

**BIOREACTORS AND  
BIOPROCESSES ENGINEERING  
LABORATORY (BBEL)**



## **I-31. Analyzing and Modeling Photobioreactors for Microalgal and Cyanobacteria Cultures**

### **A. Problem definition**

Microalgae and cyanobacteria cultures have been recognized for their potentials in many industrial applications, such as high value compounds (e.g., polyunsaturated fatty acid) production, wastes' treatment, CO<sub>2</sub> fixation, and renewable bioenergy, etc. Due to the prohibitively high cost of production, however, only a few industrial photobioreactors (PBR) for mass producing microalgae or cyanobacteria have been built and operated with most of them failed in a few months. To cut production costs, successful design and scale-up of PBRs to maximize the areal or volume productivity is crucial.

The major problem for PBR design and scale-up is light: its availability and its use efficiency. Light energy is usually supplied from reactor surfaces, and its intensity decreases exponentially from the illuminated surface to the center. Although the overall effects are photolimitation due to the large dark center, photoinhibition along the highly illuminated surface, where light energy concentrates, could significantly lower the light use efficiency. It was found that mixing can greatly enhance productivity by improving the light use efficiency due to the induced beneficial flashlight effects. These effects are caused by moving cells between the illuminated surface and the dark center, which is determined by the local characteristics of hydrodynamics. However, these local characteristics remain unclear because most conventional measurement techniques neither provide in-depth knowledge nor be applied in opaque PBRs. As a result, the mechanism of how flashlight (in other words, hydrodynamics) interacts with photosynthesis is not clear. Moreover, most current studies for photobioreactor performance evaluation resort to static photosynthetic rate models due to the limited hydrodynamic information. Based on these studies which ignore the flashlight effects and can only be applied for specific conditions, the design and scale-up of PBRs require extensive, costly and labor-intensive empirical efforts.

### **B. Research Objectives**

The overall objective of this study is to advance the understanding of hydrodynamics' role in photobioreactor performance, and to develop a fundamentally based modeling approach for PBR performance evaluation.

### **C. Research Accomplishments**

Using both CARPT and CT technique, a comprehensive study was carried out to study the local hydrodynamic characteristics in a draft tube airlift column reactor in air-water and in microalgae culturing systems. These CARPT and CT experiments focus on investigating macro- and micro-mixing and the liquid flow field in the fully developed flow region, as well as in the Top and the Bottom regions. The effects of selected geometrical and operating parameters (i.e., the superficial gas velocity and the sizes of the Top and Bottom regions) on the hydrodynamics of the airlift column reactor are also investigated.

Based on the findings from the CARPT measurement, we proposed a mechanism for the interactions between the flow dynamics and photosynthesis. The temporal irradiance patterns were calculated from the CARPT measured particle trajectories using an appropriate irradiance distribution model. These patterns contain a cascade of light fluctuations with different frequencies due to the chaotic nature of flow dynamics. Based on the principles of how flow dynamics interact with photosynthesis, a concept of over-/under- charged cycle was proposed. This concept was also applied to quantitatively characterize the light availability and fluctuations delivered to the cells by three parameters: the time averaged irradiance, the frequency of the over-/under- charged cycles, and the dimensionless relaxation time.

A novel dynamic modeling approach was developed for PBR performance evaluation. This general approach integrates first principles of photosynthesis, hydrodynamics, and irradiance distribution within the reactor. It can be extended to include other physiologically based photosynthesis rate models and irradiance distribution models. Hence, this approach provides a direct and comprehensive tool for photobioreactor analysis, which should be essential for proper and efficient reactor design and scale-up for large-scale biomass production.

An extensive verification of the developed dynamic growth rate model was conducted. A red marine alga, *Porphyridium sp.*, was cultured in three types of airlift column reactors, i.e., draft tube, split, and bubble columns. The physical properties, multiphase flow dynamics, irradiance distribution inside the reactor, evolution of the biomass concentration, and photoinhibition effects were examined. The developed dynamic growth rate model successfully predicted the reactor performances measured in this study (Figure 1) and the performance measured by Merchuk et al. (2000) (Figure 2). These results demonstrated the robustness of the developed dynamic growth rate model and indicated its potential applicability in industrial interested conditions (i.e., high incident light intensity and biomass concentration).

A computationally promising CFD simulation model has been identified to study the multiphase flow dynamics in an internal loop airlift column reactor under the bubbly flow regime. This model is based on 3D steady state simulations and uses the  $k-\varepsilon$  turbulent model, Ishii-Zuber's drag force correlation, and Lopez de Bertodano's turbulent dispersion force with a coefficient of 0.3. This model satisfactorily captured the mean multiphase flow field, but considerably under-estimated the turbulent intensity in the studied airlift column.

#### **D. Reference**

1. Luo, H.-P., Al-Dahhan, M.H. (2004) Analyzing and Modeling of Photobioreactors by Combining First Principles of Physiology and Hydrodynamics. *Biotechnology and Bioengineering*. 85(4), 382
2. Luo, H.-P., Kemoun, A., Al-Dahhan, M.H., Fernandez, J.M. & Molina, E. (2003) Analysis of Photobioreactors for Culturing High Value Microalgae and Cyanobacteria Via an Advanced Diagnostic Technique: CARPT. *Che.Engg.Sci.*, 58(12), 2519

3. Merchuk, J.C., Gluz, M. & mukmenev, I. (2000). Comparison of PBRs for cultivation of the microalga *Porphyridium sp.* *J. Chem. Technol. Biotechnol.* 75(12), 1119-1126

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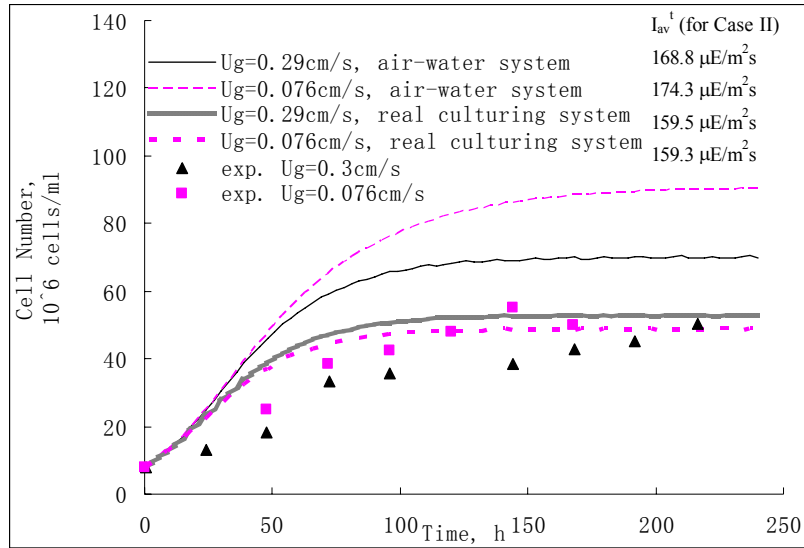


Figure 1. Dynamic simulation of the reactor performance measured by Merchuk et al. (2000) using CARPT data obtained from *Porphyridium sp.* culturing system. The prediction made in Chapter 5 based on CARPT data obtained from an air-water system is also shown. The time-averaged light intensities were calculated using Lambert-Beer law for conditions of External Irradiance= $250 \mu E m^{-2} s^{-1}$  and Cell concentration= $8 \times 10^6$  cells/ml.

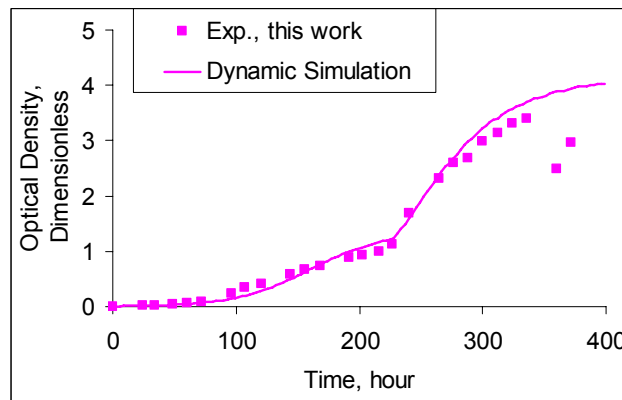


Figure 2. Dynamic simulation of the reactor performance measured in this study using the CARPT data obtained in *Porphyridium sp.* culturing system and justified model parameters.



## **I-32. Shear Mapping in Impeller Mixed Anaerobic Digester Using CARPT**

### **A. Problem Definition**

Mixing plays several essential roles during anaerobic digestion of sludges (e.g., animal waste and waste activated sludge, WAS). These roles include enhancing substrate contact with the microbial community, improving pH and temperature uniformity, preventing stratification and scum accumulation, facilitating the removal of biogas from the digestant, and aiding in particle size reduction. The most conventional way of mixing in full-scale reactors treating sludges is through mechanical methods using an impeller. The intensity at which mixing occurs, and thus the shear rate, has an effect on performance of anaerobic digestion. Generally, it is thought that the more energy one puts into mixing, the more efficient the process will be. Indeed, high mixing intensities resulted in particle size reduction and diffusion limitation reduction, which increased processing capacity for a digester treating WAS. However, it has also been hypothesized that a high shear rate may be harmful to anaerobic digestion because it may disrupt spatial associations between syntrophic microorganisms. Several studies have shown that high mixing intensity and duration has a detrimental effect on the reactor performance, but it has not been experimentally verified if disruption of syntrophic relationships is the cause. A better understanding of the role of mixing in anaerobic digestion will result in better design and operation, leading to a reduction in failure rate and increased utilization of anaerobic technology on the farm.

### **B. Research Objectives**

The overall objective of this project is to study the effect of mixing intensity (i.e., applied shear) on digester performance, microbial ecology, and syntrophic relationships in continuously stirred anaerobic digesters treating cow manure. This part only deals with the quantification of the shear stress in the impeller mixed digesters using a non-invasive Computer Automated Radioactive Particle Tracking (CARPT) technique (Karim et. al. 2004). The evaluation of effect of shear stress on the performance of the digester and the factors mentioned above was carried out in a separate performance study (Hoffman, 2005). The results of the both the studies will be combined together to accomplish the desired objective. The digesters used in the shear mapping study were similar to the digesters used in the performance studies along with the same operating conditions.

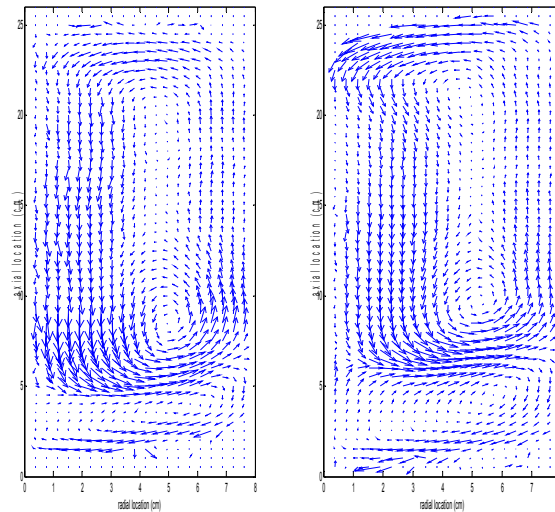
### **C. Research Accomplishments**

Three impeller speeds, 50, 250, and 500 rotations per minute (rpm), were used to create mixing within the 6 inch diameter digester (4 L working volume) with 25° hopper bottom. The digester was mixed by a 62 mm diameter axial flow impeller (Lightnin A-310) with a motor at different speeds as listed above. The effluent from the digesters running for performance studies at a given impeller speed was used in the respective CARPT experiments at a particular impeller speed.

CARPT provides valuable hydrodynamic information including flow pattern, velocities, dead zones and turbulence parameters like shear stresses, turbulent kinetic energies and eddy

diffusivities. The results are shown here only for digester with impeller speeds of 250 and 500 rpm, the flow created at 50 rpm in the digester was too weak to give any useful data or results using CARPT.

Figures 1 a & b show the flow pattern obtained in the digester with impeller speeds of 250 and 500 rpm, respectively. They show the direction and magnitude of the velocities inside the reactor, the circulation loops are also clearly visible. Lightnin A310 is an axial flow impeller; it means that it should create an axial flow in the upward or downward direction based on the direction of rotation. These figures show two circulation loops, a loop with weak downward flow at the bottom and another loop with strong upward flow at the top. The downward velocity vectors merging towards the center at the top of the digester are the indication of vortices. Vortices are formed due to absence of baffles. The flow pattern remains the same for both the impeller speeds of 250 and 500 rpm. However the magnitudes of the velocities are different.



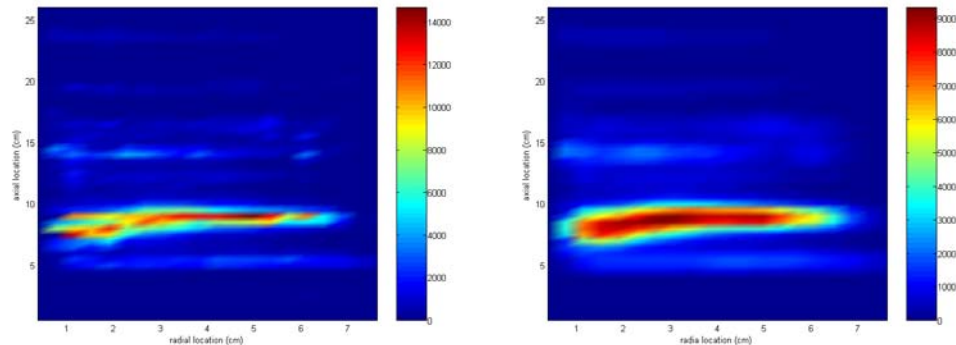
**Figure 1 a & b.** Velocity vector plot in the digester with impeller speeds of 250 and 500 rpm, respectively

As expected the radial and azimuthal velocities are highest at the plane of the impeller. The velocities obtained at 500 rpm speed are relatively higher than the speed of 250 rpm.

The velocities calculated here are the time averaged and azimuthally averaged values. The CAPRT data is processed to obtain the instantaneous values of velocities which are then processed further to obtain the time averaged velocities and other hydrodynamic parameters. The shear stresses viz. Reynolds stresses and normal stresses are obtained from these instantaneous velocities.

Figures 2 a & b show the mapping of azimuthally averaged radial shear stress in the digester operated at impeller speeds of 250 and 500 rpm, respectively. Radial shear stress is higher in the high velocity region near the impeller and the value of radial shear stress at 500 rpm is greater than that at the 250 rpm shear stress value. The maximum value of radial shear stress at 500 rpm is over 14,000 dynes/cm<sup>2</sup> whereas for 250 rpm its about 9000 dynes/cm<sup>2</sup>. Axial

shear stress is approximately 10 orders of magnitude lower than the radial shear stress. The shear stress values reported here are the azimuthally averaged values of normal shear stress. Reynolds stresses can also be obtained from the CARPT data.



**Figure 2 a & b.** Mapping of azimuthally averaged radial shear stress in dynes/cm<sup>2</sup> at 250 and 500 rpm impeller speed, respectively.

#### **D. Conclusions and Recommendations**

It was possible to visualize the flow inside the digester for different impeller speeds using CARPT. CARPT also provided values of velocities inside the digester. As expected the velocities were higher near the impeller region. The Lightning A310 impeller, which is axial flow impeller, produced a strong upward axial flow for both the speeds. The absence of baffles inside the digesters caused vortices. The radial shear stress was noticeably higher than the axial shear stress and the higher values of shear stresses were obtained near the high velocity impeller region. The flow produced at impeller speed of 50 rpm was too low to obtain any meaningful results from CARPT study. The mapping of shear inside the digester provided the estimate of the shear stress values. These values can be related to the performance studies to study the effect of shear on the microorganisms.

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#### **E. Reference**

1. Karim K., Varma R., Vesvikar M., Al-Dahhan M. (2004). Flow Pattern Visualization of a Simulated Digester. *Water Research* 35(7): 1817-1827.



### **I-33. Hydrodynamic Study of Gas Recirculation Bioreactors Using Radioactive Particle Tracking and Gamma Ray Tomography**

#### **A. Problem Definition:**

Anaerobic degradation of biological matter is a natural process that occurs in the absence of oxygen, the end result of which is the production of methane. Unsafe and improper disposal of biological matter (e.g., animal waste from cattle, poultry and hog farms, bagasse from sugarcane, wheat and rice straw, etc.) results in environmental pollution like contamination of ground and surface water, foul odor and increase in the Biological oxygen demand of water resulting in the loss of aquatic fauna.

Methane generated by anaerobic digestion of animal waste has been proved to be promising option for converting animal waste (biomass) to useful and sustainable energy in the form of methane (Gosh, 1997). Hence, using anaerobic bioreactors to capture the methane turns an environmental pollution liability into an asset. Hydrodynamics, like in any conventional chemical reactor, plays an important role in the performance of such reactors. A detailed study of the role of mixing induced by the hydrodynamics has not been clearly understood.

Aspects of the anaerobic digesters design and scale up, and effect of the hydrodynamics on mixing need further investigation. With the advances in imaging science and radioactive particle tracking, it is possible to develop noninvasive methods to investigate the hydrodynamic behavior of these systems, despite them being opaque. Computer Tomography is technique that can give valuable insight in the holdup distribution of the different phases in such a system, and radioactive particle tracking gives information on the velocity profile of the phase being tracked. Studies have been carried out with the aid of single radioactive source Computer Tomography and computer automated radio active particle tracking to determine the effect of holdup distribution of gas phase on the velocity profiles (hence hydrodynamics) in a anaerobic bioreactor.

#### **B. Research Objective**

The objective of this work is to compare the effect of gas phase distributor design on the flow field of the lab scale gas recirculated anaerobic bioreactor. The distribution of the gases phase determines the bouncy forces in the system, which in turn affects the liquid phase circulation. Studies were carried out using single orifice gas sparger system and a multi orifice gas sparger system for the same superficial gas velocity.

#### **C. Research Accomplishments**

Studies were carried out with a lab scale digester of 6' and a °25 flanged and angled bottom (Figure 1). A 5% (weight by volume basis) slurry of animal waste was used for the studies. It was found that the slurry with 5% solids concentration attenuates the gamma rays as much as pure water does. Hence this system was assumed to be a two phase system with the slurry and water as one phase and the gas as the other for the tomography studies. Biogas recirculation rate was maintained at 1 liter/min, 3 lit/min and 5 lit/min. Single orifice and multi orifice spargers were used. The tomographic scans were carried out at two axial

locations in the reactor. It was observed that gas was uniformly distributed in the draft tube for multi-orifice (Figure 2A), this in turn aids the liquid recirculation (Figure 2B). Hence the use of a sparger is recommended in such a system.

For the single orifice system, the results suggest channeling of the gaseous phase in the draft tube (figure 2). This renders the mixing ability of the gaseous phase in a gas mixed reactor, like this one, ineffective. The multi orifice system ensures that the gas phase is distributed in the draft tube region of the reactor, and hence improve the liquid recirculation.

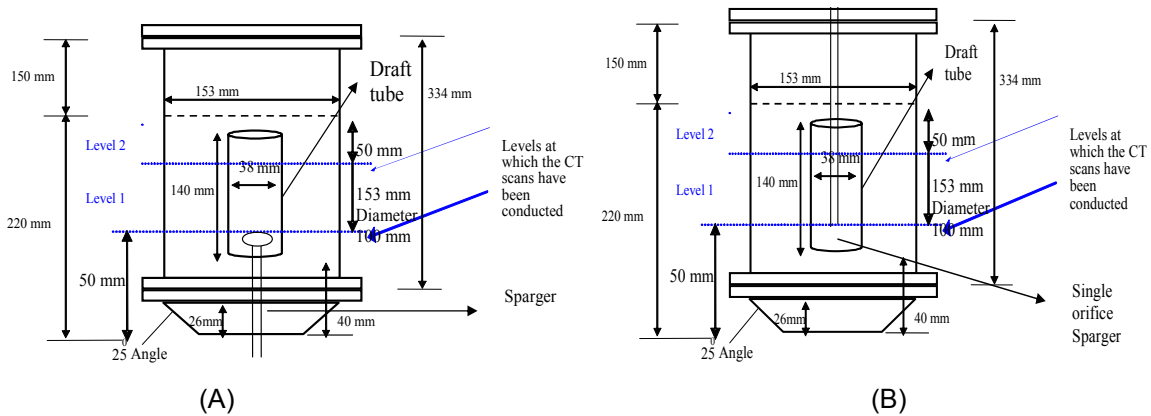


Figure 1: Schematic of the bioreactor used. (A) with multi orifice (B) with single orifice

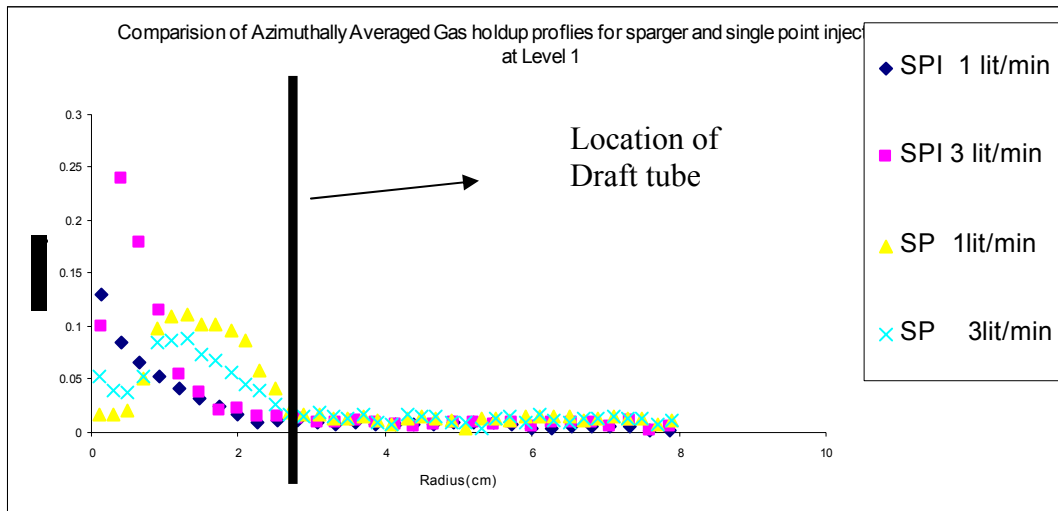


Figure 2: Comparison of azimuthally averaged gas Hold up profiles for Single point injection system (SPI) and sparger system (SP), al level 1.

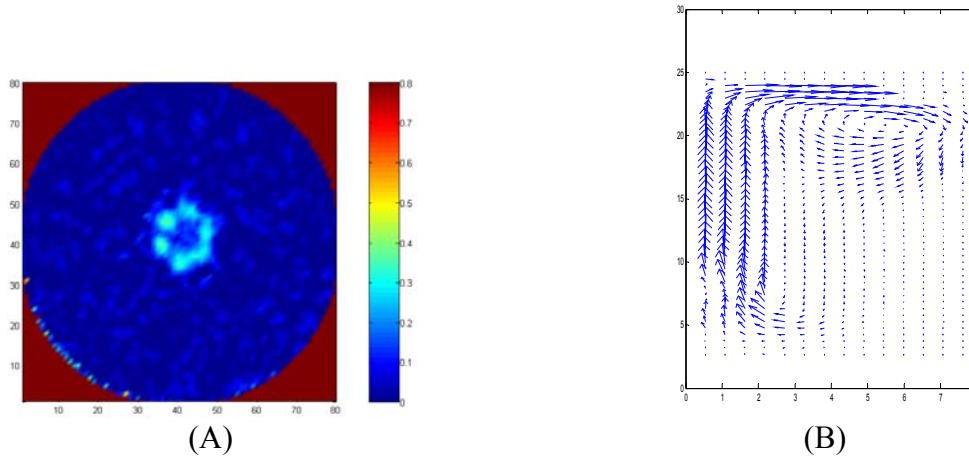


Figure 3: (A) Gas holdup distribution in anaerobic bioreactor (cross sectional view).  
 (B) Time averaged longitudinal (r,z) liquid velocity profile in anaerobic digester.

#### D. References

1. Casey T. J., "Requirements and methods for mixing in anaerobic digesters", *Anaerobic digestion of sewage sludge and organic agricultural wastes*, Elsevier App. Sci. Pub., 1986, 90-103.
2. Ghosh, S. (1997) *Anaerobic Digestion For Renewable Energy and Environmental Restoration*. The 8th International Conference on Anaerobic Digestion, Sendai International Center, Sendai, Japan, Ministry of Education Japan.
3. Roy, S., Abdenour Kemoun, Muthanna Al-Dahhan and M. P. Dudukovic (2001) "A method for estimating the solids circulation rate in a closed-loop circulating fluidized bed." *Powder Technology*, **121**(2-3), 213-222.

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## **I-34. Performance Study of a Pilot Scale Anaerobic Digester**

### **A. Problem Definition**

Mixing in anaerobic digester is required for number of important reasons viz. to provide efficient utilization of entire digester volume, to prevent stratification and temperature gradients, to disperse metabolic end products and any toxics contained in the feed, to maintain intimate contact between the bacteria and the substrate, to prevent foaming and scum formation and to avoid solids settling. In short, adequate mixing provides a uniform environment, one of the keys to good digestion.

In spite of the crucial role played by mixing in digester's operation, contradictory findings are reported in the literature about the necessity of mixing and the required mixing intensity to enhance the digester performance. There are many reasons for the controversies and uncertainties about the mixing effect on anaerobic digesters. One of them is, mixing is not adequately quantified and characterized in these systems. Another important reason is most of these digester performance studies are performed in small laboratory scale reactors and/or using low solids concentration. These approaches do not contribute greatly in understanding the mechanisms by which mixing influences anaerobic digestion performance and neither does it provide criteria on which full scale digester design or strategies of operation can be based.

Laboratory scale reactors are valuable in estimating kinetic parameters, in estimation of nutrient and alkalinity requirements and discovering potential problems like toxicity. This is possible because small scale digesters are easy to control, efficient mixing and uniform environment can be guaranteed. On the other hand, experimentation on a large scale digester is necessary to elucidate the operational problems and difficulties like effects of improper mixing, clogging of feed and outlet ports, solids accumulation, foaming and so on.

### **B. Research Objectives**

1. To study the effect of mixing on the pilot scale digester.
2. To demonstrate the effect of digester size on the role of mixing by comparing the lab scale and pilot scale digester performance.

### **C. Research Accomplishments**

A stainless steel pilot scale digester with working volume of 97 liters (18 inches in diameter) was designed and developed for this study. The unit was geometrically similar to the laboratory scale digester of 4 liters working volume (6 inches in diameter) used in the study performed by Karim et al. 2005. Digester was mixed by recirculation of biogas, the digester was equipped with a draft tube and a sparger. The biogas recirculation rate was calculated as 9.07 liter per minute, resulting in an input energy density of  $8 \text{ W/m}^3$ , which corresponds to 1 l/min biogas recirculation rate in the 6-inch laboratory scale (4-litres) unit at same energy input rate. For gas mixed condition, gas was pumped out from the top of the reactor by a pump and returned to the digester at the lowest point of the draft tube. For unmixed

condition, simply the recirculation of gas was stopped by switching off the pump. The digester was operated using cow manure collected from a local dairy farm in the Oak Ridge, TN area. The raw sludge was processed and diluted with water to obtain 6.6% total volatile solids (total solids of about 12-13%) concentration. The pilot scale digester was operated in a manner similar to the small scale units to maintain the similarity in the process. Every other day, 12 L of reactor content was removed and 12 L of feed slurry was added to the top of the digester. This feeding rate corresponds to a hydraulic retention time of 16 days. Gas samples were analyzed for methane and carbon dioxide content. Liquid samples were analyzed for total solids (TS), total volatile solids (TVS), total Fatty acids (TFA), and total alkalinity (TA).

The digester operation was started with biogas recirculation. After 70 days of operation of the digester in mixed condition, biogas recirculation was stopped and it was operated in unmixed condition for more than 70 days. Again the biogas recirculation was started after this and digester was operated in mixed condition for more than 12 days, this was done to check the reproducibility of the results obtained.

Approximately 0.55 L biogas (or 0.4 L CH<sub>4</sub>) per L digester volume per day was produced for initial mixed condition. For the unmixed condition the biogas (methane) production rate gradually decreased to significantly lower values. These rates increased after starting the mixing again. The increase in the gas production rates after switching to mixing condition from unmixed was enough indication of positive effect of mixing on the digester performance. The composition of the biogas from the digester is approximately 65% CH<sub>4</sub> and 35% CO<sub>2</sub> for mixed condition. For the unmixed condition the composition of biogas changed lowering the methane content to approximately 55%.

Since the destruction of organic matter is the primary objective of anaerobic digestion, fraction of VS and TFA consumed during the digestion process indicates the degree of stabilization or efficiency of digester performance. Considerable reduction in TS and TFA was observed for both mixing conditions, whereas the TS and TFA reached the feed value by the end of run for unmixed condition.

Cumulative methane production rates for three operations are illustrated in Figure 1. All three sets of data were fitted linearly; the slope of the line represents the average daily methane production rate (L/day). The slope of line for initial and repeated mixed condition is approximately the same (around 40 L/day). The slope of the line for unmixed condition (20 L/day) is significantly lower than the mixed condition. This confirms that the methane production rate for mixed condition is significantly higher than the unmixed condition. And the reproducibility of the slopes within acceptable range confirms that the change in the gas or methane production was due to change of mixing condition.

The small scale digesters of Karim et al. (2005) did not show any difference between performance of mixed and unmixed conditions. Also the small scale reactors performed better than the pilot scale digesters in terms of gas production and reduction of TS and VFA. Since bioreaction is slow, even the mixing produced by the motion of evolving gas bubbles

and the addition of feed in the unmixed digester was sufficient for efficient operation of the laboratory scale digester.

As the size of the reactor increases, difficulty in achieving complete mixing increases, the mixing time scales decreases whereas the reaction time scale is unaffected. Thus for the pilot scale digester the mixing time constants and reaction time constants were comparable and the mixing affected the digester performance. Therefore the performance of pilot scale was dramatically different for the mixed and unmixed condition, where as mixing showed no effect in small scale units.

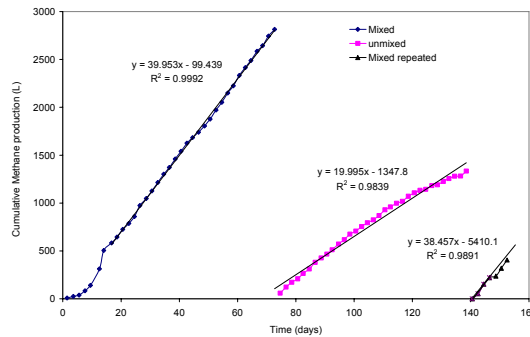


Figure 1. Cumulative Methane production in pilot scale digester.

#### D. Conclusions and Recommendations

Significant differences between the results obtained for mixed and unmixed condition in the used pilot-scale digester were observed. Mixing provided in the digester results in its efficient operation and avoids its failure. Significant differences in the performance due to different modes of mixing provided were not obtained at laboratory scale units. At the smaller scale the mixing created by the evolution of gas bubbles is sufficient for proper operation of the unit. Any additional amount of mixing does not benefit the digesters to create more gas, necessarily because the bioreaction is slow. Excessive amount of mixing is also not recommended as mixing needs energy and spending more energy will not be cost efficient or profitable. This concludes that large scale operation of digester is necessary to obtain meaningful results and findings that can be used for proper design of commercial scale units.

#### E. Future Work

The following essential question arises: what is the best or optimum mixing intensity to ensure efficient performance or less energy input to maximize the energy output obtained from the anaerobic digester as biogas. This question is yet to be answered and it needs further investigation using large scale digesters. The findings in the pilot scale digester and their comparison with those obtained with 6-inch digester suggest that laboratory scale digesters are of no use to determine the optimum mixing intensity needed for efficient digester performance.

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## **E. Reference**

Karim K, Klasson T, Hoffman R, Drescher SR, Depaoli DW, Al-Dahhan MH. (2005). Anaerobic Digestion of Animal Waste: Effect of Mixing. *Bioresource Tech.* In press.

## **I-35. Effect of Shear on Performance and Microbial Community in Anaerobic Digesters Treating Cow Manure**

### **The Problem**

The United States is home to approximately 100 million head of cattle that produce 1.8 million metric tons of wet waste annually. Cattle waste is often improperly stored and handled leading to several environmental problems, such as surface and groundwater contamination and emissions of atmospheric pollutants and greenhouse gasses. If properly handled, this waste can be a valuable resource to produce bioenergy.

### **How can Anaerobic Digestion Help?**

Anaerobic digestion is a waste treatment alternative for both industrial and agricultural wastes in which microorganisms breakdown organic material in the absence of oxygen to create biogas. Biogas consists of approximately 65% methane and 35% carbon dioxide, with traces of dinitrogen gas and gaseous sulfur compounds. With such a high methane content, biogas is used as an energy carrier. Besides reducing greenhouse gas emissions and producing a renewable energy carrier, anaerobic digestion also reduces odor, protects water quality, and controls ammonia release. Anaerobic digestion has several advantages over aerobic treatment, including higher organic removal rates, lower sludge production, and a net production of energy. Although industrial wastes have successfully utilized anaerobic digestion to reduce organic pollutants in waste streams for over 30 years, implementation of anaerobic digesters on farms for the purpose of treating animal manure and farm wastes has had high failure rates. Such high failure rates are believed to be mainly due to poor design and construction (Lusk, 1998).

### **The Role of Mixing**

Mixing plays several essential roles during anaerobic digestion of sludges (e.g., animal waste and waste activated sludge [WAS]). These roles include enhancing substrate contact with the microbial community, improving pH and temperature uniformity, preventing stratification and scum accumulation, facilitating the removal of biogas from the digestant, and aiding in particle size reduction (Stenstrom et al., 1983). The most conventional way of mixing in full-scale reactors treating sludges is through mechanical methods using an impeller. The intensity at which mixing occurs, and thus the shear rate, has an effect on performance of anaerobic digestion (Stenstrom et al. 1983). Generally, the more intense the mixing, the more efficient the process will be. Indeed, high mixing intensities resulted in particle size reduction and diffusion limitation reduction, which increased processing capacity for a digester treating WAS (Lanting 2003). On the other hand, it has also been hypothesized that a high shear rate may be harmful to anaerobic digestion because it may disrupt spatial associations between syntrophic microorganisms. Several studies have shown that high mixing intensity and duration has a detrimental effect on the reactor performance (Dague et al. 1970; Hansen et al. 1999; Angenent et al. 2001; McMahon et al. 2001; Stroot et al. 2001), but it has not been experimentally verified if disruption of syntrophic relationships is occurring. A better understanding of the role of mixing in anaerobic digestion will result in better design and operation, leading to a reduction in failure rate and increased utilization of anaerobic technology on the farm.

### How Do We Determine the Effects of Mixing?

The objective of this research is to study the effect of mixing intensity (i.e., applied shear) on digester performance, microbial ecology, and syntrophic relationships in continuously stirred anaerobic digesters treating cow manure. Four impeller speeds, 50, 250, 500, and 1,500 rotations per minute (RPM), were used to create a large mixing range, and thus applied shear, within the digesters. Performance of each digester was monitored for parameters indicating stability and performance, including total solids (TS), volatile solids (VS), soluble chemical oxygen demand (SCOD), ammonia, alkalinity, and volatile fatty acids (VFAs) by the Standard Methods (APHA, 1998). Gas production was monitored daily, and methane content was determined weekly by gas chromatography (Gow-Mac Instruments, Co., Bridgewater, NJ, USA) with a thermal conductivity detector. Together, these parameters give insight into the health of the digester, indicate digester upsets, and signify the level of digester stability. In order to define the hydrodynamic parameters in each digester, a non-invasive technique, Computer Automated Radioactive Particle Tracking (CARPT) (Karim 2004), was performed on the 500, 250 and 50-RPM digesters to acquire information on the shear stresses within each digester along with the flow patterns, velocities, and other turbulence parameters. The limitations of this method did not allow for the 1500 RPM digester to be evaluated. This technique uses a number of scintillation detectors track a Scandium 46 particle having a density equal to the waste. The results found using this technique are part of another student's work, and can be reported elsewhere. Fluorescent *in situ* hybridization (FISH) was performed according to Angenent *et al.* (2002a; 2002b) to determine the effect of mixing on the juxtaposition between Archaea and Bacteria. The probes used were EUB 338, targeting virtually all Bacteria, and ARCH 915, targeting virtually all Archaea. Finally, membrane hybridization will be performed on samples taken at intervals during the study to investigate whether different microbial communities develop in response to different shear rates, and to track population changes that may occur within each digester. Membrane hybridization was performed according to Raskin *et al.* (1994), and the probes used are shown in Table 1.

Table 1 Oligonucleotide probes, target reference groups, and original references

Probes	Target Group	Reference
S-*-Univ-1390-a-A-18	Virtually all organisms	(Zheng <i>et al.</i> , 1996)
S-D-Bact-0338-a-A-18	Virtually all Bacteria	(Amann <i>et al.</i> , 1996)
S-D-Arch-0915-a-A-20	Virtually all Archaea	(Stahl and Amann, 1991)
S-O-Mmic-1200-a-A-21	Methanomicrobiales	(Raskin <i>et al.</i> , 1994)
S-F-Mbac-0310-a-A-22	Methanococcaceae	(Raskin <i>et al.</i> , 1994)
S-G-Msar-0821-a-A-24	Methanosarcina spp.	(Raskin <i>et al.</i> , 1994)
S-S-M.con-0381-a-A-22	Methanosaeta concilii	(Zheng and Raskin, 2000)

## Reactor Operation

The experiments were conducted in four laboratory scale reactors having a working volume of 4.5 L with a 25° slope angle hopper bottom. The reactors were inoculated with primary anaerobic digester sludge collected from the St. Louis Metropolitan Sewer District's Cold Water Creek facility, Florissant, MO. Feed was prepared by diluting fresh cow waste, and screening through a 2-mm sieve. The product was then diluted to achieve a solids concentration of 50 g VS/L. The reactors were maintained at a temperature of 34±1°C and were continuously mixed. The reactors were fed every day by first removing an appropriate amount of effluent and then adding the same volume of prepared manure waste. To avoid overloading of the reactors at start-up, the initial loading rate was 20% of the target loading rate of 3.3 g VS/L-d. The loading rate was periodically increased after a minimum of one sludge retention time when steady-state gas production rates were reached.

## What was the Effect of Mixing?

With such a broad range of applied RPM, very different mixing conditions existed within each digester. Vortexes in the 1500 and 500-RPM digesters created a 35% and 10% jump in relative height of the liquid inside the digester, respectively, while a little vortex developed in the 250-RPM digester and none in the 50-RPM digester. Despite such a large range of applied RPMs, the methane yield of all four digesters was found to be similar during steady-state periods with an average methane yield of all four digesters of 0.367±0.0134 L CH<sub>4</sub>/g VS fed. However, during start-up, the most intensely mixed digester produced little or no gas between day 10 and 25 (see Figure 1), and accumulated VFAs greater than 4000 mg/L as acetic acid (see Figure 2). FISH analysis showed that the microbial flocs in the 1500-RPM digester had severely diminished in size during week one of start-up as compared to the other digesters. FISH images from week one (see Figure 3) show a definite relationship between floc size and the applied shear within the first week of digester operation. However, in later weeks, it was observed that all the digesters contained approximately the same median floc size. The 1500-RPM digester began to recover between day 29 and day 45, showing peaks in biogas production which corresponds with a drop in levels of volatile fatty acids. Similar observations were made in the 500-RPM digester after the first increase in loading rates from 20% to 30% of the target; between day 52 and day 78 the gas production from the 500-RPM digester remained lower than that of the 250 and 50-RPM digesters. On day 78, the VFA levels in the 500-RPM digester reached a peak and then began to rapidly decrease, causing the gas production to rise to that of the other digesters. This phenomenon indicates that intense mixing during start-up can have detrimental effects on reactor performance.

Similar feeding errors were made on day 150 for the 500-RPM and 250-RPM digesters, yielding information regarding the differences in the ability of the digesters to handle a shock. It is clear from Figure 1 and 2 that the 500-RPM digester was able to handle the shock much better than the 250-RPM digester, consuming almost all of the excess substrate in four days with only a small jump in VFA concentration. In contrast, the 250-RPM digester required almost seven days to stabilize, and showed a much larger jump in VFA accumulation. This may indicate the development of a microbial community that is able to handle process instabilities better in the 500-RPM digester as compared to the 250-RPM digester. Membrane hybridizations are currently being performed, to verify this theory.

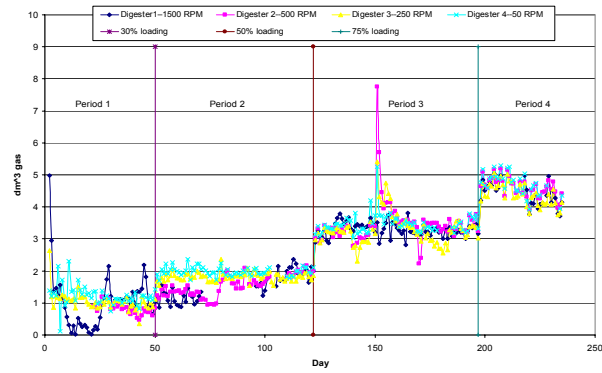


Figure 1. Daily biogas production of digesters with different applied RPM

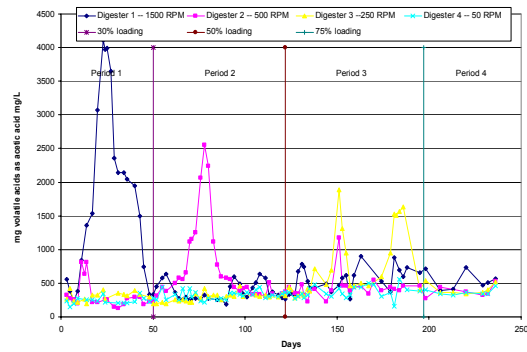


Figure 2. Volatile fatty acid levels of digesters with different applied RPM

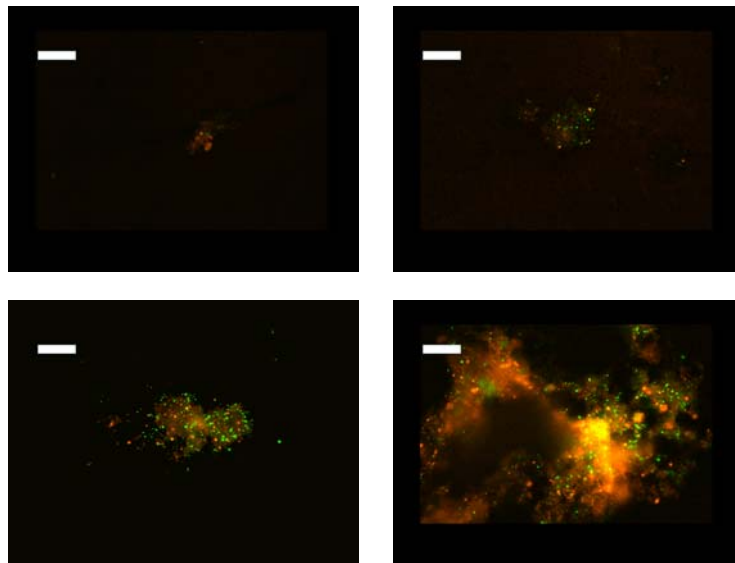


Figure 3 Week 1 FISH pictures with 1000X magnification. A) 1500 RPM B) 500 RPM C) 250 RPM D) 50 RPM.

Gray bar represents 10 micrometers.

## Summary

The knowledge gained from this study is invaluable as it specifically shows that large energy inputs to achieve higher mixing levels in anaerobic digesters are not needed, and in fact, can be very detrimental during start-up. The data presented shows that even over such a broad range of mixing intensities all digesters perform the same during periods of steady-state operation with an average methane yield between all four digesters of  $0.367 \pm 0.0134$  L CH<sub>4</sub>/g VS fed. However, decreased performance of the two high-shear rate digesters as compared to the two low-shear rate digesters during start-up and during the first increase in loading rate, make lower mixing intensities more desirable. FISH analysis did confirm that floc size decreased with increasing shear, but only during the first week of digester operation.

## References

1. Amann, R.I., et al. (1990). "Fluorescent-oligonucleotide probing of whole cells for determinative, phylogenetic, and environmental studies in microbiology." Journal of Bacteriology **172**(2): 762-770.
2. Angenent, L. T., et al. (2001). Mixing intensity in anaerobic sequencing batch reactors affects reactor performance and microbial community structure. 9th World Congress: Anaerobic Digestion 2001, Antwerp, Belgium, Technological Institute vzw and the International Water Association.
3. Angenent, L. T., et al. (2002a). "Methanogenic population dynamics during startup of a full-scale anaerobic sequencing batch reactor treating swine waste." Water Research **36**(18): 4648-4654.
4. Angenent, L. T., et al. (2002b). "Microbial community structure and activity in a compartmentalized, anaerobic bioreactor." Water Environment Research **74**(5): 450-461.
5. APHA. (1998). Standard Methods for the Examination of Water and Wastewater. 20<sup>th</sup> edition. Washington, D.C.: American Public Health Assoc.
6. Dague, R. R., et al. (1970). "Solids retention in anaerobic waste treatment systems." Journal of the Water Pollution Control Federation **42**: R29-R46.
7. Hansen, K. H., et al. (1999). "Improving thermophilic anaerobic digestion of swine manure." Water Research **33**(8): 1805-1810.
8. Karim, K., Varma R., Veskivar M., Al-Dahhan M.H. (2004). Flow pattern visualization of a simulated digester. Water Research, 38 (17), 3659-3670.
9. Lanting, J. (2003). Optimization of Biological Activity for Anaerobic Sludge Digestion, Los Angeles, Water Environment Federation.
10. Lusk, P. (1998). "Methane Recovery from Animal Manures." The Current Opportunities Casebook. National Renewable Energy Laboratory, Golden, CO.
11. McMahon, K. D., et al. (2001). "Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions: II. Microbial population dynamics." Water Research **35**(7): 1817-1827.
12. Stahl, D.A., and Amann, R.I. (1991). Development and application of nucleic acid probes. In: Nucleic acids techniques in bacterial systematics. Stackbrandt E., and Goodfellow M. New York, John Wiley & Sons, Inc., pp. 205-248.
13. Stenstrom, M., et al. (1983). "Anaerobic digestion of municipal solid waste." J. Environ. Eng. **109**: 1148-1158.

15. Stroot, P. G., et al. (2001). "Anaerobic codigestion of municipal solid waste and biosolids under various mixing conditions: I. Digester performance." Water Research **35**(7): 1804-1816.
16. Raskin, L., et. al. (1994). "Group-specific 16S rRNA hybridization probes to describe natural communities of methanogens." Applied and Environmental Microbiology **60**(4): 1232-1240.
17. Zheng, D., et. al. (1996). "Characterization of universal small-subunit rRNA hybridization probes for quantitative molecular microbial ecology studies." **62**: 4504-4513.
18. Zheng, D., and Raskin, L. (2000). "Quantification of Methanosaeta species in anaerobic bioreactors using genus- and species-specific hybridization probes." Microbial Ecology **39**: 246-262.