160. Correlation and Optimization Studies of different parameters for biogas production from Sugarcane Molasses - a Sugar Mill Waste

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Abstract

Batch studies were carried out for 10 days to optimize maximum biogas yield from diluted molasses medium used as substrate. Different parameters including pH, Total solids (TS), Volatile solids (VS) and Carbon to Nitrogen (C/N) Ratio were optimized at mesophilic temperature range (30–37 ^{0}C). Also a correlation was developed between different parameters to see their interdependency. Maximum biogas yield at pH 6.5–7.2, TS up to 10%, VS 77% of TS, and C/N Ratio 30 with 73% COD reduction, was found 1.377 dm^3/l of substrate (diluted molasses medium) with 64% methane content. High percentage reduction in volatile solids resulted in high biogas yield. As more and more volatile solids utilized, more reduction in COD and C/N ratio was observed. Reduction of volatile solids, in turn, depends upon pH of the substrate. In this way, all these parameters are correlated with each other and so for optimized for maximum biogas yield.

Keywords: *Correlation; Parameters; Biogase yield/Prduction; TS; VS; C/N Ratio; Molasses*

1. Background

Molasses is obtained during the extraction of sugar from sugarcane and sugar beet. It is produced as by-product in sugar mill. The annual production of molasses in Pakistan was 2,034,555 ton during 2010 – 11 [1] that is increasing every year. The disposal of this sugar industry waste can cause sever hazardous problem in environment. So, there is a need to treat molasses as eco-friendly specie. It can be used for bio-energy production and also for waste disposal by carrying out anaerobic digestion process. Anaerobic digestion of cattle slurry produced biogas energy on commercial scale for many years. Anaerobic digestion of molasses as waste is gaining importance day by day in Pakistan. Biogas production from organic part of waste can be carried out by anaerobic digestion technique on batch as well as semi pilot scale in third world countries like Pakistan, Bangladesh, India, Nepal and Bhutan etc. Anaerobic digestion on batch scale is simple and economically feasible to assess bio-energetic potential of the system

Different researchers have been used a variety of ways to produce biogas from different waste sources. B. Pound et al [2] demonstrated biogas yield by anaerobic digestion form mixture of pressed sugarcane stalk and cattle slurry. M. Rao et al [3] produced bioenergy from municipal solid waste garbage and claimed methane content of biogas in range of 62-75%. E. T. Iyagba et al [4] stated that co-digestion of rice husk with cow dung can be used to produce biogas on lab scale and demonstrated that rice husk does not have potential for biogas production and co-digestion with cow dung does not improve gas yield. On contrast to this, Z. Lei et al [5] studied methane production by carrying out anaerobic digestion of rice straw and observed biogas or (0.33–0.35) m^3/kg-VS methane yield and found 75.9–78.2%methane contents on average basis. In 2011, D. Giraldi et al [6] introduced the concept of Bio-cells (small anaerobic digestion plant) for the production of biogas. B. Abubakar et al [7] studied biogas potential by anaerobic process of cow dung and investigated average cumulative biogas yield was 0.15 L/kg VS added. Biogas potential of molasses was stated by C. Y. Lin et al [8] in Proceedings of the WHEC (2010) and demonstrated bioenergy (hydrogen and methane) can be produced by the fermentation of molasses (condensed) using

two stage anaerobic process. In the same year, I. Polematidis et al [9] studied biogas production from desugarized molasses and investigated that about 0.118 $m^3$of methane at STP was produced from 1 kg of raffinate (desugarized molasses). They also concluded that every metric ton of raffinate can generate 4300 MJ of thermal energy by the combustion of methane produced in the bio-reaction. 1n 2014, K. Iqbal et al [10], produced biogas from diluted molasses medium and reported 6.55 dm^3 biogas/kg TS generating 0.144 kWh electrical energy [10].
Production of biogas in a batch or continuous reactor depends upon on various factors including pH, total solids, volatile solids, carbon and nitrogen contents, temperature in the reactor, volatile acid production rates, COD reduction rates etc. Various efforts have been put forth for best optimization of conditions to obtain maximum yield. In this view,A. H. Igoni et al [11] studied the of effect of total solids on biogas production from municipal solid waste and demonstrated that increase in percentage total solids corresponds to geometric increase in biogas produced.I. Dioha et al [12] studied the effect of C/N ratio on various feedstocks to assess biogas yield and stated that C/N ratio is an important factor that controls the pH of the feedstock. They found optimal range of C/N ratio 20–30.1 for maximum biogas yield for different feedstocks. In our work, we adopted the method reported by K. Iqbal et al[10] for biogas production from sugarcane molasses and studied the co-relation of different parameters and proposed the optimized conditions for maximum biogas yield.

2. Material and Methods

Molasses, the main precursor of the study was obtained from a sugar industry. It was first characterised for physical as well as chemical parameters as shown in (table 1.) The nutrients were determined following APHA 2005 [13]. For each batch study, molasses was freshly diluted for the required total solid concentrations per batch volume. Diluted molasses medium acting as substrate was kept at 4^0C if it required to run experiment after some time otherwise used immediately. This diluted molasses medium was poured into quick fit conical flask (bioreactor) along with inoculum as methanogen source. The flask is fitted with a rubber cork having outlet and inlet passages as shown in fig. (--) and connected to a graduated cylindrical water jar which is joined to another receiving flask. The gas is collected over water and equivalent portion of water is displaced to receiving flask.

Table 1. Physical and chemical properties of pure molasses obtained from Sugar Industry

Characteristics	Outcomes
pH at 25 ^{0}C	5.95
Density	1.07 g/ml
Specific Gravity	1.097
Brix	85 degree
Moisture	33.68 %
Total Solid Contents	66.32 %
Total Volatile Contents	79.4 % of TS
Total Fixed Solids (Ash)	13.66 %
Total Sugar Contents	48.3 %
Organic Carbon	27.84 %
Nitrogen (TKN)	0.84 %
C/N Ratio	33.1
COD	67.62 %

The preparation of biogas from molasses involved the following steps;

2.1 Preparation of Substrate

Pure molasses (Table 1) was diluted to attain required concentration of TS per batch volume. This diluted molasses medium acts as substrate for anaerobic digestion and if needed, small calculated quantity of fresh cow dung or sewage sludge or other type of waste was added to adjust specific TS & C/N ratio. Twelve batches were constructed (table 3) studied for optimization. For each batch, total volume was kept about 2000 ml along with inoculum volume.

2.2 Preparation of Inoculum

Inoculation of substrate (diluted molasses medium) with anaerobic microbes is effective for biogas production. For this purpose, common practices involved to obtain microbes from the material in which they are already populated. These materials included different types of wastes like sewage sludge, animal manure and cattle slurry. Animal manure is the best source because it provided nutrients that are necessary for the growth of microbes, later used forbiogas production. In this way, such sources of microbes are valuable and inexpensive. Manure can also neutralized pH of the medium and hence improved buffer capacity of digester [14].

All the ingredients listed weighed accurately and dissolved in distilled water. pH of the medium was neutralized and whole media was autoclaved. 10 g of fresh cow dung was added as methanogens source. The media is effective for the growth of already existing microbes. The media was then incubated under anaerobic conditions at 37^0C. This media along with microbial culture used as inoculum for biogas production. Bacterial count test was conducted to observe the viability and growth of methanogens. In this study, approximate 10 % inoculum size was optimized to carry out anaerobic digestion process.

Table 2. Nutrients for the growth of methanogens

Sr. No.	Nutrients	Required Quantity (g/l)	Sr. No.	Nutrients	Required Quantity (g/l)
1.	Yeast Extract	2.0	13.	Sodium Molybdate ($Na_2MoO_4.2H_2O$)	0.001
2.	Peptone	2.0	14.	Sodium Tungstate ($Na_2WO_4.2H_2O$)	0.001
3.	Ammonium Chloride (NH_4Cl)	1.0	15.	Zinc Sulphate ($ZnSO_4.7H_2O$)	0.001
4.	Magnesium Sulphate ($MgSO_4.7H_20$)	0.4	16.	Aluminium Chloride ($AlCl_3.6H_2O$)	0.001
5.	Calcium Chloride ($CaCl_2.2H_2O$)	0.4	17.	Ferrous Chloride ($FeCl_2.4H_2O$)	0.001
6.	Ferrous Ammonium Sulphate $Fe(NH_4)_2(SO_4)_2.6H_2O$	0.002	18.	Sodium Acetate (CH_3COONa)	1.36
7.	Cobalt Chloride ($CoCl_2.6H_2O$)	0.001	19.	Cysteine / Boric Acid	0.01
8.	Manganese Chloride ($Mn\ Cl_2.4\ H_2O$)	0.001	20.	Sodium Sulphide ($Na_2S.\ 9H_2O$)	0.3
9.	Copper Sulphate ($CuSO_4.5H_2O$)	0.001	21.	Potassium Iodide (KI)	0.01
10.	Nickle Chloride ($NiCl_2.6H_2O$)	0.001	22.	Sodium Bicarbonate ($NaHCO_3$)	5.0
11.	Sodium Sillicate (Na_2SiO_3)	0.001	23.	Potassium Chloride (KCl)	0.04
12.	Nitrilo Tiacetic Acid	0.015	24.	Diammonium Biphosphate $(NH_4)_2HPO_4$	0.4

2.3 Physical and Chemical characterization

Physical and chemical analysis of the medium was carried out by monitoring pH, TS, VS, C/N ratio and COD. The tests were conducted according to APHA 2005 [13] at initial stage as well as after specific period of time by taking out approx. 10–20 ml of mixture. pH was monitored and adjusted on the daily basis while other parameters were analyzed after every 48 hours of time. Biogas yields were also noted on daily basis by measuring quantity of water displaced.

Table 3. Batch study characterization

Batch No.	**Batch Description** (Parameter Studied)	**Batch Size** (ml)	Batch Characteristics				
			pH	**TS** (%)	**VS** (% of TS)	**C/N Ratio**	**COD** mg/L
1	pH	2000	5.0	10.08	--	29.89	--
2	--do--	--do--	6.5	9.96	--	30.19	--
3	--do--	--do--	7.5	10.12	--	30.01	--
4	--do--	--do--	8.0	10.18	--	29.92	--
5	TS	--do--	6.5–7.5	6.18	77.62	29.71	150412
6	--do--	--do--	--do--	9.69	76.41	30.11	190455
7	--do--	--do--	--do--	11.24	77.58	29.86	209321
8	--do--	--do--	--do--	14.28	79.15	29.53	225632
9	--do--	--do--	--do--	16.27	78.49	30.09	245805
10	C/N Ratio	--do--	--do--	10.24	--	33.16	--
11	--do--	--do--	--do--	9.84	--	26.78	--
12	--do--	--do--	--do--	9.96	--	29.89	--

Note: Values of TS & C/N ratio in batch 1–4 and of pH & C/N ratio in batch 5–9 and of pH & TS in batch 10–12 were tried to maintain according to optimized conditions of said parameters (pH 6.5 – 7.5, TS 10% and C/N ratio 30). In the table, these values are not exactly according to optimized values because these are obtained by the actual results of batch characterization and considered as optimized.

2.4 Digestion Process

Digestion process was started in the bioreactor as the substrate was inoculated. The process was carried out under mesophilic temperature conditions (30–37^{0}C). pH was noted daily and adjusted between 6.5–7.5 to ensure maximum biogas production. In the bioreactor, substrate was homogenized with inoculum by carrying out slow mixing occasionally. This is helpful to avoid temperature gradients, crust formation and dead spaces that affect the process of digestion in the bioreactor [10]. Each batch was studied for 15 days and data was evaluated for the biogas production.

3. Results and discussion

In this study, it has been observed that production of biogas from diluted molasses medium depends upon certain conditions of parameters including pH, TS, VS, C/N ratio and COD while operating at mesophilic temperature range (30–37 ^{0}C). Different experiments were run to optimize a parameter for maximum yield. So, each parameter was discussed as under

3.1 pH

Four batches (table 3) were run at pH i.e 5.0, 6.5, 7.5, 8.0 with initial concertation of TS of about 10% and C/N ratio 30 and noted the effect pH on biogas yield. It was found that after adjusting initial specific pH of the batch, pH tends to decrease as the reaction starts in bioreactor producing biogas. However, a specific pH was continuously monitored and regularly maintained by injecting specific volume of saturated solution of NaOH. Decreasing in pH may be perhaps due to increase of non-dissociated VFA's in an anaerobic digestion [15]. The concentrations of total solids, volatile solids were also decreased with the passage of time continuously along with reduction of chemical oxygen demand (COD). Biogas yield was found maximum and almost identical at pH 6.5 and 7.5 (2.73dm^3& 2.74dm^3 respectively) as compared to biogas yield at pH 5.0 and 8.0 (1.516dm^3& 0.84dm^3 respectively (fig. 1). It is because that below pH 6.3 and above pH 7.8, the methanogenic bacteria become very slow in their activity [16] and further filamentous bacteria grow at lower pH and higher pH causes accumulation of unionized ammonia [17]. pH more than 8.0 will show toxic effects on the methanogenic bacteria population. [18]. Hence maximum biogas production from diluted molasses medium can be carried out at pH range 6.5 – 7.5.

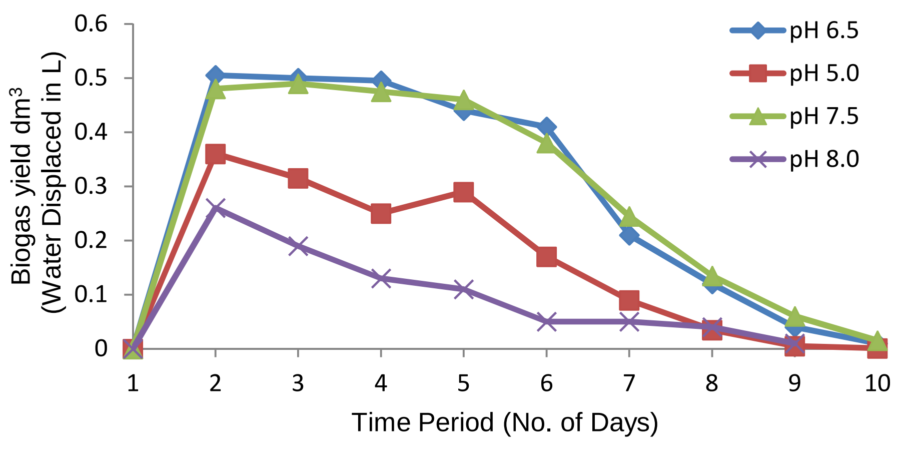

Fig. 1. Effect of pH on biogas yield

3.2 Total Solids

Five batches (No. 5-9, Table 3) were conducted to evaluate the effect of TS on biogas production. It was observed that increases in total solid contents directly increase the biogas production. Solid contents gradually decrease day by day (fig. 3) and resulted decline in biogas production (fig. 2). We started our experiments with initial total solid concentration at 6.81%, 9.69%, 11.24%, 14.28% and 16.27%

maintaining a pH at 6.5–7.5 and C/N ratio about 30. The batches with initial TS concentration at 9.69% and 11.24% showed better results (biogas yield 2.73dm^3& 2.79dm^3 respectively) as compared to the batches with initial TS concentration at 6.81%, 14.28% and 16.27% (biogas yield 1.34dm^3, 2.655dm^3& 2.54dm^3 respectively) fig. 2. The batch with initial TS 6.81% produced biogas in low quantity because of low initial value. The batches with initial TS values 14.28% and 16.27% produced biogas in lesser amount because higher TS concentration results more formation of volatile fatty acids thus inhibiting the methanogens responsible for the production of biogas. Total solid contents less than 13% produced more biogas by making readily access of bacteria to the substrate. [10]. Increasing total solid content decreases the digester volume due to lower water requirements [19]. Furthermore, higher TS concentrations produce acidic effects on the medium than that of lower concentration of TS resulting decrease in pH [20]. It is as observed that higher the initial concentration of TS, more will be the biogas production rate as the bioconversion started. That is why; using higher concentration of TS result early completion of reaction but overall biogas production is decreased (fig. 2). Although overall consumption of TS batches (with initial high concentration) is more than of TS batches (with initial low concentration) fig 3. This is probably due to more conversion of TS into VFA's resulting decline in pH and inhibiting the reaction. Fig. 3 shows the decreasing trend of TS from the first day to last. The percentage reduction for batches with initial TS concentrations at 6.81%, 9.69%, 11.24%, 14.28% and 16.27% was found to be 19.09%, 19.30%, 27.58%, 38.58% and 40.29% respectively. Low percentage reduction result trend line to become parallel with axis (fig. 3). It indicates slower but effective utilization of solid contents by the microbial community in bioreactor enhancing biogas production. Also very low consumption of solids decreased the gas production. So, maximum biogas production occurred at an optimum level. In our studies, this optimum range was found to be about 10% initial concentration of TS. The biogas produced at 10% solid content was 1.377dm^3 (Table 5)

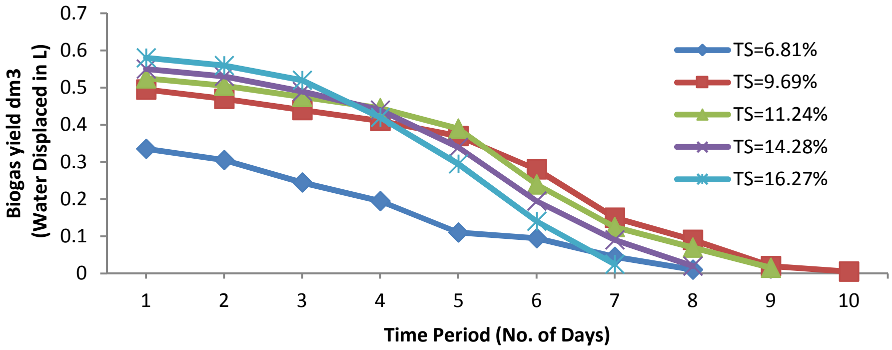

Fig. 2. Effect of TS concentration on biogas production

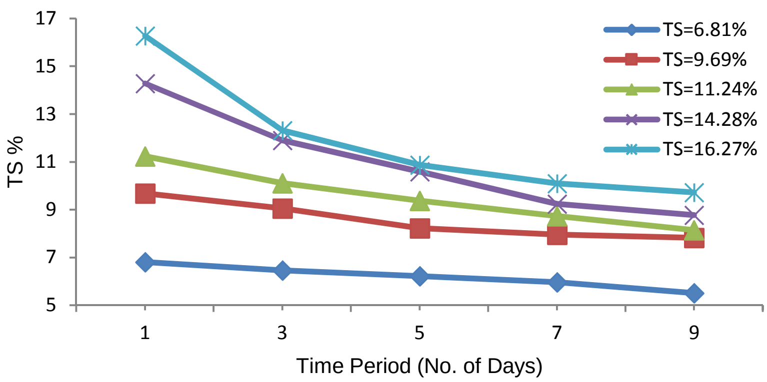

Fig. 3. Decrease in Total Solid Concentration as biogas produced

3.3 Volatile Solids

Volatile solids represent the organic nature of medium and are measured as solid contents minus ash contents. Their concentration also decreased like TS as the reaction is proceeding and hence, reducing the biogas yield. In our experiments, effect of VS was studied along with TS (batches no. 5-9, table 3) and observed that reduction trend just like TS. The curve of VS runs parallel to TS (fig. 4). Higher value of VS represent more water displaced directly related to more biogas evolution (fig. 2). Results are strengthened by the study of [21]. Also more quantity of volatile solids ensures more formation of volatile fatty acids. Fig. 4 shows the decreasing trend of VS from start of Experiment to end. The percentage reduction for batches with initial TS values at 6.81%, 9.69%, 11.24%, 14.28% and 16.27% was found to be 19.12%, 19.47%, 27.18%, 38.69% and 40.64% respectively. Percentage reduction was found similar with that of TS, VS consumed depending upon TS.In figure 4, all the curves started from same point because all batches have same VS initial concentration with slight difference. After that, curves separated because VS reduced as percent function of TS. But like TS, curves for lower initial concentrations of VS seem to be parallel.

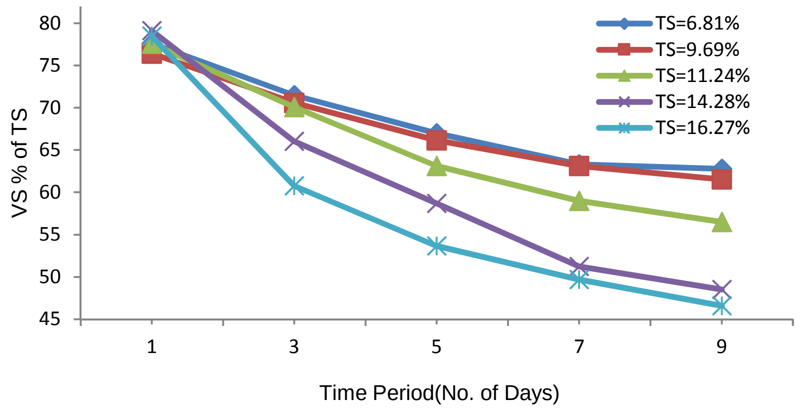

Fig. 4. Decreasing trend in volatile solids

3.4 Effect of Carbon to Nitrogen (C/N) Ratio

C/N ratio is also another significant factor to assess the ability of a bioreactor to produce biogas. The batch no. 10–12 (table 3) were conducted to observe the efficacy of C/N ratio on biogas yield. The study was carried out at different C/N ratios (33.16, 26.78, 29.89) keeping the TS concertation to about 10% and pH at 6.5–7.5 (table 3). The maximum biogas yield was found at C/N ratio 29.89 (2.78dm^3) than the other C/N values 33.16 (2.14dm^3) and 26.78 (2.565dm^3) fig. 5. So, the optimum C/N ratio was found to be 29.89 (almost 30) and hence, all other trials (for effect of pH & TS) were conducted to about C/N ratio 30 (table 3). As the bioconversion continued, C/N ratio decreased because carbon compounds are utilized by the acidogenic bacteria into methanogenic substrates and volatile fatty acids [22]. Lowering the C/N ratio would result in liberation of ammonia raising the pH of the medium. According to P.Shanmugam et al [23], C/N ratio determines both the NH_3-H and VFA values observed in the digester. Increasing ammonia nitrogen raised pH while by contrast lowering of pH is governed by VFA which neutralized HCO_3^{-1}, CO_3^{-2} and acetate ions. Nevertheless ammonia can be toxic to digestion process. F. Callaghan et al [24] and E. A. Salminen et al [25] accustomed a tolerance level for NH_3 in an anaerobic bioreactor to treat high protein wastes.

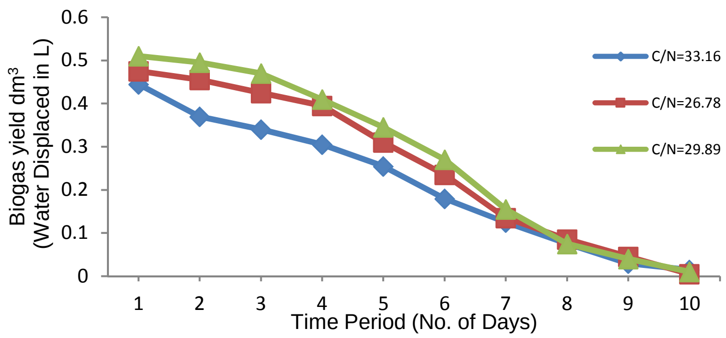

Fig. 5. Effect of C/N Ratio on biogas production

3.5 Chemical Oxygen Demand (COD)

COD effect was observed in the batch study from 5–9 (table 3) and found a gradual reduction regularly to the end of the process (Fig. 6). Reduction in COD is seen parallel to reduction in VS and TS. These results are supported by the studies of F. Liotta [26], in which they investigated that the COD degradation occurred under all TS conditions. On the other hand, according to P.Shanmugam et al [23], bio-kinetic yield is estimated using COD data for the design of rector and hence COD dataprovide a better alternate of experimental COD for design purposes. The reduction in COD may be due to the bioconversion of organic matter into biogas (mixture of methane and carbon dioxide) that is produced in the bioreactor. COD is not the primary factor like TS, VS and C/N ratio that directly affect the biogas production rather than it may be the factor that is itself affected by biogas evolution. That is why; COD values could not be rationalized to assess biogas evolution in an anaerobic digestion process.

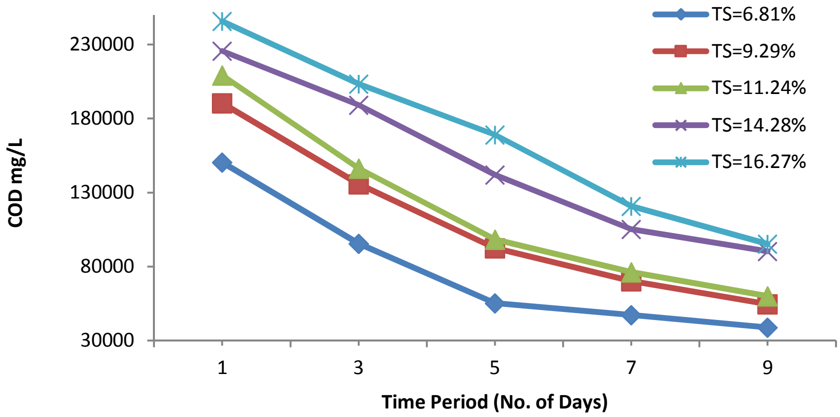

Fig. 6. Reduction in COD

3.6 Parameter Optimization and Correlation

Twelve batch studies were run to optimize the operational conditions of pH, TS, VS, and C/N ratio for maximum biogas yield using mainly diluted molasses medium as substrate. The maximum biogas production was found 2.73dm^3 & 2.74dm^3 at pH 6.5 and 7.5 respectively, and 2.73dm^3& 2.79dm^3 at TS 9.69% and 11.24% respectively and 2.78dm^3 at C/N ratio 29.89. By rounding off data, it was concluded that maximum biogas can be obtained at pH 6.5–7.5, TS 10% and C/N ratio 30. So, these are the optimum conditions for biogas evolution from diluted molasses medium.

In our studies, it is observed that parameters (pH, TS, VS & C/N ratio) are correlated with each other by depending upon one another. Concertation of VS is dependent on TS, mean more TS, more will be the VS. The production of biogas in an anaerobic bioreactor is due to the digestion of organic compounds by the activity of microbes (Aicidogens, Methanogens etc) while in the bioreactor, organic strength is measured by the quantity of VS which in turn are dependent on TS. So, in this study, biogas yield is rationalized by changing the concentration of TS in different batches. In the batch study, it is seen that decreasing trend of VS is just like TS (fig. 4)

TS also related to pH as higher initial concentration of TS would result accumulation of VFA and decline the pH of the medium thus reducing biogas yield. (Fig. 2). pH is also dependent on C/N ratio as low C/N ratio would cause release of ammonia decreasing pH of the system [23].

So, all parameters responsible for the production of biogas are correlated with each other. Therefore, before the start of anaerobic digestion of a substrate, optimization of parameters is the first to obtain biogas yield.

In our work, maximum biogas produced 1.377 dm^3 /litre of diluted molasses medium (table 4)

Table 4. Description of Batches with maximum biogas yields

Sr. No.	Batch Description	Characteristics					Biogas Yield (dm^3/L batch size)
		Batch Size (ml)	**pH**	**TS %**	**VS %**	**C/N Ratio**	
1.	Batch Study was conducted to observe the effect of pH	2000	6.5	9.96	--	30.19	1.365
2.	Batch Study was conducted to observe the effect of pH	--do--	7.5	10.12	--	30.01	1.37
3.	Batch Study was conducted to observe the effect of TS	--do--	6.5-7.5	9.69	76.41	30.11	1.365
4.	Batch Study was conducted to observe the effect of TS	--do--	6.5-7.5	11.24	77.58	29.86	1.395
5.	Batch Study conducted to observe the effect of C/N Ratio	--do--	6.5-7.5	9.96	--	29.89	1.39
	Proposed optimized batch constructed from 1-5	**2000**	**6.5-7.5**	**10**	**77**	**30**	**1.377**

4. Conclusions

1. Different batch were made using diluted molasses as substrate by maintaining specific values of pH, TS %, VS % and C/N ratio.
2. Biogas yield (dm^3) was measured by water displacement method on 1 atmospheric pressure.
3. Different parameters (pH, TS, VS, C/N ratio) were optimized for maximum biogas yield
4. Maximum biogas production 1.377dm^3 per litre diluted molasses medium was obtained on optimum conditions of pH 6.5-7.5, TS 10%, VS 77% and C/N ratio 30.
5. All parameters (pH, TS, VS, C/N ratio) are correlated with each other by inhibiting/enhancing mode.
6. There is need to optimize parameters before starting any anaerobic digestion process to evaluate maximum biogas yield.

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