# Biomethanation of black liquor in fluidized-bed bioreactor

Sk. M. Hossain[1,*], M. Das[2]

[1] *Centre for Advanced Studies and Research, Younus College of Engineering & Technology, Vadakkevila, 691010 Kollam, India*
[2] *Department of Chemical Engineering, University of Calcutta, 92 A P C Road, 700009 Kolkata, India*

Attempts were made to optimize bioprocess parameters for maximum production of methane and removal of chemical oxygen demand (COD) and biological oxygen demand (BOD) from black liquor in the process of biomethanation in three-phase fluidized bioreactor. The optimum hydraulic retention time (HRT) is 8 h and optimum initial pH of the feed was observed to be 7.5. The optimum feed temperature is 40°C and optimum feed flow rate is 16 L/min at organic loading rate (OLR) of 45.158 kg COD·m$^{-3}$·h$^{-1}$ respectively. The maximum methane constitutes 64.82% (v/v) of the total biogas generation. The maximum biogas yield rate is 0.723 m$^3$/kg COD·m$^{-3}$·h$^{-1}$ with methane yield rate of 0.530 m$^3$/kg COD·m$^{-3}$·h$^{-1}$ respectively at optimum biomethanation parameters. The maximum COD and BOD remediation of the black liquor are 79.65% (w/w) and 81.54% (w/w) with maximum OLR of 11.686 kg COD·m$^{-3}$·h$^{-1}$ respectively.

**Key words:** anaerobic**,** biogas, biomethanation, black liquor, fluidized-bed, optimum.

## INTRODUCTION

The energy crisis of the early 1970s brought into sharp focus the vital importance of the biomass energy in the face of destabilized global trade in fossil energy. Much of the present-day technology is fuelled by biomass of carboniferous era. To a varying extent, this fossil biomass energy resource is supplemented all over the world by energy obtainable from extant biomass. The unique ability to capture photons from solar radiation and to store the energy in the form of sugars, starch, cellulose materials, *etc*. implies renewable energy supply given appropriate conversion technologies.

A reassessment of conventional biomass energy production and conversion technologies is pertinent at this stage. The bulk of biomass energy is currently derived from agricultural crop wastes [1−7]. Biogas production is of major importance for the sustainable use of agrarian biomass as renewable energy source. In a few instances, municipal wastes and 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 to use industrial cellulose wastewaters and effluents to satisfy the ecological balances and pollution abatement [15−32].

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 inter-dependent. They all perform under anaerobic conditions, *i.e.* in the absence of molecular oxygen at highly 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 chemical oxygen demand (COD) and total organic carbon (TOC) using 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 the anaerobic digestion of wastewater from a distillery industry having very high COD and biological oxygen demand (BOD), which was fed in a continuous upflow fixed film column reactor using different support materials such as charcoal, coconut coir and nylon fibres 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 appeared to be a

* To whom all correspondence should be sent:
E-mail: skmhossain@yahoo.co.in

cost effective and promising technology for mitigating the problems caused by distillery effluent. Jantsch *et al.* [20] investigated anaerobic biodegradation of fermented spent sulphite liquor. Batch experiments with diluted liquor and pretreated liquor indicated a potential of 12−22 l of methane per litre liquor, which corresponds to 0.13−0.22 l of methane $(gVS)^{-1}$ and COD removal of up to 37%. COD removal in a mesophilic upflow anaerobic sludge blanket (UASB) reactor ranged from 10% to 31% at an organic loading rate (OLR) of 10−51 $g \cdot (L \cdot d)^{-1}$ and hydraulic retention time from 3.7 to 1.5 days. The biogas productivity was 3 $l \cdot (L_{reactor} \cdot d)^{-1}$, with an yield of 0.05 l gas $(gVS)^{-1}$.

Encouraged by the above work, continuous investigations are being undertaken to develop an effective anaerobic biomethanation of black liquor (wastewaters from craft pulping) using actively digested sludge from a sewage plant for biogas generation in three-phase fluidized-bed bioreactor. Attempts are also made to optimize hydraulic retention time (HRT), initial feed pH, 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 black liquor wastewaters.

## EXPERIMENTAL

*Collection of seed and suspension culture preparation.* Activated sewage sludge collected from a local sewage plant constitutes the ideal "seed" material. It is 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 are filtered through several layers of sterile adsorbent cotton. The mixed bacterial population is counted [33] as $7.1 \times 10^8$ number of cells per ml of the suspension culture (Luckey Drop Method). The suspension culture media contained the following constituents per liter: $KH_2PO_4$ − 20 g, $MgSO_4.7H_2O$ − 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, $AlK(SO_4)_2.12H_2O$ − 0.01 g, $Na_2MoO_4.2H_2O$ − 0.01 g.

### Collection and analysis of black liquor

The black liquor wastewater sample was collected from indigenous sources and stored at 4°C. The sample was analyzed [34]. The chemical oxygen demand (COD) is 28.565 mg/l and biological oxygen demand (BOD) is 17.750 mg/l respectively.

### Experimental setup

The experimental setup of three-phase fluidized-bed bioreactor (Appex Innovations Ltd.) is shown in

Fig. 1. The wastewaters enter at the bottom and pass through the fluidized-bed bioreactor and leave at the top. The flow has a velocity sufficient to expand the bed without necessarily causing vigorous agitation, which results in complete mixing of the wastewaters 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. Wastewaters flow 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 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.
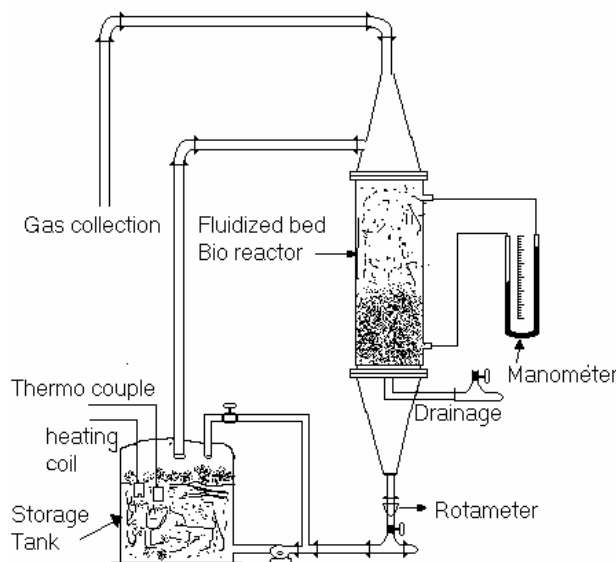


Fig. 1. Experimental setup of fluidized-bed reactor.

### General method

The anaerobic biomethanation of black liquor is studied in a three-phase fluidized-bed bioreactor of 18.6 l capacity. Experiments are carried out in 50 l plastic tank containing 20 l of raw wastewaters as feed to be digested for biogas generation. 20 l of suspension mixed activated bacterial culture as inoculums are added to the feed tank. The inoculum is taken from a seven-days-old suspension culture. 2.0 L of suspension culture media is added to the feed tank contents. The initial pH of feed in tank is 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 at 30°C by means of heating coil fitted with off/on temperature controller. The temperature of the feed is measured by a thermocouple. The feed

is pumped to three-phase fluidized-bed bioreactor from the feed tank. The initial feed flow rate is 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 are measured at regular interval of time. 50 ml of digested feed is taken out after 2, 4, 6, 8, and 10 h of HRT, filtered, followed by analysis of COD and BOD respectively.

*Effect of initial feed pH.* The general method is repeated at various initial pH values of the feed in the tank such as 6.5, 7.0, 7.5 and 8.0 respectively to optimize initial pH. The concentrations of methane gas are measured at optimal HRT of 8 h at each pH value. 50 ml of digested feed is taken out at optimal HRT, filtered, followed by analysis of COD and BOD at each pH value respectively.

*Effect of feed temperature.* The general method is repeated at different temperatures of the feed in the tank such as 35, 40 and 45°C respectively. The initial pH of the feed in the tank is maintained at optimum of 7.5. The methane gas concentrations are measured at optimal HRT of 8 h at each temperature. 50 ml of digested feed is taken out at optimal HRT, filtered, followed by analysis of COD and BOD respectively at each temperature.

*Effect of feed flow rate.* The general method is repeated at different feed flow rates (organic loading rate) in the three-phase fluidized-bed bioreactor such as 12, 14, 16 and 18 l/min respectively. The corresponding organic loading rates (OLR) are 33.867 kg COD·m$^{-3}$·h$^{-1}$, 39.513 kg COD·m$^{-3}$·h$^{-1}$, 45.158 kg COD·m$^{-3}$·h$^{-1}$ and 50.803 kg COD·m$^{-3}$·h$^{-1}$ for 12, 14, 16 and 18 l/min respectively. The initial pH value of the feed in the tank is maintained at optimum of 7.5. The temperature of the feed in the tank maintained at optimum 40°C. The methane (CH$_4$) gas concentrations are measured at optimal HRT of 8 h for each feed flow rate. 50 ml of digested feed is taken out after optimal HRT, filtered, followed by analysis of COD and BOD respectively at each flow rate.

### Analysis of methane in biogas

The analysis of biogas [35] containing methane gas is carried out by the Flame-Ionization Detector (FID). The eluate coming from the column is 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 tempe-

rature (1800−1900°C) 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 positively charged 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 its 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 detector's response is also proportional to the number of carbons in the sample component molecule [35]. The FID responds only to such 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 (CH$_4$) gas generation from black liquor and bioremediation of pollution load in fluidized-bed bioreactor with mixed activated sludge is shown in Figures 2 and 3 respectively.
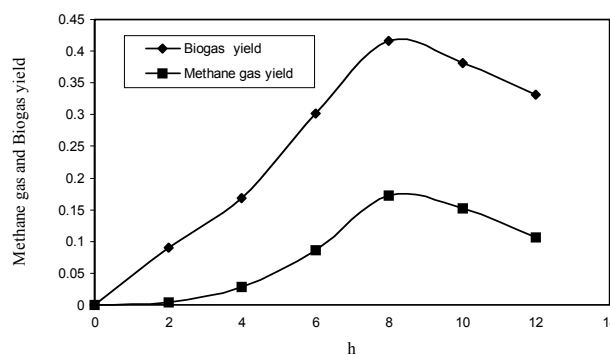


Fig. 2. Effect of hydraulic retention time.

The concentration and yield of methane gas increase with increase of HRT up to 8 h and then both decline (Fig. 2). The concentrations and yields of methane gas from black liquor are proportional to optimal HRT of 8 h. It is observed that maximal biogas yield from black liquor is 0.416 m$^3$/kg COD·m$^{-3}$·h$^{-1}$ at optimal 8 h HRT (Fig. 2). The

maximal methane gas concentration is 41.53% (v/v) at optimal 8 h HRT in the biogas (Fig. 3). The recycling time is also included in the HRT measurements. Maximal methane gas yield is 0.172 $m^3$/kg COD·$m^{-3}$·$h^{-1}$ at an optimum of 8 h HRT (Fig. 2). It is also noticed that the maximal removal of COD and BOD from black liquor are 49.53% (w/w) and 56.72% (w/w) respectively at an optimum of 8 h HRT (Fig. 3). After 8 h of HRT, the removal of COD and BOD from wastewaters decreases (Fig. 3) and yields of biogas and methane gas also decline (Figs. 2 and 3). Therefore, HRT of 8 h is taken as an optimum for further studies in the fluidized-bed bioreactor to optimize other biomethanation process parameters.
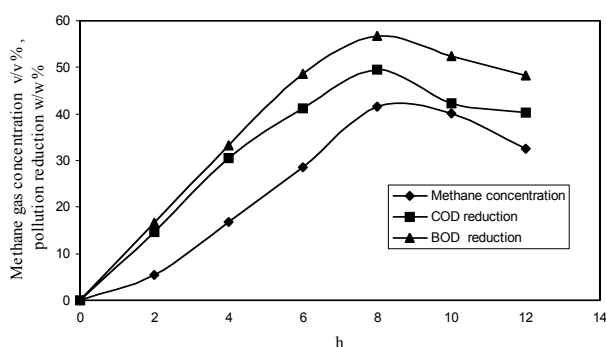


Fig. 3. Effect of hydraulic retention time.

It is evident from the Figures 2 and 3 that as the HRT increases, the yields and concentrations of methane gas by the mixed bacteria increase up to optimal value, then both decrease. This is because of bacterial populations in the reactor that can affect biomethanation of black liquor. 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 (Figs. 2 and 3). The extent of lag-phase is dependent on feed compositions, which initially contain high values of COD and BOD, respectively. Lag-phase time is required for adaptation to black liquor media for proper growth of the mixed bacteria [36−37]. The transition of bacterial growth from the lag-phase to exponential phase (maximum growth) led to a notable increase in methane gas, which proceeded in the same way until it reaches maximum at optimal HRT of 8 h as well (Figs. 2 and 3).

### Effect of initial feed pH

The effect of initial feed pH on the anaerobic biomethanation of black liquor in three-phase fluidized-bed bioreactor is shown in Figures 4 and 5 respectively. Initial pH of feed is taken both in acidic and basic medium range. The optimal HRT of 8 h is maintained for the optimization of feed pH.

The increase in yields and concentrations of methane gas are observed with increase in initial feed pH up to 7.5 and then both declined. It is observed that maximal biogas yield from sugar industry wastewaters in fluidized-bed bioreactor is 0.618 $m^3$/kg COD·$m^{-3}$·$h^{-1}$ at optimal feed pH of 7.5 (Fig. 4).
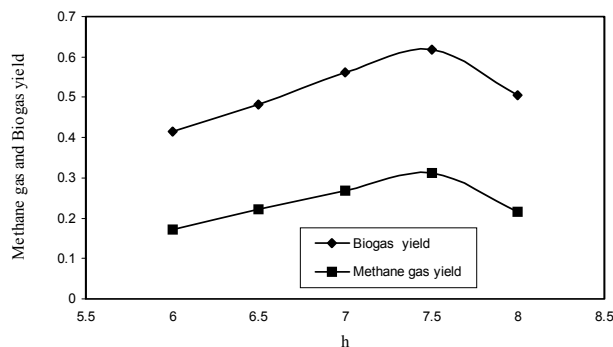


Fig. 4. Effect of feed pH.

The maximum methane gas concentration is 50.76% (v/v) at optimum feed *p*H of 7.5 with mixed activated sludge bacteria (Fig. 5). The maximal methane gas yield is 0.313 $m^3$/kg COD·$m^{-3}$·$h^{-1}$ at optimal feed pH of 7.5 (Fig. 4). With increase in feed pH value beyond 7.5, the concentrations as well as the yields of methane gas decrease sharply (Figs. 4 and 5). It is also observed that maximal COD removal from black liquor in the biomethanation process is 58.75% (w/w) at optimal feed pH of 7.5 (Fig. 5).
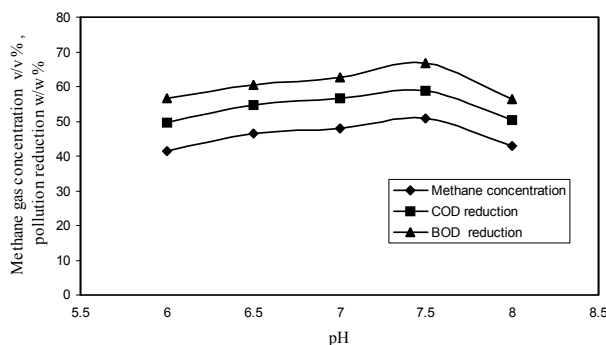


Fig. 5. Effect of feed pH.

Maximal BOD removal from wastewaters in the biomethanation process is 66.85 % (w/w) at optimal feed pH of 7.5 (Fig. 5). The removals of COD and BOD decrease after optimal feed pH (Fig. 5). Therefore, the initial feed pH of 7.5 is the optimum for maximum yield of methane gas and the removal of COD and BOD from black liquor in a three-phase fluidized-bed bioreactor and it is taken for further optimization of biomethanation parameter studies.

Variations in the pH of the feed result in changes in the activity of the mixed bacteria and hence the

bacterial growth as well as in the methane gas generation. Methagenic bacteria are very active over a certain pH range. When pH differs from the optimal value, the maintenance energy requirements increase [36−37] that leads to decrease in bacterial population and biogas yields.

### Effect of feed temperature

The effect of feed temperature on the anaerobic biomethanation of black liquor in three-phase fluidized-bed bioreactor is shown in Figures.6 and 7 respectively. The feed temperature is in the mesophilic range. With increase in feed temperature, the yields and concentrations of methane gas increase up to temperature of 40ºC and then both decrease. It is noticed that maximal biogas yield from black liquor in fluidized-bed bioreactor is 0.686 $m^3$/kg COD·$m^{-3}$·$h^{-1}$ at optimal feed temperature of 40ºC (Fig. 6). The maximum concentration of methane gas is 56.37% (v/v) at optimal feed temperature of 40ºC (Fig. 7). The maximal methane gas yield in fluidized-bed bioreactor is 0.386 $m^3$/kg COD $m^{-3}$·$h^{-1}$ at optimal temperature of 40ºC (Fig. 6). It is also observed that maximal COD removal from the wastewaters is 67.82% (w/w) at optimal feed temperature of 40ºC (Fig. 7). Maximal BOD removal from the waste waters is 70.91% (w/w) at optimal feed temperature of 40ºC at optimal biomethanation process parameters (Fig. 7). With increase in feed temperature beyond 40ºC, the biogas and methane gas yields and the removal of COD and BOD from wastewaters decline as well (Figs.6 and 7). Therefore, feed temperature of 40ºC is the optimum for maximum yield of methane gas and removal of COD and BOD from black liquor in a three-phase fluidized-bed bioreactor and it is taken for further optimization of biomethanation process parameter studies.
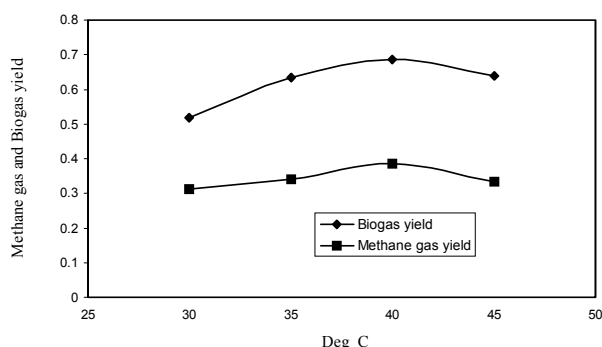


Fig. 6. Effect of feed temperature.

Every type of bacteria has an optimal, minimal and maximal 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 [36−37], 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.
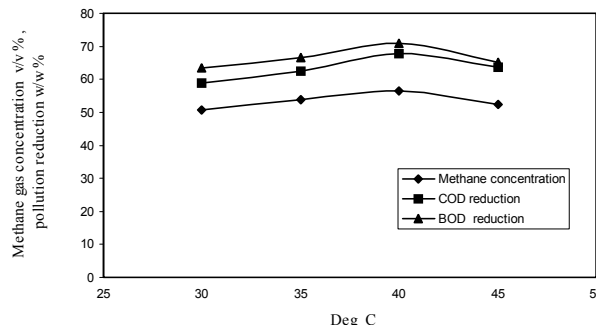


Fig. 7. Effect of feed temperature.

### Effect of feed flow rate

The effect of feed flow rate (organic loading rate) on the anaerobic biomethanation of black liquor in three-phase fluidized-bed bioreactor is shown in Figures 8 and 9, respectively. The organic loading rates (OLR) are calculated on the basis of COD inlet in the bioreactor with different feed flow rates. With increase in feed flow rate, the yields and concentrations of methane gas increase up to 16 l/min and then both decrease. It is noticed that maximal biogas yield from wastewaters in anaerobic fluidized-bed bioreactor is 0.748 $m^3$/kg COD·$m^{-3}$·$h^{-1}$ at optimal feed flow rate of 16 l/min (Fig. 8).
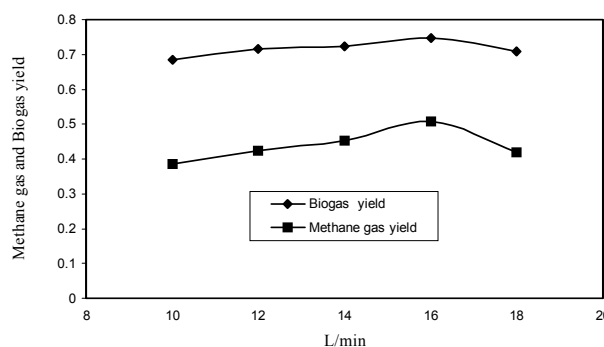


Fig. 8. Effect of feed flow rate.

The maximal concentration of methane gas is 64.82% (v/v) at optimal flow rate of 16 l/min respectively (Fig. 9). The maximal methane gas yield in anaerobic fluidized-bed bioreactor is 0.508 $m^3$/kg COD·$m^{-3}$·$h^{-1}$ at optimal feed flow rate of 16 l/min (Fig. 8). With increase in feed flow rate as well as OLR beyond 16 l/min (the corresponding OLR is 45.158 g COD·$m^{-3}$·$h^{-1}$), the yield and concentration of methane gas decline (Fig. 9). It is also observed that the maximal COD removal from the

wastewaters is 79.65% (w/w) at optimal feed flow rate of 16 l/min (Fig. 9). It is noticed that maximal BOD removal from the wastewaters is 81.54% (w/w) at optimal feed flow rate of 16 l/min (Fig. 9). With increase in feed flow rate beyond 16 l/min, the methane gas yield and concentration and the removal of COD and BOD from wastewaters decrease as well (Figs. 8 and 9). Feed flow rate of 16 l/min is the optimum for maximal yield of methane gas and maximal bioremoval of COD and BOD from black liquor in the process of biomethanation in a three-phase fluidized-bed bioreactor.
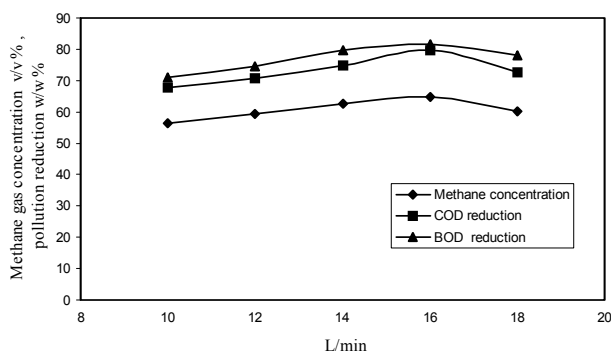


Fig. 9. Effect of feed flow rate.

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 elaborate shape of enzyme molecule of the bacteria to such a degree that denaturation of the protein occurs and it deactivates the bacterial growth [36−37]. Therefore, the maximal yields of methane gas and removal of pollution load decrease with increase in feed flow rate beyond 16 l/min as well. 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 [36−37]. In addition extensional flow, cavities, metal contamination and surface denaturation at cavities may influence bacterial growth [36−37] causing a decrease in population of viable bacterial cells as well as in methane yield and pollution load, respectively.

## CONCLUSION

Generation of methane ($CH_4$) gas from black liquor in fluidized-bed bioreactor using activated sewage sludge mixed bacteria is an effective biomethanation process. The optimal HRT is 8 h and optimal initial pH of feed is found to be 7.5 respectively. The optimal flow rate of feed in fluidized-bed bioreactor is 16 l/min with organic loading rate (OLR) of 45.158 kg $COD \cdot m^{-3} \cdot h^{-1}$ respectively. Optimal temperature of feed is 40ºC. The maximal biogas yield rate is 0.748 $m^3$/kg $COD \cdot m^{-3} \cdot h^{-1}$ with $CH_4$ yield rate of 0.530 $m^3$/kg $COD \cdot m^{-3} \cdot h^{-1}$ respectively at optimal biomethanation parameters. The maximal concentration of methane ($CH_4$) gas is found as 64.82% (v/v) at optimal biomethanation parameters in the fluidized-bed bioreactor. The maximal COD and BOD removals from black liquor are 79.65% (w/w) and 81.54% (w/w) at optimal anaerobic bio-methanation parameters respectively.

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БИОМЕТАНИЗАЦИЯ НА ЧЕРНА ЛУГА В БИОРЕАКТОР С ФЛУИДИЗИРАН СЛОЙ

Ск. М. Хосаин[1,*], М. Дас[2]

[1] *Център за съвременни изследвания, Юнус Инженерен и технологичен колеж, Вадакевила, 691010 Колам, Индия*
[2] *Департамент по инженерна химия, Университет на Калкута, 92 А. П. С. шосе, 700009 Колката, Индия*

(Резюме)

Извършени са опити по оптимизирането на параметрите за максимално продуциране на метан и намаляване на химическия потребен кислород (ХПК) и на биологически потребния кислород (БПК$_5$) на черна луга при биометанизацията в трифазен биореактор с флуидизиран слой. Оптималното времепребиваване (HRT) е 8 часа, а оптималната стойност на рН – 7.5. Оптималната температура на захранващия поток е 40°C, а оптималния му дебит – 16 L/min при натоварване с органична материя (OLR) съответно от 45.158 kg ХПК·m$^{-3}$·h$^{-1}$. Максималното съдържание на метан в биогаза е 64.82 об.% . Максималният дебит на добивания биогаз е 0.723 m$^3$/kg ХПК·m$^{-3}$·h$^{-1}$ с добив на метан от 0.530 m$^3$/kg COD·m$^{-3}$·h$^{-1}$ при оптималните определени параметри. Максималното понижение на ХПК и БПК$_5$ на използваната черна луга са съответно 79.65% (тегл.) и 81.54% (тегл.) при максимално натоварване с органична материя от 11.686 kg COD·m$^{-3}$·h$^{-1}$.