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Anaerobic biogas generation from sugar industry wastewaters in three-phase fluidized-bed bioreactor

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The studies are undertaken to develop an effective anaerobic continuous digestion process for biogas generation from sugar industry wastewaters using actively digested sludge from a sewage plant, in three-phase fluidized bed bioreactor. Attempts are made to optimize hydraulic retention time (HRT), initial feed *p*H, feed temperature and flow rate of feed (organic loading rate) for maximum production of methane gas and maximum removal of chemical oxygen demand (COD) and biological oxygen demand (BOD) of sugar industry wastewaters. The optimum conditions for the system are: digestion time, 8 h; initial *p*H of feed, 7.5; feed temperature, 40ºC; feed flow rate, 14 L/ min with maximum organic loading rate (OLR), 39.513 kg COD m^{-3} h^{-1}. The organic loading rates (OLR) are calculated on the basis of COD inlet in the bioreactor at different flow rates. The maximum expansion of the bed is observed as 23.67 m at optimum feed flow rate of 14 L/ min. The maximum methane gas concentration is 63.56% (*v/v*) of the total biogas generation at optimum process parameters. The maximum biogas yield rate is 0.835 m^3/kg COD m^{-3} h^{-1} with maximum methane gas yield rate of 0.530 m^3/kg COD m^{-3} h^{-1} (63.56% *v/v*) at optimum process parameters. The values for maximum reduction of COD and BOD are 76.82% (*w/w*) and 81.65% (*w/w*) with maximum OLR of 39.513 kg COD m^{-3} h^{-1} at optimum conditions.

Keywords: Activated bacteria, Anaerobic**,** Biogas, Biomethanation, Fluidized- bed, Methane

Biogas production is of major importance for the sustainable use of agrarian biomass as renewable energy source. The bulk of biomass energy is currently derived from agricultural crop residues[1-7]. In a few instances, municipal wastes and such sources as peat form additional sources of biomass energy[8-14]. Attempts are being made to exploit other forms of biomass such as seaweeds, and algae. While these other sources could add substantially to the world biomass energy supply, their exploitation could lead to ecological disasters. A more possible alternative is the use of industrial cellulosic wastewaters, wastewaters and effluents to satisfy the ecological balances and pollution abatement[15-32]. Economic biogas production depends on high methane gas yields. A reassessment of conventional biomass energy production and conversion technologies is pertinent at this stage.

The conversion of complex organic matter to methane and carbon dioxide is accomplished in general by four groups of bacteria[1-7] namely hydrolytic, acetogenic, acetoclasic and hydrogen-utilizing respectively. The various groups of bacteria essential to the biomethanation are interdependent. They all perform under anaerobic conditions, i.e. in the absence of molecular oxygen at high negative redox potential, but the activity of each group depends on the activities of the others. The actual ratio of methane to carbon dioxide (CO_2) varies with the substrate, temperature (mesophilic or thermophilic) and bioprocess conditions[1-7, 26-32].

Perez *et al.*[18] examined the effect of organic loading rate (OLR) on the removal efficiency of COD and total organic carbon (TOC) in anaerobic thermophilic fluidized bed reactor (AFBR) in the treatment of cutting-oil wastewaters at different hydraulic retention time (HRT) conditions. Acharya *et al.*[19] studied on anaerobic digestion of wastewater from a distillery industry having very high COD and BOD fed in a continuous upflow fixed film column reactor using different support materials such as charcoal, coconut coir and nylon fibers under varying hydraulic retention time (HRT) and organic loading rates (OLR) respectively. This study indicated that fixed film biomethanation of distillery spent wash using coconut coir as the support material appears to be a cost effective and promising technology. Jantsch *et al.*[20] investigated on anaerobic biodegradation of fermented spent sulphite liquor. Batch experiments with diluted liquor and pretreated liquor indicated a potential of 12-22 L methane per liter liquor, and COD removal of up to 37%.

Encouraged by the results of the above studies for economic utilization of wastewaters for production of biogas, the present investigations were undertaken to develop an effective anaerobic biomethanation of sugar industry wastewaters using actively digested sludge from a sewage plant for biogas generation in three-phase fluidized bed bioreactor. Attempts were made to optimize hydraulic retention time (HRT), initial feed *p*H, feed temperature and feed flow rate to obtain maximum methane gas generation and bioremoval of chemical oxygen demand (COD) and biological oxygen demand (BOD) from sugar industry wastewaters.

Experimental Procedure

Seed and suspension culture

Activated sewage sludge collected from a local sewage plant was used as seed material. It was transferred to suspension culture media prepared earlier and incubated in an incubator at 30°C for 7 days for sufficient bacterial growth. The resulting mixed bacterial cell suspensions were filtered through several layers of sterile absorbent cotton. The suspension culture had a mixed bacterial population[33] of 7.1×10^{8} cells per mL (Luckey Drop Method). The culture media contained the following constituents per liter: KH_2PO_4, 20 g; $MgSO_4.H_2O$, 5.0 g; $CaCl_2$, 1.0 g; $MnSO_4.7H_2O$, 0.05 g; $FeSO_4.7H_2O$, 0.10 g, $CaCl_2.6H_2O$, 0.10 g, $AlK(SO_4)_2$ $.2H_2O$, 0.01 g and $Na_2MoO_4.2H_2O$, 0.01 g.

Analysis of wastewater

The sugar industry wastewater collected from M/S Madura Sugar Mills Ltd, Madurai was stored at 4°C. The sample was analyzed for total dissolved solids (TDS), total suspended solids (TSS), chemical oxygen demand (COD) and biological oxygen demand (BOD) by standard methods[34]. The results are shown in Table 1.

Experimental setup

The experimental setup of three-phase fluidized-bed bioreactor (Appex Innovations Ltd) is shown in Fig. 1. The wastewater enters at the bottom and passes through the fluidized-bed bioreactor and leaves from top. The flow has a velocity sufficient to expand the bed without necessarily causing vigorous agitation, which results in complete mixing of the wastewater and mixed activated sludge bacteria. The increase in effective surface area of the medium achieved by fluidizing and expanding in the bioreactor bed provides an opportunity for higher organic loading rates, greater yield of cell mass and greater resistance to intimidators. Wastewater flows in expanded bed only. Recycle of the feed is done (Fig. 1). The biogas is collected in a gas holder. The gas holder is normally an airproof steel container which floats like a ball on the fermentation mix and cuts off the flow of air to the reactor and collects the gas generated. It is fitted with a Flame- Ionization Detector (FID). After each operation, the effluents (digested feed) are discharged through a valve.

General method

The anaerobic biomethanation of sugar industry wastewaters has been studied in a three-phase fluidized bed bioreactor of 18.6 L capacity. Experiments were carried out in 50 L plastic tank containing 20 L of raw wastewater as feed to be

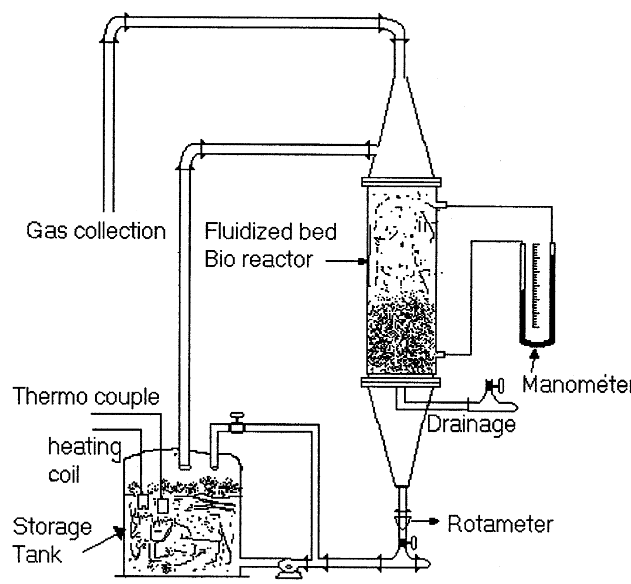

Fig. 1—Experimental set-up of fluidized bed bioreactor

Table 1— Analysis of sugar industry wastewater

Sl. No.	Constituents	Concentration (mg/L)
1	Total suspended solid (TSS)	48.755
2	Total dissolved solid (TDS)	34.570
3	COD	95.785
4	BOD	72.655

digested for biogas generation. Equal volumes (20 L) of suspension mixed activated bacterial culture as inoculum were added to the feed tank. Inoculum is taken from a seven days old suspension culture. The initial mixed bacterial population was counted as 7.1×10^8 cells per mL of the suspension culture. 2.0 L of suspension culture media is added to the feed tank contents. The initial *p*H of feed in tank was maintained at 6.0 by using 0.1 N H_2SO_4 acid and/or 1 M $CaCO_3$ slurry. The temperature of the feed is maintained by means of heating coil fitted with off-on temperature controller. The temperature of feed is measured by a thermocouple. The feed is pumped to three-phase fluidized-bed bioreactor form the feed tank. The initial feed flow rate was maintained at 10 L/min (OLR is 28.224 kg COD m^{-3} h^{-1}) through a rotameter (Fig. 1). Outlet digested feed is recycled to the feed tank. The biogas is collected in the gas holder.

Effect of hydraulic retention time

The concentrations of methane gas in the generated biogas were measured at a regular interval of time. Digested feed (effluents) (50 mL) was taken out after 2, 4, 6, 8, and 10 h of HRT, filtered, followed by analysis of COD and BOD.

Effect of initial feed pH

The general method was repeated for various initial *p*H values of the feed in the tank such as 6.5, 7.0, 7.5 and 8.0, etc. to optimize initial *p*H. The concentrations of methane gas were measured at optimum HRT of 8 h for each *p*H value. Digested feed (50 mL) was taken out at optimum HRT, filtered, followed by analysis of COD and BOD for each *p*H value.

Effect of feed temperature

The general method was repeated for different temperatures of the feed in the tank such as 35, 40 and 45°C. The initial *p*H of feed in the tank was maintained at 7.5. The methane gas concentrations were measured at optimum HRT of 8 h for each temperature. Digested feed (50 mL) was taken out, filtered, followed by analysis of COD and BOD for each temperature.

Effect of feed flow rate

The general method was repeated for different feed flow rates (organic loading rate) of 12, 14, 16 and 18 L/min. The corresponding organic loading rate (OLR) were 33.867, 39.513, 45.158 and 50.803 kg COD m^{-3} h^{-1} for 12, 14, 16 and 18 L/ min respectively. The initial *p*H value of feed in the tank was maintained at 7.5 while temperature of the feed was maintained at 40°C. The methane gas concentrations were measured for each feed flow rate. Digested feed (50 mL) was taken out at optimum HRT, filtered, followed by analysis of COD and BOD for each flow rate.

Analysis of methane in biogas

The analysis of biogas[35]containing methane gas was carried out in the Flame- Ionization Detector (FID). The eluate coming from the column was mixed with hydrogen (the fuel) and then burned in a stream of air (the oxidant) to form a combustible mixture in FID (Ametek Process Instruments, Inc). The ignited mixture yields a flame which provides the energy to ionize sample component in the eluate. The temperature (1800-1900ºC) of the air-hydrogen flame is used to ionize only carbon compounds. The positive ions thus formed during ionization in the flame are attracted to a negative "Collector" electrode and repelled by a positive "Repeller" electrode. The repeller electrode is either the metal burner or an electrode placed near the base of the flame. Upon striking the collector electrode, the positive ions cause a current to flow in the external circuit connecting the positive and negative electrodes. The current is amplified and recorded. Because the hydrogen-air flame itself generates relatively few ions, it has a non-zero base line. The current flowing through the circuit is proportional to the number of ions striking the collector, which in turn is proportional to the amount (concentrations) of methane gas entering the flame. Since the number of the positive ions formed in the flame is proportional to the number of carbon atoms in the sample component, the dectector's response is also proportional to the number of carbons in the sample component molecule[35]. The FID responds only to the substances which can be ionized in the air-hydrogen flame. For that reason the FID does not respond to most inorganic components present in biogas including carbon dioxide, hydrogen sulphide, etc.

Results and Discussion

Effect of hydraulic retention time

The effect of hydraulic retention time (HRT) on methane gas generation from sugar industry wastewaters and bioremoval in pollution load (COD and BOD) is shown in Figs 2 and 3 respectively. The concentration and yield of methane gas increase with increase of HRT up to 8 h and then both decline. It is observed that maximum biogas yield from wastewater is 0.682 m^3/kg COD m^{-3} h^{-1} at optimum 8 h HRT while the maximum methane gas concentration is 0.326 m^3/kg COD m^{-3} h^{-1} [47.85% (*v/v*)]. The recycling time is also included in the HRT measurements. It is also noticed that the maximum removal of COD and BOD from sugar industry wastewater are 54.96% (*w/w*) and 57.65% (*w/w*) respectively at optimum of 8 h HRT. After 8 h of HRT, the removal of COD and BOD from wastewater decreases and yields of biogas and methane gas also decline. Therefore, HRT of 8 h is taken as optimum for further studies in the fluidized-bed bioreactor to optimize other biomethanation process parameters.

It is evident from Figs 2 and 3 that as the HRT increases, the yields and concentrations of methane gas increase upto optimum value, then both decrease. This is because of bacterial populations in the reactor can affect the biomethanation. At the early stage of biomethanation, which coincided with lag-phase of bacterial growth, the removal of COD and BOD and yield of methane gas are very low. The extent of lag-phase is dependent on feed compositions which initially have high values of COD and BOD. Lag-phase time is required for adaptation to new environment for proper growth of the mixed bacteria[38,39]. The transition of bacterial growth from the lag-phase to exponential phase (maximum growth) led to a notable increase in methane gas, which proceeded propotionally until it reached maximum at optimum HRT of 8 h.

Effect of initial feed *p*H

The effect of initial feed *p*H on anaerobic biomethanation of sugar industry wastewater is shown in Figs 4 and 5. Initial *p*H of feed is kept both in acidic and basic medium range. The increase in yields and concentrations of methane gas are observed with increase in initial feed *p*H upto 7.5 and then both decline. The maximum biogas yield was found to be 0.718 m^3/kg COD m^{-3} h^{-1} at optimum feed *p*H of 7.5 and optimum HRT of 8 h. The maximum methane gas concentration was 52.36% (*v/v*) at optimum feed *p*H of 7.0 The maximum methane gas yield was 0.375 m^3/kg COD m^{-3} h^{-1} at optimum feed *p*H of 7.5 (Fig. 4). With increase in feed *p*H value beyond 7.5, the concentrations as well as the yields of methane gas sharply decrease. The maximum COD removal from sugar industry wastewater was 63.80% (*w/w*) at

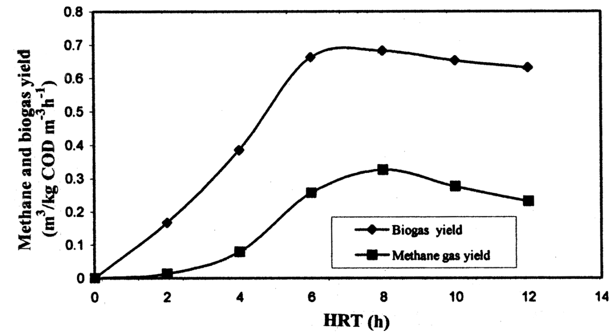

Fig. 2—Effect of hydraulic retention time on methane and biogas yield

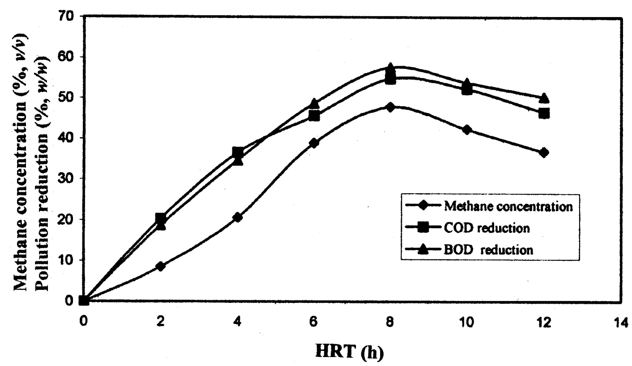

Fig. 3— Effect of hydraulic retention time on methane concentration

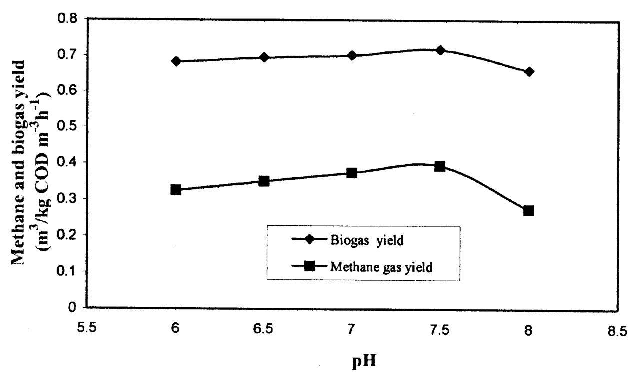

Fig. 4—Effect of feed *p*H on methane and biogas yield

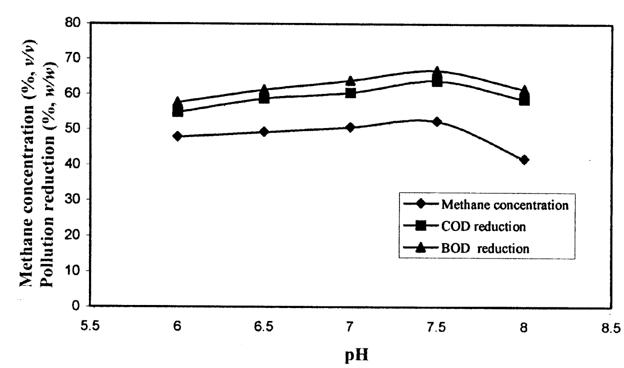

Fig. 5— Effect of feed *p*H on methane concentration

optimum feed *p*H of 7.5 (Fig. 5) while the maximum BOD removal was 66.72% (*w/w*). The removals of COD and BOD decrease after optimum feed *p*H. Therefore, the initial feed *p*H of 7.5 is the optimum for maximum yield of methane gas and the removal of COD and BOD and it is taken for further optimization of biomethanation parameter studies.

Variations in *p*H of the feed result in changes in the activity of the mixed bacteria and hence the bacterial growth as well as the methane gas generation. Methagenic bacteria are very active over a certain *p*H range. When *p*H differs from the optimal value, the maintenance energy requirements increase that leads to decrease in bacterial population and biogas yields.

Effect of feed temperature

The effect of feed temperature on anaerobic biomethanation of sugar industry is shown in Figs 6 and 7. The feed temperature is in the mesophilic range.. With increase in feed temperature, the yields and concentrations of methane gas increase upto temperature of 40ºC and then both decrease. The maximum biogas yield was found to be 0.741 m^3 /kg COD m^{-3} h^{-1} at optimum feed temperature of 40ºC (Fig. 6) at optimum HRT of 8 h and optimum *p*H of 7.5. The maximum concentration of methane gas was recorded as 57.26% (*v/v*) at optimum feed temperature of 40ºC (Fig. 7). The maximum methane gas yield was 0.424 m^3 /kg COD m^{-3} h^{-1} at optimum temperature of 40ºC (Fig. 6). The maximum COD removal from the wastewaters was 69.83% (*w/w*) while the maximum BOD removal from the wastewater was 74.45% (*w/w*) at optimum feed temperature of 40ºC, HRT of 8 h and pH of 7.5. With increase in feed temperature beyond 40ºC, the biogas and methane gas yields and the removal of COD and BOD decline. Therefore, feed temperature of 40ºC is taken as the optimum temperature for maximum yield of methane gas and removal of COD and BOD for further optimization of process parameters.

Every type of bacteria has an optimum, minimum and maximum growth temperature. Temperatures below the optimum for growth depress the rate of metabolism of bacterial cells. Above the optimal temperature, the growth rate decreases and thermal death may occur. At high temperature, death rate exceeds the growth rate[38,39], which causes a net decrease in the populations of viable bacterial cells with lowering of methane gas generation as well as COD and BOD removal.

Effect of feed flow rate

The effect of feed flow rate (organic loading rate) on anaerobic biomethanation of sugar industry wastewater is shown in Figs 8 and 9. The HRT of 8 h, *p*H of 7.5 and feed temperature of 40ºC were maintained for the optimization of feed flow rate. The organic loading rates (OLR) are calculated on the basis of COD inlet in the bioreactor with different feed flow rates. The organic loading rates (OLR) were found to be 28.224, 33.867, 39.513, 45.158 and 50.803 kg COD m^{-3} h^{-1} for corresponding feed flow rates of 10, 12, 14, 16 and 18 L/min respectively.

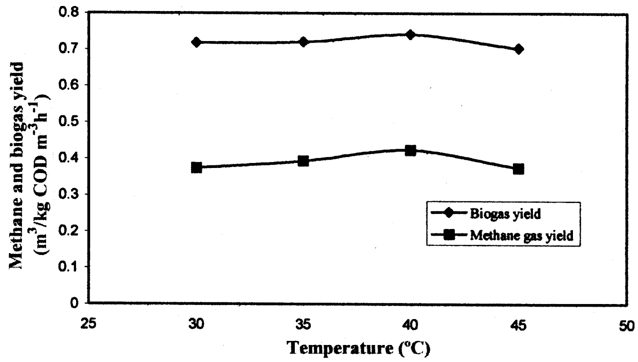

Fig. 6— Effect of feed temperature on methane and biogas yield

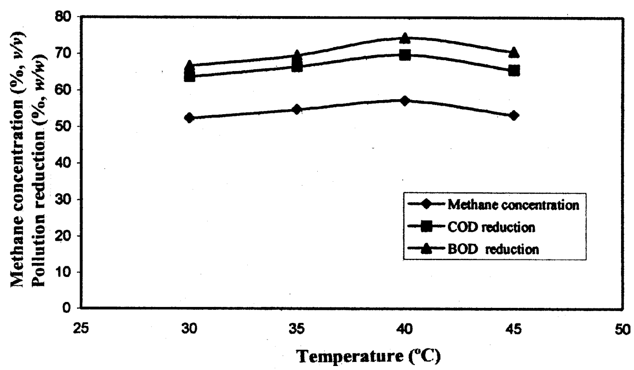

Fig. 7— Effect of feed temperature on methane concentration

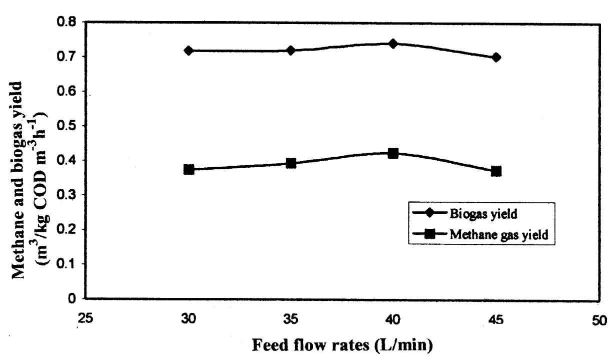

Fig. 8— Effect of feed flow rate on methane and biogas yield

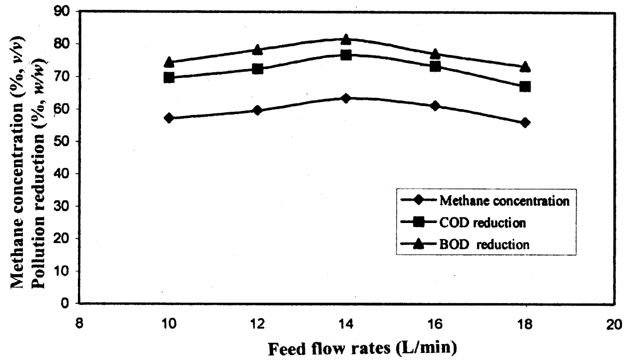

Fig. 9— Effect of feed flow rate on methane concentration

With the increase in feed flow rate, the yields and concentrations of methane gas increase upto 14 L/min and then both decrease. The maximum biogas yield was 0.835 m^3 /kg COD m^{-3} h^{-1} at feed flow rate of 14 L/ min (Fig. 8) while the maximum concentration of methane gas was 63.56% (*v/v*) at the same flow rate (Fig. 9). The maximum methane gas yield was 0.530 m^3 /kg COD m^{-3} h^{-1} at optimum feed flow rate of 14 L/ min. With increase in feed flow rate as well as OLR beyond 14 L/min the yield and concentration of methane gas decline. The maximum COD removal from the wastewater was 76.82% (*w/w*) while maximum BOD removal was 81.65% (*w/w*) at optimum feed flow rate of 14 L/ min. With increase in feed flow rate beyond 14 L/min, the methane gas yield and concentration and the removal of COD and BOD decrease. The feed flow rate of 14 L/min with corresponding OLR of 39.513 kg COD m^{-3} h^{-1} was the optimum for maximum yield of methane gas and maximum bioremoval of COD and BOD. The maximum expansion of the fluidized bed is observed as 23.67 m at optimum feed flow rate of 14 L/ min.

In the three-phase fluidized-bed bioreactor, there exists a pressure drop between inlet and outlet of the feed. Increase in mechanical forces (increase in flow rates) can disturb the shape of enzyme molecule of the bacteria to such a degree that denaturation of the protein may occur and deactivate the bacterial growth. Therefore, the maximum yields of methane gas and removal of pollution load decrease with increase in feed flow rate beyond 14 L/min (OLR, 39.513 kg COD m^{-3} h^{-1}). The characteristic mechanical fragility of bacteria may impose limit on the fluid forces which can be tolerated in fluidized- bed reactor. Since the surface tension of the interface between methane gas and water is high, it causes denaturation of proteins adsorbed at the methane-water interface. In addition extensional flow, cavities, metal contamination and surface denaturation at cavities may influence bacterial growth[38,39] which may decrease the population of viable bacterial cells as well as methane yield and pollution load.

Conclusion

Generation of methane gas from sugar industry wastewaters in anaerobic three-phase fluidized-bed bioreactor using activated sewage sludge mixed bacteria is an effective biomethanation process. The optimum value for HRT is 8 h while the optimum initial *p*H of feed and optimum temperature are found to be 7.5. and 40°C respectively. The optimum value for flow rate of feed is 14 L/min with organic loading rate (OLR) of 39.513 kg COD m^{-3} h^{-1}. The maximum expansion of the bed is observed as 23. 67 m at optimum feed flow rate. The maximum concentration of methane gas at optimum biomethanation process parameters is found as 63.56% (*v/v*). The maximum biogas yield rate is 0.835 m^3 /kg COD m^{-3} h^{-1} with maximum methane gas yield rate of 0.530 m^3 /kg COD m^{-3} h^{-1} at optimum biomethanation parameters. The maximum COD and BOD removal from the sugar industry wastewaters are 76.82% (*w/w*) and 81.65% (*w/w*) respectively, at optimum biomethanation parameters.

References

1 McInerney M J & Bryant M P, In *Fuel Gas Production from Biomass*, Vol. 1, edited by D L Wise (C R C Press, Boca Raton, FL, USA), 1981, 19.

2 McInerney M J & Bryant M P, In *Anaerobes and anaerobic Infections*, edited by P N Gottschalk & S Werner (Gustaro Fischer Verlag, Stuttgart, Germany), 1980, 117.

3 Lamptey J, Moo-Young M & Sullivan H F, In *Bioenergy*, edited by A V Desai (Wiley Eastern Ltd, International Development Research Centre, Ottawa, United Nations University, Tokyo, Japan), 1990, 6.

4 Brown N L & Tata P B S, In *Bioenergy*, edited by A V Desai (Wiley Eastern Ltd, International Development Research Centre, Ottawa, United Nations University, Tokyo, Japan), 1990, 67.

5 Vandan B L & Kennedy K J, In *Energy from Biomass and Waste VI* (The Institute of Gas Technology, Chicago, IL, USA), 1982, 401.

6 Klass D I, In *Energy from Biomass and Waste VII* (The Institute of Gas Technology, Chicago, IL, USA), 1983, 1.

7 Higgin J & Krieger J H, *Chem Eng News*, 71(11) (1993) 28.

8 Henze M & Harremoes P, *Water Sci Technol*, 28(8) (1996) 1.

9 Lastella L, Testa C, Cornacchlia G, Notornicola M, Voltasio F & Sharma V K, *Energy Conserve Manage*, 43(1) (2002) 63.

10 Biswas J, Chowdhury R & Bhattacharya P, *Enzyme Microbial Technol*, 38(3-4) (2006) 493.

11 Chun-Sheng W, Ju-Sheng H & Hsin-Hsien C, *Water Res*, 40(1) (2006) 126.
12 Pol L H & Lettinga G, *Water Sci Technol*, 18(2) (1986) 41.
13 Vandan Berg L & Kennedy K J, *Water Sci Technol*, 15(8,9) (1982) 359.
14 Neves L, Goncalo E, Oliveira R & Alves M M, *Waste Manage*, 26(6) (2008) 965.
15 Gangagni A & Bapat A N, *Bioresource Technol*, 97(18) (2006) 2311.
16 Isci A & Demirer, *Renewable Energy*, 32(5) (2007) 750.
17 Chanakya H N, Reddy B V V & Modak J, *Renewable Energy*, 34(2) (2009) 416.
18 Perez M, Rodriguez-Cano R, Romero L I & Sales D, *Bioresource Technol*, 98(18) (2007) 3456.
19 Acharya B K, Mohana S & Datta M, *Biomass Bioenergy*, 31(10) (2007) 247.
20 Jantsch T G, Angelidaki I, Schmidt J E, Braña de Hvidsten B E & Ahring B K, *Chem Eng J,* 106(1) (2005) 53.
21 Lettinga G, Van Velsen A F M, deZeeuw W & Hobma S W, *Biotechnol Bioeng*, 22(4) (1980) 699.
22 Zawiejn I, Wolny L & Wolski P, *Desalination*, 222(1-3) (2008) 186.
23 Manjunath D L, Mehrotra I & Mathur R P, *IAWPC Tech Annual*, 15 (1988) 23.
24 Patel H & Madamwar D, *Process Biochem*, 36(7) (2001) 613.
25 Olthof M & Oleszkiewicz J, *Chem Eng J*, 89(23) (2005) 121.
26 Gohil A & Nakhia G, *Bioresource Technol*, 97(16) (2006) 2141.
27 Buzzini A P & Pires E C, *Bioresource Technol*, 98(9) (2007) 1838.
28 Bjorklund J, Geber U & Rydberg T, *Resour Conserv*, 99(17) (2008) 293.
29 Verma V K, Singh Y P & Rai J P N, *Bioresource Technol*, 31(4) (2001) 7941.
30 Nand K, Devi S S, Viswanath P, Deepak S & Sarada R, *Process Biochem*, 26(1) (1991) 1.
31 Cantrell K B, Ducey T, Ro K S & Hunt P G, *Bioresource Technol*, 98(8) (2007) 1664.
32 Ferrer I, Poush S, Vazquez F & Font X, *Biochem Eng*, 42(2) (2008) 186
33 Trivedy R K & Goel P K, *Chemical and Biological Methods for Water Pollution Studies* (Environmental Publications, Karad, India), 1986, 125.
34 Sawyer C N, McCarty P L & Parkin G F, *Chemistry for Environmental Engineering*, 4th edn (McGraw-Hill Inc, New York, U S A), 1994.
35 Braun R D, *Introduction to Chemical Analysis* (McGraw-Hill, Inc, Aucland), 1982, 410.
36 Sivaguru K, Hossain S M, Anantharaman N & Begum K M M S, *Indian J Environ Protec*, 26(9) (2006) 788.
37 Hossain S M & Anantheraman N, *Indian J Chem Technol*, 13 (2006) 591.
38 Shulter M L & Kargi F, *Bioprocess Engineering Basic Concept* (Parentice-Hall of India Pvt Ltd, New Delhi, India), 2000.
39 Pclczar M J, Chan E C S & Kring N R, *Microbiology*, 5th edn (Tata McGraw-Hill Publishing Co. Ltd, New Delhi, India), 2004.