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RECOVERY OF UPFLOW ANAEROBIC PACKED BED REACTOR FROM HIGH ORGANIC LOAD DURING STARTUP FOR PHENOLIC WASTEWATER TREATMENT

Biodegradation of synthetic wastewater containing phenol by upflow anaerobic packed bed reactor (UAPB) was studied in this work. The reactor was operated at a hydraulic retention time (HRT) of 24 h under mesophilic (30±1 °C) conditions. The startup operation was conducted for 150 days; split into 4 phases. The phenol concentration was stepwise increased. The concentration of phenol in phases 1, 2, 3 and 4 were 100, 400, 700 and 1000 mg/l, respectively. In phase 1, the reactor reached steady state conditions on the 8th day with a phenol removal efficiency and biogas production rate of 96.8 % and 1.42 l/d, respectively. For an increase of the initial phenol concentration in phase 2, a slight decrease in phenol removal efficiency was observed. Similar trends were observed in phases 3 and 4 of startup. Due to the high phenol concentration, a sudden decrease in removal efficiency and biogas production was observed. The surviving microorganisms were gradually adapted and acclimated to high phenol concentrations. In phases 3 and 4, the phenol removal efficiencies at steady state conditions were 98.4 and 98%, respectively. The maximum biogas production was observed at day 130 with a value of 3.57 l/d that corresponds to phenol concentration of 1000 mg/l.

Keywords: phenol; upflow anaerobic packed bed; biogas; biodegradation, startup.

Nowadays, rapid industrialization has resulted in disposal of toxic and hazardous effluents into water bodies [1]. Among these hazardous materials, phenolic compounds are known as mutagenic, carcinogenic and teratogenic, which are reported to be either toxic or lethal to aquatic life at concentration of 5-25 mg/l [1-3]. Phenol and its derivatives are aromatic compounds that have been designated as priority pollutants by USEPA [3]. These compounds are found in industrial wastewaters such as manufacturing of synthetic chemicals, pesticides, pulp-paper, pharmaceuticals, coal gasification, dye manufacturing, petroleum refineries, and petrochemicals [4-7]. Thus, elimination

of phenolic compounds from industrial effluents is a necessity and of great concern [8,9].

Removal of phenol has been achieved by various physicochemical methods such as oxidation, adsorption and solvent extraction. Physical and chemical methods have proven to be costly and produce secondary toxic compounds such as hydrocarbons and chlorinated phenols. Thus, biological processes are preferred as suitable methods and alternatives for phenol degradation due to their ability to destroy the pollutants rather than chemically oxidizing or physically transferring pollution from one phase to another [1,10-14].

Aerobic removal of phenol has been discussed by several investigators [11,15,16]. A sequential aerobic sludge blanket reactor has removed phenol concentration of 500 to 0.2 mg/l [17]. Moussavi *et al.* [11] have reported that a novel MSBR was capable of treating highly concentrated phenolic wastewaters. They also obtained 99% phenol removal with an organic loading rate (OLR) of 83.4 g/m³ h and a hyd-

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raulic retention time (HRT) of 40 h. Bajaj *et al.* [15] have studied biodegradation of high phenolic wastewater in an aerobic fixed bed reactor. At OLR of 15.3 g COD/l.d, a HRT of 0.95 days and a phenol concentration of 4.9 g/l, phenol removal rate of 2.3 g/l.d has been achieved [15].

Anaerobic biological systems were highly recommended for treatment of wastewaters containing high concentrations of hazardous aromatic compounds [4,9,18-20]. These systems do not require high energy for aeration but also produce energy in the form of methane [13,21-23]. In recent years, anaerobic treatment of industrial wastewater containing phenolic compounds has become a viable technology. Several industrial cases have been reported in the literatures on anaerobic biodegradation of phenolic compounds [2,4-7,10,24,25]. Anaerobic biofilm-based processes, such as sludge blanket (UASB) reactors and fixed film bioreactors were effectively capable of treating phenol and its derivatives [1,6,7,10,12,24]. Fixed film bioreactors have a great potential to increase resistances of microorganisms to toxic and inhibitory compounds that improve the reactors stability while the organisms were acclimated to phenol. These reactors possess many advantages including short startup periods, no washout of biomass or short hydraulic retention times [4,5,23,26-28].

In order to overcome the slow growth of anaerobic microorganisms, a novel technology is required to maintain a reasonable reactor volume, low hydraulic residence times and biofilm production. Anaero-

bic packed bed reactors most probably meet such requirements [28-30].

In recent years, the effect of startup period in aerobic and anaerobic bioprocesses has been studied by several investigators [1,15,18,27,31-35]. High concentrations of toxic chemical compounds imposed inhibitory effects on the living organisms. Thus, a stepwise increase of the concentration of toxic compounds is essential for the better performance of the reactors. During this period, the microorganisms gradually adapted to the toxic compounds and developed the ability to degrade these compounds [15,34]. Pre-adaptation of the organisms to high phenol concentrations lead to tolerances against high phenol concentrations and to utilize phenol in cell catabolism [26,35,36]. The main objective of the present research work was to study the biodegradation of phenol and recovery of the bioreactor after organic shock loading during the startup period. The present work has been carried out in an upflow anaerobic packed bed reactor in a continuous mode of operation.

MATERIALS AND METHOD

Experimental setup

Figure 1 presents the schematic diagram of the pilot scale UAPB bioreactor. The plexiglas reactor was fabricated with an internal diameter of 6.2 cm and a height of 100 cm. The total and working volume of the reactor were 3.02 and 2.8 l, respectively. A 1000 ml funnel shaped gas separator was used to

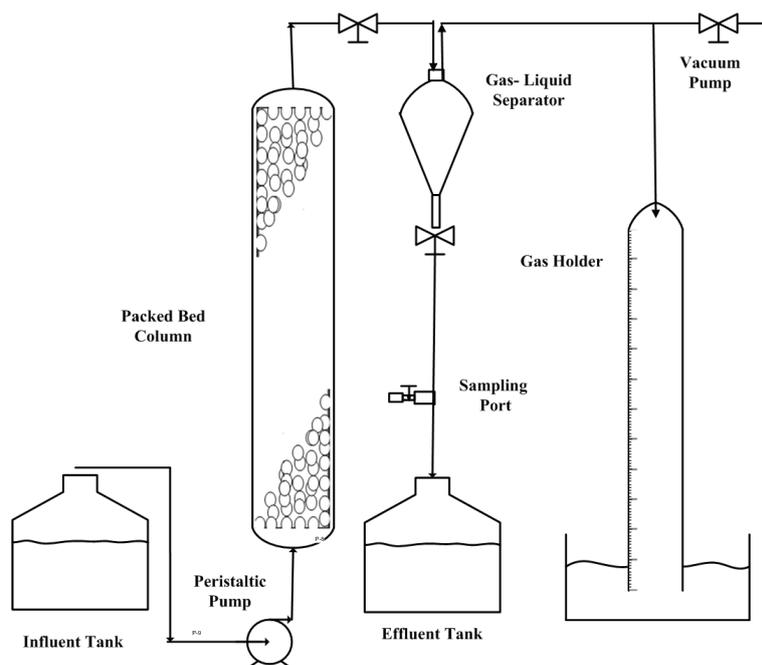


Figure 1. Schematic diagram of upflow anaerobic packed bed reactor.

liberate the generated biogas from the effluent, and then the gas was led to a gas collector tank. The gas tank was a cylindrical glass pipe with an internal diameter of 80 mm and height of 1 m. The liberated gas was frequently measured at constant HRT and the gas volume was recorded with respect to time. The volume of liberated gas was determined by the displacement of water in the gas tank. The UAPB reactor was operated for the startup period at 30 ± 1 °C for 150 days. The temperature was kept constant by a thermostat heating device. Synthetic phenol solution was continuously fed from the bottom using a peristaltic pump (SR25 adjustable flow rate, Thomas, Germany). The effluent samples were collected from the top of the column in a 20 l polyethylene container.

Preparation of seeding inoculum

The seed sludge was taken from The Pulp and Paper Factory, Sari, Iran. Acclimation of the sludge to phenol lasted 2 months. It was carried out in 250 ml Erlenmeyer flasks. The acclimation was started at a low phenol concentration (50 mg/l). The seed sludge was incubated at 30 °C under anaerobic condition. Upon complete removal of phenol within 10 days, the sludge was exposed to stepwise increased concentrations of phenol up to 500 mg/l to enrich phenol-degrading microorganisms. At the end of the acclimation period, the microorganisms were able to remove 93% of 500 mg/l phenol. The acclimated inoculum was used for further experiments in continuous operation.

Biofilm production

In order to establish a thick layer of biofilm on solid support material used as packing in UAPB, the reactor was operated for 20 days in full recirculation mode with a feed phenol concentration of 50 mg/l. At the end of this period a significant layer of biofilm was built on the polyethylene rings. The acclimated organisms were introduced once at startup. Fresh feed was added during recirculation.

Wastewater characteristics

The synthetic wastewater was prepared daily by diluting phenol and nutrient stock solutions with distilled water. Phenol served as the sole carbon source. The wastewater was supplemented with micro- and macro-nutrients as summarized in Table 1. In order to maintain the pH near neutral (6.9 ± 0.1), the wastewater was buffered with 500 mg/l NaHCO_3 . All chemicals used for the preparation of the feed solutions and sample analyses were supplied by Merck, Germany.

Table 1. Composition of bioreactor feed

Component	Concentration, mg/l
NH_4Cl	200
KCl	200
NaCl	200
$\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$	150
$\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$	100
K_2HPO_4	30
KH_2PO_4	20
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	10
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	5
$\text{NaMoO}_4 \cdot 2\text{H}_2\text{O}$	0.6
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	0.2
ZnCl_2	0.2
CuCl_2	0.2
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.2
H_3BO_3	0.2
$\text{CoCl}_2 \cdot 2\text{H}_2\text{O}$	0.2
Phenol	0-1000 ^a

^aAs indicated for respective experimental conditions

Analytical procedure

The chemical oxygen demand (COD) of the effluent was determined by the closed reflux method. Phenol analysis was carried out using 4-aminoantipyrene colorimetric method [37]. The volatile fatty acids (VFA) and total alkalinity (TA) were measured by direct titration [38]. The influent and effluent pH values were determined by a pH meter (HANNA, Germany). Gas production was measured by water displacement. The COD and phenol concentrations of the effluent were measured every other day, and the VFA and TA were determined every five days. Influent and effluent pH and the volume of gas produced by the reactor were measured daily.

Reactor operation

Phenol concentration in influent was stepwise increased from 100 to 1000 mg/l with an increment of 300 mg/l. The HRT was adjusted at 24 h. During stepwise increase of the phenol loading, sudden drops of the phenol removal efficiency occurred temporarily. The reactor was gradually able to achieve its stability. The performance of the reactor was closely monitored to check the steady state condition. The startup period lasted 150 days and was split into 4 phases. Phase 1 started from day 0 to day 10, where the phenol concentration and OLR were 100 mg/l and 0.25 g COD/l.d, respectively. In phase 2 (day 11 to day 35) the initial phenol concentration was increased to 400 mg/l (OLR of 1 g COD/l.d). In phase 3 the phenol concentration was kept constant at 700 mg/l (OLR of

1.75 g COD/l.d) for the next 50 days. In phase 4 of startup, the phenol concentration was increased to 1000 mg/l (OLR of 2.5 g COD/l.d) for 65 days. The UAPB bioreactor was able to reach steady state conditions in the last 25 days of operation with a phenol removal efficiency of 94%.

RESULTS AND DISCUSSION

Reactor performance

The UAPB bioreactor performance during startup was investigated for duration of 150 days. The obtained data are shown in Figure 2. During this period, the reactor was fed stepwise with increasing phenol concentrations as sole energy source ranging from 100 to 1000 mg/l.

Phase 1 of the startup operation was performed for 10 days. The initial phenol concentration and corresponding COD concentration were 100 and 250 mg/l, respectively. On the first day of operation the reactor was capable of degrading the supplied phenol concentration to 17 mg/l in the effluent. At day 10,

only 3.2 mg/l of phenol was observed in the effluent. At the end of phase 1, the reactor was able to produce more than 1.48 l/d biogas with a phenol removal efficiency and a corresponding COD removal efficiency of 96.8 and 80%, respectively.

Phase 2 (from day 10 to 35) of the startup period was performed with a phenol feed concentration of 400 mg/l. This phase lasted for 25 days. A slight decrease in phenol and COD removal efficiencies and biogas production indicated that the microorganisms could not degrade the increased phenol concentration. Although the microorganisms used throughout the study were well adapted to 500 mg/l phenol, the sudden increase of the initial phenol concentration resulted in an organic shock load. During the first day of phase 2, the COD and phenol removal efficiencies decreased drastically to 36 and 57.2%, respectively. At day 15 of the startup period, the biogas production rate was increased approximately two folds. On the 30th day of operation, the effluent phenol concentration reached 16 mg/l with COD and phenol removal efficiencies of 80 and 96%, respectively. At the end of

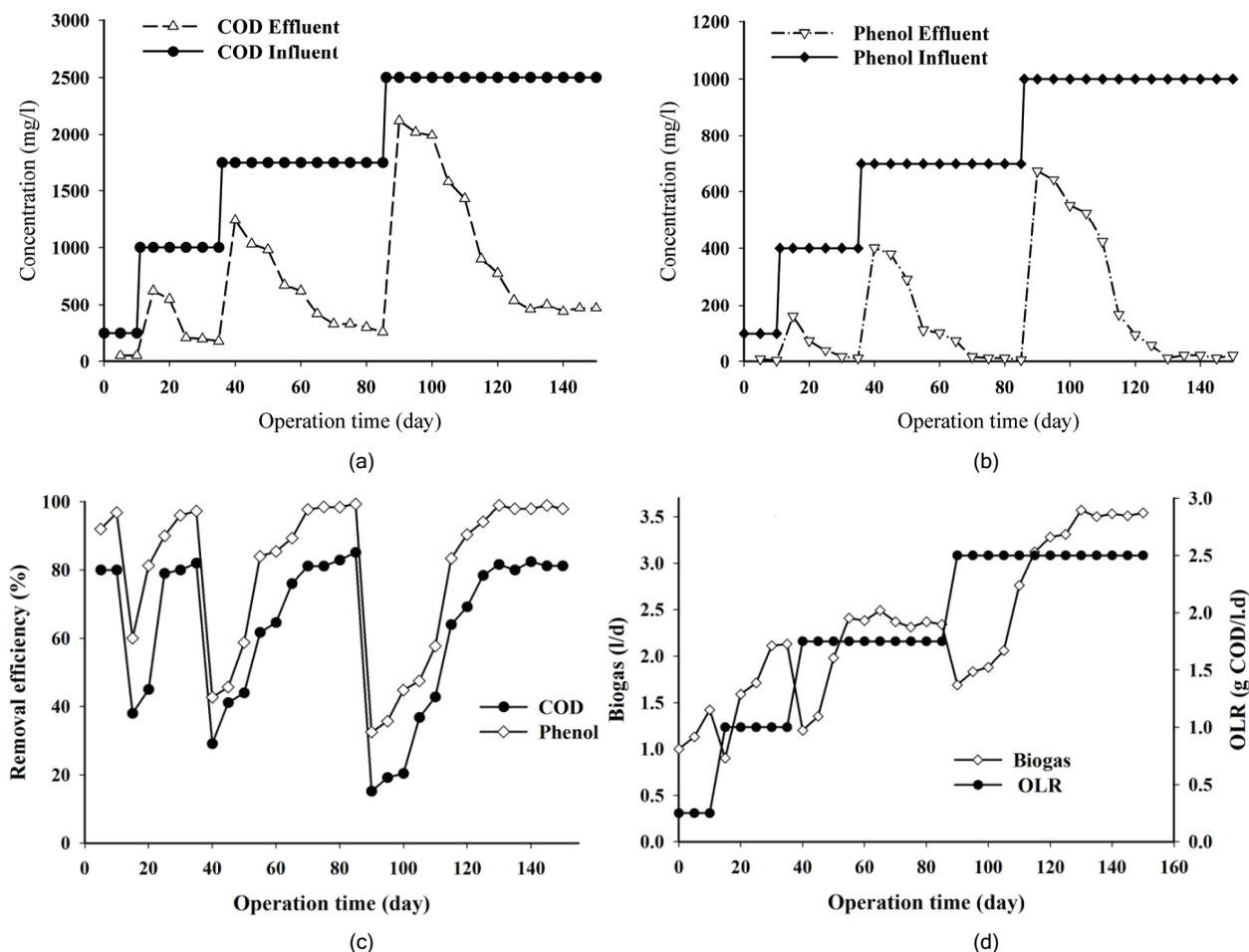


Figure 2. Performance of the UAPB bioreactor during the startup period of phenol degradation.

phase 2 the biogas production was maintained at 2.13 l/d. During phase 2, growth of a biofilm on the support material was noticed. Due to the acclimation of the microorganisms to phenol, the biofilm was rapidly growing. At the end of phase 2, a thick layer of biofilm was established on the packing bed.

Phase 3 of startup began on the 35th day with a phenol concentration of 700 mg/l. Due to the high concentration of phenol, the microorganisms in the reactor slowly acclimated and the degradation of phenol gradually increased day by day. In the first day of phase 3, the COD and phenol removal efficiencies dramatically decreased to 28 and 38.4%, respectively. The biogas production decreased to 1.2 l/d. The COD and phenol concentrations in the effluent on the 35th day were 1260 and 423 mg/l, respectively. The COD and phenol concentration on the 50th day decreased to 980 and 289 mg/l, respectively. After 5 days of operation the biogas production had recovered and the amount increased to 1.35 l/d. Within 15 days of operation in phase 3, the biogas production increased to 1.98 l/d. The reactor operation reached to steady state conditions on the 70th day with a phenol removal efficiency of 97.7% and biogas amount of 2.37 l/d. At the end of phase 3, the COD and phenol concentration in the effluent reached values of 260 and 5 mg/l with a removal efficiency of 85 and 99%, respectively.

Phase 4 of the startup period started at day 85. In this phase, the phenol concentration was stepwise increased from 700 to 1000 mg/l. An increase of the phenol concentration adversely affected on reactor

startup performance which had resulted in an increase of the phenol concentration in the effluent. The removal efficiency of phenol was sharply decreased to 30%. That decrease in phenol degradation was due to toxicity and inhibitory effect which was resulted from high phenol concentration. Thus, microorganisms required an adequate incubation time to adjust to high phenol concentrations of 1000 mg/l. Also, biogas production drastically dropped to 29% from 2.34 l/d on the 85th day to 1.69 l/d on the 90th day. The microorganisms were able to slightly recover and adapt to the high phenol concentration of 1000 mg/l within 25 days with COD and phenol removal efficiencies of 42.8 and 57.7%, respectively. On day 115, the phenol removal efficiency was sharply increased to 83.4% which corresponded to an effluent phenol concentration of 166 mg/l. The biogas production increased from 2.06 l/d on day 105 to 3.12 l/d on day 115. During the period of days 130-150, the biogas production and phenol removal efficiency remained constant at 3.5 l/d and 98%, respectively. During this period, a steady state condition was achieved with a COD removal efficiency of 82% and an effluent COD concentration of 465 mg/l. At the end of the startup period, the system was perfectly acclimated to high phenol concentrations. The reactor was effectively capable to handle high phenol concentration.

A comparison of phenol degradation reported in the literature with the results of the present work is summarized in Table 2. Ramakrishnan *et al.* [1] demonstrated that acclimation of sludge to 752 mg/l

Table 2. Comparison of phenolic compound biodegradation in different bioreactors

Phenolic compound	Influent			HRT h	Temp. °C	Type of bioreactor	Removal efficiency, %		Reference
	Phenolic compound, mg/l	Phenolic COD, mg/l	OLR, gCOD/l.d				Phenolic	COD	
Phenol m-, o-, p-Cresol 2,4-, 2,5-, 3,4- and 3,5-Dimethylphenol	490 123.0, 58.6, 42 6.3, 6.3, 4.4, 21.3	2240	2.24	24	27±5	UASB ^a	93±1	88±1	[1]
Phenol	630	1500	0.6-1.28	28-60	55	UASB	99	–	[2]
Phenol	50 to 1,200	–	–	12	30±1	HAIB ^b	99	98	[4]
Phenol	3764	9000	5.3	60	37±2	AFBR ^c	90	93.7	[26]
Pentachlorophenol	5 to 21	–	–	18	30±2	HAIB	100	77-100	[23]
Phenol	1600	2100	5.03	10.5	32±2	AFBR ^d	95	–	[5]
4-Chlorophenol	10-200	5000	2	60	20±2	UAFBR ^e	< 90	–	[39]
Catechol	100-1500	–	0.57-2.86	8	35±2	UASB	–	≥95	[13]
2-Nitrophenol	2-30	–	1.6-4.2	12-30	27±4	UASB	≥99	–	[40]
4-Nitrophenol 2,4-Dinitrophenol							≥99 89-96		
Phenol	500-1000	5000	1-2	12	9.5-15	EGSB ^f	99	90	[41]
Phenol	100-1000	250-2500	0.25-2.5	24	30±1	UAPB ^g	94	88	Present work

^aUpflow anaerobic sludge blanket; ^bhorizontal anaerobic immobilized bioreactor; ^canaerobic fixed bed reactor; ^danaerobic fluidized bed reactor; ^eupflow anaerobic fixed bed reactor; ^fexpanded granule Sludge bed; ^gupflow anaerobic packed bed

phenolic compounds in an UASB bioreactor was capable of reducing phenolic compounds to 55 mg/l with a corresponding COD removal efficiency of 88%. Similar results were obtained by Fang *et al.* in another UASB bioreactor [2]. At an influent phenol concentration of 630 mg/l and a HRT of 40 h, a removal efficiency of 99% was achieved. Biodegradation of phenolic compounds in other anaerobic bioreactors were also reported in literature by several investigators [1,13,23,39,40]. Fixed bed reactors were effectively capable of biodegradation of phenol. In an anaerobic fixed bed reactor, biodegradation of high concentrations of phenol was reported by Bajaj *et al.* [26]. At a phenol concentration of 3764 mg/l, corresponding to a COD of 9000 mg/l and at a HRT of 60 h, 90% of phenol was removed during 221 days of operation.

Removal of VFA

In the course of phenol biodegradation the variation of effluent VFA was monitored. Figure 3a shows phenol and VFA concentrations. After each stepwise increase of the phenol concentration, the VFA concentrations increased and later on decreased. This indicated that increased phenol concentrations were more toxic for acetogenic and methanogenic bacteria of the consortium than for the phenol degraders, which still produced VFA from phenol. The VFA varied between 34 and 64.7 mg/l. The maximum VFA concentration was achieved at day 95, due to the increase of the phenol concentration from 700 to 1000 mg/l. This indicated that syntrophic degradation of fatty acids by syntrophic interaction of acetogens and methanogens may become the rate limiting step in phenol degradation. The VFA/TA ratio during the entire operation was between 0.08 and 0.15. Chuo and Huang [8] reported that at high phenol concentration (2180 mg/l) in a UASB bioreactor, VFA values

were 1.8 and 82 mg/l at OLR of 7.9 and 13.8 kgCOD/ m^3 d, respectively.

Alkalinity and pH

During the startup period, 500-600 mg/l $NaHCO_3$ was added to provide a pH value between 6.9 ± 0.1 . Figure 3b shows the variations of alkalinity and pH in the UAPB bioreactor. The effluent pH varied between 6.9 and 7.0. The pH value below 6.5 and 7.1 may be harmful for methanogens [12]. The alkalinity during 150 days of startup period varied between 330 and 472 mg/l as $CaCO_3$. The pH and alkalinity was a favorable environment in UAPB bioreactor for methanogenesis. The addition of $NaHCO_3$ is essential for maintaining proper pH and alkalinity while treating phenol in the startup period. In anaerobic process, due to generation of organic acids and VFAs the pH of the reactor may drop to acidic condition.

CONCLUSIONS

An upflow anaerobic packed bed reactor was successfully capable of degrading high phenolic wastewater at HRT of 24 h and mesophilic (30 ± 1 °C) conditions. A stepwise increase in influent phenol concentration resulted in a short term dramatic decrease in the removal efficiency and biogas production. The microorganisms were, however, able to effectively acclimate to high phenol concentrations. At the end of the 150 days of the startup period, the removal efficiencies of phenol and COD with an influent phenol concentration of 1000 mg/l and a corresponding COD of 2500 mg/l were 98 and 82%, respectively. By stepwise increase of the phenol concentration, a sudden decrease in biogas production was observed. The maximum biogas production was achieved on 130th day, with a phenol concentration of 1000 mg/l and a corresponding COD concentration of 2500 mg/l. The

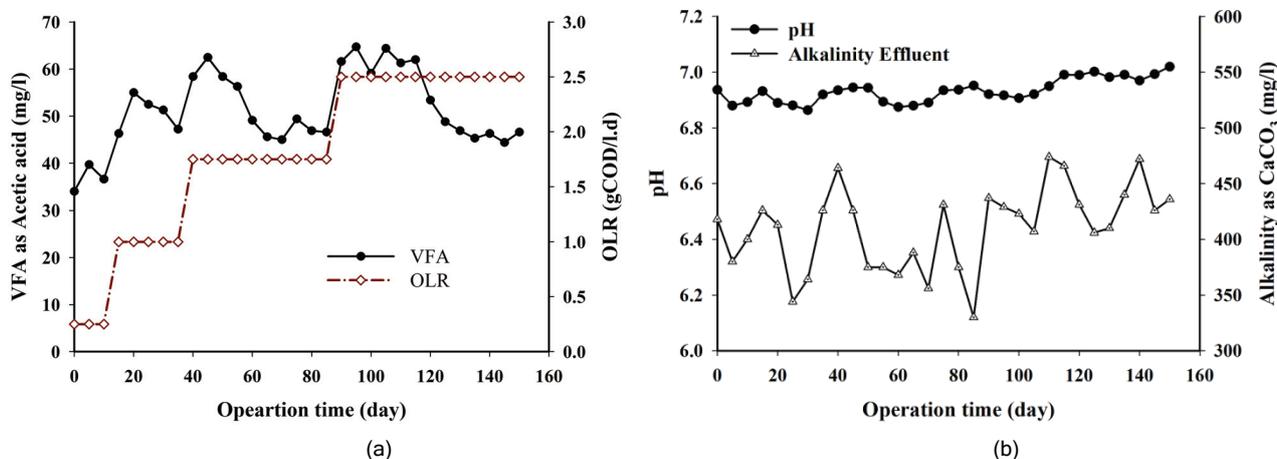


Figure 3. Variation of VFA, alkalinity and pH during the startup period in UAPB bioreactor.

variation of VFA concentrations in the reactor was between 34 and 64.7 mg/l during the entire startup period. The effluent pH varied between 7.0 and 6.8. In imbalanced anaerobic processes, the pH of the reactor may drop considerably due to accumulation of organic acids. Addition of NaHCO₃ may stabilize the pH of the media at neutral condition. Thus, the addition of NaHCO₃ or of other pH-stabilizing chemicals is essential for the control of pH and alkalinity in the reactor.

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NAUČNI RAD

OPORAVAK ANAEROBNIH REKATORA SA PAKOVANIM SLOJEM I VERTIKALNIM TOKOM OD VISOKOG SADRŽAJA ORGANIKA PRI POKRETANJU PROCESA TRETMANA OTPADNIH VODA KOJE SADRŽE FENOL

U radu je proučavana biodegradacija sintetičke otpadne vode sa fenolom u anaerobnom reaktoru sa pakovanim slojem i proticanjem naviše (UAPB). Reaktor je radio pri hidrauličom vremenu zadržavanja 24 h pod mezofilnim uslovima (30 ± 1 °C). Startovanje reaktora je trajalo 150 dana, podeljeno u četiri faze. Koncentracija fenola je stupnjevito povećavana. Koncentracije fenola u četiri faze je bila 100, 400, 700 i 1000 mg/l. U prvoj fazi, reaktor je dostigao stacionarno stanje osmog dana uz efikasnost izdvajanja fenola i brzinom produkcije biogasa 96,8% i 1,42 l/d, redom. Posle povećanja početne koncentracije fenola u drugoj fazi zapaženo je blago smanjenje efikasnosti izdvajanja fenola. Slični trendovi su uočeni u trećoj i četvrtoj fazi startovanja postrojenja. Zbog visoke koncentracije fenola, uočeno je oštro smanjenje efikasnosti izdvajanja i produkcije biogasa. Preživeli mikroorganizmi su se postepeno prilagođavali i aklimatizovali visokim koncentracijama fenola. Efikasnost izdvajanja fenola u stacionarnim uslovima treće i četvrte faze je bila 98,4 i 98%, redom. Maksimalna produkcija biogasa je zapažena stotridesetog dana sa vrednošću 3,57 l/d, što odgovara koncentraciji fenola 1000 mg/l.

Ključne reči: fenol; anaerobni pakovani sloj sa proticanjem naviše; biogas; biodegradacija; startovanje.