

**BEE 6570**  
**Mixed-Culture Engineered Systems: Bioenergy and Microbial Ecology**  
**SPRING 2012**

**Instructor of record.** Lars Angenent, Ph. D.  
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**Course Webpage.** <http://angenent.bee.cornell.edu/BEE6940.html>

**Credits.** 3

**Time of Class.**            Lectures: Tuesdays and Thursdays from 10:10 - 11:25 am

**Location of Class.**      Room 105, Riley-Robb Hall

**Instructors.**

- Lars Angenent (214 RR, 5-2480, la249 [at] cornell.edu)
- Jeff Werner (jjw58 [at] cornell.edu)

**TA.**

- Elliot Friedman (esf59 [at] cornell.edu)

**Office Hours:** Setup a meeting with me via e-mail or during the lecture.

**Pre-requisite.** Be a graduate student.

**Course Description.** During this course, we will perform an in-depth analysis of classic or the latest peer-reviewed publications that describe undefined mixed cultures in engineered systems. We will especially discuss different organic waste treatment options, such as anaerobic digestion, aerobic digestion, composting, bioelectrochemical systems (such as microbial fuel cells), and carboxylic-acid fermentation systems. After reading about more classical techniques, we will focus on the latest and most powerful molecular biology techniques (e.g., 16S rRNA gene surveys, metagenomics, proteomics, metatranscriptomics) and how statistics can inform us about community structure-function relationships. Some bioinformatic and microbial ecology tools will also be discussed and used. After completing this course, you should be able to critically read and evaluate scientific papers that show results obtained with molecular techniques from engineered systems. More specifically, you should be able to know the limitations of the utilized techniques and be able to give other techniques that may complement or improve the knowledge gained from the study.

**Required Text.** No textbook required, research papers and reviews will be posted as password-protected pdf files on the course web page. The instructor will give the password to you during the first lecture. You are expected to read the posted paper before coming to class, your involvement in discussing the paper is necessary and this will determine for the largest part your grade. Some helpful books to lookup information relevant to this course:

- Brock Biology of Microorganisms, tenth edition, Madigan, Martinko, and Parker, Prentice Hall, Pearson Education, Inc. Upper Saddle River, NJ.
- Analysis of Genes and Genomes, John Wiley and Sons, Ltd, Chichester, West Sussex, England.
- Environmental Molecular Microbiology: Protocols and Applications. Edited by Paul A. Rochelle, Horizon Press, Norfolk, England.
- Molecular Biotechnology: Principles and Applications of Recombinant DNA, third edition. Glick and Pasternack, ASM Press, Washington, DC.
- Microbial Genetics, David Freifelder, Jones and Bartlett Publishers, Boston, MA (1987).

**Phylogeny Projects.** Two phylogeny projects will be performed with small groups of students. The result will be a phylogenetic (evolutionary relationships) tree with additional text describing how the tree was obtained (approximately 1-page description). This description will be performed in a format that is similar to a materials and methods section of a peer-reviewed paper.

**Teaching of a Molecular Technique + Term Paper.** You will teach a lecture about a Molecular Technique for 30-45 minutes and will then discuss a research paper. First the basic mechanisms of the technique need to be taught and subsequently a peer-reviewed paper will be discussed. The student is expected to choose a paper that uses a molecular technique to solve an engineering-related problem that uses an undefined mixed culture; if the technique is exciting and can be translated, we can be flexible on this. A 5-page, single space term paper needs to be handed in before the lecture starts. This term paper needs to summarize, discuss, and critically evaluate the chosen peer-reviewed research paper. For example, the limitations of the utilized molecular technique needs to be assessed and other techniques that may complement or improve the knowledge gained from the study may be discussed.

**Grading:**

Phylogeny Projects:	30% (10% of grade per project part)
Teaching of Molecular Technique + Term Paper:	30%
Class Participation and Professional Evaluation	40%

**Turnitin.com:**

Students agree that by taking this course all required papers may be subject to submission for textual similarity review to Turnitin.com for the detection of plagiarism. All submitted papers will be included as source documents in the Turnitin.com reference database solely for the purpose of detecting plagiarism of such papers. Use of Turnitin.com service is subject to the Usage Policy posted on the Turnitin.com site.

**PLAGIARISM AND CHEATING:**

Except when explicitly told otherwise, you are expected to work independently. Plagiarizing from either the primary literature (by copying and pasting text) or from another student (or from yourself from a previous course) will result in a failing grade, or, in more egregious cases, an appearance before the Academic Integrity Committee. For example, cheating on an exam will result in an immediate failing grade for the exam, an appearance before the Academic Integrity Committee, and potentially a failing grade for the course. We do not have an exam, but here your five-page paper and the discussion of the method must be executed according to the above rules.

**Academic Integrity.** Each student in this course is expected to abide by the Cornell University Code of Academic Integrity. Students must in no way misrepresent their work, fraudulently or unfairly advance their academic status, or be a party to another student's failure to maintain academic integrity. A full statement of this code may be found at:  
<http://cuinfo.cornell.edu/Academic/AIC.html>.

**Accommodations for Students with Disabilities.** In compliance with the Cornell University policy and equal access laws, Lars Angenent is available to discuss appropriate academic accommodations that may be required for student with disabilities. Except for unusual circumstances, requests for academic accommodations must be made during the first three weeks of the semester so that appropriate arrangements can be made. Students are encouraged to register with Student Disability Services to verify their eligibility for appropriate accommodations.

**Tentative Course Outline:**

<b>Date</b>	<b>To do, etc.</b>	<b>Topic</b>
Th., Jan. 26  Tu., Jan. 31 Th., Feb. 2	<u>Lecture 1 (L1)</u> <b>Read paper 1</b> Intro to class and lab <b>Review ms</b> <b>Present work</b>	<b>INTRODUCTION AND BACKGROUND</b> 1. Introduction to course 2. Intro to the Angenent Lab, webpage 3. Discuss 2011 Trends in Biotechnology paper (1) 4. Review a manuscript; write a summary and review comments 5. 5 min. presentation about your research with 2-min questions (cut off at 7 min); objective: learning how to summarize in public what you are working on and what your interests are.
Tu., Feb. 7 Th., Feb. 9 Tu., Feb. 14 Th., Feb. 16 Tu., Feb. 21 Th., Feb. 23 Tu., Mar. 28 Th., Mar. 1	<u>L2: Read paper 2 &amp;3</u> <u>L3: Read paper 3 &amp;4</u> <u>L4: Read paper 5</u> <u>L5: Read paper 6</u> <u>L6: Read paper 7</u> <u>L7: Read paper 8 &amp;9</u> <u>L8: Read paper 10</u> <u>L9: Read paper 11</u>	<b>MOLECULAR CHARACTERIZATION and UNDEFINED CULTURES</b> 1. 16S rRNA gene survey with full-length sequences (Sanger sequencing) (2) and FISH (fluorescence <i>in-situ</i> hybridization) (3) 2. Northern blotting (3, 4) 3. Quantitative PCR (5) 4. PhyloChip (6) 5. DGGE (denaturing gradient gel electrophoresis) (7) 6. DNA SIP (stable isotope probing) (8) and RNA SIP (9) 7. FISH – NanoSIMS (secondary ion mass spectroscopy) (10) 8. Confocal Raman Spectroscopy (unpublished paper here) (11)

<b>Date</b>	<b>To do, etc.</b>	<b>Topic</b>
Tu, Mar. 6 Th., Mar. 8 Tu., Mar. 13 Th., Mar. 15 Tu., Mar. 20 Th., Mar. 22	<u>L10: Read paper 12</u> <u>L11: Read paper 13</u> <u>L12: Read paper 14</u> <u>L13: Read paper 15</u> Spring break Spring break	<b>PHYLOGENY (evolutionary relationship) and bioinformatics</b> 1. Introduction on phylogeny, alignments, RDP, and UniFrac (comparing communities) (12) 2. Active learning: QIIME (13) 3. Ordination (paper from Applied Microbiology here) (14) 4. Highly-parallel sequencing: 16S rRNA surveys to compare communities and build structure-function relationships (15)
Tu., Mar. 27 Th., Mar. 29 Tu., Apr. 3	<u>L14: Read paper 16</u> <u>L15: Read paper 17</u> <u>L16: Read paper 18</u>	<b>MOLECULAR TECHNIQUES AND ENGINEERING APPLICATIONS</b> 1. Highly-parallel sequencing: metagenomics (16) 2. Environmental proteomics (17) 3. Highly-parallel sequencing: metatranscriptomics (see webpage) (18)

Date	To do, etc.	Topic
Th., Apr. 5	<b>First project due and L17: Read paper 19</b>	<b>METAGENOMICS and bioinformatics</b> Active learning: dealing with shot-gun sequences with MG-RAST (19)
Tu., Apr. 10 Th., Apr. 12 Tu., Apr. 17 Th., Apr. 19 Tu., Apr. 24 Th., Apr. 26  Tu., May 1 Th., May 3	<b>Read paper 20 &amp; 21</b> <b>Read paper 22</b> <b>Read paper 23</b> <b>Read paper 24</b> <b>Read paper 25</b> <b>Second project due and Read paper 26</b> <b>Read paper 27</b> <b>No Class</b>	<b>STUDENT LECTURES: molecular techniques and bio-engineered systems</b> Michaela's lecture ( <i>see website for paper</i> ) Katharina's lecture ( <i>see website for paper</i> ) Catherine's lecture ( <i>see website for paper</i> ) Devin's lecture ( <i>see website for paper</i> ) Sarah's lecture ( <i>see website for paper</i> )  Chistian's lecture ( <i>see website for paper</i> ) Joe's lecture ( <i>see website for paper</i> )
<b>40.5 hours</b>		<b>NO FINAL EXAM.</b>

**Papers to read (posted on course web site as protected pdf files):**

1. M. T. Agler, B. A. Wrenn, S. H. Zinder, L. T. Angenent, Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. *Trends Biotechnol.* **29**, 70 (2011).
2. Y. Sekiguchi *et al.*, Phylogenetic diversity of mesophilic and thermophilic granular sludges determined by 16S rRNA gene analysis. *Microbiology* **144**, 2655 (1998).
3. R. E. Ley *et al.*, Unexpected diversity and complexity of the Guerrero Negro hypersaline microbial mat. *Appl. Environ. Microbiol.* **72**, 3685 (May, 2006).
4. L. T. Angenent, S. Sung, L. Raskin, Methanogenic population dynamics during startup of a full-scale anaerobic sequencing batch reactor treating swine waste. *Water Res.* **36**, 4648 (2002).
5. L. M. Steinberg, J. M. Regan, mcrA-targeted real-time quantitative PCR method to examine methanogen communities. *Appl. Environ. Microbiol.* **75**, 4435 (Jul, 2009).
6. K. H. Wilson *et al.*, High-density microarray of small-subunit ribosomal DNA probes. *Appl. Environ. Microbiol.* **68**, 2535 (2002).
7. N. Boon, W. De Windt, W. Verstraete, E. M. Top, Evaluation of nested PCR-DGGE (denaturing gradient gel electrophoresis) with group-specific 16S rRNA primers for the analysis of bacterial communities from different wastewater treatment plants. *FEMS Microbiol. Ecol.* **39**, 101 (FEB, 2002).
8. C. O. Jeon *et al.*, Discovery of a bacterium, with distinctive dioxygenase, that is responsible for in situ biodegradation in contaminated sediment. *Proc. Natl. Acad. Sci. U. S. A.* **100**, 13591 (Nov 11, 2003).
9. T. Lueders, B. Pommerenke, M. W. Friedrich, Stable-isotope probing of microorganisms thriving at thermodynamic limits: syntrophic propionate oxidation in flooded soil. *Appl. Environ. Microbiol.* **70**, 5778 (Nov, 2004).
10. T. Li *et al.*, Simultaneous analysis of microbial identity and function using NanoSIMS. *Environ Microbiol* **10**, 580 (Mar, 2008).
11. Unpublished Confocal Raman Spectroscopy paper.
12. C. Lozupone, R. Knight, UniFrac: a new phylogenetic method for comparing microbial communities. *Appl. Environ. Microbiol.* **71**, 8228 (Dec, 2005).
13. Z. Liu, C. Lozupone, M. Hamady, F. D. Bushman, R. Knight, Short pyrosequencing reads suffice for accurate microbial community analysis. *Nucleic Acids Res* **35**, e120 (2007).
14. Ordination Microbiology Paper.
15. J. J. Werner *et al.*, Bacterial Community Structures are Unique and Resilient in Full-Scale Bioenergy Systems. *Proc. Natl. Acad. Sci. U. S. A.* **108**, 4158 (2011).
16. H. G. Martin *et al.*, Metagenomic analysis of two enhanced biological phosphorus removal (EBPR) sludge communities. *Nat Biotech* **24**, 1263 (2006).

17. J. J. Werner, A. C. Ptak, B. G. Rahm, S. Zhang, R. E. Richardson, Absolute quantification of *Dehalococcoides* proteins: enzyme bioindicators of chlorinated ethene dehalorespiration. *Environ Microbiol* 11, 2687 (Oct, 2009).
18. Recent Metatranscriptomics paper.
19. F. Meyer *et al.*, The metagenomics RAST server - a public resource for the automatic phylogenetic and functional analysis of metagenomes. *BMC Bioinformatics* 9, 386 (2008).
- 20-27. Students will pick topics and papers.