

**THE UNIVERSITY OF WESTERN ONTARIO
BIOLOGICAL AGENTS REGISTRY FORM**
Approved Biohazards Subcommittee: February 18, 2011
Biosafety Website: www.uwo.ca/humanresources/biosafety/

This form must be completed by each Principal Investigator holding a grant administered by the University of Western Ontario (UWO) or in charge of a laboratory/facility where the use of Level 1, 2 or 3 biological agents is described in the laboratory or animal work proposed. The form must also be completed if any work is proposed involving animals carrying zoonotic agents infectious to humans or involving plants, fungi, or insects that require Public Health Agency of Canada (PHAC) or Canadian Food Inspection Agency (CFIA) permits.

This form must be updated at least every 3 years or when there are changes to the biological agents being used.

Containment Levels will be established in accordance with Laboratory Biosafety Guidelines, 3rd edition, 2004, Public Health Agency of Canada (PHAC) or Containment Standards for Veterinary Facilities, 1st edition 1996, Canadian Food Inspection Agency (CFIA).

Completed forms are to be returned to Occupational Health and Safety, (OHS), (Support Services Building, Room 4190) for distribution to the Biohazards Subcommittee. For questions regarding this form, please contact the Biosafety Officer at extension 81135 or biosafety@uwo.ca. If there are changes to the information on this form (excluding grant title and funding agencies), contact Occupational Health and Safety for a modification form. See website: www.uwo.ca/humanresources/biosafety/

PRINCIPAL INVESTIGATOR	<u>Dr. Argyrios Margaritis</u>
DEPARTMENT	<u>Chemical and Biochemical Engineering</u>
ADDRESS	<u>TEB 377</u>
PHONE NUMBER	<u>519-661-2146</u>
EMERGENCY PHONE NUMBER(S)	<u>" "</u>
EMAIL	<u>amarg@uwo.ca</u>

Location of experimental work to be carried out: Building(s) SEB, TEB Room(s) 2035, 2033 & 313

*For work being performed at Institutions affiliated with the University of Western Ontario, the Safety Officer for the Institution where experiments will take place must sign the form prior to its being sent to the University of Western Ontario Biosafety Officer (See Section 15.0, Approvals).

FUNDING AGENCY/AGENCIES: NSERC
GRANT TITLE(S): NOVEL BIOPROCESSES FOR THE PRODUCTION OF BIOPOLYMERS AND BIOFUELS

UNDERGRADUATE COURSE CODE (IF APPLICABLE): _____

List all personnel working under Principal Investigators supervision in this location:

<u>Name</u>	<u>UWO E-mail Address</u>	<u>Date of Biosafety Training</u>
<u>Peter Kilonzo</u>	<u>pkilonz2@uwo.ca</u>	

Incomplete training?

Please explain the biological agents and/or biohazardous substances used and how they will be stored, used and disposed of. Projects without this description will not be reviewed.

Solventogenic Clostridia (e.g., *C. acetobutylicum* and *C. beijerinckii*) are received from freeze-dried vials obtained from ATCC then grown in shake flasks after steam sterilization of liquid medium. All preparation work is done within the laminar sterile hood Biosafety cabinet level 1. After use, the petri-dishes that have the remaining Clostridia cells are autoclaved and then stored in special yellow bags for disposal. Prior to disposal, spent liquid media are autoclave at 121°C and 1 atm for 60 min to destroy all cells and spores present.

Please include a one page research summary or teaching protocol.

Abstract:

Clostridium acetobutylicum ATCC 824 was grown on a variety of different sugars [D-(+)-glucose, D-(+)-xylose, D-(+)-mannose, D-(+)-galactose, D-(-)-arabinose, and D-(+)-cellobiose] found in agricultural waste hydrolysates and assayed for acetone, butanol, and ethanol (ABE) production. The order of sugar utilization by the culture was D-(+)-glucose > D-(+)-cellobiose > D-(+)-mannose > D-(+)-xylose > D-(-)-arabinose > D-(+)-galactose. The high D-(+)-glucose utilization of 98% resulted to higher cell density of 2.49 g/L with the highest growth rate of 0.293 h⁻¹, than other sugars. In this system, ABE concentration of 18.3 g/L, was triggered by a total acid concentration of 11.5 g/L, but growth cessation took place at a total butanol and acid concentration of 24.1 g/L. Although the culture utilized 92% sugar from D-(+)-cellobiose and 86% from D-(+)-xylose, the resultant biomass concentration (1.53 and 1.82 g/L) was very low. In these system, a total acid concentration between 14 and 15 g/L triggered 19.0 and 13.9 g/L ABE g/L from D-(+)-cellbiose and D-(+)-xylose, respectively. Relatively high yield (0.33 g/g), productivity (0.47 g/L.h), and low growth rate of 0.069 h⁻¹ resulted from the D-(+)-cellobiose system. In both systems, growth cessation occurred at a total butanol and acid concentration between 25 and 27 g/L. Culture grown on mixed sugars utilized 70-90% total sugars. A total acid concentration between 23 and 28 g/L triggered only 13-16 g/L ABE, with relatively high yield and productivity of 0.37 and 0.43 g/L.h, whereas growth inhibition occurred at a total butanol and acid concentration between 30 and 39 g/L.

Keywords: solvents, acetone-butanol-ethanol, butanol, acids, individual sugars, mixed sugars, C.

acetobutylicum ATCC 824

1.0 Microorganisms

1.1 Does your work involve the use of biological agents? YES • NO
 (non-pathogenic and pathogenic biological agents including but not limited to bacteria and other microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)? If no, please proceed to Section 2.0

Do you use microorganisms that require a permit from the CFIA? YES NO
 If YES, please give the name of the species. _____

What is the origin of the microorganism(s)? _____

Please describe the risk (if any) of escape and how this will be mitigated:

Please attach the CFIA permit.

Please describe any CFIA permit conditions:

N/A

See E-mail

1.2 Please complete the table below:

Name of Biological Agent(s)* (Be specific)	Is it known to be a human pathogen? YES/NO	Is it known to be an animal pathogen? YES/NO	Is it known to be a zoonotic agent? YES/NO	Maximum quantity to be cultured at one time? (in Litres)	Source/ Supplier	PHAC or CFIA Containment Level
BACTERIA	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	<input type="radio"/> Yes <input checked="" type="radio"/> No	210mL	ATCC	<input checked="" type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3

2.3 Please indicate the type of established cells that will be grown in culture in:

Cell Type	Is this cell type used in your work?	Specific cell line(s)*	Containment Level of each cell line	Supplier / Source of cell line(s)
Human	<input type="radio"/> Yes <input type="radio"/> No			
Rodent	<input type="radio"/> Yes <input type="radio"/> No			
Non-human primate	<input type="radio"/> Yes <input type="radio"/> No			
Microorganism	<input type="radio"/> Yes <input type="radio"/> No	Bacteria	1	ATCC

*Please attach a Material Safety Data Sheet or equivalent from the supplier. (For more information, see www.atcc.org)

2.4 For above named cell types(s) indicate PHAC or CFIA containment level required • 1 2 2+ 3

3.0 Use of Human Source Materials

3.1 Does your work involve the use of human source materials? YES NO

If no, please proceed to Section 4.0

3.2 Indicate in the table below the Human Source Material to be used.

Human Source Material	Source/Supplier /Company Name	Is Human Source Material Infected With An Infectious Agent? YES/UNKNOWN	Name of Infectious Agent (If applicable)	PHAC or CFIA Containment Level (Select one)
Human Blood (whole) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Blood (fraction) or other Body Fluid		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (unpreserved)		<input type="radio"/> Yes <input type="radio"/> Unknown		<input type="radio"/> 1 <input type="radio"/> 2 <input type="radio"/> 2+ <input type="radio"/> 3
Human Organs or Tissues (preserved)		Not Applicable		Not Applicable

4.0 Genetically Modified Organisms and Cell lines

4.1 Will genetic modifications be made to the microorganisms, biological agents, or cells described in Sections 1.0 and 2.0? YES NO If no, please proceed to Section 5.0

4.2 Will genetic modification(s) involving plasmids be done? YES, complete table below NO

Bacteria Used for Cloning *	Plasmid(s) **	Source of Plasmid	Gene Transfected	Describe the change that results from transformation or tranfection

* Please attach a Material Data Sheet or equivalent if available.

** Please attach a plasmid map.

4.3 Will genetic modification(s) of bacteria and/or cells involving viral vectors be made?

YES, complete table below NO

Virus Used for Vector Construction	Vector(s) *	Source of Vector	Gene(s) Transduced	Describe the change that results from transduction

* Please attach a Material Safety Data Sheet or equivalent.

4.4 Will genetic sequences from the following be involved?

- ◆ HIV YES, please specify _____ NO
- ◆ HTLV 1 or 2 or genes from any Level 1 or Level 2 pathogens YES, specify _____ NO
- ◆ SV 40 Large T antigen YES NO
- ◆ E1A oncogene YES NO
- ◆ Known oncogenes YES, please specify _____ NO
- ◆ Other human or animal pathogen and or their toxins YES, please specify _____ NO

4.5 Will virus be replication defective? YES NO

4.6 Will virus be infectious to humans or animals? YES NO

4.7 Will this be expected to increase the containment level required? YES NO

5.0 Human Gene Therapy Trials

5.1 Will human clinical trials be conducted involving a biological agent? YES NO
(including but not limited to microorganisms, viruses, prions, parasites or pathogens of plant or animal origin)
If no, please proceed to Section 6.0

5.2 If YES, please specify which biological agent will be used: _____
Please attach a full description of the biological agent.

5.2 Will the biological agent be able to replicate in the host? YES NO

5.3 How will the biological agent be administered? _____

5.4 Please give the Health Care Facility where the clinical trial will be conducted: _____

5.5 Has human ethics approval been obtained? YES, number: _____ NO PENDING

6.0 Animal Experiments

6.1 Will live animals be used? YES NO If no, please proceed to section 7.0

6.2 Name of animal species to be used _____

6.3 AUS protocol # _____

6.4 Will any of the agents listed in section 4.0 be used in live animals YES, specify: _____ NO

6.5 Will the agent(s) be shed by the animal: YES NO, please justify:

7.0 Use of Animal species with Zoonotic Hazards

7.1 Will any animals with zoonotic hazards or their organs, tissues, lavages or other body fluids including blood be used (see list below)? YES No If no, please proceed to section 8.0

7.2 Will live animals be used? YES No

7.3 If yes, please specify the animal(s) used:

- ◆ Pound source dogs YES NO
- ◆ Pound source cats YES NO
- ◆ Cattle, sheep or goats YES, please specify species _____ NO
- ◆ Non-human primates YES, please specify species _____ NO
- ◆ Wild caught animals YES, please specify species & colony # _____ NO
- ◆ Birds YES, please specify species _____ NO
- ◆ Others (wild or domestic) YES, please specify _____ NO

7.4 If no live animals are used, please specify the source of the specimens:

8.0 Biological Toxins and Hormones

8.1 Will toxins or hormones of biological origin be used? YES NO If no, please proceed to Section 9.0

8.2 If YES, please name the toxin(s) or hormones(s) _____
Please attach information, such as a Material Safety Data Sheet, for the toxin(s) used.

8.3 What is the LD₅₀ (specify species) of the toxin or hormone _____

8.4 How much of the toxin or hormone is handled at one time*? _____

8.5 How much of the toxin or hormone is stored*? _____

8.6 Will any biological toxins or hormones be used in live animals? YES, Please provide details: _____ NO

*For information on biosecurity requirements, please see:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/Biosecurity_Requirements.pdf

9.0 Insects

9.1 Do you use insects? YES NO If no, please proceed to Section 10.0

9.2 If YES, please give the name of the species. _____

9.3 What is the origin of the insect? _____

9.4 What is the life stage of the insect? _____

9.5 What is your intention? Initiate and maintain colony, give location: _____
 "One-time" use, give location: _____

9.6 Please describe the risk (if any) of escape and how this will be mitigated:

9.7 Do you use insects that require a permit from the CFIA permit? YES NO
If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

10.0 Plants

10.1 Do you use plants? YES NO If no, please proceed to Section 11.0

10.2 If YES, please give the name of the species. _____

10.3 What is the origin of the plant? _____

10.4 What is the form of the plant (seed, seedling, plant, tree...)? _____

10.5 What is your intention? Grow and maintain a crop "One-time" use

10.6 Do you do any modifications to the plant? YES NO

If yes, please describe: _____

10.7 Please describe the risk (if any) of loss of the material from the lab and how this will be mitigated:

10.8 Is the CFIA permit attached? YES NO

If YES, Please attach the CFIA permit & describe any CFIA permit conditions:

11.0 Import Requirements

11.1 Will any of the above agents be imported? YES, please give country of origin _____ NO
If no, please proceed to Section 12.0

11.2 Has an Import Permit been obtained from HC for human pathogens? YES NO

11.3 Has an import permit been obtained from CFIA for animal or plant pathogens? YES NO

11.4 Has the import permit been sent to OHS? YES, please provide permit # _____ NO

12.0 Training Requirements for Personnel Named on Form

All personnel named on the above form who will be using any of the above named agents are required to attend the following training courses given by OHS:

- ◆ Biosafety
- ◆ Laboratory and Environmental/Waste Management Safety
- ◆ WHMIS (Western or equivalent)
- ◆ Employee Health and Safety Orientation

As the Principal Investigator, I have ensured that all of the personnel named on the form who will be using any of the biological agents in Sections 1.0 to 9.0 have been trained.

SIGNATURE *D. J. Smith, MSc 29/2015*

13.0 Containment Levels

13.1 For the work described in sections 1.0 to 9.0, please indicate the highest HC or CFIA Containment Level required. 1 2 2+ 3

13.2 Has the facility been certified by OHS for this level of containment?
 YES, date of most recent biosafety inspection: MARCH 01/2011
 NO, please certify
 NOT REQUIRED for Level 1 containment

13.3 Please indicate permit number (not applicable for first time applicants): _____

14.0 Procedures to be Followed

14.1 Please describe additional risk reduction measures will be taken beyond containment level 1, 2, 2+ or 3 measures, that are unique to this agent.
NO ADDITIONAL RISK REDUCTION REQUIRED

14.2 Please outline what will be done if there is an exposure to the biological agents listed, such as a needlestick injury or an accidental splash:
NO CONCERN FOR THE BIOLOGICAL AGENT LISTED

14.3 As the Principal Investigator, I will ensure that this project will follow the UWO Procedures Manual for Containment Level 1 & 2 Laboratories (see Appendix 3 projects). I will ensure that UWO faculty, staff and students are trained in the Hazard Communication Form, found at <http://www.wph.uwo.ca>.



SIGNATURE *[Signature]* Date: March 29/2011

15.0 Approvals

1) UWO Biohazards Subcommittee: SIGNATURE: _____
Date: _____

2) Safety Officer for the University of Western Ontario
SIGNATURE: _____
Date: _____

3) Safety Officer for Institution where experiments will take place (if not UWO):
SIGNATURE: _____
Date: _____

Approval Number: _____ Expiry Date (3 years from Approval): _____

Special Conditions of Approval:

Bacteria

ATCC® Number: **824™** [Order this Item](#) Price: **\$205.00**

Organism: *Clostridium acetobutylicum* McCoy et al. emend. Keis et al. deposited as *Granulobacter pectinovorum* (Stormer) Beijerinck

Designations: [CCRC 10639, CCUG 42182, DSM 792, IAM 19013, IFO 13948, JCM 1419, KCTC 1790, L.S. McClung 2291, LMG 5710, McCoy and McClung strain W, NCCB 29024, NCCB 84048, NCIMB 8052, VKM B-1787]

Isolation: plant-derived foodstuff (corn meal)

Depositor: ER Weyer

Biosafety Level: 1

Shipped: freeze-dried

Growth Conditions: ATCC medium 2107: Reinforced clostridial broth (modified)

Temperature: 37.0°C

Atmosphere: Anaerobic

Permits/Forms: In addition to the MTA mentioned above, other ATCC and/or regulatory permits may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please click here for information regarding the specific requirements for shipment to your location.

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Bacteria

ATCC® Number: **858™** [Order this Item](#) Price: **\$255.00**

Organism: *Clostridium beijerinckii* Donker emend. Keis et al.
 Designations: LMD 25.10 [NCIB 11373, NCTC 2264, VPI 11896]
 Isolation: pasteurized garden soil
 Depositor: J van der Toorn
 History: ATCC <<--J van der Toorn<<--H.J.L. Donker 7 (<<--- A.J. Kluver <<--- H.J.L. Donker)
Biosafety Level: 1
 Shipped: freeze-dried
 Growth Conditions: ATCC medium38: Beef liver medium for anaerobes
Temperature: 37.0°C
 Duration: anaerobic
 Permits/Forms: In addition to the [MTA](#) mentioned above, other [ATCC and/or regulatory permits](#) may be required for the transfer of this ATCC material. Anyone purchasing ATCC material is ultimately responsible for obtaining the permits. Please [click here](#) for information regarding the specific requirements for shipment to your location.
 References: 5543: Donker HJLBijdrage tot de Kennis der Botersuur-, butylacohlen acetonigistingen Ph.D. thesis, Delft Univ. Technol., 1926

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Subject: Re: Biological Agents Registry Form: Margaritis
From: "Dr. Argyrios Margaritis" <amarg@uwo.ca>
Date: Fri, 01 Apr 2011 16:25:26 -0400
To: Jennifer Stanley <jstanle2@uwo.ca>

Hi Jennifer,

1. There are no others.
2. Thanks for the information.



Sorry I missed the previous Biosafety Committee meeting. When is the next meeting?

Cheers!

Argyrios(Gerry).

A. Margaritis, B.A.Sc., M.S., Ph.D., P.Eng., F.C.I.C., Order Hon. PEO, F.E.C.,
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----- Original Message -----

From: [Jennifer Stanley](#)
To: [Argyrios Margaritis](#)
Sent: Friday, April 01, 2011 4:18 PM
Subject: Biological Agents Registry Form: Margaritis

Hi Dr. Margaritis -

Thank you for your updated form - I received it today.

I have a few questions:

1. Under Table 1.2, for "bacteria" I know that you use *Clostridium acetobutylicum* (ATCC 824) and *Clostridium beijerinckii* (ATCC 858) - are there any others?

2. For Section 14.2, please note the standard first aid measures in Section 3.5 of the Biosafety Manual:

http://www.uwo.ca/humanresources/docandform/docs/healthandsafety/biosafety/biosafety_manual.pdf

Regards