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EFFECTS OF ORGANIC LOADING RATE AND HYDRAULIC RETENTION TIME ON TREATMENT OF PHENOLIC WASTEWATER IN AN ANAEROBIC IMMOBILIZED FLUIDIZED BED REACTOR

Roya Pishgar[a], Ghasem D. Najafpour[b], Bahram Navayi Neya[a], Nafise Mousavi[a], Zeinab Bakhshi[a]

[a]*School of Civil Engineering, Babol Noshirvani University of Technology, Babol, Mazandaran, Iran*
[b]*Biotechnology Research Center, Faculty of Chemical Engineering, Babol Noshirvani University of Technology, Babol, Babol, Iran*

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Abstract. Treatability of phenolic wastewater in an anaerobic immobilized fluidized bed reactor (AIFBR) in consequence of stepwise increment in phenolic load as well as decrease in hydraulic retention time (HRT) was investigated. The experimental data indicated that high degradation efficiencies of phenol and COD in the bioreactor at low HRTs and high organic loading rates were obtained. At constant HRT of 16 h with increase in influent phenol concentration from 98 to 630 mg/l, the average phenol and COD removals were 96 and 88%, respectively. However, further increase in phenol concentration in the feed stream to 995 mg/l resulted in decrease in phenol and COD removal efficiencies to 84 and 79%, respectively. For influent phenol concentration of 995 mg/l, the biogas production rate of 4.55 l/l.d was obtained. As HRT decreased from 3 to 0.15 day, the system showed high stability; influent phenol and COD were removed and reached to average values of 17 and 173 mg/l correspond to the removal efficiencies of about 97 and 90.5%, respectively. The bioreactor experienced a failure with further decrease in HRT to 0.1 day. Biogas production was gradually decreased from 7.04 l/l.d at HRT of 3 days to 2.23 l/l.d at HRT of 0.1 days. The value of the ratio of volatile fatty acids to total alkalinity (VFAs/TA) ranged from 0.03 to 0.24 during the entire course of operation.

Keywords: biotechnologies in environmental engineering, wastewater management.

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Introduction

One of the most important challenges of modern world is to preserve the environment against diverse pollutions. Progressive industrialization is the major reason of releasing different types of contaminants into ecosystem (Kwon *et al.* 2009; Mousavi *et al.* 2011). Phenol and its derivatives which are known as toxic carcinogenic aromatics abundantly occur in environment by disposal of phenol-containing effluents of number of industries. Some of these industries are petroleum refineries, steel plants, pulp and paper, pharmaceuticals, manufacturing of synthetic chemicals, coal conversion, coke oven, etc (Arutchelvan *et al.* 2006; Fang *et al.* 2006; Carbajo *et al.* 2010). High concentrations of phenol (up to 17,500 mg/l) have been detected in various industrial wastewaters (Carbajo *et al.* 2010). The reported range of phenol concentrations in various industrial wastewaters are summarized in Table 1 (Carlo *et al.* 2008). Specific low concentration of phenol poses significant health and environmental risks for human (Rittmann *et al.* 2001; El-Naas *et al.* 2009). Hence, phenolic compounds have been regarded as priority organic pollutant by US Environmental Protection Agency (U. S. EPA). In addition, World Health Organization (WHO) has defined permissible level of 1 μg phenol per liter of drinking water (Nuhoglu *et al.* 2005; Yan *et al.* 2006). Considering noxious affects of phenol, phenolic wastewaters must be treated before being discharged to the surrounding environment (Yan *et al.* 2005).

Different physicochemical or conventional biological methods can be employed to reduce phenol content below permitted level (Gonzalez *et al.* 2001a; Carlo *et al.* 2008). Bioprocesses are proven to be cost-effective environmentally-friendly approaches in which complete mineralization of organic contaminants may occur

Corresponding author: G. D. Najafpour
E-mail: najafpour@nit.ac.ir

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Table 1. Phenol concentration in various industrial wastewaters

Wastewater	Phenol concentration (mg/l)
Refineries	6–500
Coking operations	28–3900
Coal processing	9–6800
Petrochemicals	2.8–1220
Pulp and paper	0.1–1600
Pharmaceutical	0.1–1600
Synthetic wastewater (this study)	98–995

(Moussavi *et al.* 2009; Firozjaee *et al.* 2011). Moreover, treatment of phenolic wastewater using physicochemical techniques may result in production of toxic by-products such as poisonous poly chlorinated phenols. Therefore, phenol biodegradation is preferred in comparison to chemical oxidizing or physical treatment as transferring pollution from one phase to another (Arutchelvan *et al.* 2006; Bakhshi *et al.* 2011).

Phenol removal in presence of oxygen is often preferred due to high phenol inhibition on anaerobic processes. However, complete degradation of phenol can happen in anaerobic systems by a well acclimated culture (Moussavi *et al.* 2009). Aerobic treatment of phenolic wastewater is extensively discussed in the literature (Gonzalez *et al.* 2001a; Gonzalez *et al.* 2001b; Alemzadeh *et al.* 2002; Tziotzios *et al.* 2005; Vázquez *et al.* 2006; Nair *et al.* 2007; Moussavi *et al.* 2009). Though, in late decades there is a widespread tendency to apply anaerobic biological treatment systems for treating different types of wastewaters (Şen *et al.* 2003; Bertin *et al.* 2004; Subramanyam *et al.* 2008; Asadi *et al.* 2009a; Asadi *et al.* 2009b; Carbajo *et al.* 2010; Saghafi *et al.* 2010; Wang *et al.* 2010). Anaerobic biodegradation is being favored to aerobic systems due to low energy and nutrient requirements and less sludge yield (Metcalf *et al.* 2003; Veeresh *et al.* 2005; Carbajo *et al.* 2010). Besides, methane can be produced throughout anaerobic digestion which is considered as an innovative energy source (Metcalf *et al.* 2003; Bakhshi *et al.* 2011). Table 2 listed the conducted researches on anaerobic biodegradation of phenolic wastewaters reported in the literature. Among several high rate biological processes, fluidized bed reactor (FBR) is an advanced technique with relatively low hydraulic retention times. Considering slow growth of anaerobic consortia, FBR as a novel technology provides appropriate bioreactor volume as well as high active biomass concentration. Due to

Table 2. Studies of anaerobic biodegradation of phenolic compounds

Substrate	Phenolic compound (mg/l)	COD (mg/l)	OLR (g COD/ l.d)	Phenolic compound removal (%)	COD removal (%)	HRT (day)	Reactor[e]	Ref.
p-nitrophenol	10–700	3000	2.89×10^{-4}	≥ 99	≥ 90	10.38	ABR	Kuscu *et al.* 2005
Resorcinol	212–1038	443–1892	1.91–12.34	81–97	78–94	0.125–0.25	FFFB	Latkar *et al.* 2003
Catechol	97–1018	188–1897	0.88–13.88	57–91	56–87			
Hydroquinone	158–903	426–1777	1.88–12.05	32–79	29–83			
OMW[a]	720–1300	10250–25500	4.34–17.70	66–75 5–32	32–65 4–18	0.042	UAPB-GAC UAPB-SB	Bertin *et al.* 2004
Phenol	100–1000	250–2500	0.25–2.5	94	88	1	UAPB	Bakhshi *et al.* 2011
Phenol	500–1000	5000	–	≥ 83	≥ 60	0.5	EGSB	Scully *et al.* 2006
CGW[b]	143–540	1000–2500	1.0–2.5	63 ≥	60 ≥	1	UASB	Wang *et al.* 2010
4-chlorophenol	40	1124–1738	1.7–5.3	82–90	≥ 90	0.25–0.67	UASB	Majumder *et al.* 2008
Catechol	100–1500	–	3.77–10.52	≥ 95	≥ 95	0.33	UASB	Subramanyam *et al.* 2007
Phenol	252–1176	1000–6500	2–18	84–99.9	74–91.3	0.33–1	UASB	Gali *et al.* 2006
Phenol	2080 ≥	–	15.46 ≥	≥ 99.9	–	0.43	AFBR	Carbajo *et al.* 2010
PCP[c]	1333 ≥	–	–	≥ 99.9	≥ 98	0.39–1.55	AFBR	Khodadoust *et al.* 1997
PCP + PAHs[d]	100 + 0.5–690	–	–	≥ 99.8	–	0.097–0.39	AFBR	Koran *et al.* 2001
Phenol	98–995	234–2390	0.35–12.25	84–98	79–91	0.15–3	AIFBR	This study

[a] Olive mill wastewater
[b] Coal conversion wastewater
[c] Pentachlorophenol
[d] Poly aromatic hydrocarbons (PAHs) such as naphthalene, acenaphthene, pyrene and benzo(b)fluoranthene
[e] ABR: Anaerobic baffled reactor, FFFB: Fixed film-fixed bed bioreactor, UAPB-GAC: Upflow anaerobic packed bed reactor filled with granular activated carbon, UAPB-SB: Upflow anaerobic packed bed reactor filled with silica beads, EGSB: Expanded granular sludge bed-based bioreactor, UASB: Upflow anaerobic sludge blanket, AFBR: Anaerobic fluidized bed bioreactor; AIFBR: Anaerobic immobilized fluidized bed bioreactor

adhesion of microorganisms to a solid support, the sludge retention time and the hydraulic residence time are uncoupled in this process. Thus, application of high organic loading rates (OLR) is feasible in such system (Şen *et al.* 2003; Carbajo *et al.* 2010; Bakhshi *et al.* 2011). FBR has been successfully used to treat a broad spectrum of either readily or hardly biodegradable wastes in addition to hazardous pollutants (Borja *et al.* 1995; Perez *et al.* 2007; Lohi *et al.* 2008; Sowmeyan *et al.* 2008; Kuyukina *et al.* 2009).

The literature survey reveals that treatment of phenolic wastewater in anaerobic immobilized fluidized bed reactor (AIFBR) is rarely carried out. In this study performance of a laboratory-scale AIFBR on treatment of synthetic wastewater containing high concentrations of phenol was evaluated. The present research was aimed at investigating the effect of increasing phenolic and organic loading rate and decreasing HRT on phenol and COD removal as well as biogas production. Simultaneously variation of pH, VFAs and alkalinity of wastewater were monitored in the entire operation period. The results may be customized for optimizing the operation of a full-scale wastewater treatment plant treating phenolic effluents.

1. Materials and methods

1.1. Fluidized bed bioreactor

Continuous biodegradation of phenol was accomplished using an anaerobic immobilized fluidized bed reactor (AIFBR). A laboratory-scale configuration was used as experimental setup (Fig. 1). The system consisted of a 7.4 cm internal diameter and 114.5 cm long cylindrical Plexiglas column to form the 5.24 l reactor. The reactor was filled with mature immobilized microorganisms entrapped in calcium alginate gel beads up to 30% of its total volume. The working volume of the reactor was about 3.5 l. The flow was continuously fed into the reactor from a conical section at the bottom that could provide a homogenous fluidization. Recycle flow was drawn from the top of the reactor by a peristaltic pump (BT600, Prefluid, China). The synthetic phenol solution along with recycle stream was fed upward into the reactor. Synthetic phenolic wastewater was transferred into reactor using a peristaltic pump (BT100, Prefluid, China). Bed expansion in the bioreactor was kept around 100% with the aid of the recycle flow. The system was embedded in a transparent talc encasement to provide constant temperature at about 25 °C. Produced biogas was released from effluent by aid of a 1000 ml separation funnel which was filled with polyethylene supports up to half of its volume. The liberated biogas was then led to a glass cylindrical collecting tube where the volume of gas was defined by displacement of inside water. The collecting tube was fabricated with an internal diameter of 15 cm and height of 150 cm. Two sampling ports were installed on AIFBR for monitoring influent and effluent characteristics. The startup and operation periods of AIFBR are illustrated below.

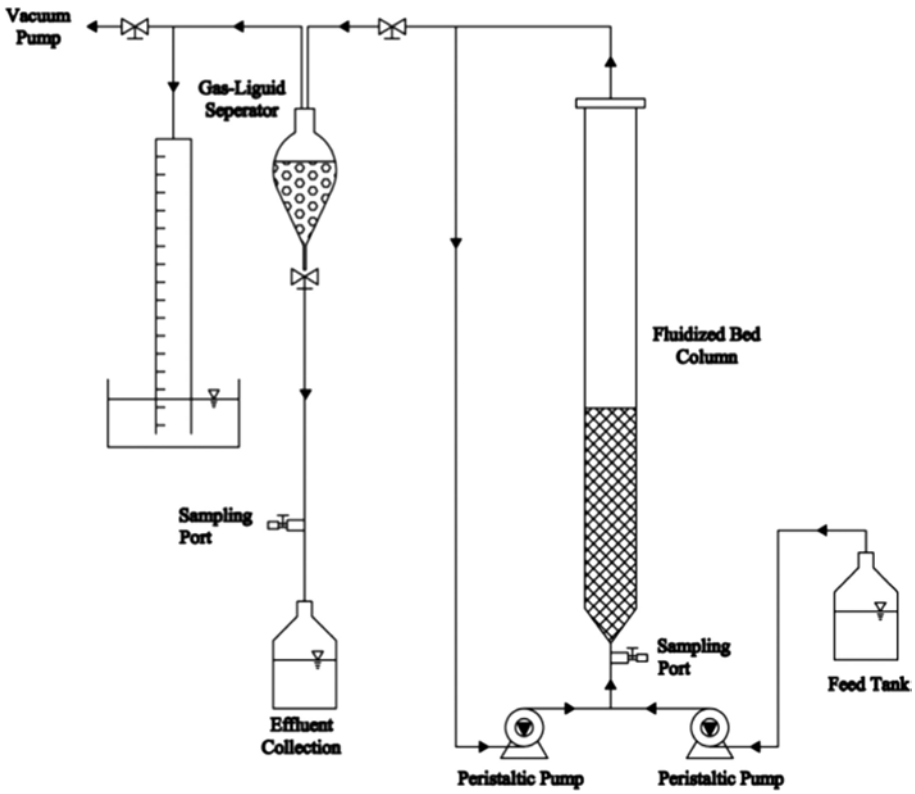

Fig. 1. Schematic diagram of a lab-scale AIFBR used as experimental setup in continuous operations

1.2. Inoculums

The seed inoculums were isolated from the effluents of two industries including coke oven, Isfahan, Iran and pulp and paper, Sari, Iran as well as activated sludge taken from wastewater treatment facility of the latter. An acclimation period of 5 months was implemented to adapt the mixed culture to phenol as sole carbon/energy source. Adaption steps were performed in 250 ml Erlenmeyer flasks under anaerobic condition in desiccators. During this period, the microorganisms were exposed to increasing phenol concentrations. At the beginning, low concentration of phenol i. e. 100 mg/l was introduced to the culture in presence of 100 mg/l glucose as co-substrate. Upon complete removal of 100 mg/l phenol within 20 days, exposure of the culture to solely phenolic media was started. As phenol removal efficiency of over 95% was observed, microorganisms were inoculated to a fresh medium containing higher phenol concentration. Inoculums concentration was about 10% (v/v). Finally, in duration of 12 days, the adapted culture was capable to degrade 96 and 10% of 700 and 1000 mg/l phenol, respectively.

1.3. Immobilization protocol

The adapted microorganisms were harvested at the stationary phase, and then entrapped and immobilized in calcium-alginate gel beads. After adding sterilized sodium alginate solution (2% w/v) into cell suspension, the mixture was introduced drop wise into sterilized calcium chloride solution (6% w/v); thus, immobilization procedure was accomplished. Sodium alginate (SIGMA) solution was prepared by adding 10 g of the alginate powder was added to 500 ml of distilled water. 60 g of anhydrous granulated form of calcium chloride (Sharlau, Spain) was separately added to 1 l of distilled water to prepare calcium chloride bath. A peristaltic pump (BT100, Prefluid, China) and a silicon tube with internal diameter of 1.1 mm were used to instill the mixture of sodium alginate solution and cell suspension in the calcium chloride bath. Spherical bioparticle beads with average diameter of 3–4 mm were ultimately obtained.

1.4. Wastewater characteristics

The synthetic phenolic wastewater was composed of phenol, yeast extract as well as a basal medium which contained all the necessary micro- and macro-elements. The composition of basal medium was (in mg/l): K_2HPO_4, 522.54; KH_2PO_4, 408.27; and NH_4Cl, 200; NaCl, 200; KCl, 200; $CaCl_2.2H_2O$, 150; $MgCl_2$, 100; $MgSO_4.7H_2O$, 5; $FeSO_4.7H_2O$, 10; $CoCl_2.2H_2O$, 0.2; $NiCl_2.2H_2O$, 0.2; $ZnCl_2$, 0.2; $CuCl_2.2H_2O$, 0.2; $MnCl_2.4H_2O$, 0.2; $NaMoO_4.2H_2O$, 0.5; H_3BO_3, 0.2; $NaHCO_3$, 400. Concentration of yeast extract was kept constant in the feed at 800 mg/l. By addition of 400 mg/l $NaHCO_3$, pH of influent was maintained at 6.9 ± 1. To prepare synthetic wastewater, phenol and nutrients stock solutions were diluted with distilled water to appropriate concentrations. All of the chemicals were supplied by Merck (Darmstadt, Germany).

1.5. Startup and operation strategies

The startup period was carried out to acquaint the immobilized culture to continuous condition in phenol removal plus evaluating the effect of increasing organic load on phenol biodegradation. During startup period, the bioreactor was run at constant hydraulic retention time of 16 h, and phenol influent concentration was gradually increased from 98 to 995 mg/l in stepwise manner (equivalent to phenol loading rate of 0.15 to 1.48 g phenol/l.d). Concentration of corresponding COD varied in the range of 234 to 2390 mg/l. The startup period was implemented in 5 operation phases for duration of 65 days. Initially, phase 1 (0–5 days) was performed, where phenol feed and OLR were 98 mg/l and 0.35 g COD/l.d, respectively. Second phase started on 6th day and lasted 8 days with influent phenol concentration of 236 mg/l and OLR of 0.85 g COD/l.d. At phase 3 (day 15 to day 27), initial phenol concentration was increased to 440 mg/l (OLR of 1.58 g COD/l.d). In the next 16 days, influent phenol concentration was kept constant at 628 mg/l which corresponds to OLR of 2.24 g COD/l.d. In the final phase between day 44 to day 65 (for the last 21 days) phenol concentration increased to 995 mg/l (OLR of 3.56 g COD/l.d). Under steady-state condition, phenol in feed stream was gradually increased while more than 96% of phenol was removed.

In the next stage, the effect of hydraulic retention time (HRT) on the process performance was evaluated. Eight experimental runs were carried out with various HRTs including 3, 2, 1, 0.5, 0.3, 0.2, 0.15, 0.1 day. This stage of experiment lasted 77 days; the values of phenol and COD in the influent were maintained at 720 and 1830 mg/l, respectively. The bioreactor was operated at OLRs of 0.61, 0.92, 1.83, 3.72, 6.15, 9.20, 12.25, 18.34 g COD/l.d, which corresponded to the above HRT values. The phenol loading rate was 0.24–7.22 g phenol/l.d. Descending order of HRT was applied in this stage of experiment. Steady-state condition was obtained when phenol concentration in the effluent remained practically constant.

1.6. Sampling and Analytical methods

Samples of influent and effluent of the bioreactor were daily analyzed in duplicate. Parameters including chemical oxygen demand (COD), residual phenol, volatile fatty acids (VFAs), total alkalinity (TA) and pH were determined. COD was measured by closed reflux method. Residual concentrations of phenol were

defined by direct photometric method using 4-aminoantipyrene and alkaline potassium ferricyanide. Direct titration was applied for determination of VFAs and TA. The above procedures were performed in accordance with Standard Methods for Examination of Water and Wastewater (Eaton *et al.* 2005). Produced gas was determined by water displacement. pH measurements were carried out with a pH meter (pH 212, HANNA, Germany).

2. Results and discussion

2.1. Startup period; effect of phenolic load

Response of the AIFBR to incremental increase of phenolic load during the startup period was evaluated. The influent and effluent phenol and corresponding COD concentrations, phenol and COD removal, biogas production, VFAs, total alkalinity, and pH are presented in Figure 2. Startup period was completed for duration of 65 days. This entire operation period was divided into five phases, where stepwise increase in phenol feed from 98 to 995 mg/l at a constant HRT of 16 h was investigated. Figure 2(a–d) shows phenol and COD removal and biogas production during the startup period.

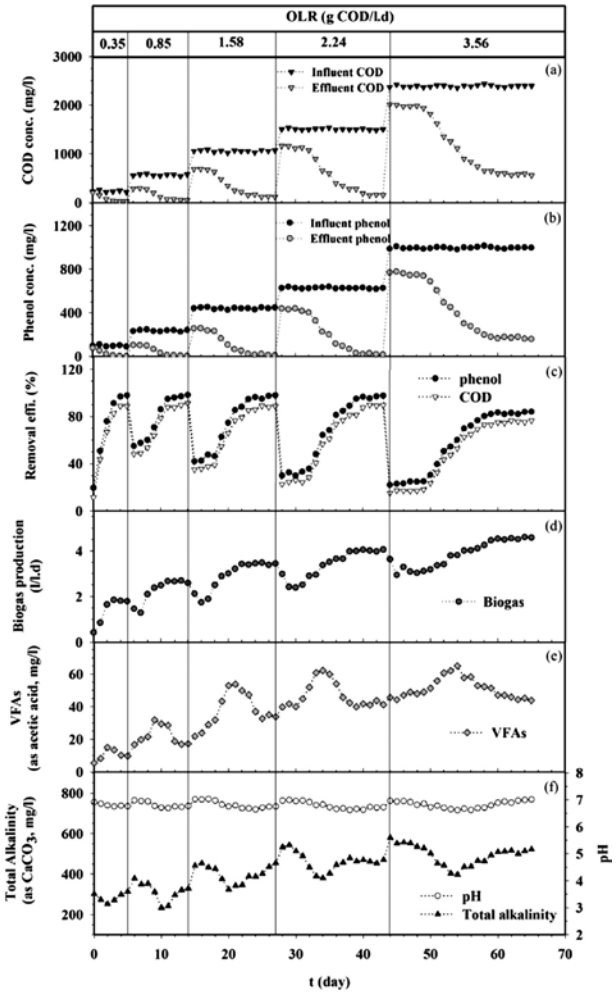

Fig. 2. Response of the AIFBR to increment of phenolic load during startup

In Phase 1 (days 1–5), the AIFBR was fed with phenol and corresponding COD of 98 and 234 mg/l, respectively. The value of OLR was 0.35 g COD/l.d. Phenol degradation was immediately started. The bioreactor could remove about 44 mg/l of input phenol at the end of the first day of the experiment. Finally, 98% of influent phenol and 89% of influent COD were removed in this phase of experiment. Maximum production of biogas was observed at the final days of the phase when phenol concentration in the effluent remained constant at about 2.6 mg/l. At day 4, the volumetric biogas rate was 1.81 l/l.d.

Phase 2 was performed within day 6 to day 14 with influent phenol concentration of 236 mg/l, correspondent to COD concentration and OLR of 556 mg/l and 0.85 g COD/l.d, respectively. Although the microorganisms were strictly adapted to phenol concentration of 700 mg/l, a lag period in degradation plus a sudden reduction in removal efficiencies of phenol and COD were diagnosed at the beginning of every single phase.

With increase in phenol concentration up to about 230 mg/l, phenol and COD removal efficiencies reduced to 55 and 48%, respectively. During initial stage of second phase (day 6 to day 9), the phenol concentration in the effluent only decreased to about 102 mg/l. Also biogas production rate decreased from 1.79 l/l.d at day 5 to 1.29 l/l.d at day 7 which is equivalent to 28% reduction.

At day 11 of experiment, phenol concentration in the effluent degraded to 12.5 mg/l with removal efficiency of 95%. At the end of this phase, COD removal of 89% and volumetric biogas rate of 2.67 l/l.d were achieved. In third phase (days 15–27), the OLR was maintained at 0.66 g COD/l.d with input phenol concentration of 441 mg/l and COD of 1050 mg/l for duration of 12 days. As the influent concentration of phenol increased, the initial lag period in phenol and COD degradation was slightly prolonged. At the initial day of this phase, phenol and COD removal efficiencies drastically decreased to 42 and 35%, respectively. During the first 4 days of 3rd phase, only 46% of influent phenol was removed; thence the phenol and COD removal efficiencies increased. Moreover, volumetric biogas rate diminished and reached 1.74 l/l.d at day 16. At day 24 of the experiment, phenol and COD removal efficiencies enhanced to 96 and 87%, respectively; accordingly the effluent concentrations of 32 mg/l phenol and 196 mg/l COD were determined in this day. In subsequence, the system experienced steady-state condition untill the day 27th of operation. At the end of this phase, low concentrations of phenol (15 mg/l) and COD (141 mg/l) in the effluent were obtained. Recovery in biogas production from 1.74 to 3.42 l/l.d occurred within days of 16 to 22 of the experiment. Biogas production

achieved the value of 3.45 l/l.d at the end of this phase. The increment in organic load obviously resulted in further growth of the microorganisms. It seemed that consequent increase in density of the alginate beads had happened. This phase required high recycle flow rate for 100% fluidization of the support beads could prove the assumption of beads weight increased.

Phase 4 of startup operation was carried out within days 28 to 44. This phase lasted 17 days; influent phenol concentration was increased to 628 mg/l. Increment in phenol feed led to corresponding COD concentration of 1520 mg/l and OLR of 2.24 g COD/l.d. In this phase, phenol degradation rate decreased in compare to previous phases. It indicated the substrate inhibition which was imposed by high phenol concentration on the metabolism of the culture. At day 29, the effluent concentrations of 439 mg/l phenol and 1165 mg/l COD were observed which were equivalent to removal efficiencies of 30 and 22%, respectively. Removal efficiencies slightly increased to 36% for phenol and 28% for COD at day 32. With further increase in removal efficiencies, 64% of influent phenol concentration was removed and reached 224 mg/l phenol in the effluent at the day 34th of experiment. Maximum phenol and COD removal of 95 and 87% were accomplished at the end of this phase. Biogas production rate represented descending trend throughout two initial days of 4th phase. However, it recovered and obtained the steady value of 4.08 l/l.d within days 37 to 43.

Final phase of startup period was started at day 44 and lasted till day 65. In this phase, the influent phenol and COD concentration were 995 and 2390 mg/l, respectively. Therefore, the value of OLR was kept constant at 3.56 g COD/l.d. Due to intense inhibition of high phenol concentration, its removal efficiencies showed a dramatic decrease to 22% at first day of this phase. At the same day, COD removal of 15% was obtained. For 7 days at the beginning of this phase, no significant reduction in phenol and COD concentration was observed. At day 56, phenol removal efficiency increased to 72% which corresponded to effluent phenol and COD concentrations of 277 and 839 mg/l, respectively. The AIFBR completed final phase in stable condition. Steady-state condition was obtained at the time interval between the days 58 to 65 with a phenol effluent concentration of 140 mg/l correspond to removal efficiencies of 84% for phenol and 79% for COD. The volumetric biogas rate of 4.55 l/l.d was achieved at the end of 5th phase which was the highest value for the startup period. Carbajo and coworkers (Carbajo *et al.* 2010) achieved the maximum volumetric biogas rate of 4.4 l/l.d with average yield of 0.28 l CH_4/g COD removed in an AFBR while the OLR was progressively increased to 15.46 g COD/l.d. However, they observed that with increase in OLR biogas composition was changed; the percentage of CO_2 increased as the CH_4 percentage decreased.

2.2. Variation of VFAs during startup period

The concentrations of effluent volatile fatty acids measured as acetic acid during phenolic waste treatment are presented in Figure 2e. Stepwise increase in influent phenol led to ascending attitude in VFAs concentration at the beginning of every operation phase; subsequently after obtaining a peak value, a gradual decrease was followed in VFAs value. From one phase to another, the peak concentration of VFAs was increased as phenolic load was added. Decrease in VFAs concentration may be correlated to the methanogenic stage when VFAs and organic compounds were utilized and stable end product of methane and carbon dioxide were formed. During startup operation, VFAs concentration varied in the range of 5.5 to 65 mg/l. At the end phase of startup, the highest value of effluent VFAs i.e. 65 mg/l was observed. Bakhshi *et al.* (Bakhshi *et al.* 2011) reported the range of 34–64.7 mg/l for VFAs concentration as the value of OLR increased from 0.25 to 2.5 g COD/l.d during phenol treatment in UAPB. Subramanyam and Mishara (Subramanyam *et al.* 2007) found out that the value of VFAs which was produced during catechol degradation in a UASB varied from 15 to 65 mg/l. However, in presence of resorcinol and catechol, VFAs concentration of 30 to 63.5 mg/l was observed in the UASB (Subramanyam *et al.* 2008); that may be due to intense toxicity of two inhibitory substrates and retardation in methanogenic activity which resulted in consequent accumulation of VFAs. In this period of the operation, the ratio of VFAs/TA varied between 0.03–0.16. It is according to the reported data in literature (Subramanyam *et al.* 2007; Subramanyam *et al.* 2008; Bakhshi *et al.* 2011).

2.3. pH and alkalinity during startup period

Figure 2f shows the variation of pH and total alkalinity (TA) in the effluent of AIFBR during startup operation period. Anaerobic systems are sensitive to pH variation. Production of organic acids and VFAs either in hydrolysis or acidogenesis periods of anaerobic digestion can lead to acidic condition (Rittmann *et al.* 2001; Bakhshi *et al.* 2011). The pH values of below or above the range of 6.5–7.1 may adversely affects the biological process by disturbance and interruption in bacterial activity, especially in methanogenesis (Subramanyam *et al.* 2008). Thus, the value of pH in AIFBR was adjusted between 6.8 ± 0.2 by adding $NaHCO_3$ with the concentration of 400 mg/l. Subramanyam and Mishara (Subramanyam et al. 2007) observed instability in the pH of a UASB degrading phenolic waste in absence of alkalinity. The effluent alkalinity as $CaCO_3$ in the AIFBR was 233 to 579 mg/l. As VFAs concentration increased at the beginning of every phase of startup operation, the effluent alkalinity contributed a diminution, though, it was enhanced later.

2.4. Effect of HRT on the AIFBR performance

In this period of operation, the AIFBR was continuously fed with a solution of 720 mg/l phenol and corresponding COD of 1830 mg/l for 77 days. The feed with phenol concentration of 700 mg/l was well treated due to high performance of the AIFBR at the similar phenolic load during the startup period. The HRT initially was fixed at 3 days with OLR of 0.61 g COD/l.d; it was progressively declined from 3 to 0.1 days in eight experimental runs between the days 65 to 142 of the experiment. As a result, the OLR ranged between 0.61–18.34 g COD/l.d. For each HRT, removal capacity of phenol and COD in the bioreactor were monitored periodically. Once phenol and COD concentrations in the effluent remained constant, HRT was decreased to a lower value.

Figure 3 shows the performance of the AIFBR at different HRTs, in terms of the influent and effluent concentrations and removal efficiencies of phenol and COD, biogas production, the effluent VFAs, pH and alkalinity. The trend of organic load reduction with respect to time in term of decreasing HRT is shown in Figure 3. Reduction of HRT means the bioreactor operates faster. In fact, this figure represents the actual data obtained with respect to HRT.

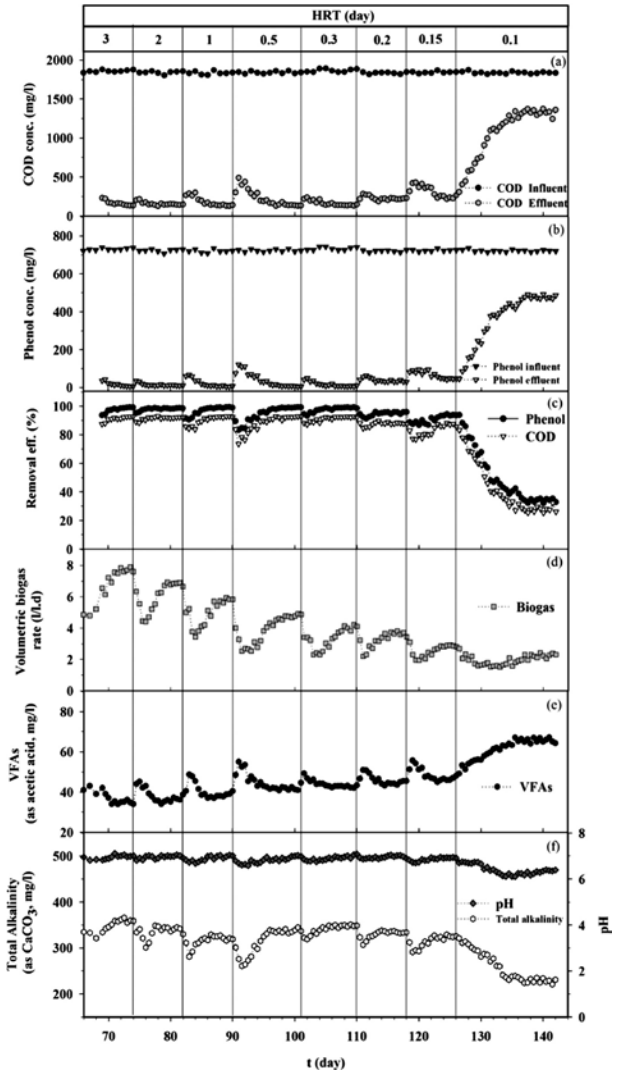

Fig. 3. Effect of decreasing retention time on the AIFBR performance

In three initial runs of the experiments when HRT varied between 1–3 days, the AIFBR presented an appropriate performance; the bioreactor immediately reached a very stable condition due to long HRTs. Finally, the effluent phenol and COD concentrations found to be less than 7 and 145 mg/l, respectively. In these three sets of experimental runs, the removal efficiencies of over 98% for phenol and above 91% for COD were achieved.

Nevertheless, the system required a prolonged time interval for removing phenol from the effluent at HRT of 0.5 day. With decrease in the value of HRT to 0.5 day, the bioreactor showed instability in 6 initial days of the phase (days 90–96). The concentration of phenol in the effluent increased from 8 mg/l at day 90 to 121 mg/l at day 91. At the same day (91st day), the effluent concentration of 488 mg/l for COD was determined. However, the AIFBR finally reached steady-state condition between the days of 96 to 101. At HRT of 0.5 day, the influent phenol and COD were removed with efficiencies of 97 and 90%, respectively. During the experimental runs of 5 to 7 (HRT of 0.3 to 0.15 day), the bioreactor was able to obtain high removal efficiencies for phenol and COD with average values of 95 and 89%, respectively. However, the stable removal efficiency of phenol and COD at the end of each run were progressively increased with further decrease in the value of HRT. For the HRT of 0.15 days (OLR of 12.25 g COD/l.d), the performance of the bioreactor was initially disturbed for 4 days; the phenol and COD concentrations of effluent were 96 and 420 mg/l, respectively. Although the system showed an appropriate behavior in phenol elimination at day 123; but a decrease in final removal efficiencies was observed for phenol (93%) and COD (86%) rather than former runs. Residual phenol of 48 mg/l and COD of 235 mg/l were determined at the HRT of 0.15 days. However, as the value of HRT was declined to 0.1 day, the bioreactor intensely experienced an unstable condition. At the end run, progressive increase in undegraded phenol and COD concentrations in the effluent was observed and biomass washout rapidly occurred. The average concentration of 17 mg/l for phenol and 173 mg/l for COD in the effluent of the AIFBR was detected for the HRTs of 3 to 0.15 days. Biogas production was continuously monitored when the HRT was decreased during 77 days of the experiment (Figure 3d). As the HRT decreased and correspondent increase in OLR happened, biogas production rate was diminished. When HRT was fixed to a lower value, a sharp drop in biogas production occurred and volumetric biogas rate went through a minimum value. However, it subsequently followed an increasing trend to a stable condition. The maximum biogas

production e.g. 7.04 l/l.d was observed in the first run with the HRT of 3 days. At the end of run, no significant biogas production (1.72–2.38 l/l.d) was observed due to severe instability in the performance of the AIFBR.

The AIFBR responded successfully to phenol biodegradation at high OLRs and low HRTs in compare to other studies in the literature (see Table 2). The long HRT of 10.38 days was applied in an ABR for p-nitrophenol degradation where removal efficiency of 99 was achieved at loading rate as high as 33.9 g p-nitrophenol /m^3.d. Khodadoust *et al.* (Khodadoust *et al.* 1997) achieved 99.9% removal of pentachlorophenol in anaerobic granulated activate carbon fluidized bed reactor (GAC-AFBR) at an empty bed contact time as low as 9.3 h. In presence of poly aromatic hydrocarbons, high degradation efficiency of pentachlorophenol (i.e. 99.8%) also occurred in the GAC-AFBR with 46.5% average conversion to its dechlorination intermediates. Also low concentrations of influent poly aromatic hydrocarbons were indentified in the effluent (Koran *et al.* 2001). Adequate treatment of phenolic wastewater was reported in an AFBR at constant HRT of 0.43 days and OLR of up to 15.46 g COD/l.d by Carbajo *et al.* (Carbajo *et al.* 2010).

2.5. Effect of HRT on the Variation of VFAs

The variation of VFAs in the effluent between the days of 65 and 142 are depicted in Figure 3e. VFAs concentration was progressively increased as the HRT decreased. Increase in the concentration of VFAs at the beginning of each run could be explained by accumulation of organic acids due to facing with a higher value of HRT. But, the system was later adapted to the new situation when HRTs ranged between 0.15–3 days and reached to a very stable condition at the end of any individual run. The effluent VFAs varied in the range of 36–66 mg/l. The maximum value of VFAs as acetic acid, i.e. 66 mg/l, was obtained at HRT of 0.1 days; that may be due to the failure in the AIFBR. The parameter VFAs/TA was between 0.09–0.2 as the HRT decreased from 3 to 0.15 days, though it increased to 0.24 at the final run which indicated high concentrations of VFAs as well as low activity of methanogens.

2.6. Effect of HRT on the pH and alkalinity

The value of pH and alkalinity relevant to decrease in HRT are shown in Figure 3f. Within the runs of 1 to 7 with HRTs of 0.15–3 days, when the system presented a favorable behavior in phenol removal, pH and alkalinity of the effluent was in the range of 6.59–7.11 and 261–362 mg/l, respectively. At the end run with HRT of 0.1 days, the value of pH dropped to 6.11 at day 135. Alkalinity concentration at day 126 decreased from 325.5 to 227 mg/l at day 140. Addition of 400 mg/l $NaHCO_3$ was accomplished for the pH stability, though, addition of this content of alkalinity could not counterbalance the acidic condition at HRT of 0.1 days because of the high accumulation of VFAs.

Conclusion

1. Phenol containing wastewater was effectively treated in as anaerobic immobilized fluidized bed reactor (AIFBR) at high organic loading rates (i.e. 0.35–12.25 g COD/l.d) with a culture entrapped in calcium alginate beads as biocatalyst.
2. At startup period in which phenolic load was stepwise increased, over 95% of phenol and 87% of COD was removed at phenol loading of 98–630 mg/l, OLR of 0.35–2.44 g COD/l.d and phenol loading rate of 0.15–0.94 g phenol/l.d. However, a decrease in phenol and COD removal to 84% and 79% at phenol feed of 995 mg/l was observed due to intense substrate inhibition.
3. The highest biogas production of 4.55 l/l.d occurred at 995 mg/l phenol during startup.
4. In this period, VFAs, pH and alkalinity in the effluent of the AIFBR varied between 5.5–65 mg/l, 6.5–7.1 and 233–579 mg/l, respectively. The value of VFAs/TA ratio was 0.03 to 0.16.
5. The AIFBR was favorably tolerated the decrease in hydraulic retention time from 3 days to 0.15 day. In this operation period, removal efficiency of 93–98% for phenol and 86–91% for COD was obtained for constant phenol loading of 720 mg/l and OLRs of 0.61–12.25 g COD/l.d. However, further decrease of HRT to 0.1 days (OLR of 18.34 g COD/l.d) led to instability and failure in reactor performance.
6. For HRT of 0.1 days, VFAs/TA was 0.24 which showed a disruption in anaerobic process due to low HRT.

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Roya PISHGAR has a Master of Science of Environmental Engineering and a Bachelor of Science of Civil Engineering at the School of Civil Engineering, Babol Noshirvani University of Technology. Has published 6 journal papers and 5 conference papers and has attended the 1st Environmental Remediation Technologies Conference. Research interests include: water and wastewater treatment, waste management, air pollution.

Ghasem D. NAJAFPOUR is a Professor of Chemical Engineering at the Babol Noshirvani University of Technology, Iran. An Associate Professor of Chemical Engineering at the Universiti of Sains Malaysia (1998–2005). An Associate Professor at the Chemical Engineering Department, University of Mazandaran, Iran (1995–1998). An Assistant Professor at the Department of Chemical Engineering, University of Mazandaran, Iran (1983–1995). Visiting Professor, Sabbatical Leave & Research Scientist at the Department of Chemical Engineering, University of Arkansas, USA (1990–1993). Teaching Assistant at the University of Arkansas, USA (1976–1980, 1981–1983). Head of Biotechnology Research Center and Editor in Chief of IJE, IJEE, MEJSR, JAES and WASJ. Has published 312 National, International ISI indexed Journals and Proceedings and 9 books.

Bahram NAVAYI NEYA is an Associate Professor of Civil Engineering at the Babol Noshirvani University of Technology, Iran. He has a PhD of Civil Engineering-Hydraulic Structures at the Moscow Power Engineering Institute, Russia; Master of Science of Civil Engineering-Hydraulic Structures; Bachelor of Science of Civil Engineering at the Amir Kabir university of Technology (Tehran Polytechnic), Iran. Has published 19 English and Persian papers, 53 national and international conference papers and 3 books. Research interests include: design and analysis of water network, elasticity and plasticity theories, plates and shells, concrete behavioral models, design and analysis of concrete dams.

Nafise MOUSAVI. Has a Master of Science of Environmental engineering and a Bachelor of Science of Civil engineering at the School of Civil Engineering, Babol Noshirvani University of Technology, Babol, Mazandaran, Iran. Has published 6 journal papers and 5 conference papers and has attended the 1st Environmental Remediation Technologies Conference). Research interests include: water and wastewater treatment, waste management, air pollution.

Zeinab BAKHSHI. Has a Master's degree of Science of Environmental engineering and a Bachelor's degree of Civil engineering at the School of Civil Engineering, Babol Noshirvani University of Technology, Babol, Mazandaran, Iran. Has published 8 journal papers and 5 conference papers. Research interests include: water and wastewater treatment, membrane technology.