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Kinetic evaluation and process performance of a fixed film bioreactor removing phthalic acid and dimethyl phthalate

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ABSTRACT

Phthalate esters are toxic organic contaminants which can enter into the environment through various industrial processes. In this study, a 6-liter fixed film bioreactor was used to examine biodegradation of phthalic acid (PA) and dimethyl phthalate (DMP) in synthetic wastewater. Effect on the process of two operating factors, namely hydraulic retention time (HRT) (at four levels ranging between 6 and 48 h) and initial phthalate concentrations (at six levels ranging from 10 mg to 500 mg/l), was investigated. The process was stable at all operating conditions, except for the condition with influent PA and DMP of 500 mg/l and HRT of 6 h. More than 95% removal efficiency was achieved for the conditions with HRT longer than 10 h. Remarkable amount of DMP (398 mg/kg of sludge) was adsorbed on the biomass due to its higher hydrophobicity compared to PA (171 mg/kg). The kinetic parameters (μ_m, K_s, Y and K_d) were determined and compared for both substrates, PA and DMP.

1. Introduction

Phthalates are an important class of chemicals manufactured for use, primarily, as plasticisers in polyvinyl resin, cellulosic and polyurethane polymers for manufacturing building materials, home furnishings, transportation, clothing, and for packaging of food and medical products [1]. Dimethyl phthalate (DMP) is typically used in cellulose-ester based plastics, such as cellulose acetate and butyrate, which are esters of 1,2-benzenedicarboxylic acid sharing a common structure made up of a benzene ring with two side chains. Phthalates with lower molecular weight are toxic to aquatic organisms (e.g. DMP, DEP and DBP) [2,3]. They are also used as additives in paints, adhesives, cardboard, lubricants and fragrances [4]. Phthalates have also been observed to have disrupting properties on the endocrine system [5].

Release of phthalate esters into the environment during manufacturing processes and by their wide use and disposal has caused serious concerns, since some of them are suspected to be mutagens, hepatotoxic agents and carcinogens [2,6]. Phthalate and its esters and degradation intermediates are suspected to cause cancer and kidney damage and, as a result, the US Environmental Protection Agency has added this class of chemicals to the list of priority pollutants [7]. If phthalates are not removed from sewage at a sewage treatment plant (STP), they may have toxic or endocrine disrupting effects on aquatic species in the receiving water bodies [3].

Since the rates of photolysis and chemical hydrolysis of such compounds are very slow, metabolic breakdown by microorganisms is considered to be one of the major routes for the environmental degradation of phthalate esters [8]. Several types of microorganisms were found to degrade phthalate esters including aerobic and anaerobic species [9]. A few studies have been reported on the biodegradability of DMP by bacteria [10], fungi [11], algae [12], activated sludge cultures [2,13,14] and bioreactors [15].

Phthalate esters are metabolized by both aerobic and anaerobic biological treatment methods [13,16–26]. The most common pathway for aerobic degradation of phthalate is through the protocatechuate pathway, followed by ring cleavage and complete mineralization to carbon dioxide and water [16].

These compounds have not shown any adverse effect on activated sludge systems and concentrations up to 900 mgL^{-1} could be tolerated by the system [27]. In a wastewater plant of a coke factory, PA esters, having a short hydrocarbon chain, could be removed by an activated sludge system with hydraulic retention time (HRT)

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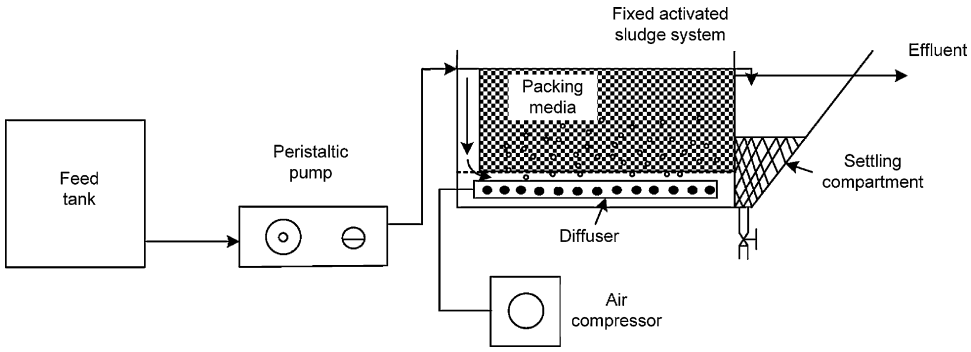

Fig. 1. Schematic diagram of the experimental rig.

from 7 to 90 d, while the removal efficiency of acid esters, having long hydrocarbon chain, is much lower at the same HRT [13].

The results obtained from a pilot scale study using activated sludge showed removal efficiency of 79–97% for PA esters with inlet amount of 100 $\mu g L^{-1}$ [28]. In another experiment, under the same conditions, the removal efficiency obtained was 71–91%. In plastic and paint industries the concentration of PA esters has been reported to be about 10–100 $mg L^{-1}$ [29].

The applicability of a fixed activated sludge system consisting of an aeration tank with a packing bed and a settling tank is developed to a higher level because of its high efficiency in removing organic compounds from urban and industrial wastewater [30,31]. Biodegradation of DMP in a immobilized cell reactor with high DMP loading and removal rates, using an acclimatized mixed bacterial culture isolated from activated sludge, was studied [32]. It showed very high removal efficiency at phthalate-loading rate of 560 g/m^3 h, indicating high affectivity of this approach in deterioration of phthalate compounds.

In the present research, the performance of a fixed film bioreactor for removing PA and DMP at various operating conditions was studied. Kinetic parameters (μ_m, K_s, Y and K_d) for both PA and DMP biodegradation processes were also determined using a mass balance model.

2. Materials and methods

2.1. Experimental setup

The schematic diagram of the experimental setup is shown in Fig. 1. An integrated system including a fixed activated sludge bioreactor and settling tank with total volume of 6 and 3 l, respectively, was designed and fabricated. The bioreactor working volume was determined 5 l. In order to provide high surface for growing the biofilm, the aeration tank was packed using PVC pieces with a specific surface area of 700 $m^2 m^{-3}$. An air compressor was applied for aerating the wastewater through a perforated column placed at the bottom of the reactor.

2.2. Feed

A synthetic wastewater solution was prepared with different concentrations of PA and DMP (Merck Co., Germany) including 10, 20, 50, 100, 200, and 500 mg/l. Nitrogen and phosphorous sources were supplied from ammounium nitrate (NH_4NO_3) and potasium dihydrogen phosphate (KH_2PO_4) (Merck Co., Germany), respectively, with a ratio of COD:N:P = 100:7:1.

2.3. Operation conditions

All experiments were carried out at lab temperature (28–32 °C). After a one-month start-up period, the bioreactor was initially operated at HRT of 48 h so that the HRT was stepwise decreased to 6 h. At each HRT, the bioreactor was fed by various concentrations of the substrates ranging from 10 to 500 mg/l. Each step was continued to a steady state condition where the variations in effluent parameters maintained constant. During the experiment, the process was continuously monitored by taking samples from influent, effluent, and sludge.

2.4. Analytical methods

The following parameters were analyzed according to Standard Methods [33]: pH, alkalinity, total suspended solids (TSS), volatile suspended solids (VSS), BOD and COD. Sodium hydroxide sollution (1 M) was used to adjust pH when pH dropped to less than 6 due to the high concentration of PA. To measure PA and DMP concentrations, a HPLC-UV system (2000 Eurochrom, Knuer Co., Germany) equiped with a column (C8, 15 cm × 46 mm) (Waters Co., USA) with packing size of 5 μm was used. The mobile phase consisted of methanol–water (65:35 v/v), so that water used contained 0.5% phosphoric acid. The flow rate of the mobile phase was adjusted at 1 ml/min. The analysis was carried out at ambient temperature. At each injection, 100 μl of the sample was taken. The limit of detection and quantity of detection were 5 and 50 μl, respectively. Methylen chloride was used to extract PA and DMP from the sludge [33]. Dissolved oxygen (DO) and temperature were monitored using a portable DO meter (B50, YSI Co., USA).

In order to determine the evaporation rates of PA and DMP, the bioreactor was operated at different HRTs and substrate concentrations in biomass-free conditions. In these experiments, the concentrations of PA and DMP at influent and effluent were periodically measured. The eliminated amounts of the compounds indicated the evaporated fraction.

2.5. Inoculum solution

In order to prepare a suitable inoculum solution, some sludge was taken from a working wastewater treatment plant (Shoosh, Tehran). The sample was then acclimatized with PA and DMP in a batch experiment. After a one-week acclimatization phase, the sample was prepared to be inoculated into the bioreactor.

3. Mathemathical modeling

The substrate utilization rate in biological systems can be modeled with the following expression for soluble substances [34]

$$r_{su} = -\frac{dS}{dt} = \frac{kSX}{K_s + S} \quad (1)$$

where r_{su} is the rate of change in the substrate concentration due to utilization, g/m^3 d, k is maximum specific substrate utilization rate,

Table 1
Reactor performance for removing PA.

Influent PA (mg/l)	HRT (h)	Effluent PA (mg/l)	PA in sludge (mg/kg)	PA evaporation rate (g/h)	Biomass concentration (g/l)	Effluent pH	Effluent COD (mg/l)	Effluent BOD_5 (mg/l)
10	6	0.05	58	–	0.9	7.5	24	17
	12	0.005	43		0.87	7.8	15	8
	24	0.005	36		0.84	7.5	14	9
	48	0.004	31		0.8	7.7	11.4	5
20	6	0.1	69	–	0.9	7.4	23	17
	12	0.031	54		0.87	7.4	15	7
	24	0.018	48		0.84	7.6	15	8
	48	0.016	39		0.8	7.6	13	9
50	6	1.5	91	–	0.9	6.7	27	23
	12	0.18	74		0.87	6.9	18	10
	24	0.034	67		0.84	7.1	17	8
	48	0.017	51		0.8	7.2	15	8
100	6	5	103	–	0.9	6.4	33	20
	12	0.94	89		0.87	6.5	28	17
	24	0.15	77		0.84	6.9	25	11
	48	0.23	63		0.8	7	20	10
200	6	27	117	–	0.7	6.5	35	22
	12	3.5	107		0.66	6.8	32	12
	24	0.38	91		0.63	7.2	26	13
	48	0.11	72		0.6	7.5	21	10
500	6	133	171	0.5	1.5	6.3	320	18
	12	16	146	0.26	1.15	6.7	30	14
	24	2.6	121	0.13	1.1	6.9	24	11
	48	0.15	89	0.07	0.95	7.0	16	9

g substrate/g microorganisms d, X is biomass concentration, g/m^3, S is substrate concentration in solution, g/m^3 and K_s is half-velocity constant, g/m^3.

The biomass growth rate is proportional to the substrate utilization rate by the synthtesis yield coefficient, and biomass decay is also proportional to the biomass present. When the substrate is being used at its maximum rate, the bacteria are also growing at their maximum rate [35]. By substituting ($k = \mu_{max}/Y$) in Eq. (1), we will have:

$$r_{su} = \frac{\mu_{max} XS}{Y(K_s + S)} \tag{2}$$

Since the reactor used in this project is an attached bioreactor, the mass balance equation is written based on the substrate (PA and DMP). In this part, in addition to the rates of substrate influent, effluent and reaction, the substrate adsorption rate in sludge and substrate evaporation rate were also considered. The equation is as follows:

$$Q_0 S_0 = QS + \frac{1}{Y}\left(\frac{\mu_{max} SX}{K_s + S}\right) V + PS_s + E \tag{3}$$

where Q_0 and Q are influent and effluent flow rates, respectively, m^3/d, S_0 and S are the influent and effluent substrate concentration, g/m^3, S_s is substrate concentration adsorbed in the sludge, g/Kg, P is sludge production rate, Kg/d, and E is substrate evaporation rate, g/d.

And also,

$$r_g = \frac{dX}{dt} = -Y\left(\frac{dS}{dt}\right) - K_d X = Y\left(\frac{kXS}{K_s + S}\right) - K_d X \tag{4}$$

by dividing both sides of Eq. (4) by the biomass concentration X, the specific growth rate is defined as follows:

$$\frac{r_g}{X} = \mu = Y\left(\frac{kS}{K_s + S}\right) - K_d \tag{5}$$

where μ is specific biomass growth rate, g VSS/g VSS d. The specific biomass growth rate (μ) can be defined as inverse of the solid retention time (SRT) [34].

$$\frac{1}{SRT} = \frac{(Q - Q_w)X_e + Q_w X_u}{VX} \tag{6}$$

Thus, Eq. (4) is rearranged as follows:

$$\frac{1}{SRT} = -Y\left(\frac{r_{su}}{X}\right) - K_d = Y\frac{Q(S_0 - S)}{VX} - K_d \tag{7}$$

where Q and Q_w are influent and waste sludge flow rates, m^3/d, X, X_e, and X_w are biomass concentrations in influent, effluent and settling tank understream, respectively, V is volume of the reactor, m^3, K_d, microbial decay rate, d^{-1}, $r_{su}/X = Q(S_0 - S)/VX$ is the specific substrate utilization rate, g COD/g VSS d.

4. Results and discussion

4.1. Process analysis

The results obtained from the experiments with PA and DMP as substrate are summarized in Tables 1 and 2, respectively. In order to evaluate the reactor performance, different quantities of PA, including effluent PA and PA accumulated in sludge, as well as effluent pH, COD and BOD_5 were measured as the process responses. From Table 1, with increase in HRT from 6 to 48 h, the responses were decreased. The process was stable at all operating conditions, except under the condition with influent PA of 500 mg/l and HRT of 6 h. In this condition, large amount of biomass was observed along with exterme foaming (Fig. 2). It might be attributed to high microbial growth and enzymal discharge by the biomass at such a high rate of organic loading (2 g PA/l d corresponding to 3.6 g COD/l d). The system was controlled by adding an antifoam (DF-433 chemco) and prepared for the subsequent step for DMP.

Similar observations were recorded with DMP as substrate (Table 2). While high removal rates with 100% efficiency of DMP removal up to the phthalate-loading rate of 560 g/m^3 h have been reported [32], in this study, the maximum phthalate-loading rate applied was 83 g/m^3 h and about 82% DMP removal was achieved. It might be due to the specific consortia (*Pseudomonas putida,*

Table 2
Reactor performance for removing DMP.

Influent DMP (mg/l)	HRT (h)	Effluent DMP (mg/l)	DMP in sludge (mg/kg)	DMP evaporation rate (g/h)	Biomass concentration (g/l)	Effluent pH	Effluent COD (mg/l)	Effluent BOD_5 (mg/l)
10	6	0.050	78	–	1.20	7.7	13.0	10
	12	0.022	62		1.15	7.8	11.0	7
	24	0.015	49		1.10	7.8	11.0	7
	48	0.010	41		1.05	7.8	9.0	7
20	6	0.320	98	–	1.20	7.5	16.4	11
	12	0.035	76		1.15	7.7	11.6	8
	24	0.027	57		1.10	7.7	11.6	8
	48	0.020	49		1.05	7.8	10.0	7
50	6	0.800	117	–	1.20	7.0	22.0	13
	12	0.080	93		1.15	7.2	15.4	11
	24	0.034	79		1.10	7.3	15.0	10
	48	0.028	65		1.05	7.5	13.0	8
100	6	1.700	137	–	1.20	6.8	29.0	13
	12	0.620	108		1.15	6.9	20.2	12
	24	0.140	104		1.10	7.1	16.0	10
	48	0.100	85		1.05	7.3	13.0	8
200	6	16.00	169	–	0.95	6.5	54.0	18
	12	2.500	149		0.92	6.8	36.0	14
	24	0.450	144		0.90	7.1	21.0	10
	48	0.350	123		0.85	7.2	15.0	8
500	6	93.00	398	0.0065	2.10	6.4	110.0	20
	12	11.00	264	0.0038	1.82	6.6	54.0	17
	24	1.500	175	0.0021	1.56	6.8	27.0	13
	48	1.000	102	0.0013	0.84	7.0	22.0	11

P. fluorescens and Micrococcus halobius) isolated in the Christopher's research work as well as special nutrient supplementation. In another research, *Comamonas acidovorans* strain Fy-1 showed high ability to degrade high concentrations of phthalate (2600 mg/l) within 48 h. Two reconstituted consortia of microorganisms, one comprising Pseudomonas *fluorescens, P. aureofaciens and Sphingomonas paucimobilis*, and the other comprising *Xanthomonas maltophilia and S. paucimobilis,* were effective in completely degrading DMP (400 mg/l) in 48–96 h [24]. Although degradation of DMP by microorganisms from different environments has been reported, few of them were able to completely degrade DMP at such a high concentration within such a short period of time [13,36,37].

Reactor performances regarding PA and DMP removal were depicted in the Figs. 3 and 4, respectively. More than 95% removal efficiency was achieved for the conditions with HRT longer than 10 h. As can be seen in the figures, drastic decrease was observed in the PA and DMP removal by increasing the substrate concentration (>300 mg/l) and by decreasing HRT (<10 h). Minimum removal efficiencies for PA and DMP at HRT of 6 h were obtained 73.4 and 81.4%, respectively. A range of 71–91% as removal efficiency for PA esters was reported by Petterson [29] and similarly 79–97% by Petrasek et al. [28]. It must be noted that the system used in the two above studies was activated sludge with municipal wastewater as substrate (containing very low PA compounds).

By comparing octanol–water partition coefficient (K_{ow}) of PA and DMP, it can be concluded that DMP is more hydrophobic than PA, implying higher tendency for adsorption and entrapment in the sludge generated in the process [38]. This claim was proved by the experimental results which showed 171 versus 398 mg PA and DMP, respectively, per kg of the dry sludge. Petrasek et al. measured 6–153 mg/kg PA esters in a fixed activated sludge system [28].

The process performance was evaluated by computing specific substrate utilization rates ($-r_{su}/x$) at different HRTs and influent substrate concentrations. Figs. 5 and 6 represent $-r_{su}/x$ and food to microorganism (F/M) ratio versus the substrate concentration. The results showed a proportional variation for both $-r_{su}/x$ and

Fig. 2. An image of foaming in the condition of PA concentration of 500 mg/l and HRT of 6 h.

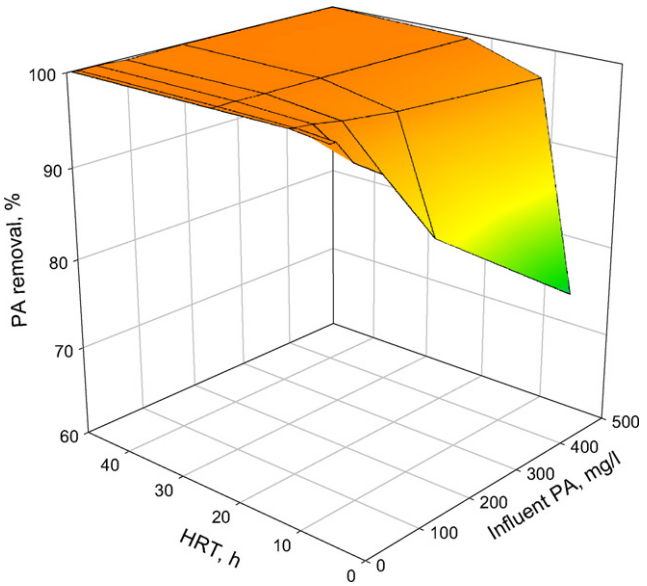

Fig. 3. Reactor performance for PA removal.

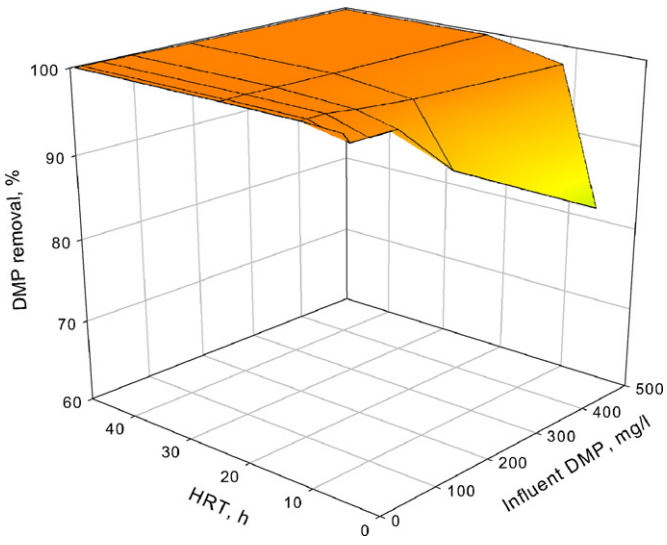

Fig. 4. Reactor performance for DMP removal.

F/M ratio as function of organic loading rate (OLR), indicating high removal efficiency at high F/M ratio. An exception occurred when the reactor was operated at HRT of 6 h and with influent PA of 500 mg/l.

In this condition, in spite of increasing the F/M ratio, $-r_{su}/x$ did not increase as expected. It was attributed to the inhibitory effect of PA at such an OLR, as more $-r_{su}/x$ was obtained at less OLR, whereas this did not occur for DMP under the same conditions, implying higher biodegradability of DMP compared to PA. Maximum substrate utilization capacity for PA and DMP were determined as 2.45 and 1.8 g CODrem/g VSS d, respectively.

Table 3
Kinetic coefficients estimated for PA and DMP.

Substrate	Y, g VSS/g phthalate	μm, h^{-1}	K_s mg phthalate/l	K_d h^{-1}
Phthalic acid	0.6112	0.0371	8	0.0047
Dimethyl phthalate	0.7875	0.0249	1.1	0.0025

4.2. Process kinetics

The kinetic coefficients (Y, μ_m, and Ks) were computed using the mass balance equation (Eq. (3)) in Sigma plot Software. The results are summarized in Table 3. It shows that the biomass production yield for DMP is greater than that for PA, implying higher accessibility of DMP for conversion to biomass compared to that of PA. It might be attributed to the molecular structure of the compounds, by the two free methyl branches in DMP providing more affinity for dissociation by the microbial consortia (smaller K_s). Less available μ_m for DMP compared to PA confirms the aforementioned reasoning, due to the raised biomass concentration in the system while the process runs with DMP as substrate.

In order to estimate the biomass decay coefficient (K_d), the relationship between the inverse SRT and the specific substrate utilization rate ($-r_{su}/X$) (Eq. (7)) for PA and DMP was plotted in the Figs. 7 and 8, respectively. In a study [39], *Chlorella pyrenoidosa* has shown an ability to accumulate and biodegrade phthalate esters. The average biodegradation rates of DMP per day were reported as 13.4 mg/L versus 1628 mg/l d, with a DMP influent concentration of 500 mg/l in this study. It was because of the multi-culture

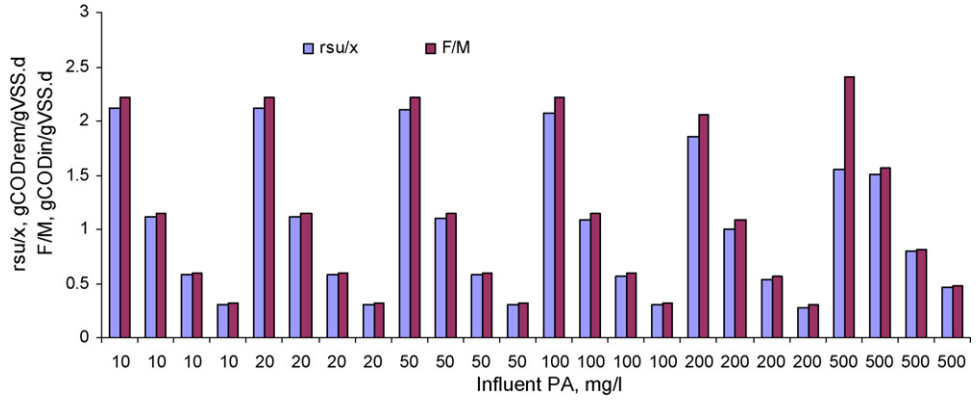

Fig. 5. Specific substrate utilization rate and food to microorganism ratio versus influent PA.

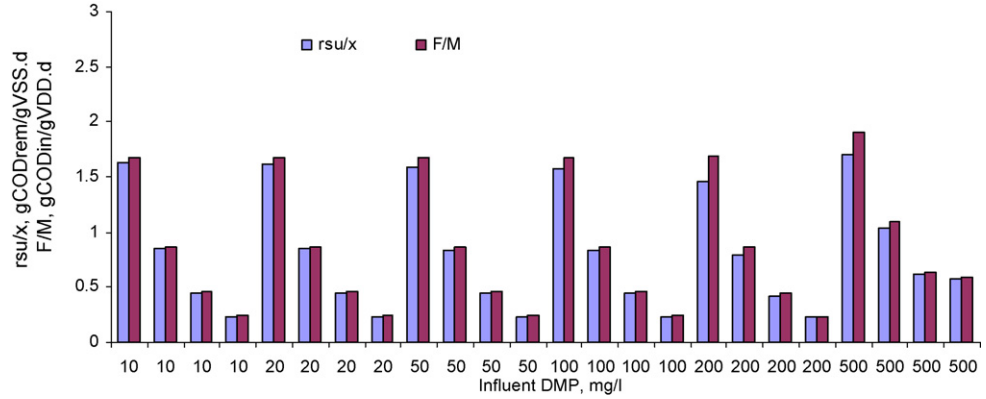

Fig. 6. Specific substrate utilization rate and food to microorganism ratio versus influent DMP.

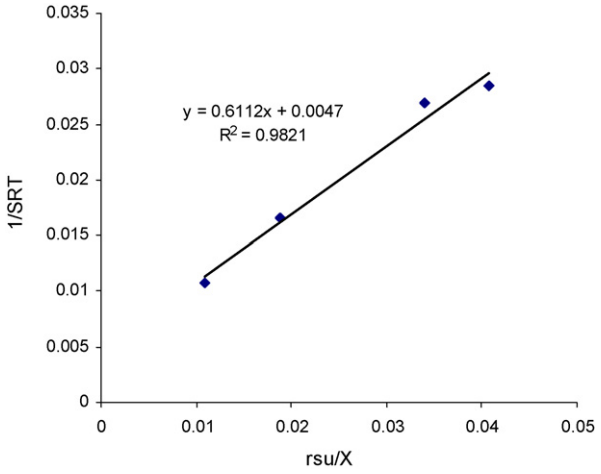

Fig. 7. Specific microbial growth rate versus specific substrate utilization rate for PA removal at influent concentration of 500 mg/l as PA.

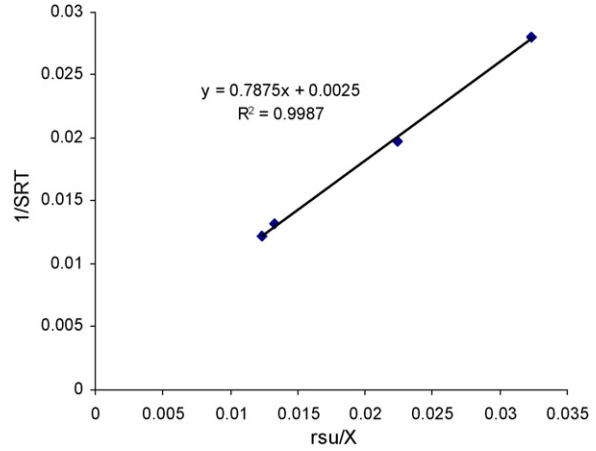

Fig. 8. Specific microbial growth rate versus specific substrate utilization rate for DMP removal at influent concentration of 500 mg/l as DMP.

which was used in the present study as well as the type of reactor (attached growth) that provided a synergistic condition among the diverse microorganisms. Furthermore, PA and DMP biodegradation rates conformed to the second-order model with determination coefficient (R^2) of 0.918 and 0.942, respectively. In this analysis, rate constants were found to be 6.88 and 21.2 $m^3\ g^{-1}\ h^{-1}$ for PA and DMP, respectively.

5. Conclusions

As conclusion, a fixed activated sludge system could easily tolerate the phthalate-loading rate from 0.009 to 4 g COD/l d. More than 95% removal efficiency was achieved for the conditions with HRT longer than 10 h. Maximum substrate utilization capacities for PA and DMP were determined at 2.45 and 1.8 g CODrem/g VSS d, respectively. The biomass production yield for DMP is greater than that for PA, implying higher accessibility of DMP for conversion to biomass compared to that of PA. PA and DMP biodegradation rates conformed to the second-order model with rate constants of 6.88 and 21.2 $m^3\ g^{-1}\ h^{-1}$, for PA and DMP, respectively.

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