

ASSESSMENT OF TOXICITY AND ANAEROBIC DEGRADATION OF ANTI-OSMOTIC DRUG BASED PHARMACEUTICAL EFFLUENT IN AN UPFLOW ANAEROBIC FLUIDIZED BED SYSTEM

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ABSTRACT

During the production of cephadrine (a main constituent of anti-osmotic drug) a large quantity of concentrated effluent was produced. The main polluting compounds in this effluent are osmotic drug, acetic acid and ammonia. The main objectives of the study were to assess the toxicity in terms of specific methanogenic activity on anaerobic degradation of cephadrine with Volatile Fatty Acid (VFA) as co-substrate in batch systems using adapted and non-adapted cultures and to study the effect of bioaugmentation with adapted and non-adapted cultures on anaerobic treatment of anti-osmotic drug based pharmaceutical effluent in continuous mode using a fluidised bed reactor. The toxicity assessment was measured in terms of specific methanogenic activity ($\text{ml CH}_4 \text{ g}^{-1} \text{ VSS d}^{-1}$), which was found to decrease with increase in cephadrine concentration and attained a maximum at 100 mg l^{-1} . The COD reduction (%) in continuous mode reached maximum of 88.5 at a Hydraulic Retention Time (HRT) of 12 h using bioaugmentation through periodic addition of 14 to 20 g l^{-1} of biomass (acclimated cells) every 2 days from an off-line enricher-reactor.

KEY WORDS: Toxicity, Bioaugmentation, Enricher reactor, Anti-osmotic pharmaceutical effluent, Anaerobic, Fluidized bed reactor.

INTRODUCTION

Pharmaceutical industries, mainly anti-osmotic and synthetic drug based industries suffer from inadequate effluent treatment due to the presence of toxic substances and complex biomass formed during the secondary treatment process. Aromatic compounds such as phenols and methylated phenyls are commonly encountered pollutants, originating typically from modern industrial activities. Because of their bacterial toxicity and

recalcitrance, when present in wastewaters, they play a key role in decreasing COD removal rates in treatment systems. As a result of their toxicity and despite their usually relatively low concentration, their removal from contaminated effluents is as important as removal of the COD fraction. Bioaugmentation refers to a method of maintaining sufficient biomass when adequate carbon substrates and nutrients are unavailable in the effluent. Bioaugmentation using purchased cells

(Babcock *et al.*, 1992) could not find evidence of successful operation with respect to anaerobic degradation of industrial effluents. The present work adapts the methodology suggested by earlier investigators (Cardinal and Stenstrom, 1991) using suspended cultures, where an off-line reactor, called an enricher- reactor, was used on-site to grow cells specifically prepared for use in the main (anaerobic fluidized bed) reactor. The enricher-reactor operates separately and under different conditions from the main reactor. The substrates being fed to the enricher-reactor can be the same as in the main reactor designed to induce the desired operation in the enricher-reactor (Babcock and Stenstrom, 1993; Babcock *et al.*, 1993). The biomass was produced within the enricher- reactor where optimum growth conditions were maintained and growth- supporting substrates were added. The cells produced in the enricher-reactor were viable in the main reactor for several generations, which enhanced the biomass concentration. The acclimated cells could be either continuously or intermittently transferred to the main reactor, and an adequate increase in biomass can be maintained. The increased biomass can be maintained to increase treatment efficiency, or less frequently augmented to prevent washout or maintain the desired organism condition of acclimation to a specific substrate or wastewater.

Anaerobic degradation of different constituents of antibiotic and synthetic drug based effluents were recently been carried out, using methanogenic organisms (Bisailon *et al.*, 1993; Haggblom *et al.*, 1990; Roberts and Fedorak, 1987; Roberts *et al.*, 1990), sulphate reducing organisms (Haggblom *et al.*, 1990; Ramanand and Suflita, 1991, 1993; Suflita *et al.*, 1989), nitrate reducing organisms (Bossert *et al.*, 1986; Tschsch and Fuchs, 1987) and iron reducing organisms (Lovely and Lonergan, 1990).

In this investigation, the assessment of toxicity was carried out in terms of specific methanogenic activity on anaerobic degradation of cephradine with Volatile Fatty Acid (VFA) as co-substrate in batch systems using adapted and non-adapted cultures. The effect of bioaugmentation was studied on anaerobic treatment of anti-osmotic drug based pharmaceutical effluent in continuous mode with adapted and non-adapted cultures using a fluidised bed reactor.

MATERIALS AND METHODS

Industrial Effluent

The pharmaceutical effluent (real effluent) was procured from M/s Orchid Pharmaceutical Private Ltd, Chennai (India). The effluent was analyzed as per the Standard Method (APHA, 1995) and the characteristics are shown in Table 1.

Carrier Material

Activated carbon particles of average size 700 μm and density 1500 kg m^{-3} were used as carrier material with static bed porosity of 0.38. The bed material of 2 kg of carbon was loaded in the reactor corresponding to a static bed height to diameter (H/D) ratio of 1.5.

Seed and Inoculum

Culture A (adapted culture) is a mixed sludge obtained from the anaerobic treatment plant (supernatant of anaerobic digester) and a secondary sludge from the anti-osmotic effluent treatment plant. Culture B (non-adapted) was obtained from an anaerobic digester treating municipal sewage located at Kodangaiyur, in Chennai, India.

Medium Composition

The medium contained 3 ml l^{-1} of a nutrient solution composed of (g l^{-1}): KH_2PO_4 (5.0), NH_4Cl (27), MgSO_4 (1.2), $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$ (0.12). It contained also 0.3 ml l^{-1} of a micronutrients solution and, where required, volatile fatty acids as substrates mixture. The mixture of VFA was composed of (final concentrations, g l^{-1}) acetate (2) propionate (0.5) and butyrate (0.5). The solution of micronutrients was composed of (mg l^{-1}): H_3BO_3 (50), $\text{FeCl}_3 \cdot 4\text{H}_2\text{O}$ (2000), ZnCl_2 (50), $\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$ (500), $\text{CuCl}_2 \cdot \text{H}_2\text{O}$ (30), $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$ (50), $\text{AlCl}_3 \cdot 6\text{H}_2\text{O}$ (90), $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$ (2000), $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ (50), $\text{Na}_2\text{SeO}_3 \cdot 5\text{H}_2\text{O}$ (100), EDTA (1000), HCl (36 %) (1 ml l^{-1}). The water used this preparation is double distilled water. The water used for all other analyses is distilled water only.

Analytical methods

Ecotoxicity of the medium was checked using a Microtox model 500. Ecotoxicity evaluation is based on the measurement of inhibition of light emission by the bacterium *Photobacterium phosphoreum* in the presence of toxic compounds in the medium. The effluent to be tested was diluted and mixed with dilution water containing *Photobacterium phospho-*

Table 1. Characteristics of pharmaceutical industrial effluent

Sl.No.	Characteristics	Value
1.	Flow rate (m ³ d ⁻¹)	350
2.	pH	3 - 4
3.	Temperature (°C)	25 - 45
4.	COD (mg l ⁻¹)	12000-15000
5.	BOD5 (mg l ⁻¹)	2000
6.	Cephadrine (mg l ⁻¹)	200-315
7.	Total Suspended Solids (mg l ⁻¹)	6000
8.	Total Dissolved Solids (mg l ⁻¹)	11000-18500
9.	Total Volatile Solids (mg l ⁻¹)	7500
10.	NH ₃ (mg l ⁻¹)	15 - 40
11.	Surfactants	2 - 20

reum and the light output was measured after 5 and 15 minutes (Microbics Corp., 1988). Light output of the bacteria was compared between the control (0 % waste, 100 % dilution water) and the dilutions. If a reduction in light output resulted, the value of EC50 (effective concentration - the concentration responsible for a 50% reduction in an observed phenomenon, which, in this case, was light output) was calculated (Arbuckle and Alleman, 1992).

Chemical oxygen demand of effluent was measured as per Standard Method (APHA, 1995). Volatile fatty acids (VFA) and the composition of the biogas were analysed by a gas chromatography (GC) on a Hewlett-Packard, model 5890 A, equipped with a flame ionization detector (FID). Cephadrine, 7-amino-3-deacetoxycephalosporanic acid (7-ADCA) and penicillic acid were determined as described in British Pharmacopoeia, 1980 using an Infrared Spectrometer (Model M- 500). The temperature during all the measurements was found to vary from 28 - 33°C.

Batch and toxicity assays

Batch activity and toxicity assays were determined in 2.5 l with 2 l working volume. Biodegradability assays, 1000 ml medium was used. Biodegradability assays were carried out to assess any residual biomass activity of the medium in terms of methane production. Methane production was measured by displacement of an aqueous solution. For toxicity assays, various concentrations of cephradine were added to the medium and measuring the methane gas produced assessed specific methanogenic activity. The specific methanogenic activity is represented in terms of the volume of methane gas produced (ml) per gram of biomass [g VSS (Volatile Suspended Solids)] per day

(ml CH₄ g⁻¹ VSS d⁻¹). Duplicate assays and several controls were also used. Controls included autoclaved inoculated flasks to check for any abiotic removal and flasks containing only distilled water instead medium but without carbon sources to estimate any residual biomass activity.

Experimental Set-up

A schematic diagram of the experimental set up is shown in Fig. 1. An anaerobic fluidized bed reactor consisted of a perspex column (4) made by joining two sections of different lengths of 750 mm internal diameter measuring to a height of 1200 mm of 5 mm thickness. Over this, an upper section of 150 mm diameter and 300 mm height was mounted to prevent carry over of suspended particles into the effluent and also to serve as a gas holder (5). The working volume of the reactor was 10 l.

Start-up of the Reactor

The reactor was loaded with activated carbon and supernatant of digested sludge (Culture A), procured from Municipal Sewage Treatment Plant of Kodangaiyur, Chennai India. Culture A was used for start-up experiments under complete recycling conditions. After this, the sludge and the diluted pharmaceutical effluent (Culture B) were mixed to get a COD concentration of 250 mg l⁻¹. The start-up experiments were also carried out with Culture B. The reactor was operated under total recycling conditions for one week before switching it over to continuous operation. The bed expansion was maintained at 25 - 30 % during the entire start-up period by maintaining the fluidization velocity at 25 m hr⁻¹ [1.9U_{mf} (1.9x fluidization velocity (U_{mf}))].

Enricher-Reactor Operation

The enricher-reactor operates as a complete recycle reactor and directly connected to the main reactor as shown in Fig. 1. Initially 7.8 l of Culture A (adapted Culture) and nutrient solution of 1.8 l were mixed and maintained in a complete recycle condition for 7 days. A mass of 14 - 20 g l⁻¹ of volatile suspended solid (VSS) was transferred from this enricher-reactor to the main reactor every 48 hrs. The cell mass (VSS) in the enricher-reactor was kept constant by replenishment of Culture A and nutrient solution (in the same initial volumetric proportion). This is similar to the experimental study (Babcock *et al.*, 1992) where a cell mass (VSS) of 14 - 20 g l⁻¹ was added every 2 days during start-up of the reactor. Adapted and non-adapted cultures were used for start-up to assess the effect of bioaugmentation. In the start-

up experiments conducted using multiple inoculum, the volume (%) of culture A was varied from 0 to 100 % with volume of feed (pharmaceutical effluent) from 100 to 0 %. The volatile fatty acid (acetic acid) was varied from 0 to 2000 mg l⁻¹. The total COD (Pharmaceutical effluent and acetic acid together) was found to vary from 250 to 4000 mg l⁻¹. A mass of acclimated culture (VSS) of 14 g l⁻¹ was transferred from enricher-reactor to the main reactor every 48 hrs with an intention to bioaugment the reactor. In the start-up experiments conducted using single inoculum, the volume (%) of culture B (viz. supernatant of anaerobic digester) was varied from 0 to 100 % with volume of feed (pharmaceutical effluent) from 100 to 0 %. The COD of the effluent was varied from 245 to 4000 mg l⁻¹. The influent and effluent COD concentrations and biogas yield were monitored daily.

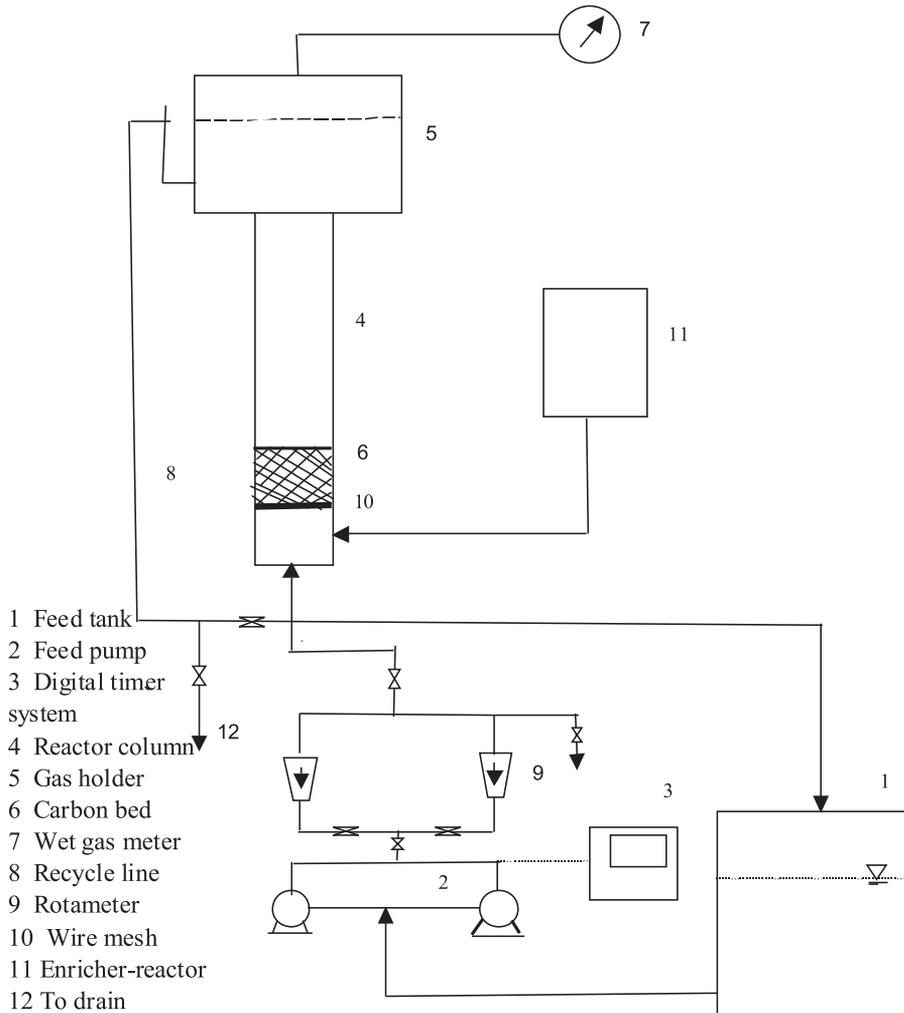


Figure 1. Experimental set-up of Anaerobic fluidized bed reactor.

Table 2. The toxicity of cephradine in batch system Microtox testing

Cephradine concentration (mg l ⁻¹)	Microtox, EC50			
	Days			
	5	15	27	45
50	N ^a	N	N	N
100	57	44	N	N
200	57	D ^b	D	D
315	57	D	D	D

N^a = no toxicity detected

D^b = Greater than 100 %

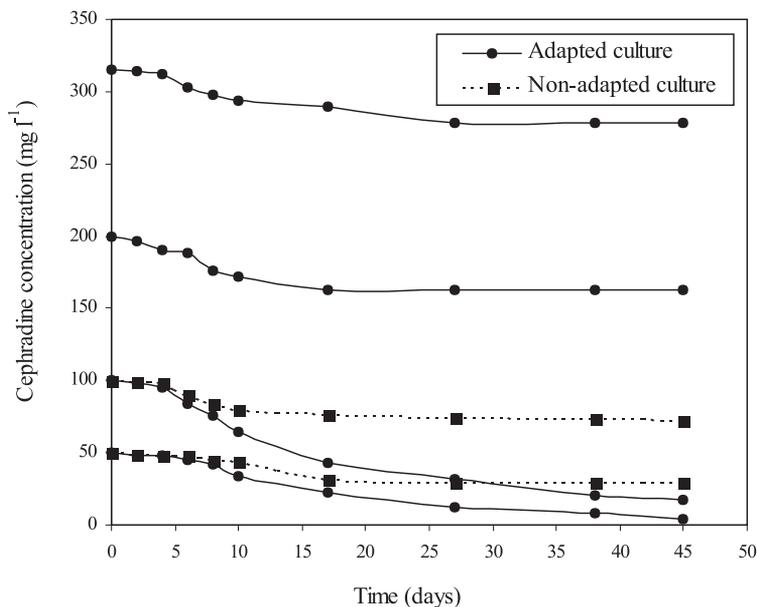


Figure 2. Variation of cephradine concentration in batch system with adapted and non-adapted cultures

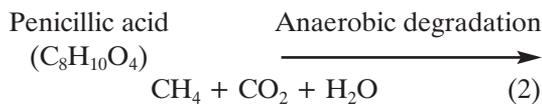
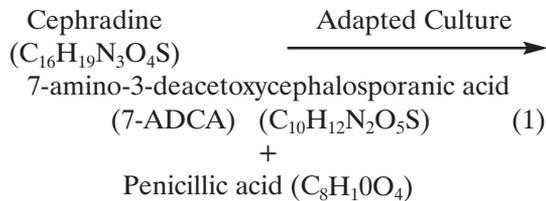
RESULTS AND DISCUSSION

Assessment of toxicity on anaerobic degradation of cephradine

The toxicity due to anaerobic degradation of cephradine was assessed in the presence of Volatile Fatty Acid (VFA) as co-substrate in batch system using adapted and non-adapted cultures. The experiment was carried out in 2.5 l bottles containing different concentrations of cephradine, 1000 ml medium and VFA inoculated with adapted and non-adapted cultures. The cephradine concentrations chosen for the experimental study included 50, 100, 200 and 315 mg l⁻¹. The adapted culture (Culture A) was able to degrade cephradine at all the concentrations with higher rate degradation at 50 and 100 mg l⁻¹ com-

pared to 200 and 315 mg l⁻¹. The non-adapted culture (Culture B) could degrade cephradine only at 50 and 100 mg l⁻¹ concentrations. The variation of cephradine in batch system with adapted and non-adapted cultures were shown in Figure 2. It was inferred from the Figure 2 that the cephradine concentrations of 200 and 315 mg l⁻¹ was found to be toxic to the adapted culture and the degradation did not take place after 27th day. The toxicity values were evaluated in terms of Microtox testing using *Photobacterium phosphoreum* and the results were represented in the form of EC50 (effective concentration - the concentration responsible for a 50 % reduction in the light output by *Photobacterium phosphoreum*). The samples of cephradine tested at different times were presented in the form of EC50 in Table 2.

The term degradation of cephradine is referred to the degradation to its intermediates namely, 7-amino-3-deacetoxycephalosporanic acid (7-ADCA) and penicillic acid in the presence of in the presence of cultures. The degradation of cephradine proceeds as per equation 1 and 2.



The intermediates of cephradine viz., 7-amino-3-deacetoxycephalosporanic acid (7-ADCA) and penicillic acid formed during the batch study under different concentrations (cephradine: 50, 100 and 200 mg l⁻¹) using adapted and non-adapted cultures were shown in Figure 3. It was observed that the intermediates were well accumulated at concentrations of 50 and 100 mg l⁻¹ in both adapted and non-adapted culture systems. The maximum concentrations of (7-ADCA) and penicillic acid were respectively found to be 13 and 12 m l⁻¹ at 100 mg l⁻¹ of cephradine. The non-adapted cultures developed resistance and did not degrade cephradine beyond 100 mg l⁻¹. The adapted culture degraded cephradine to only 7-ADCA even at 315 mg l⁻¹ concentra-

tion and did not produce penicillic acid and hence no yield of methane was observed. Hence, the specific methanogenic activity was found to cease at 315 mg l⁻¹ concentration. The specific methanogenic activity is represented in terms of the volume of methane gas produced (ml) per gram of biomass [g VSS (Volatile Suspended Solids)] per day (ml CH₄ g⁻¹ VSS d⁻¹). The specific methanogenic activity measured for different concentrations of cephradine (50, 100 and 200 mg l⁻¹) using adapted and non-adapted cultures were shown in Figure 4. It was observed from the figure that the specific methanogenic activity was found to increase with increase in cephradine concentration and attained a maximum of 375 ml CH₄ g⁻¹ VSS d⁻¹ at 100 mg l⁻¹ using adapted culture. The activity was reduced at 200 mg l⁻¹ concentration and completely ceased at 315 mg l⁻¹ concentration and hence no curve was shown for 315 mg l⁻¹ concentration.

Start-up Experiments

Effect of Culture Composition

As it can be seen from Figures 5 and 6, the anaerobic fluidized bed reactor reached a steady state at the end of 147 days with respect to maximum COD removal (63 %) and biogas yield (18 l d⁻¹) using non-adapted culture and at the end of 107 days with a maximum COD removal (70 %) and biogas yield (22.5 l d⁻¹) using adapted culture. This clearly showed that the start-up of the reactor carried out using adapted culture attained a faster steady state

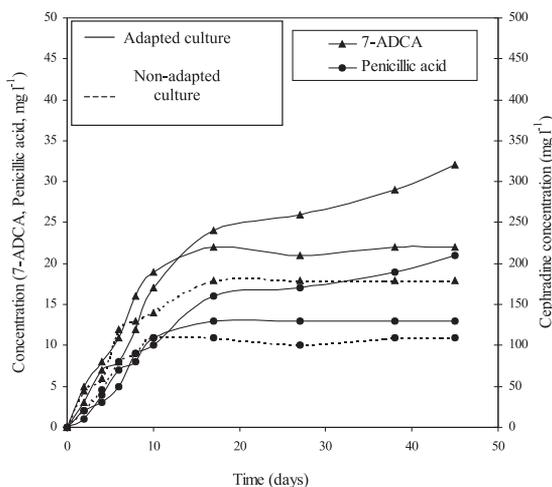


Figure 3. Biodegradation of cephradine in batch system with adapted and non-adapted cultures

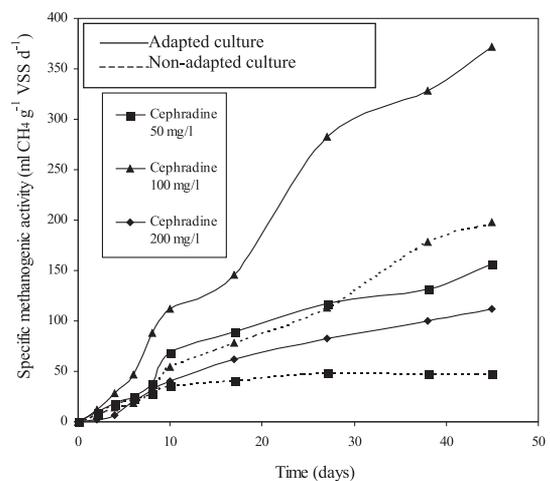


Figure 4. Specific methanogenic activity for cephradine degradation in batch systems.

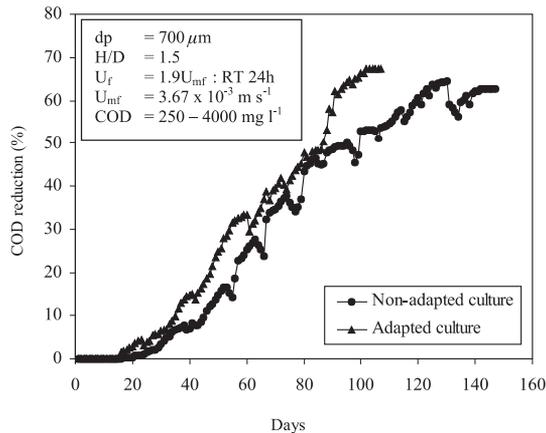


Figure 5. Variation of COD reduction (%) during start-up of the reactor

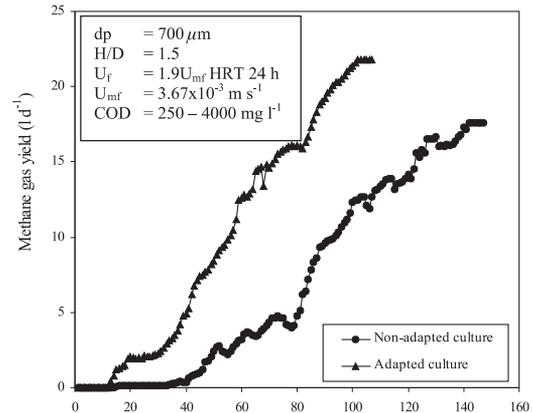


Figure 6. Variation of the methane gas yield during start-up of the reactor

with a higher COD removal (%) compared to the reactor started using non-adapted culture.

The study of Kennes *et al.*, (1997) reported a start-up period of 187 days using non-adapted culture and 122 days using adapted culture for synthetic para-cresol based effluent.

Continuous Experiments

Bioaugmentation

The term "bioaugmentation" refers to a method of maintaining sufficient biomass when adequate carbon substrates and nutrients are unavailable in the effluent (Babcock *et al.*, 1992). The optimum value of COD/VSS ratio was decided based on the batch results of cephradine and VFA studied for toxicity assessment. The COD/VSS ratio studied for the bioaugmentation study included 0.15, 0.2, 0.3 and 0.35. The maximum concentration of the effluent was varied from 2000 - 4000 mg l⁻¹. The effect of biomass addition in terms of COD/VSS ratio on COD removal (%) is presented in Table 3 and in Figures 7 and 8 and the corresponding methane gas yields in Figures 9 and 10 using adapted and non-adapted cultures.

The decrease in the trend of the all the curves at 48 hours showed the addition of biomass at every 48 hrs in the main reactor from enricher-reactor. The COD reduction (%) was found to be higher in adapted culture with relatively low retention time as compared to non-adapted culture. The COD reduction (%) and methane gas yield were found to be 88.5 and 1.9 l d⁻¹ respectively at an

HRT of 12 hr using adapted culture with a COD/VSS ratio of 0.15 for a maximum COD concentration of 4000 mg l⁻¹. For the same COD concentration, in case of non-adapted culture, COD reduction (%) and methane gas yield were observed to be 78.5 and 1.5 l d⁻¹ at an HRT of 12 hr for a COD/VSS ratio of 0.15. This was in general agreement with the experimental study of Kyoung *et al.*, (1997) for the degradation of 1-naphthylamine and Babcock *et al.*, (1993) for the degradation of phenolic compounds (COD reduction (%) 76.2 using adapted culture. In the study of Babcock *et al.* (1993), the maximum COD reduction (%) and methane gas yield were respectively found to be 82.3 and 1.91 l d⁻¹ for non-adapted culture (for a COD/VSS ratio of 0.15) and 91.2 and 5.62 l d⁻¹ for adapted culture or a COD concentration of 4000 mg l⁻¹. The results showed that higher removal efficiency could be achieved at relatively higher concentrations in the presence of acetic acid as a co-substrate.

CONCLUSION

The assessment of toxicity was carried out in terms of specific methanogenic activity on anaerobic degradation of cephradine with Volatile Fatty Acid (VFA) as co-substrate in batch systems using adapted and non-adapted cultures. The effect of bioaugmentation was studied on anaerobic treatment of anti-osmotic drug based pharmaceutical effluent in continuous mode with adapted and non-adapted cultures using a fluidised bed reactor.

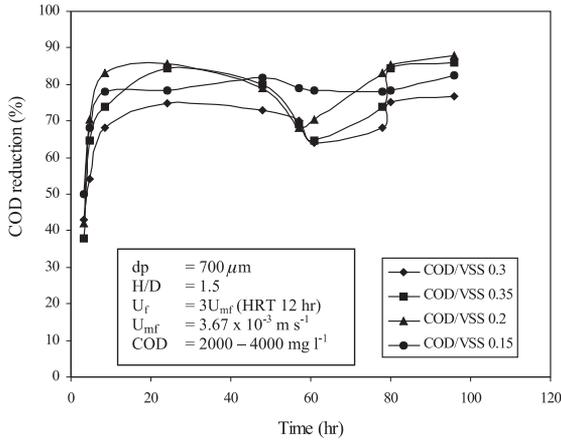


Figure 7. Effect of bioaugmentation on COD reduction (%) using adapted culture.

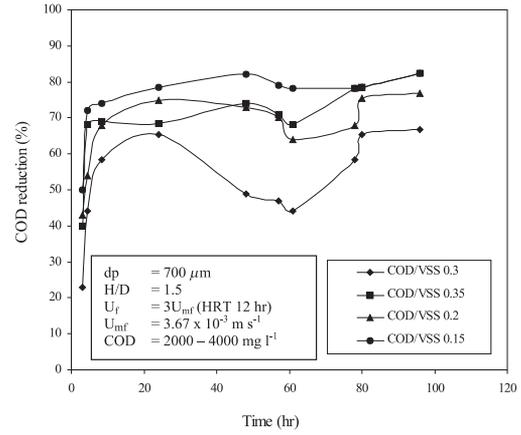


Figure 8. Effect of bioaugmentation on COD reduction (%) using non-adapted culture.

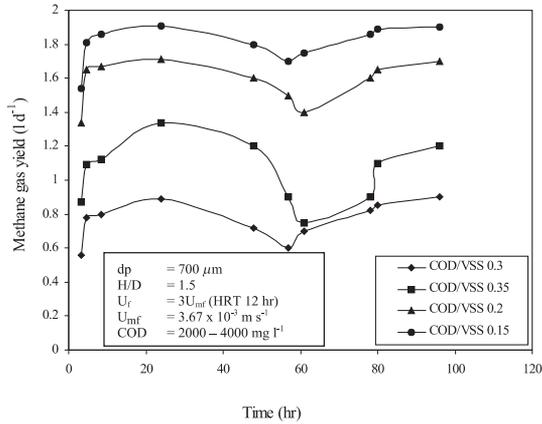


Figure 9. Effect of bioaugmentation on methane gas yield using adapted culture.

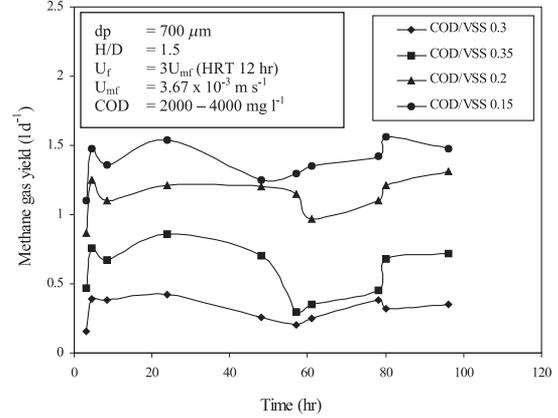


Figure 10. Effect of bioaugmentation on methane gas yield using non-adapted culture

Table 3. The effect of HRT on biomass growth using adapted and non-adapted cultures

Sl.No	HRT (hr)	U_f^a	Initial pharmaceutical influent concentration COD (mg l ⁻¹)	Biomass added (mgVSS l ⁻¹)	(COD/VSS) ratio	Maximum COD removal (%)	
						Adapted culture	Non-adapted culture
1	3.2	$5.8U_{mf}^b$	2000	13332	0.15	70	80.3
2	4.6	$4.8U_{mf}$	2500	12500	0.2	74	83.4
3	8.4	$3.8U_{mf}$	3200	10665	0.3	76.2	86.2
4	11.6	$3.1U_{mf}$	4000	11426	0.35	78.5	88.5
5	24	$1.5U_{mf}$	2000	13332	0.15	83.2	91.2

^a U_f = Fluidization velocity

^b U_{mf} = Minimum fluidization velocity

The study has demonstrated that the use of off-line enricher-reactor to continuously inoculate the continuous flow reactor, overcomes the difficulty of growing adequate biomass on inhibitory or toxic substances or waste with too little carbon and nutrients to sustain a viable biomass, or other conditions that may not be conclusive to treatment. The results of the study conclusively demonstrated that bioaugmentation improves removal efficiency and reactor stability. The toxicity assessment was measured in terms of specific methanogenic activity ($\text{ml CH}_4 \text{ g}^{-1} \text{ VSS d}^{-1}$), which was found to decrease with increase in cephradine concentration and attained a maxi-

mum at 100 mg l^{-1} . The COD reduction (%) in continuous mode reached maximum of 88.5 at a Hydraulic Retention Time (HRT) of 12 h using bioaugmentation through periodic addition of 14 to 20 g l^{-1} of biomass (acclimated cells) every 2 days from an off-line enricher-reactor.

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