

# Anaerobe 2010

The 10th Biennial Congress of the  
Anaerobe Society of the Americas

Philadelphia, PA USA • July 7-10, 2010

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### **ANAEROBES: HISTORY, INDUSTRY, AND THE BIRTH OF BIOTECHNOLOGY**

Cox, M.\*

Anaerobe Systems, Morgan Hill, CA USA

The first anaerobe was described by Louis Pasteur in 1861. This is considered the birth of the field of microbiology. Sergei Winogradsky showed that *Clostridium pasteurianum* could fix nitrogen in 1895. Fritz Haber patented a process to produce ammonia for hydrogen and nitrogen in 1909. Chaim Weizmann used *Clostridium acetobutylicum* to produce acetone from carbohydrate in 1915. This is considered the birth of biotechnology. Large quantities of hydrogen and carbon dioxide were produced as by-products of the acetone, butanol, ethanol fermentation process. Commercial Solvents purchased Weizmann's patents at the end of World War I. Commercial Solvents went on to produce a wide range of alcohols, solvents, and other building block for organic synthesis. The first commercial production of crystalline penicillin was carried out at Commercial Solvents. Anaerobic bacteria offer many opportunities to produce fuel, chemical feed stocks, fertilizers, and other valuable products through fermentation of biomass. As we exit the fossil fuel era, anaerobes will play an important part in agriculture, industry, and energy. There is a pressing need for formal training and educational programs to prepare scientists for this now expanding field, as well as new opportunities for microbiologists trained in working with anaerobes.

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## ANAEROBES & BIOTECHNOLOGY: OPPORTUNITIES FOR MICROBIOLOGISTS

### ANAEROBES: A PIECE IN THE PUZZLE FOR ALTERNATIVE BIOFUELS

Lawson, P.A.\*; Caldwell, M.E.; Allen, T.D.; Tanner, R.S.

Department of Botany & Microbiology, University of Oklahoma, Norman, OK USA

Increasing attention is being directed towards alternative “green” sources of energy to reduce both the reliance on petroleum and the foreign sources of this commodity. Most bioethanol produced in the U.S. is from the fermentation of corn starch, resulting in a direct competition with a commodity food and therefore is not viewed as a long-term sustainable process. Cellulosic ethanol production is a desirable alternative but current technologies have not overcome obstacles (e.g. pretreatment and waste disposal) associated with the direct fermentation of cellulosic material. An alternative to direct fermentation is the indirect fermentation of lignocellulosic biomass to ethanol, a very efficient process that can use a wide range of starting materials with little waste matter. Using this method, biomass is pyrolyzed to produce synthesis gas ( $\text{CO}:\text{CO}_2:\text{H}_2$ ) which is then converted by bacteria to ethanol through anaerobic fermentation. However,  $\text{CO}$  fermentation to ethanol is limited to relatively few bacteria, leading our laboratories to search for microorganisms that can undertake the aforementioned process. Using a variety of anaerobic enrichment and cultivation methods, a number of candidate organisms were recovered. Phylogenetic investigations using the 16S rRNA gene analysis in tandem with phenotypic and chemotaxonomic methods revealed several organisms that produce ethanol and other desirable products. Our studies demonstrate that these organisms show a remarkable taxonomic diversity including several novel *Firmicutes*, representing the families *Eubacteriaceae* (cluster XV), *Lachnospiraceae* (cluster XIVa) and *Ruminococcaceae* (cluster III). Due to the level of ethanol produced, temperature and pH optima, as well as ethanol tolerance, these organisms have shown potential for use in industrial applications for cellulosic ethanol production.

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### MOLECULAR- AND CULTIVATION-BASED INVESTIGATIONS OF ORGANISMS ASSOCIATED WITH MICROBIALY INFLUENCED CORROSION (MIC) OF OIL RESERVOIR INFRASTRUCTURE ON THE NORTH SLOPE OF ALASKA

Johnson, C.N.\*; Stevenson, B.S.; Drilling, H.; Lawson, P.A.

Department of Botany & Microbiology, University of Oklahoma, Norman, OK USA

Corrosion of gas and liquid pipelines is expensive both in terms of time and money, costing \$7 billion per year and resulting in severe disruptions and delays in fuel delivery. The scientific community is becoming increasingly aware that reservoir microorganisms can not only degrade hydrocarbons but also actively participate in the microbially influenced corrosion (MIC) of pipelines and other associated metal infrastructure. However, little is known about the microbial populations responsible for these activities, which often exist as complex biofilms. Samples were obtained from a pipeline undergoing physical corrosion mitigation (pigging) on the North Slope of Alaska. Molecular surveys were conducted to elucidate a census of community composition through terminal restriction fragment length polymorphism (T-RFLP), sequencing of 16S rRNA gene clone libraries, and 454 pyrosequencing. Thus, by using these molecular inventories as a “roadmap”, organisms of interest have been targeted for anaerobic enrichment cultivation using differing substrates and electron acceptors. In order to understand the interactions of the microbial consortia inhabiting this environment, one must identify physiological properties of particular species suspected in biocorrosion. A number of known and novel members of the *Firmicutes* and *Bacteroidetes* were isolated under strict anaerobic conditions and identified using 16S rRNA gene sequencing. Two isolates represented dominant taxa (~11%) in the molecular inventories derived from systems of high corrosion and correlate to a shift in composition not seen in other oil production wells. Phenotypic characterization, along with physical and metabolic relationships with other microbes and their capacity to cause MIC are now being investigated; which is an important step in understanding the metabolic capabilities and corrosive mechanisms of the microbial communities in these unique extreme ecosystems. The knowledge gained through these studies could lead to better mitigation strategies to combat deleterious microbial processes.

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### **AN ECONOMIC ANALYSIS OF ANAEROBIC DIGESTION TO REMOVE FOOD WASTE ON A MID-SIZED UNIVERSITY CAMPUS**

Johnson, J.D.;<sup>\*1</sup> Johnson, S.;<sup>2,3</sup> Eames, J.M.<sup>1</sup>

<sup>1</sup>Loyola University Chicago, Chicago, IL USA

<sup>2</sup>Hines VA Hospital, Chicago, IL USA

<sup>3</sup>Loyola University Medical Center, Chicago, IL USA

Over 25% of prepared food in the U.S. is discarded and represents a burden on society, as it takes up space in landfills and contributes to global warming. Anaerobic digestion is a process whereby naturally occurring anaerobic bacteria break down organic matter in a series of reactions ultimately producing a gaseous mixture called biogas (~60% methane) and a liquid sludge, or digestate. Technology using this process is increasingly being adopted as a waste management tool, primarily in the agricultural sector.

We conducted an analysis of the economic feasibility of installing an anaerobic digester system at our University to treat food waste generated on campus (>10 tons/week). Currently, discarded food is hauled to a landfill and costs \$43 to 48/ton through 'tipping-fees'. Potential sources of revenue to be realized include; avoided waste-hauling costs, renewable energy from biogas, and fertilizer sales from the digestate.

By removing 10 tons of food from the waste stream per week, a savings of \$23,000 per annum in tipping-fees can be achieved. One ton of food waste will produce 3300 ft<sup>3</sup> of methane gas and we estimate production of 1,748.78 mbtu of methane per annum. The biogas/methane could be combusted immediately and stored in the form of hot water, effectively offsetting the purchase of natural gas and producing an energy offset of \$8,743/annum. By partnering with a fertilizer company, the digestate could become a third revenue source. Based on existing applications of similar sized digesters to estimate construction and maintenance costs, we determined that tipping fee offsets and biogas energy production would produce a net annual benefit of \$18,000 for the University, but would require an initial investment of \$148,000. Assuming a 20 year project life, we estimate an 11% internal rate of return (IRR) and a payback period of 7.9 years.

Anaerobic digestion is an attractive financial investment. Future developments, like the institution of a "cap and trade" system, rising energy prices, or the development of a market for digestate, have the potential to dramatically increase the payback from such an investment.