kg, the cake was assembled

using forklifts and smashed the previous record of 8,000 kg. Students from Prof. Diosady's group had provided their professional expertise in the lead-up to the event to devise the plan for constructing this monster ice cream snack.

March Break Open House

The Faculty of Applied Science and Engineering's (FASE) annual March Break Open House provides prospective undergraduate students an opportunity to visit our labs and interact with students to learn about the exciting career and research opportunities in engineering. Graduate students Patrick Hyland, Nikolaos Anesiadis, Julie-Anne Gandier, Alan Lam, Camila Londono, Jine Jine Li, Anna Zila, Christopher Tran, along with Director Elizabeth Edward and Communications Manager Christina Heidorn led tours of BioZone during the events for approximately 100 high school students, to showcase our bioprocess engineering, tissue engineering and bioremediation labs.

Convocation Tours

Tours of BioZone were hosted as part of the Dept. of Chemical Engineering and Applied Chemistry's pre-convocation tours. Graduate student Pratish Gawand led groups of 15-30 graduates and their families through our facilities.

Genomics in the Park

Emma Master and Elizabeth Edwards took part in this event at Ontario's provincial legislature in Toronto in Nov. 2010, with the goal of providing Ontario MPPs and Ministry staff an opportunity to learn about genomics research. Over 100 government representatives attended.

Guest Speakers

BioZone PIs hosted a number of guest speakers throughout the year, many as part of the Lectures at the Leading Edge seminar series:

Meanachem Elimelech, Yale University (Edwards)
Jim Liao, University of California, Los Angeles (Mahadevan)
Yi Tang, University of California, Los Angeles (Mahadevan)
Dave Mooney, Harvard University (McGuigan)
Howard Stone, Princeton University (McGuigan)
Kun Lin Yang, National University of Singapore (Edwards)

Social & Fundraising

Tea Time

Every Tuesday at 3 pm, interested students, postdocs, staff and professors gather over pots of tea and plates of cookies at this popular event that fosters friendship, collaboration and discussion among BioZone's many researchers and labs. Birthdays are celebrated with cake!

BioZone Winter Camping Trip

In February 2011, a dozen BioZone members braved the elements for the first annual winter camping trip, this time at MacGregor Point Provincial Park.

Holiday Party

The BioZone holiday party was organized by students and post-docs and included a potluck with international flair due to the diversity of people in BioZone.

BEEM Summer Social

In June 2011, BioZone researchers and PIs who are part of the BEEM project held a summer social at the Bedford Academy restaurant.

BioZone Summer Camping Trip

Organized by students, the second annual summer camping took place at Selkirk Provincial Park and Point Pelee National Park. Over 20 BioZone members and their families enjoyed a fun-filled weekend of tents, beach and campfire cooking.

Fundraising for Important Causes

BioZone members are a passionate bunch and frequently step up to the plate to support causes that have a personal significance.

- Students organized a bake sale and raised over $1300 for disaster relief in Japan after the earthquake and tsunami of March 2011, which raised over $1300.
- In what has become a yearly tradition, BioZone once again fielded a team to participate in the World Wildlife Fund's CN Tower Climb to raise funds for environmental protection. The team raised over $1950.

We wish to thank our industry and government partners for their support.

UNIVERSITY OF TORONTO
FACULTY OF APPLIED SCIENCE & ENGINEERING

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