BIOREACTORS AND BIOPROCESSES ENGINEERING LABORATORY (BBEL)

I-30. Enzymatic Dewatering of Distiller Dried Grains (DDGs)

A. Problem Definition

There are three main reasons why the interest in ethanol has been renewed in the United States since the 1970s: 1) Oil supply disruptions in the Middle East, 2) Environmental concerns over the use of lead as a gasoline octane booster, and 3) the phase-out of the use of MTBE in gasoline. Ethanol can be used as an oxygenate in gasoline and as a blended mixture to reduce the gasoline demand. However, for ethanol to be successful and readily accepted, the cost of ethanol must be close to the wholesale price of gasoline. This can currently be achieved by government subsidies, but unless the cost of ethanol is reduced, it will not be able to compete with gasoline by itself. There are currently 97 corn to ethanol processing plants in operation in the United States, with the overall capacity of producing 4486 million gallons of ethanol per year. In January of 2005, the cost of one gallon of ethanol was around $1.75, but the department of energy seems to think that that cost could be reduced to 60 cents per gallon by 2015. The only way that this goal will be achieved is by advances in biotechnology and process modifications in an attempt to increase the efficiency of the corn to ethanol process.

One way to do that is to decrease the energy requirements of the corn to ethanol dry grind process. Current energy requirements make up 18% of the cost to produce one gallon of ethanol. Two main operations in the process, the jet cooker and the drier, utilize a large portion of that energy. The drier is used to dry up the dried grains that are removed and centrifuged after distillation and used as cattle feed; 38% of the energy cost to produce one gallon of ethanol comes from running the drier (based on 40 mmgpy dry grind corn to ethanol plant). Decreasing the energy requirement of the drier is one way to decrease the production cost of ethanol from corn.

B. Research Objectives

The objective of this work is to identify and evaluate enzymes that will aid in the dewatering of DDGs during centrifugation thus decreasing the moisture content of the DDGs that are sent to the drier in a dry-grind corn to ethanol process. This would result in a decrease in the energy requirement of the drier which would in turn translate into savings in the production cost of ethanol.

C. Accomplishments

Three experiments were run to evaluate and quantify which enzymes were able to degrade the corn kernel cell wall and release bound water found within those cells. Once the enzymes degraded the cell wall, the water could be released during centrifugation of the distiller grains. The second experiment was used to determine whether the enzyme load had an effect on the amount of water being released, and if so, how large of a role would the enzyme load play in the process as a whole. Lastly, the third experiment was done to determine whether or not the experiment could be replicated.

Fourteen enzymes were studied and screened in experiment one. Fifteen bioreactors (14 enzyme treatments and a control) that contained a liquefied and saccharified mash of corn and water were allowed to ferment using *Saccharomyces cerevisiae* for 92 hours. Then a 50ml sample was taken from each bioreactor and centrifuged for 15 minutes in a bench-top centrifuge. The liquid phase was then filtered using a vacuum filtration system and weighed while the solid phase was also weighed. The weights of the liquid and solid phases for each bioreactor were compared to the control and the 6 highest liquid weights and smallest solid weights as a percentage of the control were said to be successful. Table 1 shows the 6 top enzyme treatments as a percentage of the control. These 6 enzymes showed an improvement of up to 18% decrease in moisture content of the distiller grains leaving the centrifuge. This has the result of making a gallon of ethanol three cents cheaper including the extra enzyme cost.

The 6 top enzymes were then rerun in a second experiment, but this time the addition of the dewatering enzyme was made in three different enzyme loads of 0.1, 0.5 and 1ml. It was observed that there was a significant difference in the amount of water removed after centrifugation between the 0.1 and 0.5ml loads but no significant difference in the results for the 0.5 and 1.0ml loads. Table 2 shows the effects of different enzyme loads on centrifugation.

The top 2 enzymes from the first experiment were then run as triplicates using an enzyme load of 0.5ml to see if the modified process could be replicated. The results (Table 3) show that there was no significant difference between the triplicates and that this process modification has the potential to be implemented at a plant scale and still give significant results.

Enzyme Tratment	Liquid Phase (% of control)	Solid Phase (% of control)
A	**110.91**	**83.59**
B	100.83	102.73
C	105.19	98.44
D	100.13	102.97
E	**109.69**	90.85
F	109.27	94.16
G	**113.26**	**85.72**
H	**112.52**	**86.12**
I	107.97	**87.80**
J	105.66	101.48
K	107.5	89.83
L	106.6	98.122
M	**112.28**	**88.81**
N	104.55	91.76
O	107.54	91.42
Control	100	100

Table 1: liquid and solid phase weights as percentage of control for various enzyme treatments and control

Enzyme Treatment	Enzyme Load (ml)	Liquid Phase (grams)
A	0.1	29.89
A	0.5	**30.14**
A	1.0	**30.19**
E	0.1	28.04
E	0.5	**29.59**
E	1.0	**30.31**
G	0.1	**30.75**
G	0.5	29.70
G	1.0	**30.39**
H	0.1	**29.82**
H	0.5	30.30
H	1.0	**29.36**
I	0.1	**30.01**
I	0.5	29.78
I	1.0	**30.44**
M	0.1	**30.44**
M	0.5	**29.90**
M	1.0	31.10

Table 2: Liquid phase weights for different enzymes loads for top 6 enzyme treatments

Enzyme Treatment	Liquid Phase (% of control)	Standard Deviation
A1	33.43	0.54
A2	35.34	
A3	34.40	
G1	34.57	0.24
G2	34.11	
G3	34.23	
Control 1	31.59	0.49
Control 2	32.17	
Control 3	29.64	

Table 3: Standard deviations for top 2 enzymes, A and G, and control run as triplicates

D. Future Work and Milestones

During the summer of 2006 a dosage experiment will be performed in order to optimize the amount of enzyme needed for dewatering. Later, the use of proteases during fermentation will also be studied in an attempt to not only improve centrifugation by also the ethanol yield achieved during fermentation. Particle size distribution effects on enzymatic dewatering will be investigated to better understand the mechanism behind enzymatic dewatering and to check for improvements in the process. A more detailed economic analysis and modeling of the centrifuge and drier processes will be done so that cost effectiveness and scale up can be better understood. Last but not least, Genencor's new enzyme, Stargen, that eliminates the need for high-energy processing of starch will be studied in conjunction with the dewatering enzymes to see if improvements can be done further to reduce the cost of ethanol production.

For further information, please contact Bia Henriques at abh1@cec.wustl.edu

E. Acknowledgements

Enzymes were gifts from Genencor International (a Danisco Company) and Novozymes Inc. (North Carolina). Corn was a single hybrid (33A14) grown at the University of Illinois during the 2004 growing season. Work was performed at the USDA headquarters in Wyndmoor, PA under with the help of David Johnston, lead scientist.

F. References

1. Johnston, David, *Composition and Economic Comparison of Germ Fractions from Modified Corn Processing Technologies*, Cereal Chemistry, 82, 8, 2005
2. Farrel, Alexander. *Ethanol Can Contribute to Energy and Environmental Goals*, Science Magazine, 311, 2006, 506
3. Shapouri, Housein. *The 2001 Net Energy Balance of Corn Ethanol*, US Department of Agriculture, Washington, DC, 2004, also available at www.usda.gov/oce/oepnu
4. DiPardo, Joseph, *Outlook for Biomass Ethanol Production and Demand*, Department of Energy, 2002, also available at www.eia.doe.gov/oiaf/analysispaper/biomass

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_2 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-ε 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

For detailed information, please contact Hu-Ping Luo at CREL:
Tel. 314-935-6042, or E-mail: **hpluo@wuche.che.wustl.edu**.

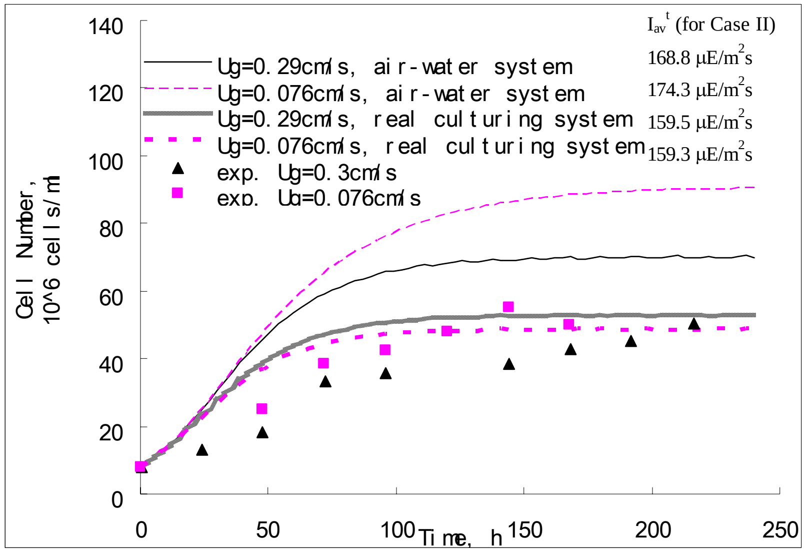


Figure 1. Dynamic simulation of the reactor performance measured by Merchuk et al. (2000) using CARPT data obtained from *Porphyridium sp.* culturing system. The prediction made in Chapter 5 based on CARPT data obtained from an air-water system is also shown. The time-averaged light intensities were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

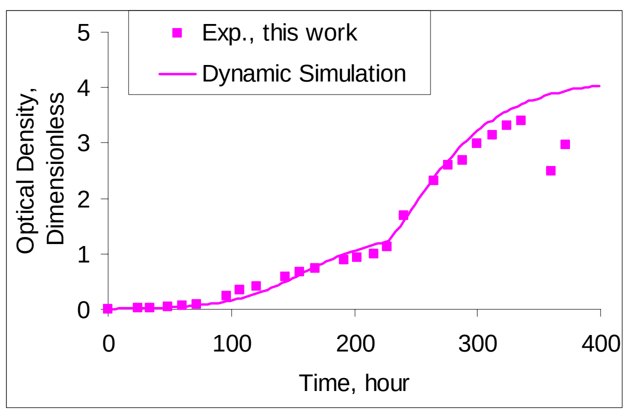


Figure 2. Dynamic simulation of the reactor performance measured in this study using the CARPT data obtained in *Porphyridium sp.* culturing system and justified model parameters.

I-32. Effect of Scale and Mixing on the Performance of Anaerobic Digesters

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 of mixing in digester 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 these controversies and uncertainties. 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 influence of mixing on digester performance or in providing criteria for full scale digester design.

Laboratory-scale reactors are valuable in estimating kinetic parameters, in estimation of nutrient and alkalinity requirements and discovering potential problems like toxicity, because they 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. Objectives

1. To study the effect of mixing on the performance of anaerobic 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. Accomplishments and Current Work

Two identical laboratory-scale digesters with working volume of 3.87 liters (6 inches in diameter) were used. One was mixed by gas recirculation at a rate of 1 l/min; digester was equipped with draft tube with diameter one fourth of digester diameter and a multipoint sparger to facilitate mixing. Another digester was unmixed; unmixed condition implies that no mixing is provided by external means, but digester is naturally mixed due to the evolution of biogas bubbles and addition of feed and effluent removal. Pilot scale digester had working volume of 97 liters (18 inches in diameter) and was geometrically similar to the laboratory-scale digester. The pilot-scale digester operation was started with biogas recirculation. After 70 days of operation of the pilot-scale 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 and the digester was operated in mixed condition for more than 12 days, this was done to check the reproducibility of the results obtained. The biogas recirculation rate in pilot-scale digester was 9.07 l/min, resulting in an input energy density of 8 W/m^3, which corresponds to 1 l/min biogas recirculation rate in the 6-inch laboratory scale unit at same energy input rate.

Both the digesters were operated in same manner using same 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. This feeding rate was maintained corresponding to a hydraulic retention time of 16 days. Gas samples were analyzed for methane and carbon dioxide content. Slurry samples were analyzed for total solids (TS), total volatile solids (TVS), Volatile Fatty acids (VFA), and total alkalinity (TA).

Table 1 shows the results of the performance results of two scales of digesters, whereas Figure 1 compares their cumulative methane production rates. Laboratory-scale digester produced more biogas with higher methane content than the pilot-scale digester. The TS, TVS and VFA content in the effluent of laboratory-scale was also lower than the pilot-scale digester. The laboratory-scale digester in mixed and unmixed condition showed same performance in terms of methane production. Pilot-scale digester in mixed condition performed significantly better than in unmixed condition with approximately 100% higher methane production. Increase in VFA in the effluent reaching the values of feed VFA indicated that unmixed pilot-scale digester was failing.

Since the rate of bioreaction is low, anaerobic digesters are kinetically controlled. But, still sufficient amount of mixing is required to maintain a uniform environment inside the digester to guarantee efficient distribution of substrate, ph and temperature. Even the small amount of mixing produced by the motion of evolving gas bubbles and the addition of feed in the unmixed digester is sufficient for efficient operation of the laboratory scale digester. Since the reaction is kinetically controlled, any additional amount of mixing does not further improve the performance of the mixed laboratory-scale digester over an unmixed digester. As the size of the reactor increases, difficulty in achieving complete mixing increases, and additional mixing is required. Since, no additional mixing was provided in pilot-scale unmixed reactor, it showed poorer performance than the pilot-scale mixed reactor.

Table 1 Effect of mixing on performance of laboratory-scale and pilot-scale anaerobic digester

Scale	Laboratory-scale (6-inch, 3.78 L)		Pilot-scale (18-inch, 97 L)	
Condition	Mixed	Unmixed	Mixed	Unmixed
Gas recirculation rate (*L/min*)	1	-	9	-
Feed/effluent rate (*L/2 days*)	0.470	0.470	12	12
Biogas production rate (*L/L/day*)	1.2	1.1	0.55	0.3
Methane content (*%*)	76	73	65	52
Cumulative methane production rate (*L/ day*)	3.3	3.1	40	20
Cumulative methane production rate per unit volume (*L/L/ day*)	0.87	0.82	0.41	0.2

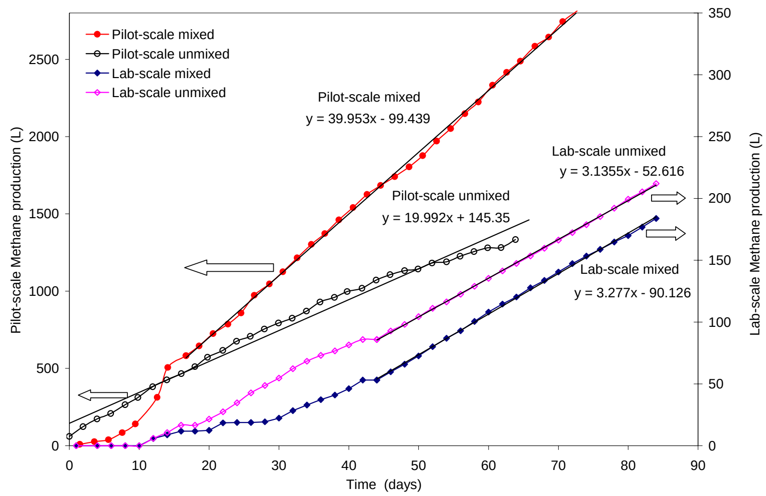


Figure 1 Comparison of cumulative methane production rates for laboratory-scale and pilot-scale digesters

Significant differences between the results obtained for mixed and unmixed condition in the pilot-scale digester were observed. Mixing provided in the digester results in its efficient operation and avoids its failure. Mixing played no significant role in the performance if laboratory-scale digesters. 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 digestion process is kinetically controlled. Excessive amount of mixing is also not recommended as mixing needs energy and spending more energy will not be 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.

D. Future Work and Milestones

The following essential question arises: what is the best or optimum mixing intensity to ensure efficient or less energy input to maximize the energy output obtained from the biogas. This question is yet to be answered and it needs further investigation using large scale digester is currently in progress. 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.

For detailed information, please contact
Mehul S. Vesvikar
msv1@cec.wustl.edu

E. Acknowledgements

DOE for financial support

F. Reference:

Mehul S. Vesvikar, Borole A., Klasson T., Karim K., Al-Dahhan M.H. 2005. Performance study of a pilot scale anaerobic digester and comparison with laboratory scale units. Sent for review to *Bioresource Technology*.

I-33. Effect of Sparger Design on Hydrodynamics of a Gas Recirculation Anaerobic Bioreactor

A. Problem Definition:

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.

Since the objective of a commercially viable anaerobic degradation is to generate net energy while disposing of waste, the amount of process energy that can be invested is limited by the quantity of energy generated from methane. Although the other byproducts such as the solid sludge, has great potential as soil conditioner and fertilizer, its commercial value is limited. Gas recirculation bioreactors are an appealing option as they have no moving parts and their energy requirements are minimal. In these reactors the biogas generated is recirculated with the aid of blowers.

Hence the objective is to develop gas mixed reactors that could maximize methane generation with minimal process energy consumption.

B. Research Objective

In this work the effect of a single orifice sparger (SOS) system, also called an ejector, on mixing and hydrodynamics has been compared with that of a multi orifice ring sparger (MORS). The gas phase distribution and the velocity profile and flow pattern studies have been performed using the same superficial gas velocity in both the systems, with the synergistic use of single source gamma ray Computer tomography (CT) and Computer Automated Radioactive Particle Tracking (CARPT) as advanced non-invasive flow measurement techniques.

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 mutli-orifice (Figure 2B), this in turn aids the liquid recirculation (Figure 2A) for the same energy input. Hence the use of a sparger is recommended in such a system.

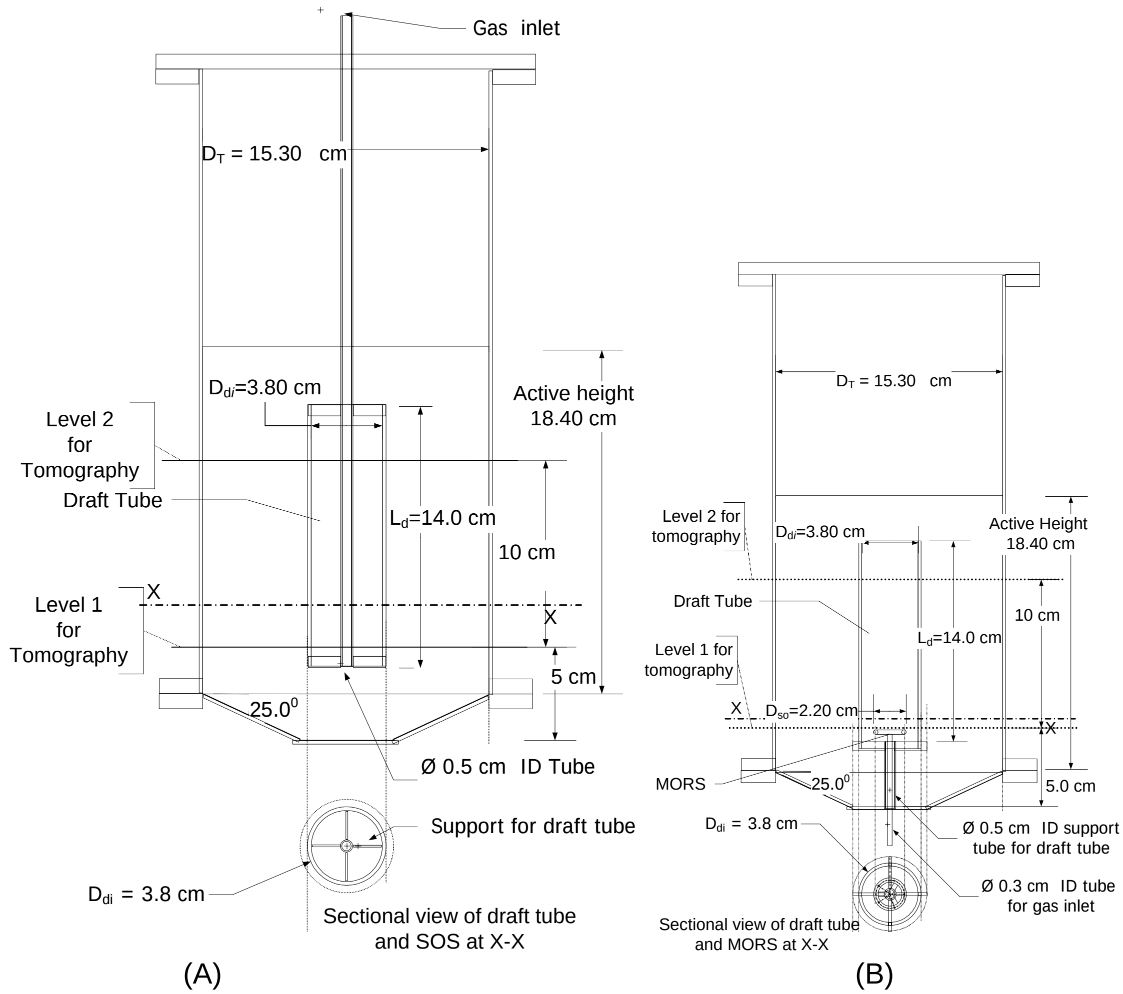


Figure 1: Schematic of the bioreactor used: (A) with single orifice (B) with multi orifice.

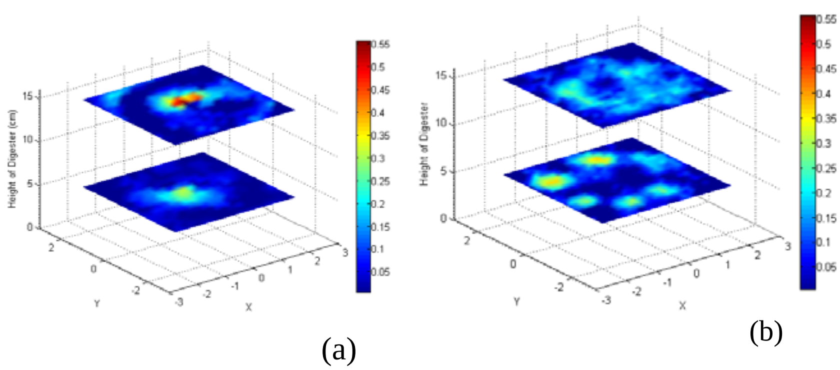
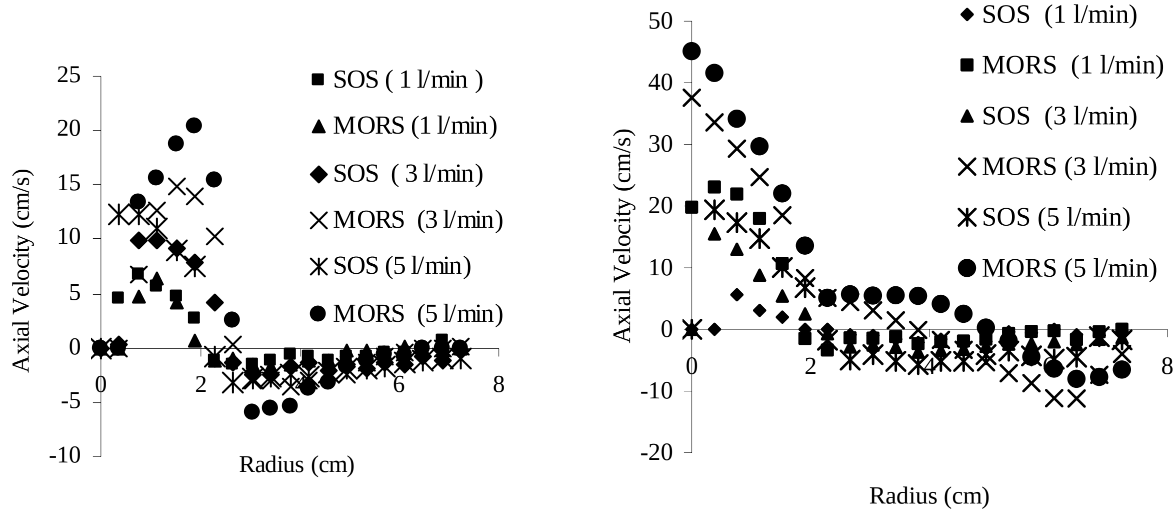


Figure 2: Time averaged gas hold up tomograms for single orifice sparger (SOS) system multi orifice ring sparger (MORS) system at levels 1and 2.



(A) (B)

Figure 3: Time averaged axial liquid velocity profile at level 1(A) and Level 2(B) in anaerobic bioreactor.

D. Future Work and Milestones:

For a given energy input, fixed by the gas flow rate, the Multi Orifice Ring Sparger (MORS) was found to gives better gas phase distribution and higher mean gas holdup at any level in the draft tube when compared to Single Orifice Sparger (SOS).

There were two loops in the circulation patterns observed in the system. It was also determined by CARPT that the entire downcomer region of the reactor is not involved in the downward flow of the liquid. Higher liquid velocity values were observed in the draft tube region for the bioreactor with MORS for a fixed gas flow rate. The same trend was observed with the RMS liquid velocity in the entire reactor. Hence the MORS system is considerably more efficient for mixing the reactor than the SOS system.This study confirms that for a given energy input, efficiency in mixing can be obtained by appropriate sparger design.

E. Acknowledgements

This work was funded by the United States Department of Energy (Identification number: DE-FC-36-01GO11054).

F. 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.

For further information please contact, Rajneesh Varma, Graduate student
E-mail:varma@wustl.edu, Phone: (314)935-9437, Fax: (314)935-7411