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Anaerobic treatment of phenolic wastewater: Effect of phosphorous limitation

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ABSTRACT

Synthetic phenolic wastewater representing an industrial synthetic wastewater was supplemented with varying amount of phosphorous (P) and treated in upflow anaerobic sludge blanket (UASB) reactor. The variation of chemical oxygen demand COD:P from 300:1 to 300:0.1 did not influence the conversion of phenol COD to methane COD. The concentration of P in the influent was reduced from 2.5 to 0.25 mg/L respectively. However, on further reducing the COD:P in the feed from 300:0.1 to 300:0 the (i) CH_4-COD decreased from 90 to 40%, and (ii) cell yield reduced to 25–50%. The average cell yield was 3.5%. Percent P in cells varied from 0.6 to 2.4% respectively. The activity of the sludge assessed as specific methanogenic activity (SMA) was found in the range of 0.15–0.66 g CH_4-COD/g VSS d^{-1}. The optimum COD: P for synthetic phenolic wastewater has been estimated to be 300:0.1.

Keywords: Phenol; Biodegradation; Phosphorous; Reactor; Sludge; Methane

1. Introduction

The release of phenol into the environment is of concern as it is toxic and potentially carcinogenic chemical. According to Indian standards the permissible limit of phenol in industrial effluents to be discharged into inland surface waters and into public sewers is 1.0 and 5.0 mg/L [1,2] respectively. The toxic effects of phenol to the biomass in a UASB reactor can be mitigated either by (i) dilution [5], (ii) effluent recirculation [5,6] or (iii) mixing with readily degradable substrate (co-substrate) [4,7,8].

The phosphorous deficient wastewaters are supplemented with phosphorous prior to biological treatment. The requirement of P depends on cell yield, percent of P in cells and nature of the wastewater. The bacterial cells bringing about methanogenesis contain 6–12% N. Phosphorous in these cells is ~14% of nitrogen. At 5% net cell synthesis (i.e. 5% of incoming COD is converted to cell COD), the requirement of P is 0.8 kg per1000 kg of COD (COD:P = 300:0.24) or 0.14 kg per 60 m^3 of methane produced [9].

The bench scale anaerobic degradation of a wide variety of wastewaters under different values of COD: P in UASB reactors as well as in other high rate anaerobic reactors has been reported in the literature [10,11]. The treated effluent from such a system is rich in nutrients. The cost of the biological treatment also increases due to addition of excess of nutrients [12]. The bioconversion of phenol to methane has carried out under a wide range of variation in the value of COD:N:P. Judicious use of nutrients in anaerobic treatment has not been systematically investigated. The optimal nutrient dosage for anaerobic treatment systems is largely unknown. Phosphorous more than the stiochiometric requirement for anaerobic degradation if added are uneconomical and excess of nutrients are subsequently released into water bodies bring about changes in the ecology of the aquatic systems. Therefore it became necessary to find out the effect of

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COD:P on metahnogenesis of phenol, a representative of Industrial waste to ascertain optimum COD:P. Britz et al. [13] however, systematically investigated the effect of nitrogen and phosphorous on anaerobic degradation anaerobic bed filters of petrochemical wastewater.

It therefore necessitated investigation of the effect of the concentration of phosphorous on the treatment of industrial synthetic wastewater. Keeping this in view, an attempt has been made to assess the minimum amount of P required for the bioconversion of phenol COD to CH_4-COD in a UASB reactor.

2. Materials and methods

Treatment of phenolic wastewater was carried out in a UASB reactor of 10 L capacity housed in a temperature controlled chamber maintained at 30±2°C. The synthetic wastewater was prepared by dissolving phenol in tap water and adding varying amount of N and P. The study was carried out in two (I–II) phases. Investigation by different researchers reveal that the treatment of phenol COD up to 2856 mg/L (phenol = 1200 mg/L) in a UASB reactor is feasible [3,4] and easily degradable without any co-substrate or recirculation. The concentration of phenols in effluents varies from 10 to 17,000 mg/L [4], therefore the phenol concentration of 420 mg/L was maintained in order to carry out the study smoothly without impairing the degradation process. Throughout the experimentation (Phase I–II) the COD of synthetic feed was maintained at 1000±20 mg/L. Britz et al. [13] have not mentioned the hydraulic detention time (HRT) value which is an important parameter in anaerobic process whereas author has conducted the study on minimum HRT of 6 h.

Since phenol is difficult to degrade, the UASB reactor was started with non synthetic phenolic wastewater of 1000 mg/L COD prepared by diluting molasses [4]. The feed was supplemented with micronutrients, NH4Cl and K_2HPO_4 as sources of N and P respectively [14]. The experimental work pertaining to reactor startup and development of granular sludge constitute the study of phase I. After the reactor startup, the amount of molasses was gradually decreased with concomitant increase in phenol. The synthetic phenolic wastewater was prepared by dissolving 420 mg/L of phenol in tap water used for feed. On day 48 the reactor was fed with 100% phenol based synthetic wastewater. In phase II the COD:P was varied from 300:1 to 300:0 at COD:N equal to 300:1. Also, HRT was reduced from 10 to 6 h. The feed P as well as HRT was always changed at pseudo steady state (PSS).

The reactor performance was assessed by monitoring several parameters. pH and COD were measured daily while microbial concentration in terms of volatile suspended solids (VSS) was measured once in two weeks, nitrogen and phosphorous were determined weekly. Biomass was digested to determine cell-N and P (%). Biogas from the reactor was collected in a gasholder filled with 10 % acidified solution. Methane content of the biogas measured thrice a week was normalized to standard temperature and pressure (STP).The values of CH_4 or CH_4-COD reported herein are at STP. All determinations were made as per procedure laid down in Standard Methods [15]. Specific methanogenic activity (SMA) of the sludge samples drawn once or twice in 15 d has been analyzed by using (i) acetate and (ii) benzoate as substrates in accordance with the procedure described by Kato et al. [16].

3. Results and discussion

The biomass in the reactor developed from molasses based synthetic wastewater was acclimatized with phenol-based synthetic wastewater of COD 1000 mg/L in 108 d. The acclimatized biomass yielded > 10 L/d of biogas and removed > 80 % COD. COD loading rates were adjusted by varying HRT from 10 h to 6 h after operating the reactor for 17 and 108 d respectively. Following this the reactor was continuously operated for nearly 1 year. The total duration of reactor operation was 349 d (Table 1). pH of the feed and effluent was 7.56±0.23 and 6.54±0.17 respectively. The reactor was regularly desludged (i) to maintain sludge bed height of 25–30% of the reactor height and (ii) to procure sludge for analysis and characterization. The regular desludging also helped in maintaining SLR in a narrow range of 0.4– 0.5 g COD/g VSS/d. The operational details along with the reactor performance of UASB reactor is presented through Fig. 1.

3.1. Reactor performance

The treatment of synthetic phenolic wastewater with varying concentration of P at fixed COD: N equal to 300:1 was undertaken in phase II. The reactor was operated at an OLR of 3.9–4.1 g COD/ L.d. The CH_4-COD and overall COD removal were 90±3% and between 95–99% respectively. The conversion of phenol to methane at COD:P of 300:0.1 was comparable with that of 300:1. Therefore, it indicates that the phosphorous input can be significantly reduced without compromising with the process efficiency.

After fixing up COD:N equal to 300:1, the amount of P was changed in stepwise manner to maintain COD:N:P from 300:1:1 to 300:1:0 (phase II) .The HRT maintained was 6 h. The COD:N:P up to 300:1:0.1 did not influence the COD removal efficiency and the biogas production. The ratio of CH_4-COD to COD fed was in the range of 0.8–0.95. However, on further lowering P in a stepwise manner from 300:1:0.1 to 300:1:0, the COD removal efficiency dropped from 95% to 30% and the biogas production reduced from 19.2 to 0.5 L/d. During phase II of the operation, phosphorous was again increased from zero to 0.33 mg/L to maintain feed COD:P of 300:0.1 and nitrogen was maintained at 3.3 mg/L, i.e. COD:N from 300:1. The

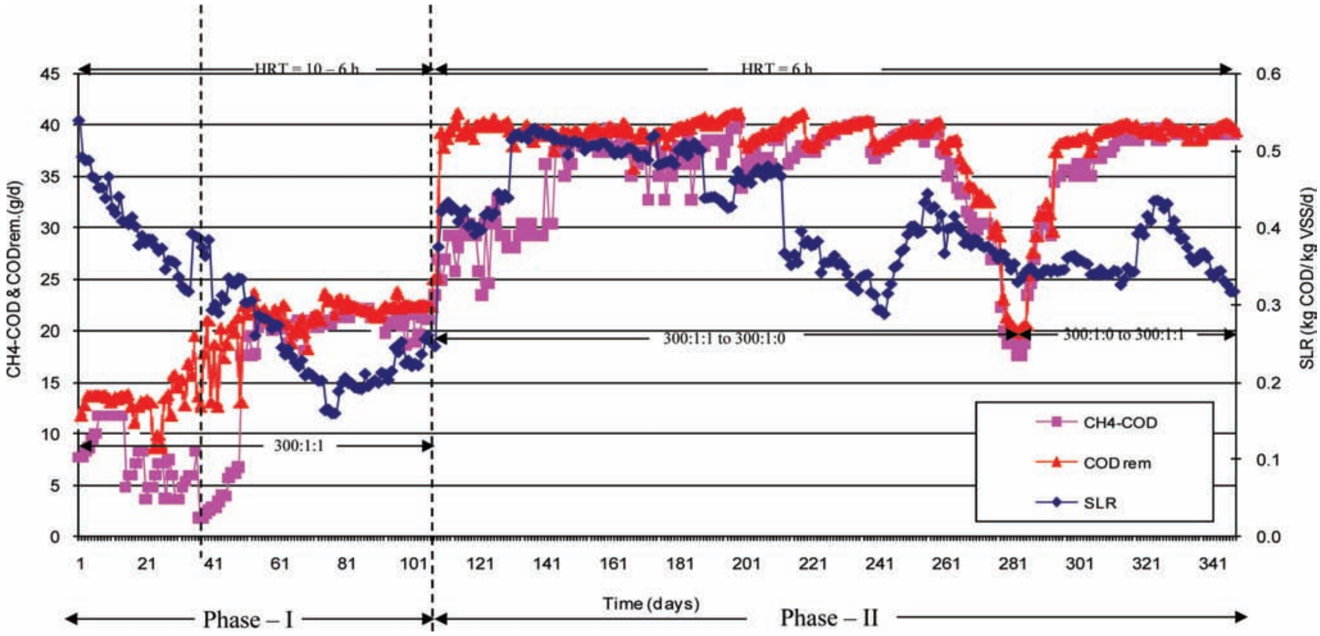

Fig. 1. Temporal variation of COD removed, CH_4-COD and SLR at different COD:P.

Table 1
Variation of UASB reactor operating parameters of the study

Phase	Days	Feed	COD:N:P	HRT (d)	OLR (g COD/L-d)	SLR (g COD/g VSS/d)
I*	1–16	100% molasses	300:1:1	10	2.4–2.8	0.24–0.34
I*	17–25	75% molasses 25% phenol	300:1:1	10	2.4–2.9	0.26–0.34
I*	26–39	50% molasses 50% phenol	300:1:1	10	2.1–2.6	0.22–0.34
I*	40–48	25% molasses 75% phenol	300:1:1	10	2.2–2.8	0.25–0.32
I*	49–75	100% phenol	300:1:1	10	2.4–2.6	0.20–0.33
II*	76–108	100% phenol	300:1:1	10	2.4–2.6	0.26–0.31
II*	109–176		300:1:0.75	6	4.0–4.1	0.42–0.51
II*	177–199		300:1:0.5	6	3.9–4.2	0.35–0.48
II*	200–219	100% phenol	300:1:0.25	6	3.9–4.0	0.32–0.38
II*	220–239		300:1:0.1	6	3.9–4.1	0.28–0.44
II*	240–259		300:1:00	6	3.9–4.1	0.33–0.42
II*	260–349		300:1:0.1	6	3.9–4.1	0.31–0.43

*COD of the feed = 1000 mg/L

optimum COD:N:P for synthetic phenolic wastewater thus has been estimated to be 300:1:0.1. These observations are in conformity with the findings of Britz et al. [13]. He reported optimum COD:N:P equal to 300:1.2:0.2 for the degradation of petrochemical wastewater in down flow fixed-bed anaerobic reactor. A few reports have emphasized effects of P limiting conditions on the performance. Fedorak and Hurdey [17] reported that omission of phosphorous caused methane formation to stop. Alphenaar [18] reported reduction in methanogenic activity in UASB reactor to 50% of the control due to phosphorous deficiency. Also Goodwin et al. [19] suggested deteorious effects of phosphate deficiency on performance of both acid-forming and methanogenic bacteria. Findings presented herein suggest that the concentration of P can also be limiting with N limiting concentration.

After having observed the reactor performance of ~90% at COD:P ≥ 300:0.1, the other parameters have not been discussed according to phase wise operation of the reactor. The observations have been broadly put into two categories; on the basis of COD:N:P values i.e. COD:N:P at which process efficiency is (i) ~90% (ii) less than 50%. Britz et al. [13] have conducted a study on petrochemical effluent and mentioned a very low quantity of phenol but author has conducted the study on pure synthetic phenolic wastewater.

3.2. COD mass balance

The fate of the substrate entering the reactor in terms of COD can be expressed by Eq. (1).

$$COD_{Inf.} = COD_{Eff.} + CH_4\text{-}COD_{Eff.\ Diss.} + CH_4\text{-}COD_{gas} + COD_{biomass} + COD \text{ for } SO_4 \text{ red} \quad (1)$$

All the terms are self explanatory. A large fraction of influent COD (~90%) is converted to CH_4-COD and ~1.5% of COD is used for sulphate reduction. Synthetic wastewater prepared from tap water contains 15–20 mg/L of sulphate. COD required for sulphate reduction is equivalent to 13–18 mg/L.

The methane COD dissolved in the effluent computed by taking Henry's constant and partial pressure of methane equal to 64 mg CH4-COD/L-atm and 0.75 atm respectively equals to 4.8% at organic loadings corresponding to HRT of 6 h. The remaining COD is the (i) COD of biomass computed from change in VSS or growth of biomass (g/d) and taking VSS COD equal to 1.42 g COD/g VSS and (ii) unaccounted COD. COD mass balance is given in Table 2. At COD:N:P of 300:1:0.1 the effluent COD ranged from 1 to 6% and methane–COD, varied from 88 to 95% of the influent COD. Perusal of data (Table 2) reveals that for a COD of 300 and P less than 0.1 the CH_4-COD is reduced with concomitant increase in the effluent COD. However, the fractionation of COD into other forms is not sensitive to P limiting conditions. The COD conversion efficiency (ratio of CH_4-COD to COD_{fed}) up to COD:N:P equal to 300:1:0.1 is comparable or more than the values reported in the literature, for example, 93% [6], 78–81% [7] and 72% [4].

3.3. Sludge growth and activity

The VSS concentration, a measure of microbial population in the reactor was analyzed weekly to ascertain the effect of P on sludge growth, sludge activity and sludge washout. In fact the reactor was regularly desludged to maintain sludge bed occupancy of 25–30% of the reactor height. This was done in accordance with the observations and computations made on short circuiting flow through the UASB reactor [20,21]. The data on VSS (sludge), effluent VSS and SMA is presented through histograms in Fig. 2. The results show a decreasing trend in the effluent VSS and increasing trend in sludge VSS with days of operation. Despite poor performance of the reactor in Phase IV, the effluent VSS concentration was between 40–60 mg/L. The results of sludge VSS are presented as an average value at each phase moreover the standard deviation as shown in Fig. 2 representing the variation of VSS in each phase. This is probably due to reduced transport of sludge from the bed to the sludge blanket in the reactor which in turn is due to the reduced gas production [22]. The increased VSS concentration shows the increase in sludge retention time (SRT) of the reactor thus confirming the stability of the reactor performance as a result of nutrient limiting conditions. In the case of domestic synthetic wastewater, however, washout of the flocculant sludge increases at poor performance of the reactor. The entire bed has been observed to be lifted [21].

The sludge growth was estimated from the concentration of VSS (i) in the sludge bed, (ii) in the effluent and (iii) the sludge removed from the reactor during desludging. Since the reactor performs satisfactorily up to COD:N:P = 300:1:0.1, it was thought logical to estimate kinetic parameters such as specific growth rates, $\mu_{0.1}$ and $\mu_{<0.1}$ at 300:0.1 ≥ COD:P ≤ 300:0.1. However, cumulative sludge growth per unit weight of sludge (g/g) over the entire duration of reactor operation is in conformity with single empirical correlation. The slope of the line is the specific growth rate (μ d^{-1}). The reduced concentration of P (phaseII) apparently does not affect specific growth

Table 2
COD mass balance: Fractionation of feed COD into different forms

S. No.	COD:N:P	COD_{Inf} (g/d)	CH_4 COD (%)	COD^{*}_{Eff} (%)	$COD^{**}_{Biomass}$ (%)	Error (%)
1	300:1:0.1	26–42	88–95	1–6	4.0–4.7	–6.2 to –0.5
2†	300:1:0.075–300:1:0	42	39–82	12–55	1.5–3.5	–3.8 to –6.2

* CH_4-COD in the effluent: 4.6, 1.6 and 0.4% of the influent COD of 42, 32 and 26 g/d
**Biomass COD = 1.42 g COD/g VSS
† P limiting

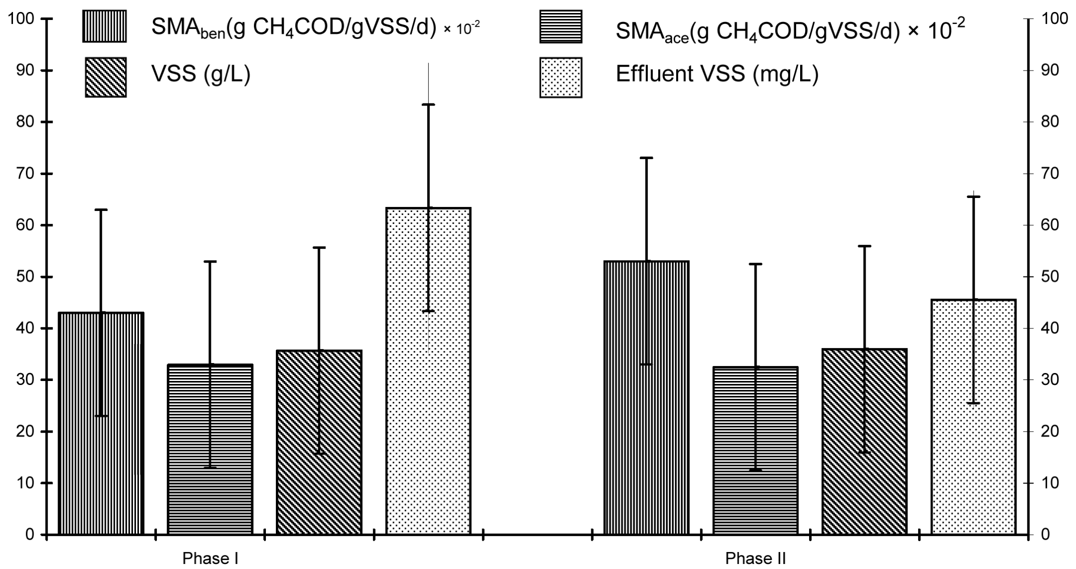

Fig. 2. SMA (acetate and benzoate), VSS (sludge) and effluent VSS at different COD:P.

rate. This is in contrast to the observations on reactor performance in terms of conversion of phenol COD to CH_4-COD. The deteriorated performance adversely affects sludge yield and not the μ. The biomass COD up to COD:N:P equal to 300:1:0.1 is in the range of 4–4.7%, where as under phosphorous limiting conditions the range is from 1.5 to 3.5% (Table 2).

The overall cell yield can be estimated from Eq. (2):

$$\mu = Yq - b \tag{2}$$

where μ = net specific biomass growth rate (d^{-1}), q = specific substrate utilization rate (kg COD/kgVSS d^{-1}), Y = biomass yield coefficient (kg VSS/kg COD), b = bacterial decay coefficient (d^{-1}).

In order to obtain the value of b, μ and q were estimated in each phase and plotted. The intercept corresponding to b (decay rate) is presented in Table 3. The decay rate does not appear to be sensitive to change in operational conditions. The cell yield, Y from $b = 6.5\times10^{-3}$/d has been estimated to be 0.037 and 0.015 kg VSS/kg COD at COD:N:P equal to 300:1:0.1 and 300:1:0 (P-limiting) respectively. The cell yield as shown in Table 3 reduced by more than 50% at reduced P concentration (phase II) is directly dependent on the performance (%) or substrate utilization rate (q). Fang et al. [23], Lay and Cheng [24] have reported cell yield ranging from 0.038 to 0.047 kg VSS/kg COD for the anaerobic treatment of synthetic phenolic wastewater with more than 80% efficiency.

The net granular sludge yield has been shown to be related to the phosphorous concentration of the influent [25]. According to Archer [10], there was no net cell growth with medium strength wastewater (COD = 250–430 mg/L) containing 1 μM phosphate (≡ 0.096 mg/L; i.e. COD: P ~300:0.1). It therefore suggests that up to a certain low concentration of phosphorous, e.g. 300:0.1 (COD:P), the net cell yield can be reduced without compromising with the process efficiency.

Table 3
Kinetic parameters of UASB reactor at different COD: P ratio

Phase	Range of COD:N:P	Y (kg VSS/kg COD)		Decay rate b^* 10^{-3} (d^{-1})	$q^\#$ (kg COD/kg VSS/d)
		Mass balance**	Computed***		
I	300:1:1	0.028–0.049	0.024–0.065	0.7–6.5	0.28–0.36
	300:1:0.75–300:1:0.1	0.03–0.032	0.033–0.037	0.2–6.5	0.42–0.56
II	300:1:0.075–300:1:0.05*	0.024–0..025	0.025–0.032	1.1–3.8	0.29–0.43
	300:1:0.025–300:1:0.0*	0.011–0.017	0.015–0.017	0.6–2.5	0.16–0.44

* P limiting **from biomass COD given in Table 2
***Sludge yield from the plot of μ vs. q, ($\mu = Yq - b$)
#q = SLR × efficiency = (kg COD_{fed}/kg VSS-d) × (kg CH_4-COD/COD_{fed})

The kinetic parameters μ and b do not appear to be sensitive to P in the feed. The sludge activity (SMA), i.e. the ability of biomass to carry out bioconversion to CH_4, might be responsible for deteriorated reactor performance at COD:P < 300:0.1. Therefore, SMA was assessed at F/M of 0.75 by the procedure laid down by Kato et al. [16]. In the case of phenol degradation the conversion of phenol to benzoate has been shown to be rate liming step [26]. Therefore SMA was determined by taking benzoate as well as acetate as substrates. The results are shown in Fig. 2.

The SMA of granular sludge using benzoate and acetate was found to vary from 0.37±0.10 to 0.49±0.14 and 0.30±0.11 to 0.37±0.13 g CH_4-COD/g VSS d^{-1} respectively in all the phases (phase I–II). The activity of the sludge grown in a feed supplemented with COD:N:P of 300:1:0.1 compared well with the values reported in literature [3,22,27–29]. SMA decreased by more than 50% during the operation period (from 0.43 to 0.15 g CH_4-COD/g VSS d^{-1}) at P limiting conditions. The author has also analyzed for the sludge kinetic parameters such as biomass yield coefficient (kg VSS/kg COD), specific methanogenic activity (SMA) of granular sludge at each step. The author has conducted the study in a upflow sludge blanket reactor as compared to fixed film bed reactor.

3.4. Phosphorous mass balance

The fate of varying amount of P (0.003–0.14 g P/d) added can be schematically presented as Fig. 3.

The influent phosphorous is fractioned into cell-P and phosphate. The distribution into effluent-P, cell-P at different COD:P values is tabulated in Table 4. The change in feed P is reflected proportionately in the effluent P. The stoichiometric nitrogen requirement for growing cells has been reported to range from 6 to 12%. The anaerobic microbial cells ($C_5H_7O_3N$) contain 11% N and 2.2% P; the experimental determinations are close to the cell formulation reported by Rittmann and McCarty [30].

Considering the cell yield of 3.5%, ~90% process efficiency, the stoichiometric requirement, a COD:N:P ratio of 300:1.67:0.3 is recommended, though the performance is satisfactory even at COD:N:P of 300:1:0.1. The additional nitrogen and phosphorous might be coming from cell-lyses or cell decay and/or from phosphorous precipitates. Decay of cells is expected to release ~11% of nitrogen and ~2.2% phosphorous. A low decay rate can perhaps further reduce the nutrients in the effluent. Alternatively, at COD:N:P equal to 300:1:0.1 cell yield is reduced. The cell yield corresponding to COD:N:P of 300:1:0.1 has been computed to be ~1%. This however is not in conformity with the experimental data giving average cell yield of 3.4%. Perusal of the data in Table 4 indicates the fact

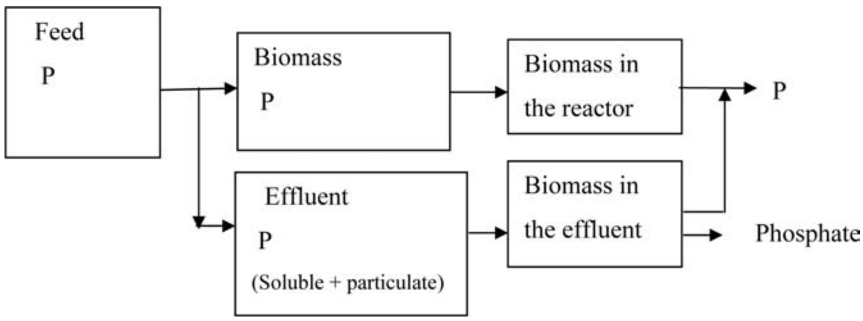

Fig. 3. Phosphorous mass balance in the anaerobic treatment system.

Table 4
Nitrogen and phosphorous mass balance at different COD:P ratios

COD:N:P	Influent (g/d)		N and P effluent (% of influent) (soluble)		N and P in cell VSS (% of influent)* Reactor		Effluent		N and P cells (%) (experimental)		Unaccounted (% of influent)	
	N	P	N	P	N	P	N	P	N	P	N	P
300:1:0.1	0.14–0.90	0.14	72–79	72–80	10–13	4.5–6	10–13	11	12–14	2.2	1–2	5–8
300:1:0.075–300:1:0†	0.14	0.003–0.011	30–40	0–49	12–16	14–18	50–52	43–86	9–11	0.6–1.4	1–2	0–8

An organic load of 42 g/d, cell yield of 3.5% and cell –P of 2.2%, the requirement of P is 0.033 g/d
*Total VSS cells produced and effluent VSS (g/d)
† P limiting

that percent N and P in cells at COD:P < 300:0.1 do not change instead cell yield reduces from 0.034 to 0.015. The present study conforms to the findings of Alphenaar [18] on phosphate uptake, even at a concentration of 0.1 mg PO_4-P/L, is beneficial.

From the above findings it is inferred that at COD:N:P ratio of 300:1:0.1 ~90% of phenol can be transformed to methane, P in effluent can be significantly reduced making the process cost effective and eco friendly.

4. Conclusions

More than 90% of phenol can be converted into CH_4 at HRT of 6 h and COD:N:P of 300:1:0.1. The effluent phosphorous is ~80% of the influent P. The P added is less than the stoichiometric requirement of 300:1.8:0.36 by the growing biomass. The additional amount of the phosphorous perhaps is released from cell decay. It therefore reduces the net cell yield without compromising with the process efficiency. The average cell yield was 3.5%. Phosphorous in the cells varied from 0.6 to 2.4 %. The reduced sludge activity (SMA) is perhaps responsible for reduced efficiency at COD:P < 300:0.1.

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Abbreviations

APHA	—	American Public Health Association
CH_4-COD	—	Methane COD, mg
COD	—	Chemical oxygen demand, mg/L
F/M	—	Food to microorganism ratio
HRT	—	Hydraulic retention time, h
IS	—	Indian standard
N and P	—	Nitrogen and phosphorous, mg/L
PSS	—	Pseudo steady state
SLR	—	Sludge loading rate, g-COD/g VSS/d
SMA	—	Specific methanogenic activity, g-CH_4-COD/g VSS/d
STP	—	Standard temperature pressure
UASB	—	Upflow anaerobic sludge blanket
VSS	—	Volatile suspended solids, mg/L

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