

ENVIRONMENTAL MICROBIOLOGY

60.432

LAB MANUAL

2003

Lab manual is available as a pdf file on the website.

Table of Contents

Lab #	Title	Page
	Environmental microbiology schedule	3
	References	5
	General instructions	6
	Lab standard operations procedure (sop)	9
	WHMIS	12
	<u>EXPERIMENTS</u>	
1	Clean water analysis by standard methods	14
	Part I Membrane Filter Technique: mFC and MI agar plates	
	Part II 3M™ Petrifilm™ <i>E. coli</i> /Coliform Count Plates	
	Part III Qualitative Coliform/ <i>E. coli</i> Detection in water samples	
2	Microbial ecology of composting	24
	Part I BIOLOG EcoPlate™ Microbial Community Analysis	
	Part II Nucleic Acid Microbial Community Analysis	
3	Microbial biodegradation of petroleum	47
	Part I Effect of amendments on petroleum (diesel fuel) biodegradation	
	Part II GC analysis of extracted alkanes	
4	Determination of terminal electron accepting processes in sediments	64
	Part I Dissolved oxygen profile of sediment (field trip)	
	Part II DAPI staining	
5	Competition between anaerobes in a wastewater treatment plant: the impact of sulfate reducers on methane production	72
	Part I Anaerobic culture preparation	
	Part II GC measurement of % methane	
	<hr/>	
	APPENDIX	78
	Field trip maps	79
	Media and Solutions	83
	Pipetman Operation	84
	Rotary Vacuum Evaporator Operation	86
	Stereoscopic Microscope Operation	87
	Epi-fluorescent Microscope Operation	89
	Accelerated Solvent Extractor (ASE) operation and GC/MS operation	90
	Sample Lab Exam	104
	Release and Indemnification Forms for field trips	106

ENVIRONMENTAL MICROBIOLOGY LAB SCHEDULE - 2003

DATE	WEEK	EXPERIMENT/PROCEDURE
Sept 11	1	Lab Introduction Lab 1 Clean water analysis by Standard Methods Part I Membrane Filter Technique: mFC and MI agar plates Part II 3M™ Petrifilm™ <i>E. coli</i> /Coliform Count Plates Part III Qualitative Coliform/ <i>E. coli</i> Detection in water samples
Sept 18	2	Lab 2 Field Trip ¹ - Brady Landfill Composting Lab 2 Microbial ecology of composting Part I BIOLOG EcoPlate™ Microbial Community Analysis Part II Nucleic Acid Microbial Community Analysis A. DNA extraction from soil B. PCR amplification
Sept 25	3	Lab 2 Microbial ecology of composting Part II Nucleic Acid Microbial Community Analysis C. MinElute PCR purification D. RFLP microbial community analysis
Oct 2	4	Lab 3 Field Trip ¹ - Microbial biodegradation of petroleum Lab 3 Microbial biodegradation of petroleum Part I Effect of amendments on petroleum (diesel fuel) biodegradation - Culture Preparation and Inoculation
Oct 9	5	Lab 4 Field Trip ¹ -Fort Whyte Centre sediment collection Lab 4 Determination of terminal electron accepting processes in sediments Part I Dissolved oxygen profile of sediment (field trip) Part II DAPI - fixing
Oct 16	6	Lab 4 Determination of terminal electron accepting processes in sediments Part II DAPI - staining and microscopy
Oct 23	7	Lab 3 Microbial biodegradation of petroleum Part II GC analysis of extracted alkanes ASE and GC/MS Tutorial & Demonstration of the rotary vacuum evaporator
Oct 30	8	Lab 5 Field Trip ¹ -Waste Water Treatment Plant Lab 5 Competition between anaerobes in a wastewater treatment plant: the impact of sulfate reducers on methane production Part I Anaerobic culture preparation
Nov 6	9	Lab 5 Competition between anaerobes in a wastewater treatment plant: the impact of sulfate reducers on methane production Part II GC measurement of methane
Nov 13	10	no scheduled lab
Nov 27	12	Lab Exam in Room 201/204 at 1:00 pm (1 hour 20 min)

¹field trip details given in class prior to scheduled date.

Lab DATA Due Dates

Report #	Date due	Comment
1	Monday, Sept 15	Hand in a COPY of data sheet
2	Monday, Sept 22	Hand in a COPY of data sheet

Lab REPORT Due Dates

Report #	Week #	Date
Lab 1	3	Sept 25
Lab 2	5	Oct 9
Lab 3 Part I Group data	8	Nov 3
Lab 4	8	Oct 30
Lab 3 Part II Class data	9	Nov 10
Lab 5 Part I Group data	10	Nov 13
Lab 5 Part II Class data	11	Nov 20

RECOMMENDED READINGS

Maier, R. M., Pepper I.L. & C.P. Gerbe. 2000. Environmental Microbiology **Chapters 8 through 13** - environmental microbiology methods New York: Academic Press. p. 177 - 318

References available in the reference binder (1 hour reserve in the Science and Technology Library)

Lab 1

- 1 Franson MH (managing), Clesceri LS, Greenberg AE, Eaton AD, editors. 1998. Standard Methods for the Examination of Water and Wastewater. 20th ed. Washington: American Public Health Association p 9.1 - 9.18
- 2 Guidelines for Canadian Drinking Water Quality - Bacteria quality June 1988 (edited February 1991), Updated October 2001,(edited January 2002) - select html (at the time accessed, June 2003, pdf file was only available for one section) <http://www.hc-sc.gc.ca/hecs-sesc/water/dwgsup.htm>
- 3 Guidelines for Canadian Recreational Water Quality 1992, Prepared by the Federal Provincial Working Group on Recreational Water Quality of the Federal-Provincial Advisory Committee on Environmental and Occupational Health. p 1-22, 62-71 http://www.hc-sc.gc.ca/hecs-sesc/water/recreational_water.htm

Lab 2

- 4 Garland, JL & AL Mills. 1991. Classification and Characterization of Heterotrophic Microbial Communities on the Basis of Patterns of Community-Level Sole-Carbon-Source Utilization. Appl.Environ. Microbiol. 57:2351-2359.
- 5 BIOLOG Microbial Community Analysis. http://www.biolog.com/mID_productLiterature.html
- 6 Maier, RM. Pepper, IL, & CP Gerba. 2000. Environmental Sample Collection and Processing. New York: Academic Press. p.181-186
- 7 Weisburg, WG, Barns, SM, Pelletier, DA, Lane DJ. 1991. 16S ribosomal DNA amplification for phylogenetic study. J. Bact. 173: 697-703.
- 8 LaMontagne, MG, Michel Jr., FC, Holden, PA, Reddy, CA. 2002. Evaluation of extraction and purification methods for obtaining PCR-amplifiable DNA from compost for microbial community analysis. J. Micro. Meth. 49: 255-264.

Lab 3

- 9 Maier, R. 2000. Microorganisms and organic pollutants. In: Maier, R, Pepper, IL, Gerba, CP. Environmental Microbiology. New York: Academic Press. p 363-380, 394-402.
- 10 Bossert, ID, Kosson, DS 1997. Methods for Measuring Hydrocarbon Biodegradation in Soils. In: Hurst, CJ., Knudsen, GR, McInerney, MJ, Stetzenbach, MV, editors. Manual of Environmental Microbiology. Washington, D.C.: ASM Press. p 738-745.
- 11 Walter, MV. 1997 Bioaugmentation. In: Hurst, CJ., Knudsen, GR, McInerney, MJ, Stetzenbach, MV, editors. Manual of Environmental Microbiology. Washington, D.C.: ASM Press. p 753-757.

Lab 4

- 12 Hofman, PAG, de Jong, SA. 1993. Sediment Community Production and Respiration Measurements: The Use of Microelectrodes and Bell Jars. In: Kemp, PF, Sherr, BF, Sherr, EB & JJ Cole, editors. Handbook of Method in Aquatic Microbial Ecology. Ann Arbor: Lewis Publishers. p 455-463.
- 13 Ravenschlag, K, Sahm, K, Knoblauch, C, Jorgensen, BB, & R. Amann. 2000 Community Structure, Cellular rRNA content, and activity of sulfate reducing bacteria in marine arctic sediments. Appl. Envir. Microbiol. 66: 3592-3602 <http://aem.asm.org/cgi/reprint/66/8/3592.pdf>

Lab 5

- 14 Zinder, SH 1998. Methanogens In: Burlage, RS., Atlas, R., Stahl, D., Geesey, G. & G Sayler, editors. Techniques in Microbial Ecology. New York: Oxford Univeristy Press. p113-132
- 15 Santegoeds, CM, Damgaard, LR, Hesselink, G, Zopfi, J, Lens, P, Muyzer, G, de Beer, D. 1999. Distribution of Sulfate-Reducing and Methanogenic Bacteria in Anaerobic Aggregates Determined by Microsensor and Molecular Analyses. Appl. Env. Micro. 65: 4618-4629. <http://aem.asm.org/cgi/reprint/65/10/4618.pdf>

GENERAL INSTRUCTIONS

Lab Instructor:	Dr. L. Cameron	Office: 414B
Lab Demonstrators:	George Golding	Lab: 413
	Heather Grover	Lab: 125

Lab Location: 201 Buller

WEBSITE: www.umanitoba.ca/faculties/science/microbiology/staff/cameron/

OR via University of Manitoba Microbiology Homepage:

https://www.umanitoba.ca/faculties/science/microbiology/course_notes.html

Information available at the website: changes/corrections, additional information, data, marks

REGULATIONS

1. Lab attendance is compulsory, both field trips and in department experimental labs. On lab days where there are field trip, the field trips starts at 1:00 pm. There will be no lecture by Dr. Londry on field trip lab days. Dr. Londry and the teaching assistant will accompany you on your field trips.
2. Students must wear a lab coat. There is no smoking, drinking, or eating in the lab.
3. Students work in pairs for the majority of the lab. For the project ONLY, two pairs will work together.

EVALUATION

1. The lab is worth 20% of the final mark:

Lab exam:	12%
Lab reports:	8%

0.2% of your mark can be subtracted from your final lab mark if poor conduct in lab or requested data not handed in.
2. Students must pass the lab to pass the course (10% of the 20% lab mark).
3. The lab exam will be held during lecture slot. The date is stated in the schedule. Exam must be written in pen (not pencil).
4. Lab reports and project (stapled, no binders) are to be handed in as stated in schedule by 4:30 pm of that day. Hand in reports through slotted drawer in room 414 ONLY. Demonstrators do not accept lab reports. If handing in lab report late, 1 mark will be subtracted for each class day late. Marked lab reports will be returned to students the next week. **A late report will not be accepted after that report has been returned to the class.**
5. Approximately two weeks prior to the lab exam, a brief outline of lab exam format and information content will be available on the website.
6. You must notify the lab instructor no later than two school days, after missing a lab exam, of your intent to write a deferred lab exam. The deferred lab exam must be rescheduled before the end of this term's classes. Failure to comply will result in a zero on your lab exam.
7. **Plagiarism (copying another student's lab report (present or previous year) or copying published literature without citing) is a violation of University regulations. Refer to the STUDENT DISCIPLINE BY-LAW in your student handbook (rule book) for action taken for plagiarism.**

WRITTEN REPORT PRESENTATION

1. Lab reports may be done as an individual effort or a group effort by the two students that carried out the experiment. The decision on the number of reports per group is totally dependent on members of the group. This decision may be changed any time during the term. Therefore for each lab report the group has the option to hand in one or two reports exclusive of what has been done before or after that particular report. Indicate on the cover page of the report if the report is a group report or an individual report. If handing in an individual report also include lab partner's name. For labs 7,8 or 9 make sure your group number is on the cover of your report. Only ONE PROJECT REPORT is accepted per group of four students.
2. A reference file is available in the science library (1 hour reserve).
3. Lab reports must be written in **pen (no pencil) or typed**. No binders. Stapled left hand corner.
4. On the front page of the report state:
 - Course name and number
 - Experiment number and Title
 - Group # and section #
 - Individual or Group name(s). If handing in an individual report, also include lab partners name.
 - GROUP report or INDIVIDUAL report
 - Date
5. Number pages.
6. Lab reports consist of data presentation, data analysis and possibly questions. The information is to be presented exactly as requested. Number sections the same as the lab manual.
7. Always include a sample of each calculation type.
8. If a group's data is not workable, borrow data from another group and reference. Non workable refers to data that cannot be plotted, used for calculations or required analysis. It does not necessarily mean the expected data.
9. Cite reference in text of lab report and record full reference at end of lab report. When should you cite and reference. The following is a good definition of plagiarism that explains when you should cite a reference. **“The unacknowledged use of another person's work, in the form of original ideas, strategies, and research, as well as another person's writing, in the form of sentences, phases and innovative terminology.”** (Spatt¹, 1983, p.438) To cite use bracketed reference number that you used when listing references at end of lab report or by bracketing first authors name and date. Quote text unless you paraphrase completely in your own words. But remember, quotes should only be a small part of your work. If you are using the name year system, list the references alphabetically. Some examples are as follows (McMillan² 1997):

¹Spatt, B. (1983). *Writing from Sources*. New York: St. Martin's Press.

²McMillan V.E. 1997. *Writing Papers in the Biological Sciences*. 2nd ed. Boston: Bedford Books: 1997. 197 p. and McMillan, V.E. 2001. *Writing Papers in the Biological Sciences*. 3rd ed. Boston: Bedford Books. 123 p.

- Binder V, Hendriksen C, Kreiner S. 1985. Prognosis in Crohn's disease - - based on results from regional patient group from county of Copenhagen. *Gut* 26:146-50.
- Danforth DN, editor. 1982. *Obstetrics and gynecology*. 4th ed. Philadelphia: Harper and Row. 1316 p.
- Petter JJ. 1965. The lemurs of Madagascar. In: DeVore I, editor. *Primate behavior: field studies of monkeys and apes*. New York: Holt, Rinehart and Winston. p 2920319.

If available only on the web:

Kingsolver JC, Srygley RB. Experimental analyses of body size, flight and survival in pierid butterflies. *Evol. Ecol. Res.* [serial online] 2000;2:593-612. Available from: Colgate University online catalog. Accessed 2000 Oct 3.

10. Personal or Professional Electronic sources²:

Cite in-text by putting the following in parentheses, author's last name or file name (if no author's name is available) and publication date or the date of access (if no publication date is available).

At the end of report list

- (i) author or organization
- (ii) publication date or date last revised
- (iii) title of Web site
- (iv) URL site in angle brackets
- (v) the date accessed.

Cameron, L. 60.344 Microbial Physiology Lab Information
 <http://www.umanitoba.ca/faculties/science/microbiology/staff/cameron/60_344.htm>.
 Accessed 2002 April 12.

11. Cite software used for statistical analysis and graphs.

Table presentation

- Table number and title (legend) presented above the table body.
- Number tables using arabic numbers, even if only one table in a report.
- Include enough information in title to completely describe table, eliminating the necessity to search elsewhere in the lab report to understand information presented in table. Table title starts with an incomplete sentence. Additional complete sentences may be included to adequately describe the table, eg. number of days of colony growth and temperature, media type, microorganism source (this also applies to figures).
- If abbreviations are used in table, indicate what abbreviations mean as a footnote. Other footnotes may be required to clarify material in the table.
- Like information should be in columns making it easier to view the table.
- Data in columns is listed under the center of each heading. Align decimal points and dashes. If a number value is less than 1 always include zero before the decimal.
- Column or Row headings should be complete and self explanatory. A heading is a separate entity from the title. It cannot be assumed information given in the title is adequate for a heading. The unit of measurement should only be included in the heading, not in column data.

- Group related column headings under larger headings.
- If information is the same for each column or row do not include but treat as a footnote.
- Make the table as concise as possible but include all necessary information. For example, when presenting a table of bacteria colony characteristics it is important to state media type, incubation time and temperature as colony characteristics vary depending on these conditions somewhere in the table.
- Tables should be properly set up with a straight edge.

Figure presentation (graphs, diagrams, photographs, films)

- Figures are to be numbered separate from tables, using arabic numbers. Include figure number even if only one figure.
- Following the figure number a figure legend should be presented below graph. The figure legend, like the table, starts with an incomplete sentence describing the graph. For example, do not repeat just the labels of the x- and y-axis but present in a descriptive manner. Additional sentences should be included if additional information is required to completely describe figure, for example, abbreviations explanation, any constant experimental conditions, etc.
- All diagrams, photographs, and films are figures and should be completely labelled. For figures of graphs, there is one dependent variable plotted and one or more independent variables plotted. The dependent variable is a function of the independent variable. It is accepted practise to plot the independent variable on the x-axis and the dependent variable on the y-axis. For example the measurement of absorbance (dependent) with increasing concentration of protein (independent). The size of the graph should fit the plot(s). The axis should not necessarily start at zero. Place graph completely within graph grid, this includes axis labels and legend. The overall size of graph should not be too large but should not be so small that information is obscured. Graph must be completely labelled (always include units). Use different symbols for each plot (not different coloured pens) on a graph. If more than one plot explain symbols in legend or in a key included in the body of the graph. Graph plots can be drawn in a number of ways (this depends on the plot): (a) best straight line, (b) join the points with a straight line, and (c) use a curved ruler or french curve.
Note: Do not drawn a free hand line.
- Completely label diagram figures. All labelling should be to one side with all labels aligned. Arrows or lines should be used to indicate what is described in diagram.

Note: When writing your lab reports you are frequently requested to present both a table and a figure for a given set of data, similar to keeping a research journal. This is not the accepted practice for papers published in journals or books. Usually either a table or a figure is presented for a given set of data and depending on nature of data, it may only be summarized in the text. How do you make a choice of data presentation? The aim is to effectively and efficiently demonstrate what you want to show, for example, correlations, comparisons, pattern, trends, etc.

LAB STANDARD OPERATIONS PROCEDURE (SOP)

Bench area: Wash bench area before and after use with savlon.

Personal safety: You must wear a lab coat. Wear coat only in the lab, transport separately outside of the lab (in a plastic bag). Wash hands with antibacterial soap before leaving the lab. No eating or drinking in the lab. Use aseptic technique for transfer of bacteria. This is to protect yourself as much as to ensure the purity of your culture. Protect hands with gloves and eyes with glasses when needed. The gloves provided in the lab are to be disposed of after use.

Biohazards: Know biosafety risk groups. Handle all cultures as potential pathogens. Never mouth pipette. Always use a pro-pipette. If you spill a culture, cover the spill with paper towels. Pour Savlon over the towels to saturate. Gather up soaked towels and discard. Wipe area to dryness with fresh paper towels. Wash hands with soap and water. Place cultures on discard trolley. All cultures are autoclaved before disposing. Dispose of ependorff tubes^a in petri plate containers. Dispose of pipetman tips^a in clear plastic lined basins along with glass or plastic Pasteur pipets, broken glassware, glass slides, brittle plastic objects, metal objects^a (not needles or blades). Bacteria dilutions may be poured down the sink and the tubes rinsed before placing on the discard trolley. Rinse sink with lots of water.

When handling level 2 microorganisms you must wear disposable gloves, make sure any cuts on your hands are covered with a bandage, and be aware of the possibility of bacteria aerosol when you flame your loop.

^a due to the multi-use nature of the teaching lab, all eppendorf tubes, pipetman tips, Pasteur pipets, brittle plastic or metal objects will be treated the same as similar items contaminated with microorganisms.

Glassware (unbroken): Remove tape and pen markings (use alcohol) from glassware before placing on discard trolley. Used glassware should be rinsed and placed on the discard trolley. Rinsed test tubes should be placed in tray provided on the discard trolley. Used glass pipettes should be placed in pipette holders.

Petri plate culture and non-sharps solid culture material disposal: use covered plastic containers lined with clear plastic bags for contaminated petri dishes or any bacteria contaminated solid non-sharps material (ependorff tubes, API strips, antibiotic strips, microtitration plates, etc)

Hazardous material disposal: Examples: radioactive material, ethidium bromide, solvents, etc. The lab demonstrator will instruct proper disposal methods for labs that contain hazardous materials. These materials must be disposed of in appropriately labelled containers and disposed via the safety office. Use fumehood when recommended. A MSDS binder available in lab gives information on all hazardous materials used in the lab. Use extreme care with flammable solvents. Alcohol used to flame spread rod should never be positioned within 40 cm of flame. Never put a very hot spread rod into a beaker of alcohol. The alcohol may catch fire. Many of the immunochemicals are preserved in 0.1% Na azide...handle with gloved hands. Handle caustic (acids and bases) solutions with care. Never discard an acid or base greater than one molar down the sink. Discard in labelled glass containers provided. Use lots of water when discard caustic solutions (< 1M). These materials are disposed of through the university safety office. Never pour solvents down the sink (eg. phenol, ether, chloroform, etc). Discard in labelled containers provided.

Sharps disposal: Dispose of all sharps (needles, syringes, razors, scalpel blades) in specified container. Dispose of syringe with needle attached - do not take apart. Do not replace the needle cap before disposing (high frequency of accidents occur when replacing cap). Sharp's containers are autoclaved before disposing.

Broken glass disposal: Dispose of broken glass in labelled plastic containers lined with clear plastic. Transferred to boxes before discarding.

Know location: Exits, fire extinguisher, eye wash, sink shower, and first aid kit. This information

is given in the first pre-lab.

Equipment operation: Know how to operate equipment before use. DO NOT use equipment unless you know exactly how to operate the equipment. The demonstrator is always available to assist.

Leave your bench area clean All equipment and supplies should be returned to original location.

LABORATORY BIOSAFETY GUIDE

Environmental samples contain mostly level 1 risk microorganisms but there are also level 2 microorganisms present. Treat all isolated microorganisms as if they were level 2 risk microorganisms. Follow standard operations procedure, SOP (see above).

The University of Manitoba Biosafety Guide (Feb 2000) and Health Canada Laboratory Biosafety Guidelines booklets are available in your lab. Biosafety information is also available at the Health Canada websites:

Guidelines: <http://www.hc-sc.gc.ca/pphb-dgsp/ols-bsl/lbg-ldmbl/index.html> Health Canada http://www.umanitoba.ca/campus/health_and_safety/
MSDS (infectious agents): <http://www.hc-sc.gc.ca/pphb-dgsp/msds-ftss/index.html>

There is no listing of level 1 agents in the guidelines or MSDS pamphlets

Risk group 1 bacteria are low individual and community risk and are unlikely to cause disease in healthy workers.

Risk group 2 bacteria are moderate individual risk and limited community risk. Bacteria in this group can cause human or animal disease but are unlikely to infect healthy laboratory workers. Effective treatment is available. Risk of spreading is limited.

CONTAINMENT LEVEL 1 (UM biosafety guide p. 11)

- microbiology lab with washable walls, countertops and hand wash sink
- established safe laboratory practices (hand washing and disinfection of countertops)
- general WHMIS safety training
- UM lab registration

CONTAINMENT LEVEL 2 (UM biosafety guide p.11)

- all of level 1 specifications
- biosafety permit
- biological safety cabinet (not required)
- biohazard signage
- a written standard operations procedure
- MSDS for the infectious agent

WHMIS

The Workplace Hazardous Materials Information System (WHMIS) is a system for safe management of hazardous materials. WHMIS is legislated by both the federal and provincial governments.

Under WHMIS legislation, laboratories are considered to be a workplace, and students are workers. By law, all workers must be familiar with the basic elements of the WHMIS system.

The WHMIS program includes:

1. Cautionary labels on containers of controlled products. Consumer products, explosives, cosmetics, drugs and foods, radioactive materials, and pest control products are regulated separately, under different legislation.
2. Provision of a Material Safety Data Sheet (MSDS) for each controlled product.
3. A worker education program

1. A. SUPPLIER LABELS

Controlled products must have a label of prescribed design which includes the following information:

PRODUCT IDENTIFIER - trade name or chemical name

SUPPLIER IDENTIFIER - supplier's name and address

MSDS REFERENCE - usually, "See MSDS supplied"

HAZARD SYMBOL - (see illustration on next page)

RISK PHRASES - describes nature of hazards

PRECAUTIONARY MEASURES

FIRST AID MEASURES

B. WORKPLACE LABELS

All material dispensed in a workplace container must be labelled with the **Product Name**, **Precautionary Measures** (simplified) and **Reference to Availability of MSDS**.

2. MSDS

Individual course MSDS are located in a binder in your lab (Room 201 binder located in 204). The main MSDS binders are located in the Microbiology preparation room, 307/309 Buller. MSDS are also available on the local area computer network (see your demonstrator, if necessary).

The MSDS will provide: relevant technical information on the substance, chemical hazard data, control measures, accident prevention information, handling, storage and disposal procedures, and emergency procedures to follow in the event of an accident.

3. SAFETY

The Laboratory Supervisor will provide information on the location and use of safety equipment, and emergency procedures.

WHMIS DIAGRAM

LAB 1 CLEAN WATER ANALYSIS BY STANDARD METHODS

OBJECT

The object of this experiment is to use standard methods to examine recreational water for the presence of microorganisms pathogenic to humans by detection of coliforms using (1) membrane filter technique and (2) defined substrate technology (DST) Colilert®.

INTRODUCTION

Most common human pathogens, including coliforms are present in the human intestine. Therefore, when coliforms are present in water it is an indication of fecal (or thermotolerant coliforms- new term) contamination, and there is the possibility of human pathogens present. The detection of coliforms is much easier to demonstrate than searching for all possible pathogens, so it is used as an indicator group. The coliform group include all gram negative, facultative anaerobic non spore-forming rods that ferment lactose with gas and acid production at 35°C before 48 h³, such as *E. coli*, *Citrobacter*, *Enterobacter* and *Klebsiella*. Another characteristic common to all coliforms is the presence of β -galactosidase. However, additional genera also have β -galactosidase and some can be eliminated by including the cytochrome oxidase test (coliforms are cytochrome oxidase negative). Thermotolerant coliforms are capable of producing blue colonies on m-FC with 24 hours at 44.5°C. *Escherichia* is the main thermotolerant coliform (~97%), along with *Klebsiella* (1.5%), *Citrobacter* and *Enterobacter* (1.7%). *Escherichia* is the only thermotolerant coliform that is exclusively of fecal origins and does not grow outside the human or animal digestive track. *Citrobacter*, *Enterobacter* and *Klebsiella* may be present in fresh feces and have the ability to persist and grow outside of the human or animal digestive track .

Quantitative Methods for measuring water quality

The membrane filter technique is a reliable method that can be used to rapidly screen large volumes of water for indicator coliforms. This method allows isolation and identification of colonies. One drawback is that the water cannot be turbid. The water sample is filtered through a 0.45 μ m filter. The filter containing the bacteria is placed on a pad saturated with a differential selective medium. Two differential selective media are used in this lab.

mFC agar

USGC Ohio District's Microbiology laboratory

<http://www-oh.er.usgs.gov/micro/fc.html> (assessed 5/20/2003)

mFC agar incubated at 44.5°C (submerged in a waterbath) for 24 hours is used to detect thermotolerant coliforms such as *E. coli*. mFC agar contains a pH indicator, analine dye, which turns blue in the presence of strong acids produced by fecal coliforms, ie. *E. coli*. The high temperature eliminates the presence other coliforms.

MI agar

³Guidelines for Canadian Drinking Water Quality - Bacteria quality June 1988 (edited February 1991), Updated October 2001,(edited January 2002 - select html (at the time accessed, June 2003, pdf file was only available for one section) <http://www.hc-sc.gc.ca/hecs-sesc/water/dwgsup.htm>

BD Diagnostic systems

<http://www.rapidmicrobiology.com/news/29h0.php> (assessed 5/20/2003)

A recently developed medium, MI agar, is USEPA (United States environmental protection agency) approved for testing drinking water. MI plates are incubated at at 37°C. MI detects and enumerates both total coliforms and *E. coli*. Like Colilert (see below) MI agar detects the presence of coliforms by the presence of β -galactosidase in all coliforms and by the presence of β -glucuronidase only found in *E. coli*. In daylight, *E. coli* colonies are bluish-grey while coliform colonies are cream colored. However, when place over UV light, the *E. coli* colonies flouresce blue-green (darker colonies) while coliforms are blue-white.

3M™ Petrifilm™ E. coli/Coliform Count Plates

3 M Microbiology products

<http://www.3m.com/microbiology/home/products/petrefilm/petripod/ecoli/intguide.html> (assessed 5/20/2003)

Although petrifilm plates were developed to monitor food quality they can also be used to measure water quality. It is possible to detect and enumerate coliforms and *E. coli* present in water samples. The dehydrated medium contains violet red bile nutrients, a cold-water-soluble gelling agent, an indicator of β -glucuronidase (5-bromo-4-chloro-3-indolyl- β -D-glucuronide, BCIG) and an tetrazolium indicator that help enumeration. *E. coli* (97%) due to the presence of β -glucuronidase produce a blue to red-blue precipitate. Confirmation of *E. coli* by gas production (95%) is demonstrated by the entrapment of gas associated with blue to red-blue colonies. Coliform colonies are red due to acid production and associated with trapped gas bubble. Petrifilm plates like all other indicator plates fail to indicate the presence of *E. coli* when the colony number is too numerous to count. Usually the plate has a homogenous combined plate color.

Qualitative methods for measuring water quality

Defined substrate technology (DST) Colilert® Test

<http://www.idexx.com/Water/Products/Colilert/index.cfm> (accessed June 2003)

Indicator coliforms are rapidly detected using Colilert® reagent. Colilert® reagent contains the indicators ortho-nitrophenyl- β -D-galactopyranoside (ONPG) and 4-methyl-umbelliferyl- β -D-glucuronide (MUG). After incubation of water sample with the Colilert® reagent a yellow color is produced when coliforms, containing β -galactosidase which hydrolyzes ONPG, are present. If the water sample fluoresces in the presence of UV light, the enzyme, β -glucuronidase is also present. This indicates that *E. coli* is present in the water sample since glucuronidase (constitutive in *E. coli*) hydrolyzes MUG to produce glucuronide, a metabolizable substrate, and methylumbelliferone, which fluoresces in the presence of UV light.

Standard Methods for examination of water

See reference binder for Standard Methods information.

PROCEDURE

First Lecture

1. Lab groups will be assigned. **Students work in pairs** unless otherwise stated.
2. Each group will receive four sterile milk dilution bottles. Each bottle contains 1 ml sterile 1% sodium thiosulfate solution to neutralize the possible presence of chlorine.

Sample Collection (student responsibility)

3. Collect ONE TYPE of water sample the day before or the day of the lab.

Selecting sample location:

a) Raw water supply (river, stream, lake, reservoir, spring or shallow well): Sample should be representative of the source. Do not sample too close or too far from edge. Do not sample too shallow or too deep from draw off point.

b) Bathing beaches, swimming pool, hot tub: Select sample from 1 M depth. There should be numerous samples taken throughout the swimming area. Not possible for this lab (take only one sample).

Method of sample collection: Remove lid, keep sterile by holding lid downward in one hand. Fill bottle with water sample to the 100 ml line. Recap. Repeat for remaining three sample bottles. You have a total of 400 ml of ONE sample type collected.

Note: The standard sample method follows but due to presence of sodium thiosulfate in your bottle the procedure is not possible. With the bottle held by the base in the other hand, plunge downward into the water to depth that you are sampling at. Slowly turn the bottle sideways towards current (if present) allowing the water to enter the bottle. If there is no current, move bottle sideways. Or attach a weight to base of the bottle and lower into water (eg. from a boat or dock). Remove and pour off excess water to 100 ml line. Recap.

Sample labelling: Label each bottle with group number, group names, sample type, sample location details and date of sampling.

Sample storage: It is best to collect sample as close to the lab period as possible. If not used within the hour, store sample at 4°C (fridge). Best to transport sample on ice (not necessary for this lab). Put in student cold box located off room 201 when you bring your samples to university. Transport time and storage in the cold should not exceed 8 hours for compliance* purposes and 24 hours for noncompliance purposes. A longer time is acceptable for this lab.

*refers to government regulations (compliance - must follow government regulations if requested by government) or if doing your own monitoring (noncompliance).

Week 1 (lab starts at 2:30 pm)

1. Bring water samples (4 milk dilution bottles, containing 100 ml each of one type of sample) to lab.

Part I Membrane Filter Technique: mFC and MI agar plates

1. Filter Set Up: Use the sterile forceps to put the 0.45 μm filter (grided white nylon, not blue protector sheets) grid side up in the filtration unit (figure 1). Unscrew the top part of the filtration unit, place the filter on filter holder GRID SIDE UP and screw the top section back on. Return sterile forceps to sterile bottle for reuse. If forceps are no longer sterile, dip in alcohol, flame before returning to sterile bottle. Attach hosing to filtration unit and attach to vacuum via a water trap clamped to a stand (one liter flask with rubber stopper containing two inserted pieces of glass tubing - attach glass tubing that goes near the bottom of the flask to the filtration unit via rubber tubing and the glass tube that is shorter to the vacuum tap via rubber tubing). Clamp both the filtration unit and water trap to a stand to stabilize in an upright position. Do not turn on vacuum yet.

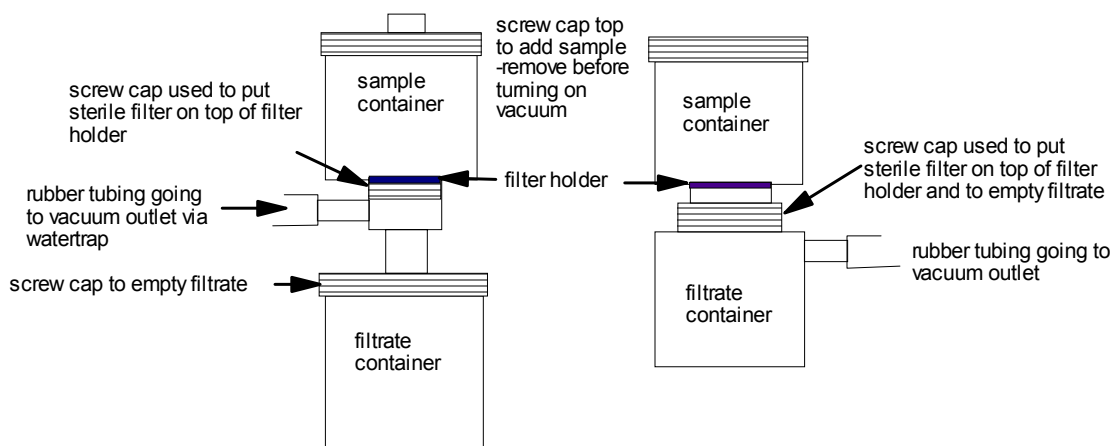


Figure 1. Water filtration unit made of transparent polysulfone which can be sterilized. Two filtration units are illustrated as two types are available in the lab. You will receive the unit sterilized with foil covering the vacuum outlet. Remove and attach rubber hosing. Also follow procedure in lab manual for putting the sterile filter in the filtration unit.

Striped pattern denotes a screw cap.



WEAR GLOVES

2. Prepare the following water samples:
- (a) 4x 100 ml of 10^{-2} dilution (1/100) your water sample. Prepare by adding 1 ml water sample to 99 ml sterile distilled water.
 - (b) 4x 100 ml of 10^{-1} dilution (1/10) your water sample. Prepare by adding 10 ml water sample to 90 ml sterile distilled water.
 - (c) 4x 100 ml undiluted water sample
 - (d) 4x 100 ml positive control (Each group dilutes their own *positive* control *E. coli*: add 10 μ l of 10^6 dilution¹ of an overnight culture of wild type *E. coli* culture to 100 ml sterile distilled water in milk dilution bottle - should give approximately 20 cells)

¹ *E. coli* dilution preparation: Prepare 100-fold serial dilutions 10^{-2} to 10^{-6} of culture in saline. Mix or vortex culture before starting serial dilution. Vortex after each transfer. Prepare dilutions in a total volume of 1 ml using 5 inch metal capped test tubes. Add 990 μ l saline to labelled dilution tubes. Transferring 10 μ l of vortexed culture to 990 μ l saline (10^{-2} dilution), vortex, then transfer 10 μ l of 10^{-2} dilution to 990 μ l saline (10^{-4} dilution), vortex, and lastly transfer 10 μ l of 10^{-4} dilution to 990 μ l saline (10^{-6} dilution), vortex.

3. Filtration: After placing filter in filtration unit, remove cover of filtration unit. Whenever removing a lid or cover in sterile technique procedure, you must keep the lid in your hand with sterile side downwards. TURN ON VACUUM (do not turn on vacuum before removing lid). Pour in your water sample. Rinse the interior surface of the funnel with approximately 60 ml sterile distilled water using partial vacuum. Filter quadruple water samples from most dilute to most concentrated. As soon as the water sample has been filtered, rinse the sides with approximately 60 ml sterile water (measure using sterile graduated cylinder) before proceeding to the next sample. **Use distilled water (500 ml amounts) in 1-liter flask to wash filter, not dilution distilled water in milk dilution bottles (MDB).**
4. Plate each sample in duplicate on mFC and MI agar: Turn off vacuum. Remove filter using sterile forceps to the appropriately labelled petri plate GRID SIDE UP atop the appropriate agar plate. Carefully place the filter on the agar by touching down one side first then slowly lower the remainder of the filter. It is important that there are no air bubbles formed between the filter and the agar. Turn off vacuum. Filter duplicate sample and place filter on mFC agar plate. After duplicates of the most dilution sample have been filtered proceed to next sample. Repeat until all samples have been plated.
5. Incubation: Place mFC agar plates in a twirl transparent polyethylene bag. Twirl top and seal edges. Place plates inside a temperature tolerant plastic container. Submerge container in a 44.5°C water bath. Incubate for 24 hours. Place MI agar plates in a sealable bag to prevent dehydration. Incubate plates in a 37°C incubator.

NEXT DAY (24 hours)

6. Record the total number of blue colonies on all countable mFC plates. If the total number of bacteria exceeds 60 record as TNTC (too numerous to count). Fill out requested information on data sheet.

7. Record colony counts as requested on all countable MI agar plates. If the total number of bacteria exceeds 150, record as TNTC (too numerous to count).
- Day light (i) record cream colored colony plate counts
(ii) record bluish-grey colored colony plate counts
- UV light (i) record blue green colony plate counts
(ii) record blue white colony plate counts
- Fill out requested information on data sheet.

Part II 3M™ Petrifilm™ *E. coli* /Coliform Count Plates (excerpt from instruction manual)
Inoculate and spread one Petriplate at a time.

1. Lift the top film and dispense 1 ml 10^{-1} dilution (1/10) of your water sample on the center of bottom film. (same dilution originally prepared for mFC plates).
2. Slowly roll the top film down onto the sample to prevent trapping air bubbles.
3. Center a hockey puck block on top of Petrifilm. Gently press downward on the center of the block. Do not slide the block when pressing. Remove block. Let Petrifilm sit for 1 min before moving to allow the gel to solidify.
4. repeat steps 1 through 3 for undiluted water sample and 10^{-6} dilution positive control *E. coli* (from dilution series originally prepared for mFC plates)
5. Incubate upright at 35°C for 48 hours under humid conditions. The TA will move the petrifilms to the 4°C student incubator on Saturday.
6. **Monday** record colony plate counts using colony counter with magnification: (i) red-blue and blue colonies with gas and (ii) red colonies with gas. If the total number of bacteria exceeds 150, record as TNTC (too numerous to count). Fill out requested information on data sheet.

Part III Qualitative Coliform/*E. coli* Detection in water samples
Defined substrate technology (DST) Colilert® Presence/Absence Test

1. Each group adds Colilert® reagent to
 - (i) one 100 ml water sample
 - (ii) one 100 ml *E. coli* sample (Each group prepares their own positive control: add 10 μ l of 10^{-4} dilution¹ of an overnight culture of *E. coli* culture to 100 ml sterile distilled water in milk dilution bottle. Use the same dilution series you prepared for mFC and MI agar procedure.
 - (iii) one 100 ml distilled water sample (negative control)
 Shake to dissolve.
2. Incubate at 35°C for 20-24 hours.

NEXT DAY

3. After 24 hours read results. Record presence or absence of yellow color. Check for fluorescence by placing bottle over a UV transilluminator*. Note: There may be fluorescence without the presence of a strong yellow color. If fluorescence is present record as weak yellow color. Discard milk dilution bottles after reading results, do not return to incubator. Fill out requested information on data sheet.

* **UV HAZARD:** High intense short waves. Wear goggles provided. Wear rubber gloves. Do not turn on the UV light without first protecting your eyes with goggles. Eye glasses are acceptable but it is advisable to also wear goggles.

DATA SHEET

Fill out requested information on data sheet. **Each group submits ONE COPY of the DATA SHEET by 4:30 pm Monday. See schedule for lab data due date. Place data through slotted drawer in filing cabinet located in room 414 or email data le_cameron@umanitoba.ca.** Keep the original copy of the data for lab report write up. Class data for Colilert results will be posted on website as soon as possible. Also, good group data will be posted if your group needs to borrow data for lab report. Cite group or website.

Lab 1 Clean water analysis by standard methods **DATA SHEET**

(available on website as a WORD and Excel document)

Date: _____

Group Number: _____ Group names: _____

Water sample type - circle one : drinking or recreational

Water sample description: _____

Coliform/ <i>E.coli</i> plate counts						
incubation time:			incubation temperature:			
agar plate type	duplicate plate counts					
	water sample			<i>E. coli</i> sample (positive control)		
	undiluted	10 ⁻¹	10 ⁻²	undiluted	10 ⁻¹	10 ⁻²
mFC <i>E. coli</i>						
MI (day light) total coliforms						
MI (day light) <i>E. coli</i>						
MI (UV light) total coliforms						
MI (UV light) <i>E. coli</i>						
Petrifilm total coliforms						
Petrifilm <i>E. coli</i>						

Note: total coliform counts include the *E. coli* countsColony description: mFC, *E. coli* - blue; MI (day light) *E. coli*- bluish grey; MI (day light) coliforms - cream; MI (UV) *E. coli* - blue green; MI (UV) coliforms - blue white; Petrifilm, *E. coli* - blue to red blue/gas; Petrifilm, *coliforms* - red /gas

Defined substrate technology (DST) Colilert® Presence/Absence Test		
Sample	Presence of Yellow color ²	Presence of Fluorescence ²
100 ml water sample		
100 ml distilled water (negative control)		
100 ml <i>E.coli</i> sample (positive control)		

² record as + (present) or - (absent)

LAB REPORT

Data Presentation and Analysis

1. Attach completed data sheet. Indicate on data sheet plate counts used to determine titer.
2. a) Tabulate coliform (if applicable) and *E. coli* (fecal coliform) titer (cells/100 ml) for water sample and positive *E. coli* control for (a) mFC agar, (b) MI agar (day light), (c) MI agar (UV light) and (c) Petrifilm™ coliform/*E. coli* count plates. To determine significant plate count range see criteria below. Footnote a sample calculation for each media.

Criteria for calculation of coliform and fecal coliform density (coliforms/100 ml) (Standard Methods, 20th edition, 1998) for drinking water and water other than drinking water quality:

- (i) Significant counts are membrane filters with 20 to 80 coliform colonies and not more than 200 bacteria colonies. The significant range of fecal coliforms per mFC membrane filter is between 20 and 60 fecal coliforms. Colonies are larger.
- (ii) In good quality water the presence of coliforms should be minimal. In the case where there is no counts greater than 20, use data with coliform counts less than 20 to calculate coliforms/100ml.
- (iii) Conclude coliform colony count as “< 1 coliform/100 ml” if there are no coliforms present on all dilutions.
- (iv) If there are no dilutions with less than 200 bacteria (total number). “Record colony density as confluent growth with (or without) coliforms.”
- (v) Water of other than drinking water quality follows the same rules as drinking water except when you can actually count the number of coliforms on membranes that have greater than 200 bacteria. Calculate the coliform density as usual but condition data with a greater than or equal sign. That is, it is acceptable to have a greater number than 200 total colonies when recording coliform density, just record with greater than or equal sign.
- (vi) Statistical reliability of results. The number of viable coliform counts may be underestimated by the membrane filter technique.

Note: The significant counting range for Petrifilm™ count plates is 15 to 150 (total coliforms including *E. coli*).

- b) State the best quantitative method (membrane filter technique) used in your lab to measure water quality. Explain why.
3. a) Attach a copy of Colilert® class data. Footnote your group’s data
b) Does your Colilert® water sample and positive control results agree qualitatively with data obtained using the quantitative methods?
c) Compared to the MI agar plate, what is the value of the Colilert® assay?
4. Conclude safety of your water sample based on Canadian guidelines. State and cite criteria to support your conclusion.

Questions

1. As stated in Standard Methods for the examination of water and wastewater, 20th edition (1998)⁴ a quality assurance programs must consider all aspects of work in the lab. One of the elements is lab equipment specifications. List **all the lab equipment** that must meet quality control for the membrane filter procedure as performed in your lab. State specifications for any 4 pieces of the equipment (one sentence each).

⁴Franson MH (managing), Clesceri LS, Greenberg AE, Eaton AD, editors. 1998. Standard Methods for the Examination of Water and Wastewater. 20th ed. Washington: American Public Health Association p 9.1 - 9.18

2. Even though *E. coli* 0157:H7 contamination of drinking water is uncommon, outbreaks do occur. Outline lab procedure to confirm suspected *E. coli* 0157:H7 contamination of drinking water.
3. In the second week of July several swimmers reported that they got sick from swimming at Winnipeg beach. Where should the samples be collected and with what frequency to assess water quality?

LAB 2 MICROBIAL ECOLOGY OF COMPOSTING

OBJECT

The object of this experiment is to study soil communities by (1) Biology Ecoplate community analysis and (2) nucleic acid based analysis.

INTRODUCTION

Refer to reference binder for additional information.

Biolog Ecoplate Soil Community Analysis

The Biology ecoplate contains 31 carbon sources in triplicate. The selection of carbon sources is based on high consumption by soil microorganisms (BIOLOG⁵ http://www.biolog.com/mID_productLiterature.html assessed June, 2003). The pattern of carbon source utilization by the soil microorganisms give a “fingerprint reaction pattern” of the soil for a particular time and condition. It has been shown that Biolog plates are a sensitive method to measure change in the soil related to the environment conditions such as temperature (1,2). Each well contains the redox dye tetrazolium violet (clear). The assay is based on the microorganism(s) ability to oxidize the carbon source and in doing so irreversibly reduces tetrazolium violet to a purple insoluble formazan.

Statistical Analysis

Use ANalysis Of VAriance (ANOVA) to determine if temperature has any effect on the microbial community diversity as assayed using BIOLOG Ecoplate. ANOVA is expressed as F-ratio. F-ratio = standard deviation of the group/expected variation of the group. If there is no effect (eg temperature) this value should theoretically be 1. If there is an effect due to temperature the value should be greater than one. How much greater depends on the significant level - 95% confidence or 0.05 significance is an acceptable significant level. <http://www.physics.csbsju.edu/stats/anova.html> , Kirkman, T. accessed June, 2003) or <http://members.aol.com/johnp71/javastat.html#Comparisons> VassarStats, accessed July, 2003). The simplest way to interpret f-test value is to convert to probability (the probability of getting a result that isn't real/true). For example, if the probability is 0.395, there is a 39.5% chance that the difference in microbial functional diversity due to temperature isn't meaningful (due to chance factors alone). In other words, there is a 60.5% probability that the difference in microbial functional diversity is due to temperature. You do not need to calculate ANOVA, just use program available at website cited above. There is a f-test function in Microsoft Excel, unfortunately only two groups can be compared at one time.

Nucleic Acid Based Soil Community Analysis

MO BIO Ultra Clean™ soil DNA kit is used to prepare PCR quality microbial genomic DNA from soil. The kit is designed to remove humic acid which inhibit the PCR reaction. The soil and suspension buffer is added to a tube containing beads. The tubes are extensively vortexed. The cells are lysed and released DNA bound by silica filter. After washing the filter, the bound DNA is eluted. Since the isolated soil DNA contains all DNA present we will use eubacteria primers fD1 (forward primer)

⁵at this website links do not appear until you pass the mouse pointer over the text

AGAGTTTGATCCTGGCTCAG and rD1 (reverse primer) AAGGAGGTGATCCAGCC to amplify bacteria DNA by PCR. fD1 and rD1 are universal bacteria primers, ie designed for most bacteria.(Weisburg et al, 1991). PCR (polymerase chain reaction) protocol is identical to procedure used for the 60.461 Molecular Genetics of Eukaryotes Lab (Court, 2001). PCR is based on repeated cycles of DNA synthesis using high temperature tolerant Taq DNA polymerase. Eppendorf tubes containing PCR reaction mixture are placed in a thermocycler. Temperature changes are controlled and repeated. For each cycle there is a high denaturation temperature where double stranded DNA (ddDNA) is denatured to single stranded DNA (ssDNA). Next the temperature changes to anneal primer and finally the temperature again changes to synthesize DNA. The cycle is repeated until enough amplified DNA is produced, in our case, 36 cycles. The PCR DNA is purified using Minielute PCR purification kit from QIAGEN company. Soil sample bacteria DNA variations are demonstrated by running restriction digested PCR DNA samples on agarose gels, staining and photographing the pattern, ie. restriction fragment length polymorphism (RFLP). If microbial diversity is present, the restriction fragment patterns will vary.

- (1) Burton, DL, Depoe S., Banerjee, MR. 1997. The functional diversity of soil microbial communities in selected Manitoba soils. Manitoba Soil Science Workshop. pp. 48-59.
- (2) Garland, JL & AL Mills. 1991. Classification and Characterization of Heterotrophic Microbial Communities on the Basis of Patterns of Community-Level Sole-Carbon-Source Utilization. *Appl. Environ. Microbiol.* 57:2351-2359.

PROCEDURE

Maximum of 6 groups.

Week 2

For this lab and all subsequent lab students are responsible for field trip information.

FIELD TRIP TO BRADY LANDFILL (CLASS 2) AND COMPOSTING FACILITY

- See appendix for map giving directions to Brady landfill
- Tour starts ~ 1:00 pm day of lab - details given in class
- Compost Sample Collection - Each group collects three different samples with respect to temperature, <40°C, 40-60°C, >60°C (each collected at a different depth).
- In lab experiment start immediately after returning from tour.

Essential student preparation for field trips

This applies to all field trips.

- bring notebook/pen/fine point permanent ink marker
- requested lab supplies
- outdoor wear for all weather, eg. hat, rainwear
- bring rubber boots or hiking boots (whatever is appropriate)
- your own camera (optional)

LAB EXPERIMENTS

Part I BIOLOG EcoPlate™ Microbial Community Analysis

The operation of the P200 pipetman will be demonstrated upon request. The appendix also gives detailed instructions on P200 and P1000 operation. Make sure you know how to read the volume setting. Confirm with the TA that you have the correct setting and know how to use the pipetman before pipetting samples.

1. Each group has three samples collected at three different temperatures (depths). Set up one EcoPlate for each temperature. Each plate contains 31 carbon sources that are inoculated in triplicate.
2. Label each plate with group # (names), sample temperature, incubation temperature*, and compost location.

<u>sample temperature</u>	<u>incubation temperature</u>
<40°C	30°C
40-60°C	45°C
>60°C	55°C

3. For each compost sample add 1.0 g to 99 ml sterile 0.2% water agar solution (10^{-2} dilution). Shake vigorously for 2 min. Transfer 1 ml of the 10^{-2} dilution to 99 ml saline solution (10^{-4} dilution). Mix by shaking.
4. For each compost sample remove the microplate from sterile foil container. Transfer exactly 150 μ l (0.15 ml) of saline diluted sample (10^{-4} dilution) to each well (total of 96 wells - triplicate of 31 carbon sources) of a Biology EcoPlate™ microplate. Be sure to shake the sample dilution frequently during inoculation of microplate. Shake dilution carefully to prevent bubble production. Work as quickly as possible to reduce contamination. Replace microplate cover and incubate at specified temperature* for ~96 hours (Monday). Place plates in a sealable plastic bag. A bucket of water has been placed in the incubators to ensure a moist environment.
5. **Monday:**
 - (i) **Record the number of positive wells** by highlighting only positive wells (+) of figure 1 (provided in triplicate for the three different samples). Carbon source figures do not need to be handed in with data sheet, only with lab report.
Criteria to determine if a well is positive or negative: Compare color density in all wells to control well, A-1. All wells resembling A-1 are negative. If there is a noticeable purple color record as a positive well. Wells with extremely faint color or small purple flecks treat as negative for calculation of functional diversity.

(ii) **Calculate functional diversity** for all triplicate samples. Functional diversity is the percentage of the 31 substrates that were used. Record requested information on DATA SHEET (also available on website as a Word or Excel document) and hand in to slotted filing cabinet in room 414 Buller or email le_cameron@umanitoba.ca before 2:30 pm Monday (Sept 23). Remember to hand in a **COPY of data**. Each group hands in only one copy of data. Biolog Ecoplate class data will be available on the website as soon as possible.

Part II Nucleic Acid Based Microbial Community Analysis

A. DNA extraction from soil

MO BIO Laboratories, Inc. UltraClean™ Soil DNA Kit instruction manual
(Components of solutions are not described in kit. However, procedure is very similar to plasmid DNA isolation. Start with a suspension buffer, add bead solution to break up soil aggregates, lyse cells either by enzyme or alkaline, solution S3 added (not sure of components), sample applied to silica gel column which binds DNA, column washed with buffered ethanol to remove salts, RNA, protein and finally the DNA eluted from the column with Tris buffer. Do not use TE buffer to elute DNA as the EDTA may interfere with PCR reaction.

Repeat the following procedure for each compost sample collected by the group (total of 3 samples, <40°C, 40 - 60°C & >60°C).

Also do a positive control using *E. coli*. Instead of adding 0.5 g soil just add 2 ml *E. coli* to the Bead Solution tube and complete the procedure exactly as described except you do not need to vortex for 10 min.

Wear disposable gloves.

1. Weigh out 0.5 g compost and put into 2 ml **Bead Solution** tube. Gently vortex to mix.
2. Add 60 µl **Solution S1** (buffered suspension solution) and invert once to mix. If the Solution S1 is precipitated put at 60°C to dissolve. Mix before using.
3. Add 200 µl **Solution IRS** (PCR inhibitor removal solution).
4. Secure bead tubes horizontally on a flat vortex. Use lots of masking tape to cross tape tubes to the top of the vortex. Turn on vortex for 10 min.
5. Microfuge (assume room temperature unless otherwise stated) for 1 min. Make sure the tops do not touch and the tubes are balanced. Usually you balance by placing equal weight tubes opposite. You may balance by forming an equilateral triangle with the tubes. Transfer supernatant to a clean microfuge tube.
6. Add 250 µl **Solution S2**. Vortex 5 sec. Put on ice for 5 min.
7. Microfuge for 1 min. Transfer 450 µl to clean microfuge tube. Do not transfer any of the pellet.

8. Add 900 μl **Solution S3**. Vortex 5 seconds.
9. Load 700 μl into **Spin Filter** and microfuge for 1 min. Discard the flow through. Add remaining sample and microfuge for 1 min. Discard the flow through.
10. Add 300 μl **Solution S4** (ethanol wash) and microfuge 30 sec. Discard the flow through. Centrifuge again for 1 min.
11. Transfer spin filter to clean microfuge tube. Add 50 μl **Solution S5** (Tris buffer) to the centre of the white filter membrane. Microfuge for 30 sec. Discard spin filter. Label the DNA sample with group numbers and compost temperature. Proceed immediately to PCR reaction and clean up.

B. PCR Amplification (derived from 60.461 lab manual, D. Court 2003)

1. Get a bucket of ice.
2. Label the lid of each 0.6 ml microfuge tube with your group # and compost temperature. Label one tube with your group # and *E. coli*. Total of 4 PCR reactions per group. You must label the lid, do not label the sides. Add 39 μl of PCR master mix* (prepared and kept on ice) to each tube and immediately put on ice. Add 5 μl of your DNA sample and 4 μl DMSO to your PCR tube, vortex briefly, and leave on ice.

* The PCR master mix contains the following components:

(you could add them individually to the tube, but to save time, they have been premixed by the lab demonstrators)

- 16.9 μl sterile distilled water
 - 5.0 μl 10X PCR buffer (from Gibco-BRL; 200 mM Tris-Cl pH 8.4, 200 mM KCl)
 - 5.0 μl dNTP solution (10 mM each of dATP, dCTP, dGTP, dTTP)
 - 3.4 μl 50 mM MgCl_2
 - 5.0 μl fD1 (forward primer) 50 pmol/ μl
 - 5.0 μl rD1 (reverse primer) 50 pmol/ μl
 - 0.4 μl *E. coli* Taq DNA polymerase (J. Switala)
 - 4.0 μl DMSO (f.c 10%)
2. The lab TA will put the reaction mixtures in the PCR thermocycler machine and collect them the next morning for storage at -20°C .
The thermocycler is programmed to carry out the PCR amplification reaction as follows:
 - step 1: 94°C 3 min (denature)
 - step 2: 94°C 2 min (denature)
 - step 3: 45°C 30 sec (anneal primers)
 - step 4: 72°C 4 min (synthesize DNA)
 - step 5: go to step 2 (repeat cycle 35 times)
 - step 6: 4°C 16 hours (cool sample until someone can put it in the fridge)

Week 3

C. MinElute PCR purification (QIAGEN Instruction Manual)

Repeat the following procedure for each PCR amplification sample.

- Column DNA Binding:** Add 5 volume **PB buffer** to 1 volume PCR reaction. Vortex. Put a MinElute column in the 2 ml collection tube provided placed in a rack. Using a pipetman place the entire sample on the MinElute column (composed of silica gel membrane for binding DNA in high salt buffer). PCR DNA bind to the column. Microfuge for 1 min. Discard flow through. Place column back in the same collection tube.

The binding buffer (BP) provides the correct salt concentration (high concentration of chaotropic salts) and pH (less than or equal to pH 7.5) for adsorption of PCR DNA (as small as 70 bp but removal of primers up to 40 nucleotides) to the MinElute column.
- Column Wash:** Add 750 μl **PE buffer** (contains ethanol) to column. Microfuge 1 min. Discard flow through. Place the column back in the same collection tube. Again microfuge for 1 min. A wide variety of contaminants can be removed at this step: primers, nucleotides, enzymes, mineral oil, salts, agarose, ethidium bromide, etc.
- DNA Elution:** Place the MinElute column in a clean 1.5 ml tube. Add 10 μl EB buffer (10 mM Tris-HCl, pH 8.5) or water to the centre of the column membrane. Wait 1 min. Microfuge for 1 min. Discard column. The flow through is approximately 9 μl purified PCR soil DNA. DNA elution is dependent on low salt buffer and the pH should be between pH 7.5 and 8.5.
- Immediately set up the following restriction enzyme digestion of each sample.

D. Part III Restriction Fragment Length Polymorphism (RFLP) microbial community analysis

- Set up the following restriction digestion in an 1.5 ml microfuge tube for each PCR-DNA sample (three different temperatures and *E. coli* positive control):

To the 9 μl purified PCR DNA sample add:

12.5 μl	sterile distilled water
2.5 μl	10X React2 buffer (Gibco-BRL; 500 mM Tris-Cl, pH 8.0, 100 mM MgCl_2 , 500 mM NaCl)
1 μl	<i>Taq I</i> restriction enzyme (~10 units) [add last]
- Mix by pipetting gently up and down. Spin for a few seconds if you have drops of liquid up the side of the tube.
- Incubate for 1 hr at 65°C.
- Add 5 μl of agarose stop buffer.
- Each group prepares a 1% agarose gel containing 5 μl ethidium bromide using mini-gel electrophoresis apparatus. In one lane add 7 μl 1 kb Plus DNA ladder (from Gibco-BRL). Load agarose gel with as much sample as possible (dependent on well size) - record gel loading pattern as the TA needs a record of loading pattern. On plan, state sample loaded in each well,

group number and student names. Leave beside your gel.

a) Prepare required percentage agarose in 1x Tris-acetate buffer. Add 50 ml* 1x TAE buffer to a 250 ml Erlenmeyer flask. Add stirring bar. Place on heater stirrer. Turn on heat full and turn on stirrer until the bar rotates at medium speed. You do not want lots of bubbles forming while dissolving the agarose. Slowly add 1 g agarose (0.02 x 50) while stirring. Cover loosely with overturned small beaker. Heat mixture until it comes to a boil and the agarose is completely dissolved. Remove from heater stirrer and cool at room temperature to ~55°C.

b) Put on gloves as ethidium bromide is a carcinogen. Add 4 µl ethidium bromide. Swirl gently to mix. Discard ethidium bromide tip in ethidium bromide waste container.

c) Pour agarose into gel holder that has been taped at ends with masking tape and well comb positioned. Allow to set at room temperature for 20 min.

d) Carefully remove well comb and masking tape. Place gel in electrophoresis unit such that the well are toward the negative electrode (black).

e) Add 1x TAE buffer until the surface of gel is covered by ~3 mm of buffer using approximately 250-300 ml 1x TAE. If required, dilute 10x TAE buffer.

f) Load agarose gel with as much sample as possible (dependent on well size). Each sample is loaded using an eppendorf micropipet by holding the pipetman just above the well and releasing sample such that it sinks to the bottom of well.

In one lane add 7 µl 1 kb Plus DNA standard/ ladder (from Gibco-BRL).

g) The power supply should be connected such that the negatively charged DNA will migrate to the positive electrode. The black designates the negative connecting wire and red the positive electrode. Connect black to black and red to red. Turn on power supply and set at constant voltage (80-90 volts). Electrophoresis for 1 to 1 ½ hours. [Most likely the TAs will finish your experiment. Photographs of gels will be available on the website as soon as possible.] TURN OFF power pack before removing agarose gel.

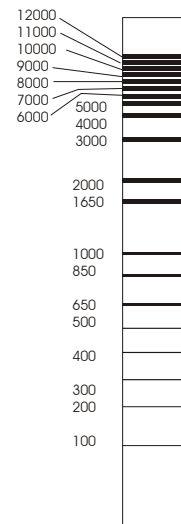


Figure . 1 Kb Plus ladder for linear double stranded DNA.

6. Electrophoresis for 1 - 1.5 hours at 80 - 90 volts.

7. Depending on available time, the demonstrator may need to record* gel electrophoresis data (RFLP). Data will be posted on website as soon as possible after photographing.

*digital photograph using BIO-RAD Gel Doc of restriction enzyme digested samples stained with ethidium bromide and view over UV light.

8. Discard agarose gel in ethidium bromide waste container.

Lab 2 Microbial ecology of composting DATA SHEET

Group Number: _____

Student names: _____

BIOLOG EcoPlate™ Microbial Community Analysis Data						
incubation temperature	replica 1		replica 2		replica 3	
	# positive wells	% functional diversity	# positive wells	% functional diversity	# positive wells	% functional diversity
30°C						
45°C						
55°C						

Include a sample of % functional diversity calculation:

Functional Diversity = Percentage of substrates giving a positive response at specified incubation time.

BiOLOG
EcoPlate™

Microbial Community Analysis

A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D-Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D-Galacturonic Acid	B4 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L-Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine
E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine	E1 α-Cyclodextrin	E2 N-Acetyl-D-Glucosamine	E3 γ-Hydroxybutyric Acid	E4 L-Threonine
F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid	F1 Glycogen	F2 D-Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L-Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1- Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl- amine	G1 D-Cellobiose	G2 Glucose-1- Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl- amine	G1 D-Cellobiose	G2 Glucose-1- Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl- amine
H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine

FIGURE 1. Carbon Sources in EcoPlate

Group #/names: _____

Date: _____

Compost Location: _____

Compost temperature: _____

Incubation temperature: _____

Compost depth: _____

Number of positive well: replica 1 _____ replica 2 _____ replica 3 _____

BIOLOG
EcoPlate™

Microbial Community Analysis

A1 Water	A2 β-Methyl-D- Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D- Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D- Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D- Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D- Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D- Galacturonic Acid	B4 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L- Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L- Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L- Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine
E1 α- Cyclodextrin	E2 N-Acetyl-D- Glucosamine	E3 γ- Hydroxybutyric Acid	E4 L-Threonine	E1 α- Cyclodextrin	E2 N-Acetyl-D- Glucosamine	E3 γ- Hydroxybutyric Acid	E4 L-Threonine	E1 α- Cyclodextrin	E2 N-Acetyl-D- Glucosamine	E3 γ- Hydroxybutyric Acid	E4 L-Threonine
F1 Glycogen	F2 D- Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L- Glutamic Acid	F1 Glycogen	F2 D- Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L- Glutamic Acid	F1 Glycogen	F2 D- Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L- Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1- Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl- amine	G1 D-Cellobiose	G2 Glucose-1- Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl- amine	G1 D-Cellobiose	G2 Glucose-1- Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl- amine
H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine

FIGURE 1. Carbon Sources in EcoPlate

Group #/names: _____

Date: _____

Compost Location: _____

Compost temperature: _____

Incubation temperature: _____

Compost depth: _____

Number of positive well: replica 1 _____ replica 2 _____ replica 3 _____

BIOLOG
EcoPlate™

Microbial Community Analysis

A1 Water	A2 β-Methyl-D- Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D- Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine	A1 Water	A2 β-Methyl-D- Glucoside	A3 D-Galactonic Acid γ-Lactone	A4 L-Arginine
B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D- Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D- Galacturonic Acid	B4 L-Asparagine	B1 Pyruvic Acid Methyl Ester	B2 D-Xylose	B3 D- Galacturonic Acid	B4 L-Asparagine
C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L- Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L- Phenylalanine	C1 Tween 40	C2 i-Erythritol	C3 2-Hydroxy Benzoic Acid	C4 L- Phenylalanine
D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine	D1 Tween 80	D2 D-Mannitol	D3 4-Hydroxy Benzoic Acid	D4 L-Serine
E1 α- Cyclodextrin	E2 N-Acetyl-D- Glucosamine	E3 γ- Hydroxybutyric Acid	E4 L-Threonine	E1 α- Cyclodextrin	E2 N-Acetyl-D- Glucosamine	E3 γ- Hydroxybutyric Acid	E4 L-Threonine	E1 α- Cyclodextrin	E2 N-Acetyl-D- Glucosamine	E3 γ- Hydroxybutyric Acid	E4 L-Threonine
F1 Glycogen	F2 D- Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L- Glutamic Acid	F1 Glycogen	F2 D- Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L- Glutamic Acid	F1 Glycogen	F2 D- Glucosaminic Acid	F3 Itaconic Acid	F4 Glycyl-L- Glutamic Acid
G1 D-Cellobiose	G2 Glucose-1- Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl- amine	G1 D-Cellobiose	G2 Glucose-1- Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl- amine	G1 D-Cellobiose	G2 Glucose-1- Phosphate	G3 α-Ketobutyric Acid	G4 Phenylethyl- amine
H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine	H1 α-D-Lactose	H2 D,L-α-Glycerol Phosphate	H3 D-Malic Acid	H4 Putrescine

FIGURE 1. Carbon Sources in EcoPlate

Group #/names: _____

Date: _____

Compost Location: _____

Compost temperature: _____

Incubation temperature: _____

Compost depth: _____

Number of positive well: replica 1 _____ replica 2 _____ replica 3 _____

LAB REPORT

Data Presentation and Analysis

Part I BIOLOG EcoPlate™ Microbial Community Analysis

Group Data

1. Append completed Biolog EcoPlate diagrams for the three samples (varying temperatures).
2. Append completed BIOLOG data sheet.

Class Data

3.
 - a) Append Class Biolog microbial functional diversity data. Class data is available as an Excel spreadsheet on website to facilitate calculations and chart (graph) presentation. Footnote your group's data. Include class AVERAGE and STDEV (standard deviation) for each temperature.
 - b) Present a bar graph figure with standard deviation^a bars for average % functional diversity vs temperature using Excel spreadsheet of class data to prepare chart. Refer to lab 2 appendix to see instructions to use excel bar graph with standard deviation bars.
 - ^a standard deviation bar length is equal to + and - standard deviation
 - c) Interpret results of average % functional diversity vs temperature graph?
4. Statistically analyze data. Use ANOVA (ANalysis Of VAriance) software available at website (<http://www.physics.csbsju.edu/stats/anova.html>, Kirkman, T. accessed June, 2003) to determine whether temperature has an effect on microbial functional diversity in the compost. Interpret probability with respect to compost temperature. See sample software printout and interpretation that follows. For statistical analysis, only include a completely labelled (experiment/parameters) website printout of **data entry (ANOVA)** and **ANOVA: results**. Highlight f-value and probability. On the results sheet include an interpretation of the probability. Does statistical interpretation support bar graph analysis? No other information is required. Do not calculate ANOVA by hand.

Part III RFLP microbial community analysis

1. Present a completely labelled figure of agarose gel of restriction digested DNA.
2.
 - a) Interpret RFLP data and relate to BIOLOG data.
 - b) What is the advantage of RFLP relative to the BIOLOG assay?
 - c) What experiments should be done to further characterize bacteria in samples from the Brady landfill?

Questions

1. There are two “approaches”⁶ to isolating total DNA from soil. What approach is used in your lab and state advantages compared to the other approach.
2. Even though the soil DNA isolation kit (MO BIO) used in your lab is designed to remove humic acid, what additional experimental step could be carried out to obtain “cleaner” DNA for PCR?

⁶Maier, RM. Pepper, IL, & CP Gerba. 2000. Environmental Sample Collection and Processing. New York: Academic Press. p.181-186

LAB 2 APPENDIX

Microsoft Excel Function procedures:

1. Determine AVERAGE. Highlight cell where you want to record average. Select paste function button, then statistics, then AVERAGE. Or use pull down menu - select Insert, function, statistics, then AVERAGE. A pop-up menu appear. Using your mouse right click the first or last¹ cell of data set. Hold down button and scroll down to the last or first cell in the data set (eg sand). Release button. Click OK on pop-up menu. The average value appears in your selected cell. Repeat for remaining data sets.
¹Best to start with last cell then box on top of data disappears, then reappears.
 OR just use toolbar button (summation sign with drop down menu containing average as automatically selects numerical values above average cell)
2. Determine STDEV (standard deviation). Highlight cell where you want to record standard deviation. Select paste function button, then statistics, then STDEV. Or use pull down menu - select Insert, function, statistics, then STDEV. A pop-up menu appear. Using your mouse right click the first or last cell of data set. Hold down button and scroll down to the last or first cell in the data set (eg sand). Release button. Click OK on pop-up menu. The standard deviation value appears in your selected cell. Repeat for remaining data sets.
 OR just use toolbar button (summation sign with drop down menu containing STDEV under more functions. Select appropriate cells to determine standard deviation.)

Microsoft Excel CHART (bar graph) procedure:

See example graph using 2001 student data.

1. Enter data in spread sheet.
2. Select CHART from INSERT pull down menu.
3. Select chart type: COLUMN. Use default chart sub-type.
4. Click next button. Defaults to data range tab.
5. Select series location, row (most likely) or column. This depends on how the spreadsheet is set up. Put cursor in data range box. On the spreadsheet put cursor on first entry of series (remember average values) and holding mouse button down, drag cursor to last entry. Release mouse button. Range is now entered in the box.
6. Select SERIES tab. Put cursor in category (x) axis labels: box. Put cursor on spreadsheet and select x labels (eg 30°C, 45°C & 55°C) by clicking, holding down button and dragging. X-axis labels are now entered.
7. Click next. CHART OPTIONS menu appears. Under default TITLES tab, enter title and axes labels. Under LEGEND tab, remove check mark from show legend. Unfortunately the title defaults to the top of the graph. After you are finished setting up graph, just select and move below graph. Mostly likely you will need to move the graph up. May require resizing.
8. Click FINISH.
9. Arrange figure title below graph. If required, select to change font size.
10. Insert STANDARD DEVIATION bars: Right click one bar of the series on the graph to select all bars in that series and to bring up menu. Click FORMAT DATA SERIES. Select Y ERROR BARS tab. Select display BOTH. Select CUSTOM. Put the cursor in the + box. On the spreadsheet put cursor on first entry of standard deviations. Holding mouse button down, drag

cursor to last entry. Release mouse button. Range is now entered in the box. Put the cursor in the - box. Repeat the same standard deviation data selection.

11. Click FINISH. Standard deviation bar appear on the appropriate columns.
12. Changes to graph may be made at any time by right clicking the appropriate area of the chart.
13. If the background is grey, double click background and replace grey with white to save ink. You may also change bar colors by double clicking bar(s).

ANOVA...page 1

ANOVA...page 2

ANOVA...page 3

ANOVA...page 4

ANOVA2 - PAGE 1

ANOVA2 - PAGE 2

ANOVA2-PAGE 3

ANOVA2-PAGE4

ANOVA2-PAGE5

LAB 3 PETROLEUM BIODEGRADATION

OBJECT

The object of this experiment is to monitor petroleum biodegradation under different nutrient conditions by following the disappearance of alkanes present in the petroleum via GC analysis.

INTRODUCTION

In this lab you will use the ratio of straight chain hydrocarbons (alkanes) to highly branched hydrocarbons to evaluate degradation of petroleum (figure 1). Midsize straight hydrocarbons, $C_{10} - C_{18}$ are readily degraded. By using the hydrocarbon ratio (straight:branched) this tells us that degradation is occurring. If there is no change in the ratio, hydrocarbon loss is abiotic. However, if there is a decreased ratio then loss is through biodegradation (1,2). Branched chain hydrocarbons are more difficult to degrade and persist longer than the corresponding n-alkanes. Microorganisms have difficulty degrading branched hydrocarbons due to steric effect, ie., microbial enzymes are unable to attach the functional site of the hydrocarbon (3).

Numerous factors affect the biodegradation of petroleum. Oxygen and inorganic nutrients (nitrogen and phosphorous) are important factors that promote biodegradation. In addition, other environmental factors such as pH, water availability, temperature, and salinity affect biodegradation (3).

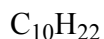
Both the effect of fertilizer (nitrogen and phosphate) and the addition of microorganisms that rapidly degrade petroleum (bioaugmentation) are investigated in your lab. "The introduced microorganisms augment the indigenous population"(4). There is some controversy as to whether the addition of microorganisms enhances biodegradation, so it should be interesting to determine if bioaugmentation works with samples from the Mid-Canada waste management site where this strategy is sometimes used.

T-test

The t-test assesses whether the means of two groups are *statistically* different from each other. The t-test considers the difference between their means relative to the spread or variability of their scores. The formula for the t-test is a ratio. The top part of the ratio is just the difference between the two means or averages. The bottom part is a measure of the variability or dispersion of the scores.

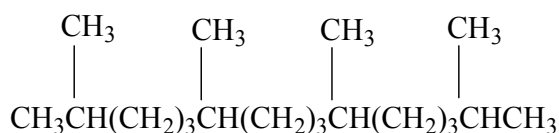
Why use the ANOVA test for Biolog data and t-test for petroleum data? Both tests are interchangeable. However, the ANOVA allows you to compare all variables and obtain one f-value and one probability for null hypothesis. If t-tests are used to determine significant difference, and the variables extensive, many t-values would be obtained and a $P=.05$ for one pair cannot be considered significant. The Biolog assayed three different samples at three different temperatures. Since we wanted to determine if temperature affected % functional diversity of compost, the best test is the ANOVA. In the petroleum biodegradation experiment, we are using one sample and varying the incubation conditions with different additives. We want to see if there is a difference for each additive. The t-test gives us the required information.

Example of straight chain hydrocarbon (alkane), decane:

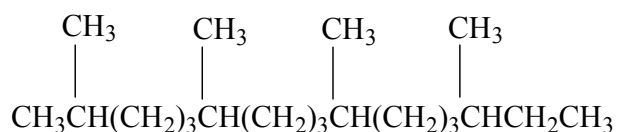


Both pristane and phytane are recalcitrant branched hydrocarbon

Pristane (2,6,10,14-tetramethylpentadecane)



Phytane (2,6,10,14-tetramethylhexadecane)



The internal standard, Squalane

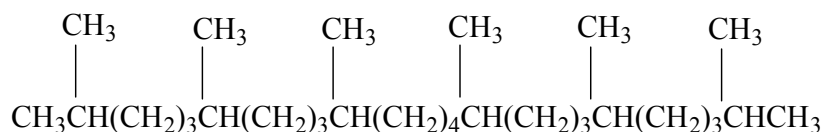


Figure 1. Straight chain hydrocarbons and branched hydrocarbons used in your experiment.

- (1) Bossert, ID, Kosson, DS 1997. Methods for Measuring Hydrocarbon Biodegradation in Soils. In: Hurst, CJ., Knudsen, GR, McInerney, MJ, Stetzenbach, MV, editors. Manual of Environmental Microbiology. Washington, D.C.: ASM Press. p 738-745.
- (2) Christensen, LB, Larsen, TH. 1993. Methods for determining the age of diesel oil spills in the soil. *Ground Water Monit. Rev* 13:142-149. [not in reference binder, not required for lab report write up]
- (3) Maier, R. 2000. Microorganisms and organic pollutants. In: Maier, R, Pepper, IL, Gerba, CP. Environmental Microbiology. New York: Academic Press. p 363-402.
- (4) Walter, MV. 1997 Bioaugmentation. In: Hurst, CJ., Knudsen, GR, McInerney, MJ, Stetzenbach, MV, editors. Manual of Environmental Microbiology. Washington, D.C.: ASM Press. p 753-757.

PROCEDURE**Students work in PAIRS.****Week 4****FIELD TRIP TO MID-CANADA WASTE MANAGEMENT LTD.**

1. Tour of soil remediation site and class 1 landfill (~5 miles south on highway #59). Tour information given in class. Tour starts at ~1:00 pm. See appendix for map directions to remediation site.
2. The class collects six varied samples of diesel-contaminated soil (use trowel to put samples in sterile mason jars). Strict sterile technique not required. Store samples in cooler.
3. In lab experiment start immediately after returning from tour.

Essential student preparation for field trips

Remember to bring:

1. bring notebook/pen/permanent fine point marking pen.
2. any special requested lab supplies
3. outdoor wear for all weather, eg. hat, rainwear, bring rubber boots or hiking boots (whatever is appropriate)
4. your own camera (optional)

LAB EXPERIMENT

Part I Effect of amendments on petroleum (diesel fuel) biodegradation

Culture Preparation and Inoculation

Equipment used is sterile but sterile technique not required. You do not need to use a Bunsen burner but do work quickly.

1. Dump all six samples into a sterile Nalgene tray. Stir with a sterile spatula (large spoon).
2. Each group works with one assigned sample dependent on group number. Set up triplicate flasks of your sample type. Add appropriate components as shown in the following table.

group #	SAMPLE TYPE	soil sample (25 g)	diesel fuel (1.0 ml)	NP ^a stock solution (2 ml)	bioremediation inoculum ^b (volume given in lab)
1	Abiotic Control (sterile). Sterilized twice with an overnight cooling between sterilizations.	✓	✓	✓	✓
2	No NP, no inoculum	✓	✓		
3	NP added , no inoculum	✓	✓	✓	
4	Inoculum added , no NP	✓	✓		✓
5	Inoculum and NP added	✓	✓	✓	✓
6	Time zero, no inoculum, no NP	✓	✓		

^a NP = nitrate, phosphate stock solution as described by P. Fedorak (personal communication)

^b bioremediation inoculum from Mid-Canada Waste Management designed to assist in the breakdown of alkanes

^c internal hydrocarbon standard

2. Incubate at 28°C for 3 weeks in dark except for group 6 - flasks will be frozen at -20°C. Sterile water will be added to all flasks by TA at 3 ml/week or as needed to maintain moisture levels.

Week 7

Part II GC analysis of extracted alkanes

1. Before proceeding with the experiment each group must observe all 6 samples (total of 18 flasks). Record color, odor, turbidity and texture for each sample. If triplicate samples are the same record only once.
2. After all groups have recorded observation data, each group takes their own flasks. Use a spatula to first mix the sample in each flask. Weigh out 5 gm of soil. Use a small beaker to weigh sample. Add 100 μ l squalane, the internal standard. Add 5 g of anhydrous sodium sulfate (soaks/binds water from soil) to the beaker while still on weigh scale. Mix well.
3. Transfer entire contents to Accelerated Solvent Extraction (ASE®) cells as demonstrated by TA. Once the class has prepared all samples take your samples to room 125 (location of ASE).

Room 125 Buller Demonstrations

4. Tutorial of ASE operation and samples loaded. See appendix for information and operation instructions for ASE. Load carousel and press start. Run samples overnight. TA takes sample (10 ml extract) and concentrates if needed. The TA is also responsible for running samples on GC.
5. After samples are loaded there is a demonstration of the rotary vacuum evaporator. See appendix for details.
6. Next there is a demonstration of GC-MS. See appendix for information and operation instructions of GC-MS.
7. Collected student GC data will be posted on the website as soon as available.
8. **Hand in Part I of lab report next week.** Group calculations (see report for details) due Monday Nov 3 by 4:30 pm to allow class data calculations and analysis.
9. Group data will be assembled and published to the website as soon as possible to allow you to do the second part of the lab report.

See Appendix for information:

- Rotary vacuum
- Accelerated Solvent Extraction (ASE®) Application Note 338
- Laboratory Standard Operating Procedure ASE 3545 2-21-01
- Saturn 2000 Benchtop GC/MS

LAB REPORT

Part I : Email of Group Calculations Due Monday Nov 3 by 4:30 pm

1. email Dr. Cameron le_cameron@umanitoba.ca a Microsoft Excel spreadsheet of your group's data and calculations (see attached sample):
 - (a) data entry (include all data)
 - (b) total hydrocarbon for each set of data (triplicate), determine average and standard deviation
 - (c) C17/pristane ratio for each set of data (triplicate), determine average and standard deviation
 - (d) C18/phytane ratio for each set of data (triplicate), determine average and standard deviation
 - (e) total diesel hydrocarbon/squalane ratio for each set of data (triplicate), determine average and standard deviation

Make sure you include clear concise headings and clearly label all information. If you use abbreviations, footnote. Remember to include your group sample parameters. Use significant figures, ie, round to smallest number of significant figures if decimals are involved - do not change whole numbers. Do not round until you have finished your calculations (this applies to ratios - when you hand in your group's calculated ratio values, they will be rounded to 2 decimal places for class data). Sometimes you need to use your own judgement when determining the number of significant figures after the decimal. For example, for ratio averages and standard deviation use 2 decimal places.

2. Report must be handed in on time as data will be collected and published to website to allow you to do the second Part II of the lab report.

Microsoft Excel PASTE FUNCTIONS (see attached sample)

1. Enter headings and data as shown on sample excel printout.
2. Determine SUM (total hydrocarbons). Highlight cell where you want to record average. Select paste function button, then math & trig, then SUM. Or use pull down menu - select Insert, function, math & trig, then SUM. A pop-up menu appear. Using your mouse right click the first or last¹ cell of data set. Hold down button and scroll to the last or first cell in the data set (eg C23). Release button. Click OK on pop-up menu. The sum (total) value appears in your selected cell. Repeat for remaining data sets or use Autosum button. Do not include squalane in total hydrocarbons.

¹Best to start with last cell then box on top of data disappears, then reappears.
OR just use toolbar button summation sign (automatically selects values in column above), press ENTER.
3. Determine AVERAGE. Highlight cell where you want to record average. Select paste function button, then statistics, then AVERAGE. Or use pull down menu - select Insert, function, statistics, then AVERAGE. A pop-up menu appear. Using your mouse right click the first or last cell of data set. Hold down button and scroll to the last or first cell in the data set (eg sand). Release button. Click OK on pop-up menu. The average value appears in your selected cell. Repeat for remaining data sets **or just copy and paste.**
OR just use toolbar button (summation sign with drop down menu containing average)
4. Determine STDEV (standard deviation). Highlight cell where you want to record standard deviation. Select paste function button, then statistics, then STDEV. Or use pull down menu - select Insert, function, statistics, then STDEV. A pop-up menu appear. Using your mouse right click the first or last cell of data set. Hold down button and scroll to the last or first cell in the data set (eg C23). Release button. Click OK on pop-up menu. The standard deviation value

appears in your selected cell. Repeat for remaining data sets **or just copy and paste.**

OR just use toolbar button (summation sign with drop down menu containing STDEV under more functions. Select appropriate cells to determine standard deviation.)

5. TO CHANGE NUMBER OF DECIMAL POINTS. Select format, cell, number, number, change to desired number of decimal points.

6. RATIO DETERMINATION: Highlight cell where you want to record ratio. Type + sign followed by numerator cell location (eg. C17) followed by division sign /, followed by denominator cell location (eg. pristane). Press ENTER. For total hydrocarbons/squalane use total hydrocarbons cell divided by squalane cell.

Lab 3 Petroleum Biodegradation

Oct 24.....

Group #: 4

Group names: Sam Johnston and Beckie Jones

Sample type/parameters: soil, diesel fuel, inoculum added, NP not added

Hydrocarbon	Area		
	A	B	C
C11	2086173	519475	1251028
C12	3545249	1509178	2435272
C13	3850571	1778241	2670107
C14	4872569	2211445	3945956
C15	3445019	1430957	2412791
C16	3780408	1788613	2369725
C17	2470002	1384435	2099670
pristine	3116492	1779758	2437705
C18	2544755	2515078	1754335
pytane	2602860	2515541	1573955
C19	2094251	1791955	1357183
C20	1759903	2194211	1175131
C21	1235114	2086497	839180
C22	584808	676946	386263
C23	329909	325879	200034
squalene	114562	189342	123423
total hydrocarbons	38432645	24697551	27031758
average	30053985		
SD	7349394		
C17/pristane	0.792558428	0.777878228	0.861330637
average	0.81		
SD	0.04		
C18/pytane	0.977676479	0.999815944	1.114603022
average	1.03		
SD	0.07		
total HC/squalene	334.4746338	129.4388408	218.0171848
average	227.31		
SD	102.83		

SD = standard deviation

HC = hydrocarbons

Part II: Class Data Presentation and Analysis Due Monday Nov 10 by 4:30 pm

1.
 - (a) Append chromatogram plots and data sheet either handed out in class or available on website. Make sure you label the chromatograms so they are easily identified.
 - (b) Append a copy of class data of peak areas for triplicate runs, total hydrocarbons and ratios with their respective averages and standard deviation.

2.
 - (a) Perform t-tests to determine whether fertilizing significantly affected the C17/Pristane ratios and the C18/Phytane ratios between treatments. (0.05 probability, 95% confidence). That is, compare group 2 ratios (no inoculum, no NP) to group 3 ratios (no inoculum, NP added). Attach t-test data entry and result sheet for each t-test. At the top of each sheet state the ratios compared. Write in parameters for Group A and Group B. Highlight t value and probability of null hypothesis (the probability that there is no difference between the groups). Interpret t-value and probability with reference to fertilizing at bottom of result sheet. See sample printout that follows.

 - (b) Perform t-tests to determine whether fertilizing significantly affected the C17/Pristane ratios and the C18/Phytane ratios between treatments. (0.05 probability, 95% confidence). That is, compare group 2 ratios (no inoculum, no NP) to group 4 ratios (inoculum added, no NP). Attach student's t-test data entry and result sheet for each t-Test. At the top of each sheet state the ratios compared. Write in parameters for Group A and Group B. Highlight t value and probability of null hypothesis (the probability that there is no difference between the groups). Interpret t-value and probability with reference to microorganism inoculum at bottom of result sheet. See sample printout that follows.

Use T-test software available at website <http://www.physics.csbsju.edu/stats/t-test.html> Kirkman, T. accessed July 2003 or <http://members.aol.com/johnp71/javastat.html#Comparisons> VassarStats, accessed July, 2003)) to determine the probability. Do not calculate T-test by hand.

3.
 - a) Which treatment demonstrated the greatest loss of total hydrocarbons? Compare total hydrocarbons for group 1,2,3,4&5 results to time zero (group 6 results). Support your answer with numerical and graphical data.

 - b) Which treatment demonstrated the greatest biodegradation, as indicated by change in C18/Phytane ratio compared to sterile control? Support your answer with data. Is this the expected result? Explain why citing literature.

 - c) What could be done to enhance bioremediation at the location where the soil was collected (Mid-Canada Waste Management Ltd)? Take into consideration what the company already does (if known). List a minimum of 4 enhancement procedures. Explain why each enhances. Indicate the procedure with the most potential for improvement.

t-test- page 3

t-test2 -page1

t-test2-page2

t-test2-page4

LAB 4 DETERMINATION OF TERMINAL ELECTRON ACCEPTING PROCESSES IN SEDIMENTS

OBJECT

The object of this experiment is to (i) monitor dissolved oxygen profile of sediment, (ii) to quantify microorganisms in sediment using fluorescent staining and (iii) correlate microorganisms with oxygen profile.

INTRODUCTION

DAPI (4',6'-diamidino-2-phenylindole) is a fluorescent nucleic acid binding dye that stains both prokaryotic and eukaryotic cells. Although the DAPI stain is not specific for a particular group of bacteria, staining of environmental samples does provide information about the localization and number of microorganisms in the soil.

For information on measurement of dissolved oxygen in sediment see page 61 (end of lab 4).

For additional information see reference binder (on 1 h reserve in the Science and Technology Library).

PROCEDURE

MAXIMUM OF 6 GROUPS

Week 5

FIELD TRIP TO FORT WHYTE CENTRE

1. Sample collection of sediment at Fort Whyte Centre from a swamp area. Possible future sampling sites, marsh or lake. <http://www.fortwhyte.org/main.htm>. Tour information given in class (map, time leaving (~1:00 pm), etc.). Each student is required to pay ~ \$2.50 entrance fee to Fort Whyte Centre.
2. In field measurement: Part I Dissolved oxygen profile of sediment using the Flett Research Ltd. Oxygen Meter. Read oxygen meter operation before attending field trip. See end of this lab, p 61. Measurement information will be given on site.

Essential student preparation for field trips

1. bring notebook/pen/permanent fine point marking pen.
2. any special requested lab supplies
3. outdoor wear for all weather, eg. hat, rain wear
4. bring rubber boots
5. your own camera (optional)

Field Trip Experiment

Week 5

Part I Dissolved oxygen profile of sediment (field trip)

Experimental details given on site.

- Take a sediment sample using a large corer.
- Next each group takes a small corer* to take subcores from the large core at 5 cm depth. From the small core extrude sediment in 5 mm increments by pushing plunger in from bottom (wrong end). Cut three 5 mm fractions off with razor blade. Put each slice into a scintillation vial containing 10 ml 2% NaCl:95% ethanol.
* Use a small syringe (~10 ml) that has been cut (pointy end), plunger removed.
- Each group is sampling an identical core.
- Measure dissolved oxygen using Flett Research Ltd. Oxygen Meter - see instructions at end of this lab. Measure dissolved oxygen of Na₂S standard (no dissolved oxygen) and a sample of water saturated with oxygen or surface water.
- Record temperature and pH of water at sampling time.
- Bring samples back to lab.

LAB EXPERIMENT

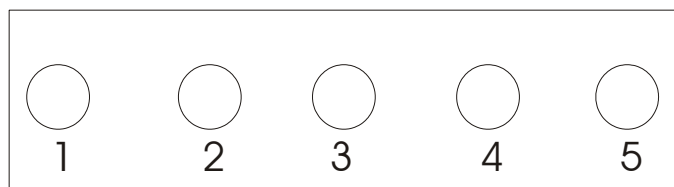
Part II DAPI staining

Use THREE sediment samples (top, middle and lowest depth) to stain and repeat the following procedure for each. Also include a positive control (either *E. coli* or *Rhodospirillum rubrum*) and negative control (sterile PBS - no stain added).

A. Preparation of sample for slide⁷.

1. Vortex sample scintillation vial. Before sediment can settle, transfer 0.1 ml sample from scintillation vial and place in an Eppendorf tube. Add 900 μ l PBS. Attach tubes horizontally to a flat top vortex. Vortex at maximum speed for 10 min.
2. Sonicate each sample for 1 min. Do not waste time when sonicating as this is the “bottleneck” step.
3. Let sit for 10 sec.
4. Remove ~0.8 ml sample from the top and place in an eppendorf tube.
5. Microfuge for 8 min at room temperature (maximum speed).
6. Aspirate off supernatant or remove with P1000. Add 1 ml sterile PBS and mix. Again microfuge for 8 min. Aspirate off supernatant and resuspend pellet in 1 ml 0.1% gelatin (made with distilled water, heated to dissolve and filter sterilized). Vortex well to mix.
7. If your sample is too concentrated, you may need to dilute your sample 2-fold before spoting.
8. Spot 15 μ l of each of the three sediment samples and controls onto three slides and dry at 37°C. Spot 1 positive control (either *E. coli* or *R. rubrum*), spots 2 - 4 top to lowest sediment, and spot 5 negative control (sterile PBS).

Spot 15 ul of each sample on slide



⁷Nierzwicki-Bauer, SA. Nucleotide Probes Workshop. Rensselaer Polytechnic Institute. Troy, NY.

B. Fixing of sample on slide.

CAUTION: All steps involving formaldehyde should be carried out in fumehood (slide staining rack over bucket).

8. Add drops of EtOH/formaldehyde (90/10, v/v) solution to slide. Add as little as possible but still cover dried sample. Incubate at room temperature for 5 min. Do not let dry.
9. Drain excess fixative.
10. Cover slide with distilled water. Let stand 2 min. Drain. Repeat once more.
11. Dry slide at 37°C. Store at -20°C.

The TA will allot TIME SLOTS for next weeks lab.

Week 6C. Staining

12. Add drops of DAPI (0.33 µg/ml 4', 6-diamidino-2-phenylindole in 50% ethanol) stain until dried cells are covered. Incubate 5 min at room temperature.
13. Rinse with distilled water. Drain. Dry slide at 37°C.

D. Epifluorescent microscope demonstration and operation as directed in lab.

14. Select one good slide and view using epifluorescent microscope (x1000 magnification). Near UV (blue filter with 515W barrier) is used for DAPI. No ring is required on filter. Cells fluoresce yellow.
OR
U2 (UV light) filter and 420 K barrier. Cells fluoresce pale blue but due to bright background a ring is required on filter to reduce the intensity of the light.
15. Using only one slide, record the degree of fluorescence as +, +/-, or - of three randomly selected views for each sample (control and three different depths). It is important that you consider only microorganisms that fluoresce, usually regular in shape. Do not consider the contribution of fluorescent debris such as pollen, plant debris, etc.
+ = lots of fluorescing cells, +/- = few cells fluorescing and - = no cells fluorescing

LAB REPORT

Data Presentation and Analysis

1. List observations taken in the field (Fort Whyte) of the core. Include change in color, consistency of core, possible odor, and other notable features.
2.
 - a) Present a figure of calibration curve, y-axis (probe signal) vs dissolved oxygen concentration of x-axis. This is a two point standard curve of known oxygen concentration relative to oxygen probe readout (probe signal). Zero oxygen is one point (1 M Na₂S bubbled with nitrogen to remove all traces of oxygen, zero oxygen) and a standard of saturated oxygen concentration is the other point. Include slope value.
 - b) Present a table of raw data (oxygen probe un-calibrated numbers, mV) and oxygen concentration (mg/L) (signal readout converted to oxygen concentration using standard curve, $y = mx+b$) correlated to depth). Printout of computer spreadsheet is acceptable. Remember to footnote sample location, date, temperature, and pH.
 - c) Present a figure of oxygen concentration with depth (ie oxygen profiles).
3.
 - a) Present a table of relative number of DAPI stained fluorescent cells/microscope field (randomly picked). For each sample you require triplicate microscope field samples. Record as +, +/- and - relative degree of fluorescent cells for all samples. Remember to include a column for sediment depth and oxygen concentration in table.
 - b) Comment on the number of DAPI stained microorganisms relative to oxygen profile. Are your results as expected? Explain why/why not.

Question

1. FISH technique is used to identify the “community structure”⁸ of specific groups of bacteria. What problems are associated with FISH technique? How did Ravenschlag et al (2000) attempt to overcome these problems when studying the community structure of sulfate-reducing bacteria in sediment?

⁸Santegoeds, CM, Damgaard, LR, Hesselink, G, Zopfi, J, Lens, P, Muyzer, G, de Beer, D. 1999. Distribution of Sulfate-Reducing and Methanogenic Bacteria in Anaerobic Aggregates Determined by Microsensor and Molecular Analyses. *Appl. Env. Micro.* 65: 4618-4629.

Instructions for Flett Research Ltd. Oxygen Meter

The instrument is designed to measure the concentration of oxygen in water and sediments. The included sensor is a 0.001 inch diameter teflon coated platinum wire which has been imbedded in a slightly flexible plastic rod. The end of the platinum wire is bare and directly contacts the aqueous medium. Since there is no covering membrane, the electrode can be repeatedly forced through most sediments with little or no damage to the electrode. The reference electrode (not included) should be a Ag/AgCl type which is often used as a reference in pH measurements (in fact people usually use the reference half of a functioning pH combination electrode). When investigating sediments, the reference electrode is positioned as close to the platinum electrode as practical (2 -20 cm) but the reference is not immersed into the sediments because sulfides may poison it and/or plug the junction. Where sulfides are present in the water column, double junction reference electrodes should be used.

The digital meter reading on the liquid crystal display (LCD) is proportional to the oxygen concentration. An analog recorder output from the meter (available via jacks on the back of the meter) can also be sent to a recorder that has an input range of 0 - 100 mv. The recorder output (in mv) will be approximately 1/10 the reading on the LCD.

The meter sensitivity can be increased by rotating the gain control clockwise - full rotation of 270 degrees will increase the meter reading by about a factor of six times. The recorder output will increase proportionally.

Typically, this meter/platinum electrode combination is used to measure oxygen gradients in sediments.

Procedure:

1. Calibration: A bottle is half filled with a sample of water overlying the core, the bottle is capped and vigorously shaken to ensure saturation with oxygen, and then both electrodes are immersed into the saturated sample and the meter reading allowed to stabilize for about 1 min. The meter reading for oxygen saturated water is recorded. The gain setting may be altered at this time to produce a more convenient reading, but, once it is adjusted, the new reading must be recorded as the saturated value. The gain setting must not be altered during subsequent measurements which are being related to the initial calibration.
2. The zero oxygen reading is often assumed to be the lowest reading obtained when measuring an oxygen profile in a core. Alternately, water from above the core can be deoxygenated by bubbling with oxygen free nitrogen and then the electrode reading taken. Most people linearly interpolate between the saturated and zero oxygen values to determine intermediate oxygen concentration observed in a depth profile.
3. Measurement: Sediment profiling for oxygen is most easily done on intact cores of 2 inches or greater diameter contained in acrylic plastic core tubes. Such cores can be firmly clamped in a stand and permit the use of other clamps to hold the electrodes (particularly the platinum) accurately at a particular core depth with no movement. Stable measurements are difficult to obtain with a shaking platinum electrode. It is possible, however, to obtain useable profiles if the electrode is slowly and steadily pushed into the sediment by an electric drive device. The profile should start at a distance of several cm above the sediment surface and continue downward until the readings become constant. When manually advancing the probe discrete distance intervals, the reading at each new depth should stabilize within about 1 minute. Low and constant readings are usually obtained by the time the platinum electrode has penetrated to a depth of several centimeters of the core.

Notes:

1. It may be necessary to standardize with oxygen saturated water between cores depending upon the particular circumstances of your measurements. If the sensitivity of the platinum electrode is significantly lower than in previous calibrations, the tip of the electrode can be wiped with a tissue, or if that doesn't increase the response, then the tip can be very lightly sanded with 600 grit sandpaper. Up to 1 cm of the probe can be sanded away before function is lost. If sanding causes an inordinate increase in sensitivity, an air bubble in the electrode's plastic body may have been exposed; further light sanding will remove the pocket and restore normal readings.
2. There is a conductimetric component to meter readings. In soft freshwater it is usually small i.e. about 10% of the saturated oxygen reading in the same water. In hard water or seawater the component may be larger. It is for this reason that saturated water calibrations should be done on the same water from which the core was taken. The conductimetric component has not been a problem in the past but it must be accounted for when using these membraneless platinum electrodes. Large changes in the distance separating platinum and reference electrodes should also cause changes in the conductimetric readings but in practice this does not appear to be a significant problem because the interprobe distance does not change much during typical measurements.

TYPICAL READINGS

When shipped, saturated tap water gave a reading of about 115 units while deoxygenated water (bubbled with commercial grade nitrogen) yielded a value of about 14 units (at 24°C and gain at minimum). Your values may differ somewhat from these but there should be large changes between oxygenated and deoxygenated water.

With the meter on but no electrodes connected, the LCD should give a result close to zero and not much dependant upon the gain setting ie 0 +/- 5 units. The reading is temperature dependant, however, and will drop about 1 unit per degree celcius drop in room temperature (at minimum gain setting). Conversely, temperature increase will cause an increase in the readings. These temperature related changes will appear larger as the gain is increased. Because of the above, it is strongly recommended that oxygen measurements be made in a reasonably constant temperature environment and that the instrument be kept out of direct sunlight.

The potential across the reference and platinum electrode jacks should be 0.66 v when measured with a high impedance voltmeter and this should remain constant from day to day.

If the LCD or voltage readings are significantly different than those listed previously, then check the batteries (5 * 1.5 v alkaline AA cells and 1 * 9 v alkaline battery). In particular, the single 1.5 v cell must be nearly fully charged because it powers a 1.22 v reference used in the polarizing circuit - replace it when the voltage falls below 1.4 volts. The other 1.5 v cells should be replaced when they fall below 1.3 volts. The 9 v battery should be replaced when potential is below 7.5 volts.

INTERNAL ADJUSTMENTS

Adjustments are to be performed in the order listed, with electrodes disconnected and meter turned on and equilibrated to room temperature.

1) If rotating the external gain control clockwise from minimum to maximum causes more than about 5 units of change on the LCD, the 10 turn potentiometer next to the 3130 integrated circuit (on the smaller printed circuit board) should be adjusted such that less than 5 units of change occur on the LCD when the is moved from the minimum to maximum. [Note: Due to its position, it is impossible to use a screwdriver to make the adjustment without unfastening the printed circuit board. Rather than unfastening the board, it will be easier and faster to gently rotate the adjustment screw on the potentiometer with a pair of needlenose pliers.] The recorder output will be similarly affected.

2) If, after performing adjustment 1 above, the meter reading departs further from zero than desired, then the 10 turn potentiometer next to the 741 integrated circuit may be adjusted so that the reading is close to zero. The external gain control should be in the fully clockwise position when making this adjustment. The recorder output is also affected by this potentiometer adjustment.

3) If the LCD meter and the recorder outputs do not correspond to the ratio of 1000 LCD units/100 mv recorder output, then the 10 turn potentiometer on the underside of the LCD circuit board may be adjusted. The adjustment screw can be seen protruding past the edge of the board in close proximity to the ON/OFF switch. This adjustment affects only the LCD meter; it has no effect upon recorder output.

ELECTRODE HOOKUP

The red wire on the platinum electrode should be connected to red jack marked 'Pt' on the rear of the meter. The green wire on the platinum electrode is a shield and should be connected to the green jack marked 'Ref'. The reference electrode should also be connected to the green jack marked 'Ref'.

LAB 5 COMPETITION BETWEEN ANAEROBES IN A WASTEWATER TREATMENT PLANT: The impact of sulfate reducers on methane production

OBJECT

The object of this experiment is study the competition between sulfate reducing bacteria and methanogens in a sample from an anaerobic digester, by examining the effects of substrates and inhibitors on methane production as measured by gas chromatography.

INTRODUCTION

For additional information refer to articles in reference binder (on 1 h reserve in the Science and Technology Library):

PROCEDURE

Week 8

FIELD TRIP TO NORTH END WASTEWATER TREATMENT PLANT.

1. Tour time, ~1:30 to 3:00 pm. Tour information given in class. See appendix for map directions to North End wastewater treatment plant site.
2. Collect 3 L sludge from an anaerobic digester.

Essential student preparation for field trips

Remember to bring:

1. bring notebook/pen/fine permanent marking pen
2. any special requested lab supplies
3. your own camera (optional)

LAB EXPERIMENT

Part I Anaerobic culture preparation

- Each group adds 8.9 ml of well mixed sludge to each of six tubes using wide mouth pipettes.
- Add 0.1 ml of resazurin indicator to each tube.
- Each group sets up two treatments in triplicate (total of 6 tubes). Add appropriate components as indicated in the following table to each of triplicate tubes. The final volume in all tubes is 120 ml.

group #	sample conditions	distilled water (0.5 ml)	2 M sodium sulfate (0.5 ml)	1 M BESA ^a (0.5 ml)	1 M molybdate (0.5 ml)	1 M sodium acetate (0.5 ml)	1 M methanol (0.5 ml)
1	control	✓ (add 1 ml water)					
	sulfate	✓	✓				
2	BESA	✓		✓			
	molybdate	✓			✓		
3	acetate	✓				✓	
	acetate + sulfate		✓			✓	
4	acetate + BESA			✓		✓	
	acetate + molybdate				✓	✓	
5	methanol	✓					✓
	methanol + sulfate		✓				✓
6	methanol + BESA			✓			✓
	methanol + molybdate				✓		✓

^aBESA = bromoethane sulfonic acid

- Seal the Balch tubes with butyl-rubber serum stoppers and then seal with aluminum crimps (metal rings).
- Incubate your tubes at 37°C for 1 week.
- Schedule a time for week 9 GC analysis(class will end early to allow groups to be scheduled).

Week 9

Part II GC measurement of % methane

Each group is assigned a time for GC analysis.

Use the GC to measure methane in the headspace of each incubation tube.

1. Prepare a syringe as per TA demonstration.
2. Inject a 0.1ml sample of the headspace in your tubes into the GC as demonstrated.
3. Repeat twice to obtain three measurements for each sample.
4. Record peak areas for each injection for each of the six tubes.

CAUTIONS:

- Wear gloves for all experimental manipulations.
- All additions to tubes must be performed in the fume hood.
- Remember to discard intact syringe with needle attached without replacing needle cap in sharp container.
- Do not take syringe and needle apart before discarding.
- Handle syringes with **extreme care**.

LAB REPORT

Part I: Group Calculations - due Nov 13 by 2:30 pm (hard copy of entire Part I plus email copy of excel spreadsheet data and calculations)

1. Append raw data (chromatogram) for the methane analysis of your samples.
2.
 - a) Tabulate Standard Curve data from the data provided (standard curve analysis performed by TA prior to lab).
 - b) Present a figure of the standard curve of peak area (y axis) vs % methane (x axis).
 - c) Determine slope and straight line formula ($y = mx + b$). (Either by hand or computer generated.)
3. Attach a Microsoft Excel spreadsheet of your group's data and calculations. Use formulae for calculations - copy and repeat - facilitates checking of calculations. See lab 3 for Microsoft Excel function operation. See sample spreadsheet of group data.
 - (i) Using the slope and intercept from the standard curve, calculate the % methane from the peak area for each of the triplicate analyses of your 36 tubes.
 - (ii) Calculate and tabulate the average and standard deviation of % methane for your group's two treatments tested.

Make sure you include clear concise headings and clearly label all information. If you use abbreviations, footnote. Remember to include your group's cultures sample conditions. Use significant figures, ie, round to smallest number of significant figures if decimals are involved - do not change whole numbers. Do not round until you have finished your calculations or if you do round to one more decimal place than significant. Sometimes you

need to use your own judgement when determining the number of significant figures after the decimal. For example, for ratio averages and standard deviation use 2 decimal places.

2. Report must be handed in on time. As data will be collected and published to website to allow you to do the second Part II of the lab report. Class data is available as an Microsoft excel spreadsheet to facilitate generation of a bar graph chart.

Part II Class Data Analysis - due Nov 20

1. Append a copy of class data, averages and standard deviations.
2.
 - a) Perform T-tests to verify if the effects of adding sulfate, BESA, molybdate, acetate or methanol on methane production are as expected¹ (total of 5 T-tests). For each T-test compare single condition to control (water). Attach only ONE example of Student's T-test data entry and result sheet. At the top of sheet state the conditions compared. Write in parameters for Group A and Group B. Do not calculate T-test by hand. Use T-test software available at website, <http://www.physics.csbsju.edu/stats/t-test.html> (Kirkman, T. accessed June, 2003) or <http://faculty.vassar.edu/lowry/ank3.html> (VassarStats, accessed July 2003)
 - b) Present a table with columns for single treatments, T-values, probabilities, and an interpret column (indicate if class data agrees with expected effect of culture condition by interpreting probability - use 95% significance).

¹Expected results:

- Adding **acetate or methanol** should increase methane production. Both methanogens and sulfate reducing bacteria use acetate.
 - Adding **sulfate** should decrease methane production because it will allow the sulfate-reducers to out compete the methanogens for common substrates.
 - Adding **molybdate** should increase methane production because it inhibits sulfate reducers, thereby allowing the methanogens to be more competitive against the sulfate reducers that are in the sludge.
 - Adding **BESA** should inhibit the methanogenesis (almost completely) since it is a selective inhibitor of methanogens.
3.
 - a) Use excel bar chart (similar to lab 2) to generate a figure of average % methane values vs treatments (all class data). Include double standard deviation bars (all data).
 - b) Analyze double treatment data from chart. For each double treatment compare to water, stating whether there is a significant increase or decreased. Also state if this agrees with expected results.

continued on next page

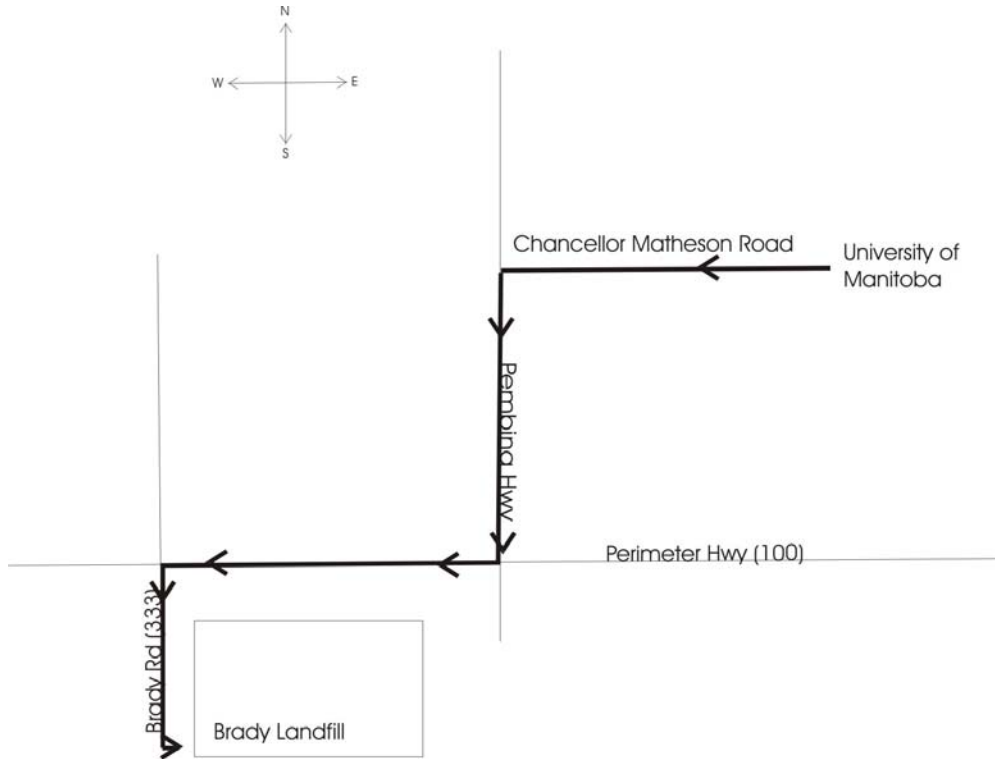
Questions

1. Santegoeds et al⁹ (1999) used a methane microsensors to detect the presence of methanogens in wastewater. Explain why they used a microsensors instead of just measuring methane production in the tube air space.
2.
 - a) Outline how to prepare a 1 M solution of methanol. Methanol has a MW = 32, density = 0.791 g/ml and available as a 99% solution. Include all details.
 - b) For culture sample condition (molybdate) determine the final concentration of molybdate in the culture tube. Show calculations.

⁹Santegoeds, CM, Damgaard, LR, Hesselink, G, Zopfi, J, Lens, P, Muyzer, G, de Beer, D. 1999. Distribution of Sulfate-Reducing and Methanogenic Bacteria in Anaerobic Aggregates Determined by Microsensor and Molecular Analyses. *Appl. Env. Micro.* 65: 4618-4629. <http://aem.asm.org/cgi/reprint/65/10/4618.pdf>

Sample page for **excel spreadsheet** of group data

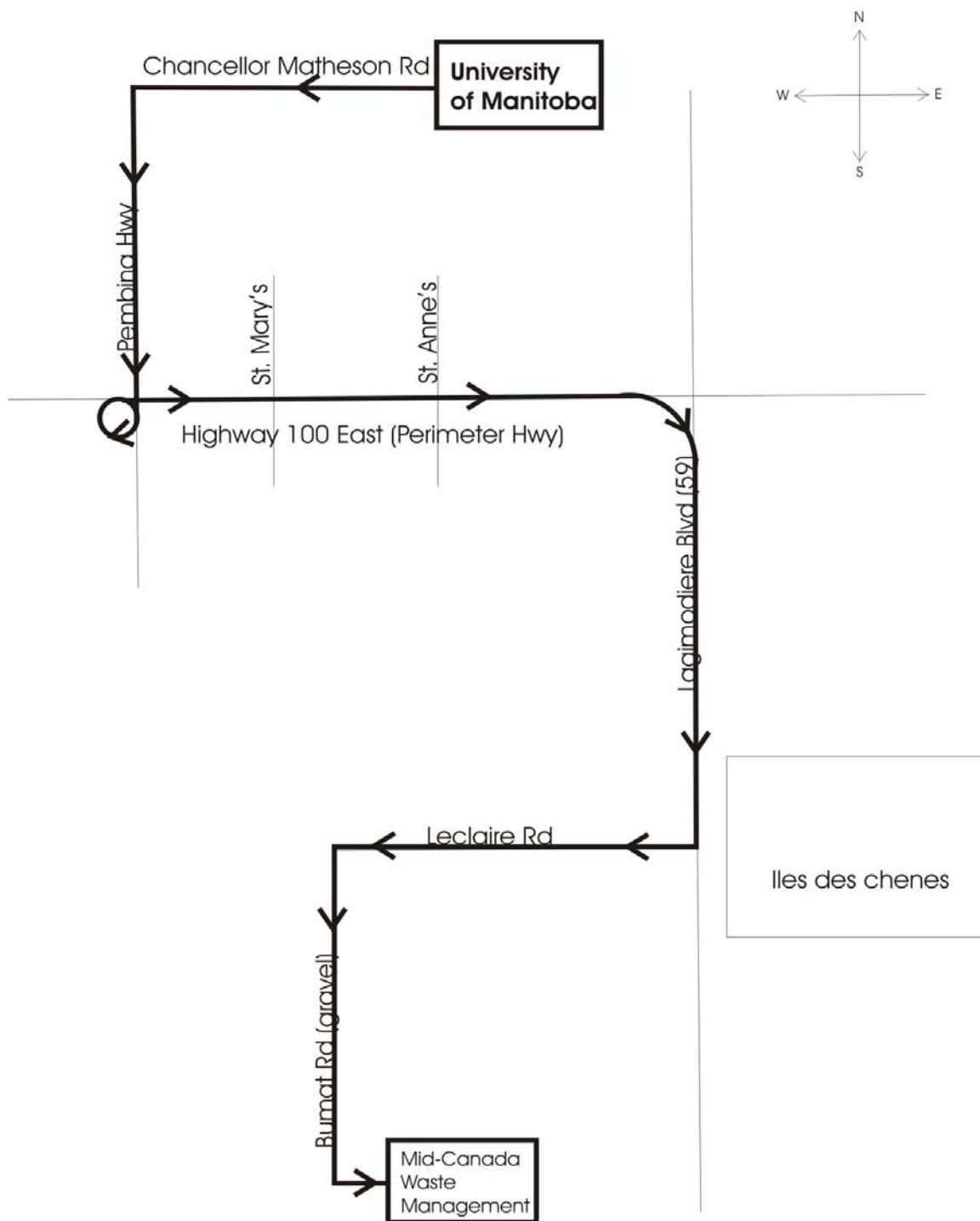
APPENDIX



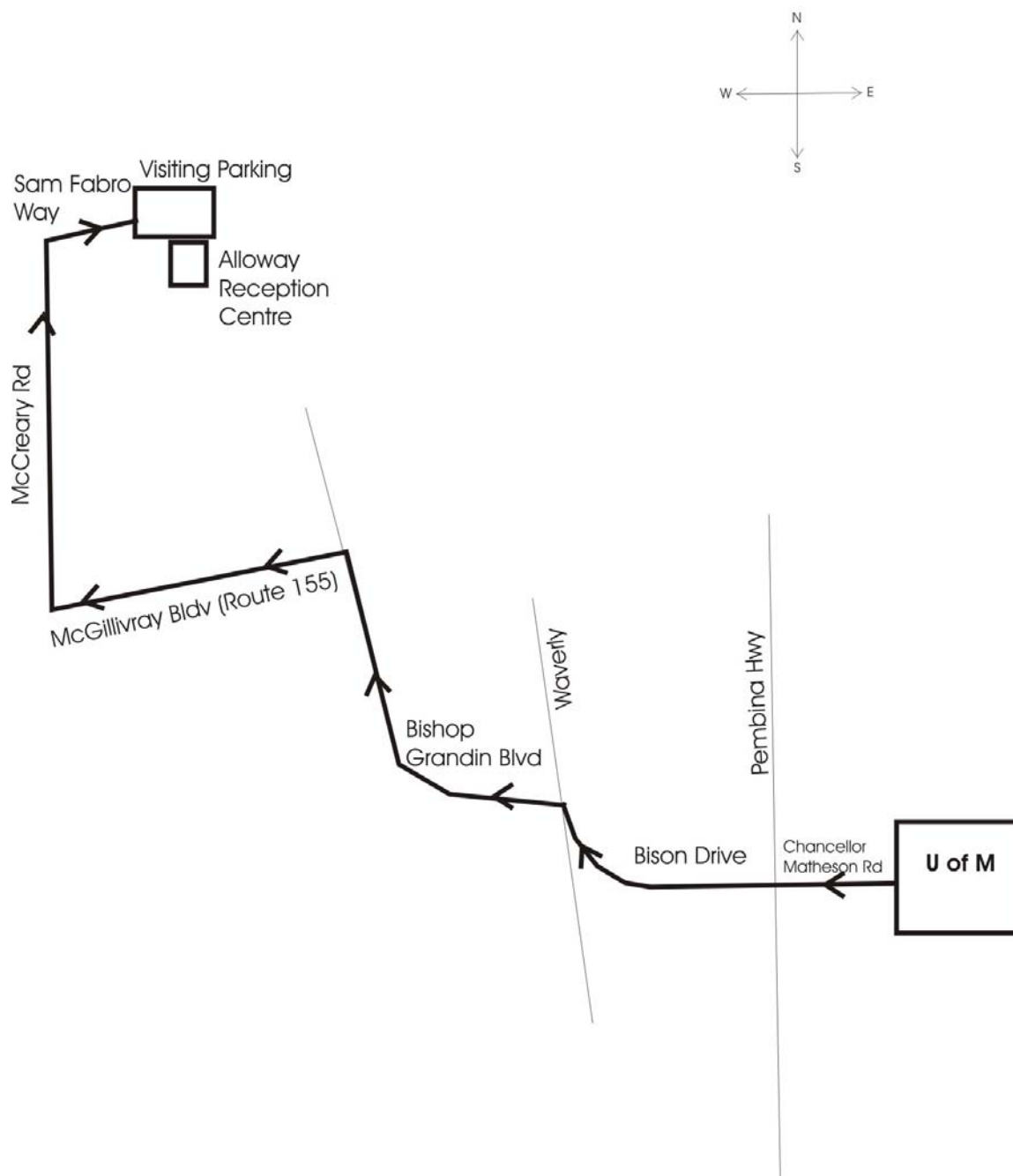
Directions to Brady Landfill

1. Go south on Pembina Highway (Hwy), go past the “log cabin” McDonalds.
2. Take Perimeter Hwy west to Brady Road, just after the landfill, you will see the landfill to your left.
3. Turn left on Brady Road, Go south until you come to the entrance of Brady Landfill - to your left.

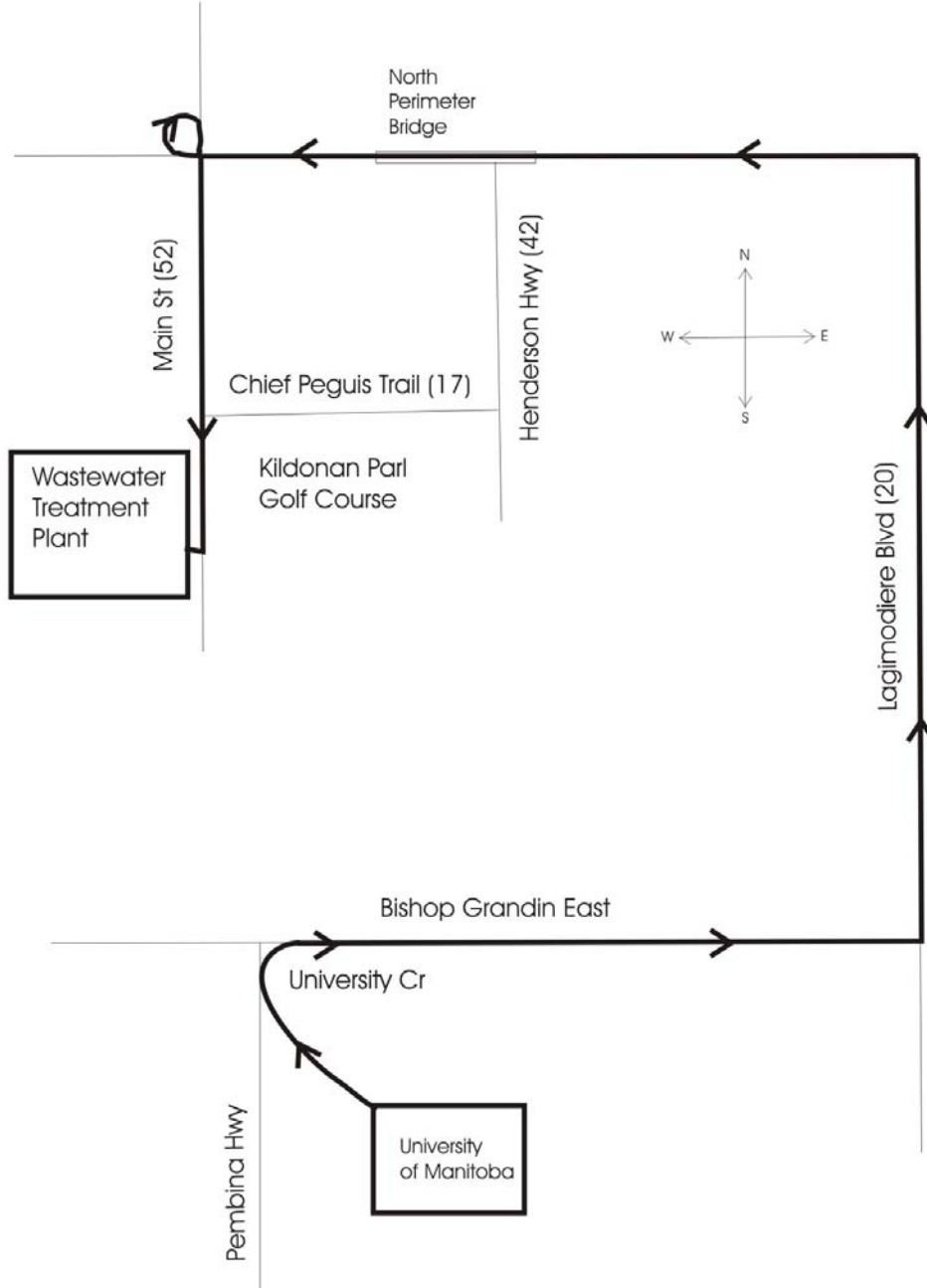
Mid-Canada Waste Management



Fort whyte Centre - 1961 McCreary Road



North End Sewage (Wastewater) Treatment Plant - 2230 Main Street



Media and Solutions

M endo Broth MF®; per liter; 1.52 g yeast extract, 5 g casitone, 5 g thiopeptone, 10 g. tryptose, 12.5 g lactose, 0.1 g sodium deoxycholate, 4.4 g dipotassium phosphate, 1.4 g monopotassium phosphate, 5 g sodium chloride, 0.05 g sodium lauryl sulfate, 2.1 g sodium sulfite, 1.05 g bacto basic fuchsin, pH 7.2. Add 2 ml per 47 mm petri plate containing absorbent pad.

m FC Agar: 10 g tryptose, 5 g proteose peptone, 3 g yeast extract, 12.5 g lactose, 1.5 g bile salts, 5 g sodium chloride, 15 g agar, 0.1 g analine dye, pH 7.4. Procedure: Suspend 52 g in 1 liter distilled water and to boiling to dissolve completely. Add 10 ml 1% Bacto Rosolic Acid in 0.2 N NaOH solution and continue to heat for 1 min. Cool to 50°C, pour plates. 5 ml/50 x 9 mm tight fitting petri plates.

Rosolic Acid pH indicator - powder, FW = 290.3, pH 6.8 (yellow to orange) - pH 8.2 (red)
 $C_{18}H_{14}O_2$

NP solution: 420 ml 10% K_2HPO_4 , 180 ml 10% KH_2PO_4 , 60 g NH_4NO_3 per liter. Should be pH 7.3. Filter sterilize.

PIPETMAN OPERATION

In your lab, you have available three different pipetmen depending on the lab. If you look at the top of the plunger it states the size of the pipetman. P20 measures accurately from 2 μl to 20 μl . P200 measures accurately from 20 μl to 200 μl . P1000 measures accurately from 100 μl to 1000 μl . Never turn the pipetman above the maximum volume; 20 μl for P20, 200 μl for P200, and 1000 μl for P1000 as this breaks the pipetman. The scale on the pipettor is read different for each type - refer to Figure 5 for an example of how to read the scale.

(Excerpted from Gilson pipetman operation manual.)

1. Setting the volume: The required volume is set on the digital volumeter by turning the knurled adjustment ring (Figure 7-2A). When the volumetric setting is increased, it is necessary to go about 1/3 of a turn above the desired setting and then come back to the exact value. When the volumetric setting is decreased the desired value may be selected directly. The volumeter display is read from top to bottom in μl for P20 and P200 and ml for P1000 (Figure 7-2).
2. Place a disposable tip on the shaft of the Pipetman. Press on firmly with a slight twisting motion to ensure an airtight seal. Depress the push-button to the first positive stop (Fig. 7-3A). While holding the Pipetman vertical, immerse the tip 2-4 mm into the sample liquid. Release the push-button slowly to draw up the sample (Fig. 7-3B). Wait 1 to 2 seconds, then withdraw the tip from the sample.
3. To dispense the sample, place the tip end at a 10-45° angle against the inside wall of the vessel and depress the push-button SMOOTHLY to the first stop (Fig 7-3C). Wait 1 to 2 seconds and then depress the push-button completely to expel any residual liquid (Fig. 7-3D). With the push-button fully depressed, carefully withdraw the Pipetman, sliding the tip along the inside wall of the tube. Release the push-button. Remove the used tip by depressing the tip ejector button (Figure 7-1F).



Figure 7: Gilson pipetman operation.

1-A, push-button; 1-B, moulded hand grip; 1-C, shaft; 1-D, built-in ejector; 1-E, tip; 1-F, ejector button; 2-A, knurled adjustment ring; 3-A, 3-B, 3-C, and 3-D as discussed in operation of push-button.

Rotary Vacuum Evaporator



Sample flask (A) contains the beef heart ubiquinone ether sample. The dial (B) is turned to rotate the sample flask to allow maximum surface exposure to vacuum. Select speed that allows the sample to coat the inner surface of the round bottom flask.

Double condenser (C) - cold water runs through coils which causes the ether to condense and fall into the round bottom collection flask(D). The condenser is connected at (G) to a water aspirator (E) via a trap flask (F). The water aspirator is the source of the vacuum. A water aspirator is used instead of the vacuum line to ensure no ether goes down the vacuum line. The trap flask prevents any water from entering your sample flask if the water aspirator incorrectly turned off. A warm water bath (30 C) may be placed beneath the sample flask to reduced the evaporation time. This method is quick (5 min) and maintains the integrity of the sample

Note: in the above diagram the rubber tubing from the water trap flask is not connected to the water aspirator but should be.

STEREOSCOPIC MICROSCOPE SMZ (for filter colony observation)

1. Turn on light source switch located on transformer. Adjust intensity with the knob and at same time adjust angle of mirror with the mirror rotation knob. The light source usually from below for petri plate observation. However, a lamp may be placed above specimen if light does not transmit through specimen.
2. Adjust interpupillary distance to allow the view field for both eyes to blend into one.
3. Adjust the diopters and focus. This microscope has two diopters one located on each eyepiece.
 - a) First match the 0 line with the index line for each diopter.
 - b) Turn the zooming knob to 5x.
 - c) Focus using the left or right focus knob.
 - d) Turn the zooming knob to 0.8x.
 - e) Only looking through the left eye, focus using the diopter ring on the left eyepiece. Next, only looking through the right eye, focus using the diopter ring on the right eyepiece. Repeat this step until the microscope stays in focus through the zooming range (0.8x to 5x).

Notes:

- (i) The microscope is set up to view microorganism culture plates. If you draw a microscope field remember to include magnification (10x eyepiece times 2x auxiliary objective times zooming magnification). For example, for 0.8x zoom the magnification is $0.8 \times 10 \times 2 = 16x$. Check to ensure that the auxiliary objective is present before calculating magnification.
- (ii) The stereoscopic petri plate microscope is in the left bench cupboards (facing north, towards center of lab). It is the student's responsibility to remove the microscope for use and replace when finished. Always carry the microscope with two hands, one holding arm and other under base. There is a separate power source for this microscope which must be attached to microscope before turning on the light source. If you have difficulty using the microscope, get help, either the TAs or lab instructor. If a light is burned out or microscope does not work, leave the microscope on top of a side bench or center bench with a note stating problem.

stereoscopic microscope

EPI-FLUORESCENT MICROSCOPE

The UV source for the epi-fluorescent microscope comes above the objectives. The UV light is directed downward (dichroic* mirror) from the objective onto the slide specimen. In addition, a barrier prevents any UV light from moving upward to the eyepiece, only allowing the longer emitted wavelength to be viewed. Various filters (specific for type of fluorescence) and matching barriers are used depending on type of sample. Never remove barrier or filter while UV light is on. The sample absorbs light at one wavelength (UV excitation) and emits at a longer wavelength. You see only the emitted light when observing the specimen. In the DAPI lab, a 485nm UV filter and a matching barrier () is used to view the methanogens. The expected color of the emission is blue. The epi-fluorescent microscope can also be used as a bright light microscope if the sliding barrier on the UV light arm is pulled out and the white light switch is turned on at the front base of the microscope. The slide prevents UV light from entering the microscope. When using as a fluorescent microscope the sliding barrier is pushed in (open circle) thus allowing UV light to enter the microscope (white light switched turned off).

*dichroic(two color) mirror reflects light wavelengths below a certain value and transmits light wavelengths above that value. Since the mirror does allow some wavelengths below the cut off to transmit, a barrier filter on the way to the eyepiece is required.

pages for ASE and GC-MS

FINAL LAB EXAM: Microbiology 60.432
DATE: Sample PAGE: 1 of 5
INSTRUCTOR: Dr. L. Cameron
Student Name : _____

ENVIRONMENTAL MICROBIOLOGY
TIME: 1 h 15 min

Student Number: _____

WRITE EXAM IN **PEN ONLY**

CONCISELY ANSWER ALL QUESTIONS on EXAM PAPER IN SPACE PROVIDED.

Answers acceptable in point form. Answer spaces have been removed.

- 1 1. When using standard methods to examine drinking water it is important to ensure the growth of stressed organisms. Explain why. What did you do in your experiment to promote the growth of stressed organisms? Explain why.
- 1 2. Outline the standard method for recreational water sample removal.
- 1 3. In your environmental lab, you used both MI agar and mFC agar membrane filter techniques to assay water quality. How do they differ?
- 1 4. What is the principle of the Colilert® test?
- 2 5. Calculate the *E. coli* density for the following duplicate membrane filter data taken from a public swimming area (procedure similar to lab manual): undiluted, 875, 822; 10^{-1} dilution, 52, 79, and 10^{-2} dilution, 2, 4. The sample is taken from only one location. How many locations should be sampled? Is the beach acceptable for public swimming? What, if any, action should be taken?
- 1 6. The “number of viable coliform counts may be underestimated by the membrane filter technique.” Explain why. Name a method that would give more accurate counts. What other problems may be encountered when using the membrane filter technique?
- 1 7. Why is it possible to use a Biolog plate to characterize the microbial community when the plate was originally designed to identify a pure culture of bacteria? What are the limitation of the Biology plate with reference to characterization of the microbial community?
- 2 8. Explain why PCR-based rRNA analysis is a good method to study microbial diversity in a compost sample. However, PCR-based rRNA analysis is not a full proof method to study microbial diversity. What pitfalls are encountered with reference to cell lysis, nucleic acid extraction, separation of PCR amplified genes, and analysis of DNA sequences?
- 1 9. Summarize results of Brady landfill compost site data.
- 1 10. Outline how the background control for the petroleum biodegradation is prepared. Explain why.
- 2 11. What ratio(s) best demonstrates the effect of NP on petroleum biodegradation? Explain what the ratio(s) mean. Explain the theory of the statistical analysis used to demonstrate the effect.

CONTINUED ON PAGE 4 . . .

FINAL LAB EXAM: Microbiology 60.432
DATE: November 29, 2001 PAGE: of 5

ENVIRONMENTAL MICROBIOLOGY
TIME: 1 h 15 min

INSTRUCTOR: Dr. L. Cameron

- 2 12. Present a schematic figure of petroleum biodegradation data with NP and inoculum added? What is the principle of the equipment used to obtain this data?
- 1 13. What does staining with DAPI demonstrate? Explain how. What other stain would be of value? Explain why.
- 1 14. When preparing the sediment sample for staining what is the function of sonication and 0.1% gelatin?
- 1 15. What data and data manipulations are required to plot the oxygen profile of your sediment samples?
- 1 16. What digester was the sample taken from at the North End Wastewater Treatment Plant? What other digesters were present at the North End wastewater treatment plant?
- 1 17. Explain the function of resazurin.
- 1 18. What are Balch tubes? Why were they used in your environmental microbiology lab?
- 2 19. What effect does acetate, molybdate, BESA and sulfate have on methanogenesis? What data and data manipulations allowed you to demonstrate this in your lab?
- 1 20. What is the initial concentration of BESA in the tubes amended with 0.5 ml 0.5 M BESA? The tubes contain 20.4 ml before adding the BESA.

RELEASE AND INDEMNIFICATION

In consideration of The University of Manitoba arranging for the opportunity to
_____ visit the **Fort Wyte Centre** _____

_____ in _____ Winnipeg, MB _____

_____ as part of Course No. 60.432 "Environmental Microbiology" and in recognition that < I am responsible for arranging my own transportation to and from the said location(s)/The University of Manitoba has arranged for my transportation to and from said location(s)> (hereinafter collectively referred to as the "activity"), I, _____, form myself, my heirs, executors, administrators and assigns RELEASE the University of Manitoba, its respective servants, agents or employees from any claims, demands, damages, actions, losses or other proceedings arising out of or in consequence of any loss, injury or damage to my person or property incurred while I am engaged in the activity notwithstanding any such loss, injury or damage may have arisen by reason of the negligence of The University of Manitoba, its servants, agents or employees.

I FURTHER AGREE TO INDEMNIFY The University of Manitoba, its servants, agents or employees from any damages which may result or claims or demands which may be made against The University of Manitoba arising out of or in consequence of the activity and/or my actions.

Date _____ Signature _____

Please print your name here: _____

The foregoing Release and Indemnification relates to the period during which the activity is conducted, it being anticipated that such period shall be from 12:30 pm Oct 9/03 to 5:00 pm Oct 9/03 inclusive.

RELEASE AND INDEMNIFICATION

In consideration of The University of Manitoba arranging for the opportunity to
_____ visit the **North End Wastewater Treatment Plant** _____

_____ in _____ Winnipeg, MB _____

as part of Course No. 60.432 “Environmental Microbiology” and in recognition that < I am responsible for arranging my own transportation to and from the said location(s)/The University of Manitoba has arranged for my transportation to and from said location(s)> (hereinafter collectively referred to as the “activity”), I, _____, form myself, my heirs, executors, administrators and assigns RELEASE the University of Manitoba, its respective servants, agents or employees from any claims, demands, damages, actions, losses or other proceedings arising out of or in consequence of any loss, injury or damage to my person or property incurred while I am engaged in the activity notwithstanding any such loss, injury or damage may have arisen by reason of the negligence of The University of Manitoba, its servants, agents or employees.

I FURTHER AGREE TO INDEMNIFY The University of Manitoba, its servants, agents or employees from any damages which may result or claims or demands which may be made against The University of Manitoba arising out of or in consequence of the activity and/or my actions.

Date _____ Signature _____

Please print your name here: _____

The foregoing Release and Indemnification relates to the period during which the activity is conducted, it being anticipated that such period shall be from 12:30 pm Oct 30/03 to 5:00 pm Oct 30/03 inclusive.

RELEASE AND INDEMNIFICATION

In consideration of The University of Manitoba arranging for the opportunity to
_____ visit **Mid-Canada Waste Management Ltd** _____
in _____ Winnipeg, MB _____

_____ as part of Course No. 60.432 “Environmental Microbiology” and in recognition that < I am responsible for arranging my own transportation to and from the said location(s)/The University of Manitoba has arranged for my transportation to and from said location(s)> (hereinafter collectively referred to as the “activity”), I, _____, form myself, my heirs, executors, administrators and assigns RELEASE the University of Manitoba, its respective servants, agents or employees from any claims, demands, damages, actions, losses or other proceedings arising out of or in consequence of any loss, injury or damage to my person or property incurred while I am engaged in the activity notwithstanding any such loss, injury or damage may have arisen by reason of the negligence of The University of Manitoba, its servants, agents or employees.

I FURTHER AGREE TO INDEMNIFY The University of Manitoba, its servants, agents or employees from any damages which may result or claims or demands which may be made against The University of Manitoba arising out of or in consequence of the activity and/or my actions.

Date _____ Signature _____

Please print your name here: _____

The foregoing Release and Indemnification relates to the period during which the activity is conducted, it being anticipated that such period shall be from 12:30 pm Oct 2/03 to 5:00 pm Oct 2/03 inclusive.

RELEASE AND INDEMNIFICATION

In consideration of The University of Manitoba arranging for the opportunity to
_____ visit **Brady Landfill** _____

in _____ Winnipeg, MB _____

as part of Course No. 60.432 “Environmental Microbiology” and in recognition that < I am responsible for arranging my own transportation to and from the said location(s)/The University of Manitoba has arranged for my transportation to and from said location(s)> (hereinafter collectively referred to as the “activity”), I, _____, form myself, my heirs, executors, administrators and assigns RELEASE the University of Manitoba, its respective servants, agents or employees from any claims, demands, damages, actions, losses or other proceedings arising out of or in consequence of any loss, injury or damage to my person or property incurred while I am engaged in the activity notwithstanding any such loss, injury or damage may have arisen by reason of the negligence of The University of Manitoba, its servants, agents or employees.

I FURTHER AGREE TO INDEMNIFY The University of Manitoba, its servants, agents or employees from any damages which may result or claims or demands which may be made against The University of Manitoba arising out of or in consequence of the activity and/or my actions.

Date _____ Signature _____

Please print your name here: _____

The foregoing Release and Indemnification relates to the period during which the activity is conducted, it being anticipated that such period shall be from 12:30 pm Sept 18/03 to 5:00 pm Sept 18/03 inclusive.