Pre-Treatment Technologies for Increasing the Biogas Potential of Agricultural Wastes

T.A. Shepherd
15 November 2006

Abstract
Anaerobic digestion (AD) is a common wastewater treatment method employed in municipal, industrial, and agricultural systems. AD requires a receiving stream with low total solids (TS) content, typically less than 8%. Low TS influent facilitates optimal conditions for microbial degradation and reduces operational cost that is associated with increased energy consumption for wastewater with high TS. Animal manures, especially beef manure, generally contain higher levels of solids that municipal and industrial wastewaters, this can limit the application of anaerobic digestion as a treatment option. Pre-treatment strategies have been employed in municipal and industrial systems to disrupt conglomerated solids and breakdown cell structures; this facilitates the reduction of TS content and has been shown to improve AD performance and increase the bio-methane potential. Investigations of pre-treatment methods have focused primarily on municipal and industrial systems; their application to agricultural systems may provide producers with an improved waste treatment system that is capable of handling higher solid influents. This paper will discuss maceration, thermal hydrolysis, chemical amendment, liquefaction, sonication, and ozonation as pre-treatment methods for optimizing anaerobic digestion and investigate their application within agricultural systems.

Key Words
Anaerobic Digestion, Agricultural Wastewater, Pre-treatment, Maceration, Thermal Hydrolysis, Chemical Treatment, Liquefaction, Sonication, Ozonation

Introduction

Conservation and management of nutrients and resources is becoming an essential factor of sustainable agriculture today. Growing concerns of water quality and land management have introduced new factors for the treatment and distribution of agricultural wastes. Government regulations on land application of animal manures, present and pending, will have a large impact on the production practices that are used today. Furthermore, energy concerns have become a high priority topic, and focus on the development and optimization of environmentally friendly bio-renewable energy sources. Technologies that are capable of achieving multiple ecologically sound goals such as conserving nutrients and producing renewable energy sources provide producers with an incentive to implement these practices into their operations.

Anaerobic digestion (AD) has long been implemented as a wastewater treatment practice in municipal, industrial, and agricultural sectors. It is the most applied technique for sewage sludge stabilization, reducing volatile solids while producing methane biogas (Tiehm *et al.* 2001). AD is capable of conserving nutrients with sludge stabilization while simultaneously producing renewable energy in the form of methane which can be used for heating or electrical generation.

AD is generally defined by a three step enzymatic and microbial process that converts substrate into methane, carbon dioxide, and stabilized biomass sludge. The primary substrates within the process are based on soluble and insoluble organics within the wastewater. The first step in the process is the hydrolysis of insoluble substrate and occurs through biological and enzymatic reactions which produce soluble substrate. Soluble substrate can then be consumed by acidogenic bacteria which produce volatile fatty acids (VFA) or anaerobically oxidized to form hydrogen. VFA and hydrogen are consumed by group specific acetogenic bacteria, producing acetate. The final step in AD is methane generation. Methanogenic bacteria consume acetate or oxidizing hydrogen to produce methane and carbon dioxide. Figure 1 provides a visual process flow of methane production from AD.

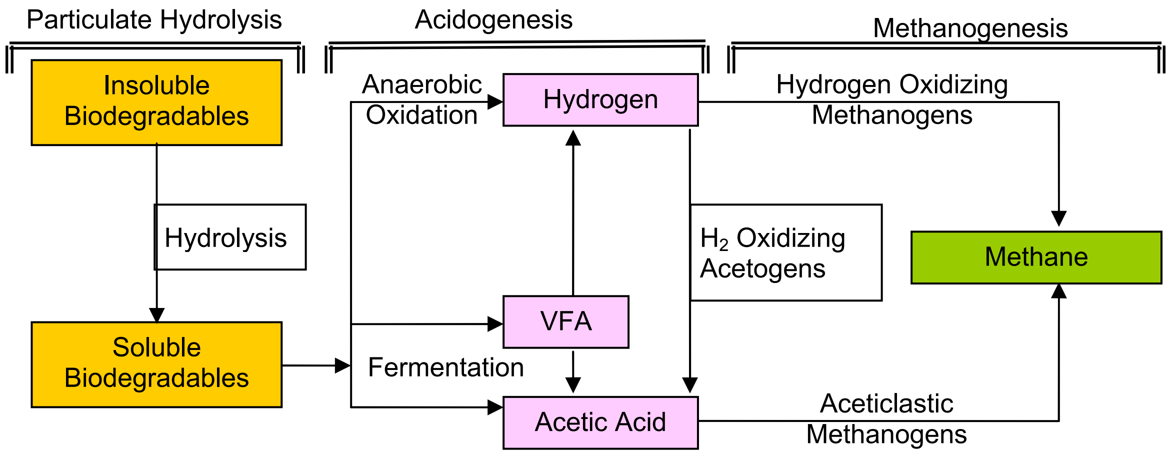

Figure 1. Anaerobic Digestion Microbial and Enzymatic Process Diagram

The hydrolysis of insoluble organic substrate has been identified as the rate-limiting step of the AD process (Eastman and Ferguson, 1981; Shimizu *et al.* 1993). Applying a pre-treatment process that hydrolyzes insoluble organics prior to AD has been shown to improve process performance and shorten the required treatment time.

Animal feeding operations often develop manures that are high in fibrous materials. The high fiber contents are mainly attributed to animal bedding and feed that is carried in the manure waste. The lignocellulose and hemicellulose within plant fibers creates the most difficulties for AD. Lignin is very resistant to enzyme hydrolysis and microbial degradation, and it creates a physical barrier preventing efficient enzyme hydrolysis of the cellulose (Angelidaki and Ahring 2000). Some pretreatment strategies are capable of separating the lignin from the readably degradable cellulose fibers, allowing for greater AD efficiency.

Energy efficiency within the AD is paramount in providing a sustainable and profitable treatment system. Losses in AD efficiency are associated with high total solid (TS) content of the influent. High TS increases the viscosity, raising the heating, mixing, and pumping requirements (Richard *et al.* 1991). Typical operating limits of TS are set at 5%. Depending on operation management practices, agricultural manure slurries have a TS range of 2-10%, limiting the application of tradition AD systems. Agricultural operations most commonly utilize lagoon systems which develop a natural anaerobic zone for treatment. Anaerobic lagoons are generally operated for solids reduction and stabilization, and in most cases do not collect the methane gases that are produced.

Anaerobic lagoons are most common in the south and have substantially lower energy demands than a traditional AD system. In Iowa and other northern states, seasonal temperatures often restrict the use of anaerobic lagoons, in this case traditional AD must be incorporated to sustain treatment. Many pre-treatment systems are capable of reducing the viscosity associated with high TS manures, making AD feasible. Coupled with the benefits of from increased solubility of insoluble organics, pre-treatment of high TS manure may create an economically beneficial AD system.

Pre-treatment technologies that are capable of reducing the time requirements for the hydrolysis of insoluble organics, increasing the availability of readily degradable organic material, and decreasing viscosity are outlined below:

- Maceration
- Chemical Treatment
- Liquefaction
- Thermal Hydrolysis
- Sonication
- Ozonation
- Biological

Pre-treatment Technologies

Pre-treatment methods have a wide range of complexity, and can be combined together to further optimize AD performance. The following sections describe the basic application of each method and the mechanisms providing the benefits to AD systems.

Maceration

Maceration is the simplest physical treatment available. It incorporates physically chopping, grinding, or blending manure. The ultimate goal of maceration is to reduce the particle size and separate lignin from the degradable biomass portion. Particle size reduction increases the surface area of fibers which allows for greater microbial access to the substrate (Angelidaki and Ahring 2000). As fiber and solid size is reduced, the methane potential increases. Sharma *et al.* (1988) indicated a diminishing return for particles sizes smaller than 30mm. Maceration equipment requires high maintenance, and energy consumption can become costly.

Chemical

Chemical amendment defines the addition of acids or bases to a wastewater, which is then allowed a specific reaction time prior to AD. Typical amendments incorporate sodium hydroxide (NaOH), ammonia hydroxide (NH_4OH), and sulfuric acid (H_2SO_4). Caustic amendment rates depend upon TS content, and generally range from 10-40% of TS mass. Caustic treatment times range typically from one hour to two days. The goal of chemical pre-treatment is the destruction of lignin bonds, providing microbes improved access to degradable organic material. In certain cases, chemical addition may create inhibitory byproducts which disrupt and decrease the performance of AD. Over application of H_2SO_4 forms hydrogen sulfide (H_2S), a primary inhibitor of the anaerobic process (Ardic and Tanner 2005). Furthermore, Na^+ is known to be an inhibitory ion to some methanogenic flora at high concentrations (He *et al.* 2006; Feijoo *et al.* 1995; Renzema *et al.* 1988). Chemical costs may prohibit the economical feasibility of its use as a pretreatment strategy.

Liquefaction

Liquefaction describes the forced explosion of cellular structures. Several methods such as decompression explosion and ammonia-freeze-explosion can be used to decrease organic particles size. The result of liquefaction produces similar physical effects as maceration on the manure slurry. Decompression explosion is achieved by pressurizing the liquid well above atmospheric pressure and then rapidly releasing the liquid into atmospheric pressure. The rapid decompression of the liquid causes cellular lysis, allowing for the dissociation of fiber and conglomerated solids. Equipment safety concerns of high pressure systems may become issues in on-farm implementation of liquefaction.

Thermal Hydrolysis

Thermal hydrolysis is defined by simply heating the wastewater to 100-200^{o}C and holding that temperature for a period of time, generally 30-120 minutes. The high temperatures disrupt cell walls and destroy lignin bonds (Mladenovska *et al*. 2006). Boiling action, similar to cavitation, causes turbulence and shear forces that disrupt sludge flocs. In certain applications thermal hydrolysis can be implemented to produce fluid pressure that can then be rapidly released, creating a decompression explosion process which enhances solids solubilization. Thermochemical pre-treatment combines chemical amendment and thermal hydrolysis in order to achieve greater lignin destruction. Thermal treatment may provide to be an economically sustainable option if the biogas collected can be utilized for the pre-heating process and AD heating.

Sonication

Sonication is the application of ultrasound waves to a confined liquid. The low frequency of ultrasound waves causes compression and expansion of material in the liquid. As this occurs, cavitation bubbles form and grow once critical bubble size is reached, they violently collapse causing shear forces and turbulence (Gonze *et al.* 1998). Furthermore intense local heating and high pressure created from the collapse of bubbles drives thermal destruction and can form radicals. Sonication increases sludge solubilization and particle size reduction allowing for greater biodegradability (Bougrier *et al.* 2006). Depending upon the sonication intensity and treatment time, different degrees of sludge dissociation can be achieved. High frequencies and long retention times can disrupt cell walls. Equipment costs and installation are the main economic concerns of implementing a sonication system.

Ozonation
Ozonation is a widely used treatment method in municipal water and wastewater treatment systems. Ozone (O_3) is a strong oxidant which readily reacts with organic matter within the wastewater. Multiple chemical reactions with O_3 and its byproduct radicals causes the reduction of organic matter which increases the solubilization, decreases the viscosity, but does not change the particle size. Ozone will react with soluble and insoluble particles as well as mineral fractions of the wastewater, and to some degree will form VFA from lipids (Bougrier et al. 2006).

Biological
Biological treatment for the enhancement AD systems are common in municipal wastewater systems. Aerobic treatment is capable of jumpstarting the breakdown of insoluble substrate, allowing for improved availability cellulose material and increasing the ratio of soluble:insoluble. Readily available soluble substrate allows for rapid consumption for methane production and improved availability of degradable cellulose decreases the hydrolysis time of insoluble substrate. The new frontier of biological treatment within AD systems is the implementation of specific cellulose and hemicellulose degrading bacteria. These bacteria specialize in the hydrolysis of cellulosic material. They are more efficient converters of insoluble substrate and are capable of speeding up the slower enzymatic hydrolysis process. As our technology improves we will be better prepared to maintain these special cultures and develop sufficient quantities economically.

Pre-treatment Comparisons and Case Studies
The bulk of research pertaining to pre-treatment for AD systems has been focus on municipal and industrial wastewater; however the principles of this work can be paralleled to agricultural operations.

Case Study 1
Bougrier *et al.* (2006 a) studied the effects of ultrasonic, thermal, and ozone pretreatments on the waste activated sludge solubilization and anaerobic biodegradability. Their work thoroughly investigated solubilization, physiological, chemical, and rheological effects, and overall AD performance of each respective pre-treatment technology with operational variations within each respective pre-treatment process. Prior to batch AD tests, they investigated the effect of each treatment method had upon the apparent viscosity; this information provides insight into the degree of sludge destruction and cellular breakdown. Figure 2 provides a compilation of data for the shear stress vs. the shear rate of treated and untreated samples.

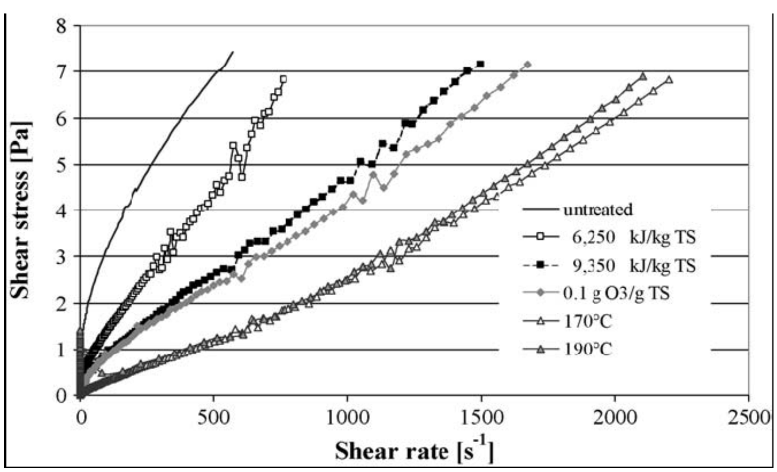

Figure 2. Shear stress versus shear rate, for treated and untreated sludge samples (Bougrier *et al.* 2006)

Waste activated sludge and manures are characterized as non-Newtonian fluids, this is displayed in Figure 2 as a non-linear relationship between shear stress and shear rate. All pretreatment process produced a more linear trend than the untreated samples, indicating a more Newtonian characteristic. All pretreatment processes reduced apparent viscosities, indicating possible reductions of energy requirements for AD heating, mixing, and pumping. This analysis showed that thermal pre-treatment at 170 and 190°C for one hour reduced the apparent viscosity the greatest, followed by sonication and ozonation.

Their next step was to test the pre-treatment in parallel AD batch reactors. This experiment provided the data shown in Figure 3, displaying the volumetric biogas production over time for all treatments and a control sample of ethanol. This information shows that thermal treatment and sonication provides the AD with influent that is significantly more biodegradable with respect to quantity of biogas produced and time required for degradation. Ozonation provided improved AD, however the magnitude of increase is significantly lower than the other pre-treatment methods.

Thermal and ultrasonic pre-treatment provided for near maximum biogas production rates at 25 days, and achieved them in a significantly shorter period of time, indicating that implementation would increase production and decrease the required retention time.

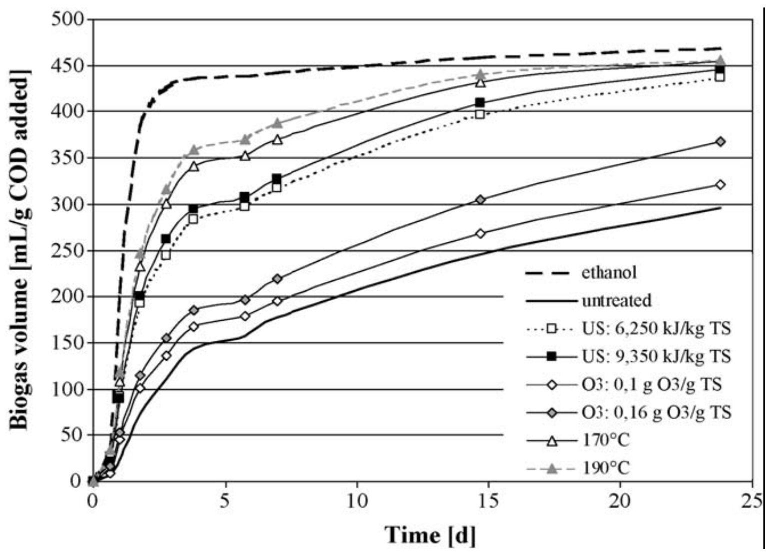

Figure 3. Batch anaerobic digestion tests (Bougrier *et al.* 2006)

Case Study 2
Ardic and Taner (2005) compared thermal, chemical, and thermochemical pre-treatments procedures on chicken manure to determine the degree of solids conversion to water soluble substrate and the overall effects on AD performance. The researchers varied the amounts of H_2SO_4 and NaOH applied to the manure (10, 15, 20% by mass of TS), soaking time for chemical reaction (1 or 2 hours), and thermal treatment (25.5 and 100°C). The pre-treatment matrix tested in this study was well developed, and cross linked all treatment processes developing 28 different procedures.

Thermal (100°C), acid, base, and thermochemical treatment increased the solubility of organics within the manure for all matrix decisions. All pretreatment methods reduced particle size, with thermal and thermochemical procedures significantly reducing particle size. Results on methane production varied significantly due to treatment. Thermal treatment for two hours provided the most consistent and highest methane production; high dose acid and base thermochemical treatment provided similar methane production rates. However, several matrix options under performed; this was attributed to high Na^+ ions and H_2S which hindered AD through inhibition of methanogenic microbial processes.

Case Study 3
Angelidaki and Ahring (2000) investigated AD performance with maceration, liquefaction, chemical amendment, and hemicellulose degrading microbe treatments on cattle manure. Their experiments encompassed batch and CSTR methods. Macerated manure was sieved and separated for determination of particle size effect upon biogas potential. Liquefaction was achieved by pressurizing manure to 100atm, with rapid decompression through a 1.2mm orifice. Chemical pretreatment incorporated NaOH, NH_4OH, and NaOH:KOH:$Ca(OH)_2$ additions at different application rates. Biological treatment was realized by the addition of hemicellulose degrading bacterium B4. Each treatment was then tested for 45-60 days in batch reactors. The results of potential methane production from the batch experiments are provided in Table 1.

As shown in Table 1, hemicellulose bacteria produced the highest methane potential with a 30% increase over untreated manure. The biomass treatment provided a methane production potential that was very near the theoretical maximum methane production rate. Addition of all bases at 40g/kg VS produced a methane increase of 20-23% and is attributed to the chemical destruction of lignocelluloses structure. Lower doses of bases provided significantly lower methane increases. Maceration and decompression explosion provided similar methane potential with an increase of 16-20% over untreated manures.

Table 1. Effect of the different treatments on the methane potential achieved from cattle manure (Angelidaki and Ahring 2000)

Treatment		**CH_4 potential increase (%)**
Maceration	< 0.35 mm	20
Maceration	2 mm	16
Decompression explosion		17
NaOH	20 g/kg VS	13
NaOH	40 g/kg VS	23
NH_4OH	<20 g/kg VS	0
NH_4OH	40 g/kg VS	23
NaOH:KOH:$Ca(OH)_2$	40 g/kg VS	20
Hemicellulose degrading bacterium B4		30

Angelidaki and Ahring (2000) continued their investigation, focusing on CSTR performance with pre-treatment strategies. They were capable of duplicating batch results, finding that macerated manure produced 17% more methane than untreated manure over one month. Analysis indicates that macerated manure enhances hydrolysis of organic macro-molecules for microbial consumption, allowing for the differences in methane potentials.

Case Study 4
Valo *et al.* (2004) and Bougrier *et al.* (2006 b) investigated thermal and thermochemical pre-treatment strategies for comparison of AD performance. Three identical semi-continuous flow reactors were operated in parallel, one for each pretreatment process and a control unit with no treatment. The authors indicate that sludge sources from two different municipal wastewater plants were tested. Four thermal pretreatment strategies were investigated: S1 (sludge from plant one) was pretreated at 170^{o}C with no chemical amendment and tested against 130^{o}C amended with potassium hydroxide, S2 (sludge from plant two) was pretreated at 170^{o}C and tested against 150^{o}C pre-treatment.

This paper reports that sludge solubilization after each thermal pretreatment process increased soluble COD from 3-7% to 49%-57%. COD destruction and methane production rate were also increased with all thermal pretreatment process, and are attributed to the increased soluble COD which allows for a bypass of the anaerobic rate-limiting step of cellular hydrolysis. Table 2 provides a comparison of methane yields for each pre-treatment and sludge source.

Table 2. Performance of semi-contnuous flow AD pretreatment methods
(Valo *et al.* 2004, Bougrier *et al.* 2006 b)

Methane Yield, L/kgVS				
Sludge	Raw	130^{o}C, KOH	150^{o}C	170^{o}C
1	128 +/- 5	220 +/- 4		228 +/- 5
2	145 +/- 5		238 +/- 4	256 +/- 7

From this data there is no significant difference of methane production between thermochemical treatment and 150^{o}C treatment, and only a slight benefit between 150^{o}C and 170^{o}C treatment. The authors provide a brief energy balance on the system to determine the cost associated with the thermal pretreatment process. Using the experimental methane production rates, they extrapolated an energy equivalence of the biogas and the energy requirements of the entire process. The energy balance shows that increased methane production achieved form raising the thermal pre-treatment from 150^{o}C to 170^{o}C is not capable of satisfying the energy requirement for this temperature increase. This experiment was established at a bench-scale and design consideration with a full-scale system may narrow the energy deficit.

Full-Scale Applications

Full-scale applications of pretreatment technologies for optimizing the anaerobic digestion process are found within municipal wastewater treatment plants. The most predominate full-scale pretreatment process is sludge disintegration via maceration or physical cell destruction. The Central Wastewater Treatment Plant in Prague incorporated a lysate centrifuge for sludge pretreatment. The lysate centrifuge developed a specific biogas production increase of approximately 7.5%, providing enough energy to meet all plant energy demand (Dohanyos *et al*. 2004).

Sonication is a growing trend for enhancing the digestion of thickened waste activated sludge (TWAS). Full-scale municipal wastewater treatment facilities in the UK, Sweden, USA, and Australia have incorporated sonication for improved TWAS treatment. Hogan *et al*. (2004) reports that sonication provides improved solids destruction, substantial biogas production increases, enhanced dewatering, decreased sludge production, reduced operating costs, and an economical payback period around two years.

Muhler et al (2004) assessed the full scale application of maceration, sonication, and ozonation pre-treatment process in full-scale operations based on sludge disintegration and system economics. This study found that each process significantly increased the solubilization of degradable material, providing improved AD, however they concluded that the investment and energy costs of these systems provided economical benefit only when the sludge disposal cost is a significant portion of total operating costs.

Discussion

With the exception of some thermochemical pretreatment strategies which developed inhibitory compounds, all treatment methods investigated were capable of enhancing AD with respect to biogas production. The case studies outlined have shown the similarities and differences between the mechanisms that create this enhancement. Implementing these systems in a farming operation could also extend the capabilities of AD to achieve satisfactory performance with high TS manures. Application and economics will become main factors determining the ultimate utilization within farming operations.

Maceration and liquefaction have similar mechanisms, relying upon physical destruction of fiberous material, sludge flocs, and cellular structures to increase soluble substrate and availability of degradable organic material to the bacteria. The AD performance enhancement is thus similar for these two methods, achieving significant improvements in methane generation. Maceration is a simple process, which most producers would be comfortable using. High energy costs will be associated with achieving the required particle size reduction of 20mm, furthermore maintenance of equipment may become intensive with the corrosive and fibrous nature of manure. Liquefaction is a more complex system that requires high pressure tanks with rapid decompression capabilities. Initial capitial investment may prevent small producer from implementing the system, however safety issues that arise from working with high pressure system is a larger influence to the widespread adoption of liquefaction as an on-farm pretreatment strategy.

The addition of acids or bases to manure slurries to instigate chemical breakdown of organic structures is another simple application that could be applied to an agricultural operation. The main advantage to this system is the ease of application, mixing and manure storage are normally available in existing systems, requiring minimal equipment expenditures. However, the quantity of caustic chemicals that would be required for large animal feeding operation raise economic and availability concerns. Safety also becomes a concern with the transportation and storage of large quantities of caustic materials. Ecological impacts and environmental regulations would need to be addressed prior to implementation as well.

Thermal pretreatment provides the greatest flexibility of application. The installation cost of this system would be minimal, with economical considerations focusing on energy demand for preheating. The case studies show some of the highest AD performance enhancement. An AD system that is efficient and capable of producing sufficient biogas quantities to sustain proper digestion and supply energy for pretreatment would make an ideal system. However, if a producer must rely heavily upon purchasing energy the economics of this system would deteriorate quickly. The integration of a thermochemical system would alleviate the energy concerns by shifting resources to the cost of chemicals.

Pretreatment via sonication provides similar benefits that thermal treatment provides, with substantial biogas generation. It is a relatively new application of technology, and the implementation cost would be much higher than a thermal system. The determining factor of economic feasibility ultimately becomes energy consumption in comparison to thermal pretreatment. Ozonation has similar economical application withdrawals, but benefits from tenure within municipal and industrial wastewater treatment systems. Safety issues with sonication and ozonation are minimal in comparison to liquefaction and chemical amendment.

Biological pretreatment, if managed properly, could become a major contributor to the enhancement of agricultural AD systems. Producers with adequate space may find that this option is the most economical requiring low energy consumption, low maintenance, and only small capital expenditures. Furthermore, advancements in biological system management and enhanced microbial workers are developed daily.

Conclusion

The driving forces of agricultural are historically based on profitability and yield, only recently have environmental concern prompted operational changes. New government regulations on environmental protection, nutrient application, and waste management strategies are beginning to impact agricultural operations. This promoted producers to rethink and redevelop their management strategies, to incorporate the technologies that will meet or exceed regulations while being capable of marinating profitability. As energy prices increase agricultural operations are looking at new sources of sustainable energy to alleviate the stress that fossil fuels are creating. AD is a developed and utilized technology in agriculture today, providing a strategy to treat and manage agricultural wastes by reducing solids, conserving nutrients, controlling odor, and developing an economical return in the methane gas that can be collected.

Pre-treatment strategies for AD increase the capital cost when integrating a AD, and very little on-farm research has been accomplished. Applications of full-scale systems in municipal plants and laboratory experiments shows that pre-treatment is capable of increasing the efficiency, productivity, and applicability of AD systems. Translating these benefits to an agricultural setting is the largest hurdle for the implementation of pre-treatment strategies. However, as the technologies improve and become affordable, energy prices increase, and environment regulations tighten economics and profitability will drive the agricultural sector implement these technologies and recover the benefits.

References

Angelidaki, I., Ahring, B. (2000) Methods for Increasing the Biogas Potential from the Recalcitrant Organic Matter Contained in Manure. *Water Science and Technology*. **41**, 3, 189.

Ardic, I., Taner, F. (2005) Effects of Thermal, Chemical and Thermochemical Pretreatments to Increase Biogas Production Yield of Chicken Manure. *Fresenius Environmental Bulletin*, **14**, 5, 373.

Bougrier, C., Delgenes, J.P., Carrere, H. (2006) Combination of Thermal Treatments and Anaerobic Digestion to Reduce Sewage Sludge Quantity and Improve Biogas Yield. *Process Safety and Environmental Protection*. **84**, B4, 280.

Dohanyos, M., Zabranska, J., Kutil, J., Jenicek, P. (2004) Improvement of Anaerobic Digestion of Sludge. *Water Science and Technology.* **49**, 10, 89.

Eastnun, J. and Furguson, J. (1981) Solubilizatoin of Particulate Organic Carbon During the Acid Phase of Anaerobic Digestion. *Journal of Water Pollution Control Federation*. **3**, 352.

Feijoo, G., Soto, M., Mendez, R., Lema, J. (1995) Sodium Inhibition in the Anaerobic Digestion Process: Antagonism and Adaptation Phenomena. *Enzyme and Microbial Technology*. **17**, 180.

Gonze, E. Gonthier, Y., Boldo, P., Bernis, A. (1998) Standing Waves in a High Frequency Sonoreactor: Visualization and Effects. *Chemical Engineering Science*. **53**, 3, 523.

He, P., Lu, F., Shao, L., Pan, X., Lee, D. (2006) Effect of Alkali Metal Cation on the Anaerobic Hydrolysis and Acidogenesis of Vegetable Waste. *Environmental Technology*. **27,** 3, 317.

Hogan, F., Mormede, S., Clark, P., Crane, M. (2004) Ultrasonic Sludge Treatement for Enhance Anaerobic Digestion. *Water Science and Technology*. **50**, 9, 25.

Mladenovska, Z., Hartmann, H. Kvist, T. (2006) Thermal Pretreatment of the Solid Fraction of Manure: Impact on the Biogas Reactor Performance and Microbial Community. *Water Science and Technology.* **53**, 8, 59.

Muller, J., Winter, A., Strunkmann, G. (2004) Investigation and Assessment of Sludge Pre-treatment Processes. *Water Science and Technology*. **49**, 10, 97.

Rinzema, A. van Lier, J. Lettinga, G. (1988) Sodium Inhibition of Acetoclastic Methanogens in Granular Sludge from a UASB Reactor. *Enzyme and Microbial Technology*. **10**, 101.

Shamara, D., Mishra, I., Sharma, M., Saini, J. (1988) Effect of Particles Size on Biogas Generation from Biomass Residues. *Biomass*. **17**, 4, 251.

Shimizu, T., Kudu, K., Nasu, Y. (1993) Anaerobic Waste-activated Sludge Digestion – a Bioconversion Mechanism and Kinetic Model. *Biotechnology and Bioengineering*. **41**, 11, 1082.

Tiehm, A., Nickel, K., Zellhorn, M. and Neis, U. (2001) Ultrasonic Waste Activated Sludge Disintegration for Improving Anaerobic Stabilization. *Water Research*. **35**, 2003.

Valo, A., Carrere, H., Delgenes, J.P. (2004). Thermal, Chemical, and Thermo-chemical Pre-treatment of Waste Activated Sludge for Anaerobic Digestion. *Journal of Chemical Technology and Biotechnology,* **79,** 1197.