Desalination and Water Treatment
www.deswater.com

2 (2009) 254–259

Phenolic wastewater: Effect of F/M on anaerobic degradation

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Received 20 December 2007; accepted revised 28 November 2008

ABSTRACT

Effect of the ratio of food to microorganism (F/M) ranging from 0.25 to 2.0 and nature of sludge on methanogenesis of phenol in the batch reactors has been evaluated. Unacclimatized flocculant sludge and acclimatized granular sludge were used as seed inocula (microorganisms) in two different sets of experiments. The degradation profiles of phenolic COD conformed to logistic growth model. Lag phases of 10 days with flocculant sludge and upto 32 days with granular sludge followed by accelerated rate of CH_4 generation were noticed. Methane generation rate constant (k) has been found to vary from 0.17 to 0.21 d^{-1} and 0.10 to 0.18 d^{-1} with flocculant and granular sludges respectively. The F/M ratio also influenced the biochemical methane potential (μ_b) and the sludge activity [mL CH_4 or g CH_4-COD formed/g VSS (sludge)]. μ_b at 80 days (μ_{b80}) with flocculant sludge ranged from 0.18 to 0.95 while μ_b at 112 days (μ_{b112}) with granular sludge ranged from 0.80 to 1.04. μ_{b80} was maximum at F/M = 0.75. The trend of sludge activity was also found similar to that of k and $\mu_{b80.}$

Keywords: Anaerobic treatment; Sludge; Phenol; Reactors; Industrial waste

1. Introduction

Phenol and its derivatives commonly found in the wastewater from the manufacturing of synthetic chemicals, pesticides, coal conversion, pulp-paper, oil-refining, etc. are toxic and potentially carcinogenic. The treatment of wastewater containing phenols and cresols has been reviewed by Veeresh et al. [1]. A few investigators [2–5] have reported stoichiometric conversion of phenol to CH_4 in batch reactors using sludge from the digester as seed inocula. The biomass 10% (v v^{-1}) has been used by Owen et al. [6], and Healy and Young [2] while 20% (v v^{-1}) by Young and Rivera [7], Fedorak and Hurdey [3]. With unadapted sludge as inocula, acclimatization time for phenol methanogenesis is concentration dependent. The acclimatization time is fairly short (15–18 days) up to phenol concentration of 500 mgL^{-1} and at concentration more than 500 mgL^{-1}, acclimatization time is ≥ 26 days) [3]. Acclimatization improves extent and rate of metabolism for phenol [7]. With phenol enriched methanogenic cultures, rate of phenol degradation has been found to be satisfactory upto initial concentration < 600 mgL^{-1}. Phenol is fermentable with acclimatization to CH_4 at concentration as high as 1430 mgL^{-1} with ~90% methane recovery [8].

Grady [9] reported that too high a concentration of F/M may cause toxic effects while too low a concentration may prevent induction of enzymes. The phenol toxicity in principal should depend on the ratio of phenol (food or substrate) to microorganisms [10]. The toxic concentration of 500 mgL^{-1} or more may prove to be non toxic provided the concentration of microorganism is high. It therefore appears that the toxicity due to high concentration of phenol can be alleviated by increasing microorganisms concentration. There are few studies conducted with reference to the effect of F/M. Sabbah et al. [10] conducted anaerobic batch experiments with different F/M ratios ranging from 0.45 to 2.4 using olive mill

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wastewater as substrate. Highest COD removal of 65% was achieved at F/M of 0.45. In a recent study, anaerobic degradability was found to depend on particulate fraction of COD and F/M [11]. The optimum value of F/M ranged from 0.57 to 0.68. Moreno et al. [12] conducted batch experiments to study the effect of F/M on anaerobic biodegradability of acetate and azo dye. He observed that mixing and initial substrate to biomass concentration had a significant effect on reaction rate and on the biomass yield coefficient. He found the minimum reaction time to be 2.3 days at F/M = 1.0. However, there has not been any systematic study on the effect of F/M on the conversion of phenol to CH_4. The nature and amount of the sludge is expected to influence the acclimatization, and rate and extent of CH_4 generation from phenol. Keeping these in view, the biodegradability of phenol as a sole substrate was assessed at different ratios of phenol (food) to sludge (microorganism, F/M).

2. Experimental methodology

Batch experiments were performed in accordance with the method proposed by Owen et al. [6]. Serum bottles of 125 mL with working volume of 100 mL were used as anaerobic batch reactors (Fig. 1). Reactors containing defined media were purged with nitrogen and carbon-dioxide in the ratio of 3:1 and charged with sludge. Two sets of experiments were performed, in first set, flocculent sludge from bench-scale upflow anaerobic sludge blanket reactor (UASBR) treating synthetic domestic wastewater was used and in the second set, granular sludge from the bench-scale UASB reactor treating phenol was added. The sludge withdrawn from the reactors was washed with tap water prior to charging into the bottle reactors. Appropriate amount of substrate was anaerobically transferred to each reactor. Operating conditions are briefly summarized in the first two columns of Tables 1a and b.

The reactors sealed with rubber stoppers were incubated in a temperature controlled chamber maintained at 30±2°C. Gas produced in each serum bottle was measured daily. Blanks (i.e. reactors without substrate) were run parallel to these reactors. pH, chemical oxygen demand (COD), microbial concentration in terms of volatile suspended solids (VSS), total suspended solids (TSS), nitrogen, and phosphorous were determined as per Standard Methods [13]. Biomass was digested to determine cell – nitrogen (N) and phosphorous (P) percentages. Biogas collected at room temperature was normalized to standard temperature and pressure (STP). Methane

Table 1a
Anaerobic degradability of phenol at different F/M (Flocculant sludge)

F/M*	Phenol conc. (gL^{-1})	Lag (d)	$C_{max.}$ (mL)	k (d^{-1})	r^2	CH_4-COD (gL^{-1})	Phenol COD fed (gL^{-1})	μ_b ($gCH_4COD/gCOD_{fed}$)
0.25	0.36	10	26	0.18	0.990	0.75	0.97	0.77
0.50	0.71	10	53	0.19	0.999	1.50	1.67	0.89
0.75	1.07	10	86	0.21	0.999	2.40	2.52	0.95
1.00	1.43	10	58	0.18	0.997	1.63	3.47	0.47
1.25	1.78	10	60	0.19	0.998	1.72	4.19	0.41
1.50	2.14	10	31	0.17	0.995	0.89	4.97	0.18
1.75	2.50	10	50	0.18	0.998	1.43	5.89	0.24
2.00	2.85	10	66	0.19	0.999	1.89	6.57	0.28

*VSS added into each serum bottles = 3.4 (gL^{-1}), Sludge N = 13.4%, Sludge P = 2.41%

Table 1b
Anaerobic degradability of phenol at different F/M (Granular sludge)

F/M*	Phenol conc. (gL^{-1})	Lag (d)	$C_{max.}$ (mL)	k (d^{-1})	r^2	CH_4-COD (gL^{-1})	Phenol COD fed (gL^{-1})	μ_b ($gCH_4COD/gCOD_{fed}$)
0.25	0.21	0	16.8	0.18	0.98	0.48	0.5	0.96
0.75	0.63	10	54.4	0.12	0.98	1.55	1.5	1.04
1.00	0.84	13	55.9	0.12	0.97	1.60	2.0	0.80
1.75	1.47	32	119.9	0.12	0.99	3.43	3.5	0.98
2.0	1.68	32	133.6	0.10	0.99	3.81	4.0	0.95

*VSS added into each serum bottles = 2 (gL^{-1}), Sludge N = 12.4 %, Sludge P = 2.02%

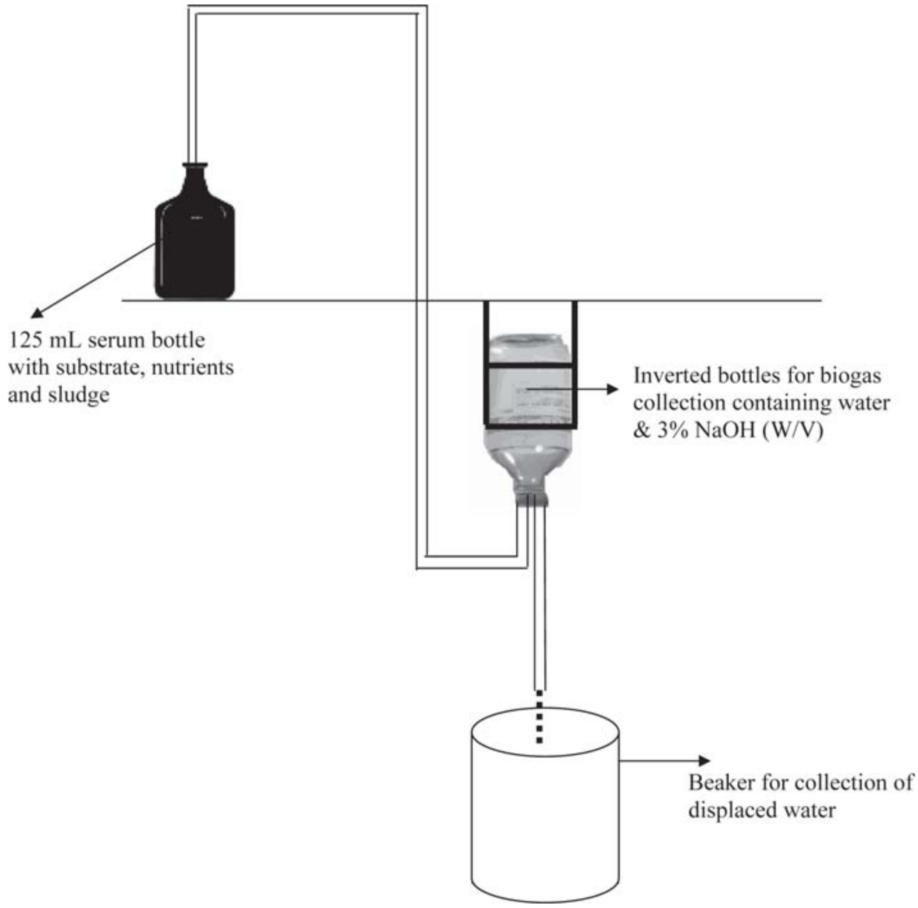

Fig. 1. Experimental set-up for batch biodegradability test with serum bottles (housed in a temperature controlled chamber at 30±2°C).

content in the biogas was measured by passing total biogas through NaOH solution. Experiments were carried out in triplicate. Values reported here in are the average values. The amount of methane or methane COD reported is after blank corrections.

3. Results and discussion

The sludge VSS, phenol COD, and percent N and P in the sludge were analyzed before the start of experiments. Values are given in Tables 1a and b. The optimum value of F/M was ascertained by carrying out degradation of varying amounts of phenol in the presence of a fixed amount of microbial population at a fixed value of COD:N:P of 300:10:1.

Data collected at different values of F/M has been analyzed to evaluate rate of phenol degradability in terms of methane generation rate constant (k, d^{-1}) and conversion of phenol COD fed to ultimate CH_4-COD (μ_b) .Sludge activity [mL CH_4 or g of CH_4-COD formed/g VSS (sludge)] a parameter derived from the observations has also been analyzed.

3.1. *Methane generation rate constant,* k

Cumulative methane at F/M ranging from 0.25 to 2.0 was monitored till gas generation ceased. It took 80 and 112 days with flocculent and granular sludge inoculums respectively. Results are presented in Figs. 2a and b. Initial lag period of 10 days was noticed with flocculent sludge, however, the lag phase with granular sludge increased with increase in F/M (0–32 days). The initial lag phase in both the cases nevertheless was followed by high rate of methane generation. The degradation of phenol thus follows an accelerative generation after an initial lag period. In a multistage sequential conversion of phenol to CH_4, the initial step is a slow process followed by fast conversion of intermediate products to CH_4 [15]. The lag may be due to the use of unacclimatized flocculant sludge and/or washing of granular sludge before use. Lag may also be due to change in micro environment, and/or slow initial step. The duration of lag in case acclimatized granular sludge has been noticed to be more than that of flocculant sludge at all F/M except at F/M = 0.25 and 0.75. There was no lag at F/M = 0.25. The phenol concentration at F/M = 0.25 was less than the reported toxic concentration [8]. The granular sludge is compact as compared to flocculant sludge [16]. The activity of flocculant sludge towards readily degradable wastewater has been found to be more than that of granular sludge [17].

Phenol is converted to methane via carboxylation, dearomatization, ring fusion and fragmentation [7]. The compact structure is likely to cause an impedence to inter and intracellular diffusion of substrate to the active sites of enzymes (bacteria).Substrate is required to induce enzyme activity in the acclimatized granular sludge that has been washed with tap water [18]. Substrate availability at the appropriate site thus becomes a slow process and a reason for an initial lag. Too high a concentration of phenol (F/M = 2) may cause toxic effects leading to long acclimatization period. Such a phenomenon i.e. increasing lag phase with increasing F/M is seen with phenol as substrate and granular sludge as inoculum. A lag phase of 5–15 days with dilute molasses (readily degradable wastewater) and granular sludge and no lag with flocculant sludge was observed [19]. With phenol and flocculant sludge a lag phase of 10 days has been observed at all F/M values (Fig. 2a).

The cumulative methane generation profiles (Figs. 2a and b) appear to follow logistic S growth curve [20]. Accordingly, the rate of methane generation from phenol depends directly on methane (C) at time t, and to its remaining room ($C_{max} - C$) for attaining the maximum methane (C_{max}) [Eq. (1)]

$$\mathrm{d}C/\mathrm{d}t = k'C\left(C_{\max} - C\right) \tag{1}$$

$$\begin{aligned} &= k'C_{\max}\left(C_{\max} - C\right)/C_{\max} \\ &= kC\left(1 - C/C_{\max}\right) \end{aligned} \tag{2}$$

k' is the constant having units of conc^{-1}.time^{-1}, and k is rate constant (time^{-1}) equal to k' C_{max}. The solution of Eq. (2) is given as under Eq. (3)

$$C_t = C_{\max}C_0 / \left[C_0 + \left(C_{\max} - C_0\right)\exp^{-kt}\right] \tag{3}$$

Eq. (2) clearly explains the fact that initially when $C << C_{max}$, the rate is proportional to C and when $C << C_{max}$ the rate is nearly zero, where C_o = initial methane concentration at time $t = 0$ (mL); C_{max} = maximum concentration of methane at maximum time (mL); C_t = concentration of methane at time t (mL).

Experimental data from phenol-flocculant sludge system and phenol granular sludge system have been used to evaluate the value of k. Time t begins after the lag period. C_0, the initial methane equal to or more than 0.1 mL after blank correction has been assumed to correspond to initial time after the lag period. The best fit curves with respect to the experimental data were obtained using nonlinear regression with the quasi-Newton algorithm. This algorithm estimates values of model parameters by minimizing the sum of squared differences between the experimental and calculated values. These are then employed in the function with nonlinear optimization using *Ky* plot software to calculate value of k. Lag period, k values, and r^2 (correlation coefficient) values are also compiled in Tables 1a and b. Perusal of the data compiled in Table 1 reveals the range of k between 0.17 and 0.21 d^{-1} for phenol flocculant sludge system. The rate constant k (d^{-1}) is comparable to the degradation of soluble wastewater prepared from sucrose and is more than the sewage and complex synthetic sewage [12]. Particulate COD in sewage causes impedence to the process.

Therefore, once the biomass is acclimatized, the rate of generation does not significantly vary and time to acclimatize is 10 days. The rate constant for the conversion of phenol to CH_4 with granular sludge at F/M = 0.25 is 0.18 d^{-1}. There is no acclimatization period, no toxicity

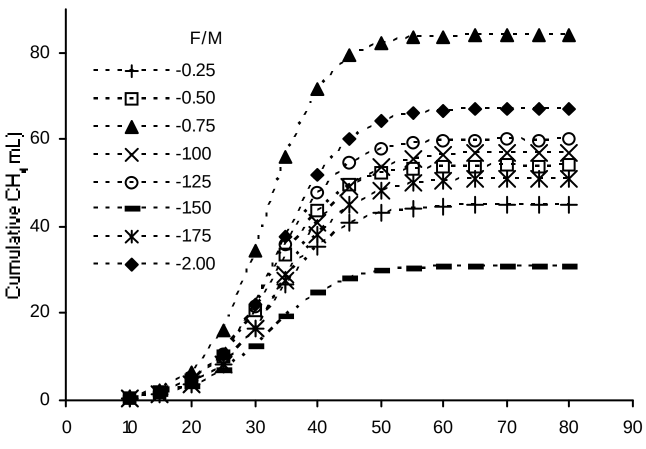

Fig. 2a. Temporal variation of cumulative CH_4 at different F/M (0.25–2.0), with flocculant sludge as seed (….. computed lines).

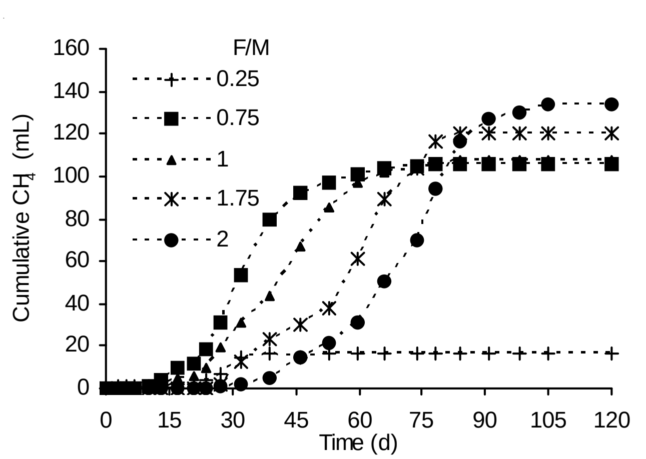

Fig. 2b. Temporal variation of cumulative CH_4 at different F/M (0.25–2.0) with granular sludge as seed (….. computed lines).

and rate of methane generation is high. The value of k at other F/M is between 0.10–0.12 d^{-1}. The increase in acclimatization period with the increase in phenol concentration (F/M) is probably due to reversible toxicity of high concentration of phenol. It can also be envisaged that the decrease in the value of rate constant *k*, 0.12–0.10 is due to toxicity. Time to recover from the toxicity directly depends on phenol concentration.

3.2. Biochemical methane potential (μ_b)

Substrate biodegradability under anaerobic conditions in a batch process can be evaluated in terms of biochemical methane potential (BMP), i.e. mL of methane produced per g of COD fed after a given period of time [6,11]. The conversion of COD fed to CH_4-COD, i.e. g CH_4-COD/g COD_{fed} (μ_b) is another expression for the process efficiency. The observations recorded here in have been analyzed in terms of μ_b. The time to attain maximum μ_b with phenol and flocculant sludge is nearly 80 days. However, with phenol and granular sludge the time to attain maximum μ_b increases with the increase in F/M. The values of μ_b for phenol-flocculant sludge and phenol granular sludge recorded after 80 and 112 days denoted as μ_{b80} and μ_{b112} respectively at different F/M have been shown as histograms in Fig. 3.

The μ_{b80}, i.e. the efficiency of phenol conversion to CH_4-COD by flocculant biomass has been found to vary significantly with variation in F/M. The μ_{b80} equal to 0.95 at F/M = 0.75 decreases at F/M < 0.75 and > 0.75. The values ranged from 0.18 to 0.95. The ratio of F/M of 0.75 thus appears to be adequate for the rate of degradation (*k*) as well as biotransformation potential of phenol COD to CH_4-COD (μ_b).The granular sludge converts 90–95% of influent COD to CH_4-COD. The time taken, however, increases with the increase in phenol. In fact, the long acclimatization period delays the process. The toxicity due to shock loads appears to be reversible in nature with granular sludge and irreversible in nature with flocculant sludge. This may probably due to diffusion. The extent of substrate (phenol)–biomass contact is more with flocculant sludge than with granular sludge.

3.3. Sludge activity

F/M affects substrate utilization which in turn depends on sludge activity. Sludge activity is expressed as mL CH_4 or g of CH_4-COD formed g^{-1} VSS (sludge). It is a parameter derived from the above data obtained by multiplying μ_b with F/M [Eq. (4)

$$\mu_b \times \left(\frac{F}{M}\right) = \frac{\text{g } CH_4 - \text{COD}}{\text{g COD}_{fed}} \times \frac{\text{g COD}_{fed}}{\text{g VSS (sludge)}} = \text{g } CH_4\text{-COD g}^{-1} \text{ VSS} \quad (4)$$

g CH_4–COD g^{-1} VSS is potential of the sludge to yield CH_4 from the substrate (phenol in this case) over a period of time. A value of $\mu_b \geq 0.95$ has been taken as 100% sludge activity in case of flocculant and granular sludges. Taking this as reference the percent sludge activity of granular sludge and flocculant sludge at other ratios of F/M is presented in Fig. 3. The trend is similar to that of μ_b shown in Fig. 4. It has been shown that F/M > 0.75 and < 0.75 reduces methanogenic activity when flocculant sludge is used as seed. However, with granular sludge reduction in μ_b and sludge activity were not as noticeable as for flocculant sludge. The compact structure is likely to cause an impedance to inter and intracellular diffusion of substrate to the active sites of enzymes (bacteria).Substrate is required to induce enzyme activity in the acclimatized granular sludge that has been used in the study

4. Conclusions

i) The methane generation from phenol started after a lag of 10 days with flocculant sludge. The lag period

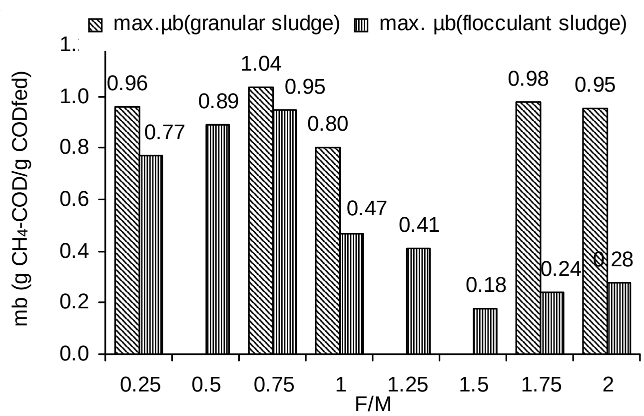

Fig. 3. Effect of F/M on maximum μ_b (granular and flocculant sludge).

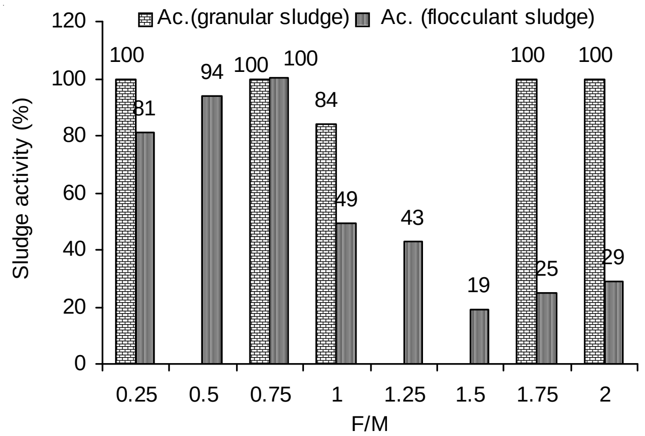

Fig. 4. Sludge activity at different F/M (granular sludge and flocculant sludge).

with washed granular sludge increased with increase in F/M.

ii) The temporal variation of cumulative CH_4 conformed to logistic growth. Values of k ranged from 0.17 to 0.21 d^{-1} and 0.10 to 0.18 d^{-1} with flocculant and granular sludges respectively.

iii) The conversion efficiency (μ_b) of phenol COD to CH_4-COD by flocculant sludge was influenced by F/M. A definite decreasing trend in the value of μ_{b80} was noticed when the F/M was increased or decreased from 0.75. The sludge activity also followed the trend similar to that of k and μ_b. However, the value of μ_b with washed granular acclimatized sludge did not vary significantly with change in F/M. The μ_b at F/M as high as 2.0 was found to be 95%.

iv) The phenol can be effectively converted to CH_4 by acclimatized granular sludge even at high F/M.

Symbols and abbreviations

μ_b	—	Substrate biodegradability, g CH_4-COD/g COD_{fed}
Ac	—	Acclimatized
BMP	—	Biochemical methane potential
CH_4-COD	—	Methane COD, mg
C_{max}	—	Maximum concentration of methane at maximum time, mL
C_o	—	Initial methane concentration at time t = 0, mL
COD	—	Chemical oxygen demand, mg/L
C_t	—	Concentration of methane at time t, mL
F/M	—	Food to microorganism ratio
IS	—	Indian standard
k	—	Rate constant, d^{-1}
N and P	—	Nitrogen and phosphorous, mg/L
NaOH	—	Sodium hydroxide
STP	—	Standard temperature pressure
TSS	—	Total suspended solids, mg/L
UASBR	—	Upflow anaerobic sludge blanket reactor
VSS	—	Volatile suspended solids, mg/L

Acknowledgements

The first and second authors received fellowship from Council of Scientific and Industrial Research (CSIR), New Delhi, India.

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