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## BIOLOGICAL TREATMENT OF PHENOLIC WASTEWATER IN AN ANAEROBIC CONTINUOUS STIRRED TANK REACTOR

*In the present study, an anaerobic continuous stirred tank reactor (ACSTR) with a consortium of mixed culture was operated continuously for a period of 110 days. The experiments were performed with three different hydraulic retention times and by varying initial phenol concentrations between 100 and 1000 mg/L. Maximum phenol removal was observed at a hydraulic retention time (HRT) of 4 days, with an organic loading rate (OLR) of 170.86 mg/Ld. At this condition, phenol removal rate of 89% was achieved. In addition, the chemical oxygen demand (COD) removal corresponds to phenol removal. Additional operating parameters such as pH, MLSS and biogas production rate of the effluents were also measured. The present study provides valuable information for the design an anaerobic ACSTR reactor for biodegradation of phenolic wastewater.*

*Keywords: phenol; biodegradation; anaerobic; mixed culture; ACSTR.*

Phenol is one of the precursors of the aromatic and toxic hazardous compounds produced by many industries and discharged to the aquatic environment. Factory processes in coke ovens, petroleum refining, resin and plastic, leather and textile manufacturing, foundry operations, pulp and paper plants, rubber reclamation plants, pharmaceuticals and agro-industrial operations emit high concentrations of phenol compounds [1,2].

Due to its toxicity and detrimental health effects, phenol has been included in the US Environmental Protection Agency (EPA) list of priority pollutants [3,4]. For these reasons, it is necessary to eliminate phenol from wastewater before it is discharged.

Wastewater with high concentrations of phenol can be treated mainly by either physico-chemical or conventional biological methods. Activated sludge with aerobic or anaerobic cultures is generally considered as one of the most attractive biological methods because of its various advantages [5–7]. The literature showed that some researchers expressed

reservations regarding the efficiency of the activated sludge process for phenol degradation. Prieto *et al.* [8] state that activated sludge usage did not appear to be entirely satisfactory for phenol removal because it has resulted in to secondary effluent problems. Similar observations were also reported by Banerjee [9]. Their studies conclude that, although phenols can be degraded in activated sludge processes, their presence in wastewaters increases the susceptibility of the biological system to upsets.

The anaerobic continuous stirred tank reactor (ACSTR) is a reactor configuration for activated sludge widely used in many industrial applications and wastewater treatment units. This type of reactor can address the above mentioned issues. In addition, it has a simple design and can be fabricated, operated easily and economically with catalyst charging and replacement. Furthermore, the well mixed nature of this type of reactor permits straightforward control over the temperature, pH of the reaction and the supply or removal of gases [10,11]. Hence, for processes involving substrate inhibition or product activation, ACSTRs are the most preferred reactors. They also have significant usage where the substrate stream contains an enzyme inhibitor, because of dilution within the reactor.

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Utilization of a mixed culture of organisms makes the activated sludge process more acceptable for the biodegradation of organic compounds [5,6,12]. The main advantage of a microbial consortium of activated sludge is microbial diversity and the interaction between all species in flocks [13]. In addition, the importance of mixed sludge usage is emphasized when the complete mineralization of toxic compounds is required [14].

Anaerobic processes degrade the complex organic matter into methane, CO<sub>2</sub> and H<sub>2</sub>O through three basic steps (hydrolysis, acidogenesis including acetogenesis and methanogenesis) in the absence of oxygen. The major advantages includes elimination of aeration costs, recovery of methane, and the production of significantly less excess biomass [15]. Since phenol is a recalcitrant compound, which causes toxicity to microorganisms during biological treatment. Therefore, anaerobic process may be a suitable technique for degrading of phenol as a sole substrate [16–19]. As a result, an anaerobic activated sludge can be appropriate candidate for degradation of phenol, because of its single basin and simplicity of the operation, relatively low cost, no sludge lost in the reaction period, better control of shock loads and does not need to return activated sludge [11,20].

The overall specific substrate removal rate can be estimated by the following relation [21]:

$$q_{dn} = \frac{S_0 - S_e}{X\theta} \quad (1)$$

where  $q_{dn}$  is the specific substrate removal rate of phenol (day<sup>-1</sup>);  $S_0$  influent concentration of phenol

(mg/L);  $S_e$  effluent concentration of phenol (mg/L);  $X$  volatile suspended solids concentration in reactor (mg/L);  $\theta$  reactor HRT (day).

The objective of this study was to evaluate the performance of ACSTR in biodegradation of phenol with a mixed culture at different operation parameters.

## MATERIALS AND METHODS

### Experimental set up

The schematic diagram of the experimental set up of anaerobic continuous stirred tank reactor (ACSTR) is shown in Figure 1. The ACSTR consisted of a cylindrical glass vessel with a working volume of 17 L, height of 600 mm and internal diameter of 194 mm. The influent was introduced at the bottom with the aid of a peristaltic pump (PreFluid™, model yz15) and effluent was collected from the top of the reactor. A 1000 mL funnel shaped gas separator was used to release the generated biogas from the effluent and then the gas was led to the gas collector tank. The gas tank was a cylindrical glass pipe with an internal diameter of 80 mm and height of 1 m. The generated gas was frequently measured for each HRT and the liberated gas volume was recorded with respect to time. The gas tank was initially filled with water which was saturated with biogas. The volume of liberated gas was demonstrated by the displacement of water in the gas tank.

### Microorganism and culture conditions

The mixed culture for the phenol degradation was obtained from the pulp and paper wastewater

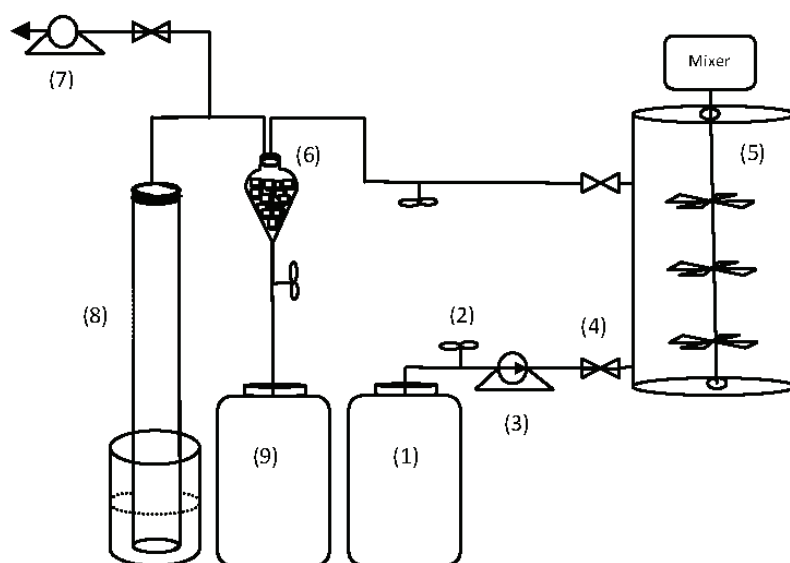


Figure 1. Schematic diagram of the ACSTR experimental set up; 1. feed tank; 2. liquid sampling port; 3. feed pump; 4. valve; 5. bioreactor; 6. gas-liquid separator; 7. vacuum pump; 8. biogas measurement tank; 9. effluent tank.

treatment plant, Sari, Iran. Phenol-degrading bacteria were adapted to phenol environment. Since phenol is an inhibitory substrate, this process required a lengthy start-up for the microbial acclimatization. In a 250 mL Erlenmeyer flask, the culture was acclimatized under anaerobic condition over a period of two-month. The media composition in the Erlenmeyer flasks (grams per liter) was 1.5 glucose, 0.8 yeast, 0.35  $\text{KH}_2\text{PO}_4$ , 0.55  $\text{K}_2\text{HPO}_4$ , 1 mL synthetic medium. The composition of the synthetic medium is given in Table 1. At first, glucose was used as a carbon source with initial concentration of 1.5 g/L; then glucose was gradually replaced by phenol up to concentration of 800 mg/L. Here, phenol was consumed as sole carbon source in the media. After preparation of the acclimated culture to phenol concentration of 800 mg/L, the bioreactor start-up was initially batch-wise for a defined period, as the volume of culture in the bioreactor was gradually increased to a defined. Once the culture volume was estimated to be sufficient based on turbidity, continuous operation was started. The bacterial activity in ACSTR was assessed as explained in the start-up section.

Table 1. Composition of synthetic medium

Component	Concentration, mg/L
$\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$	6
$\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$	5
$\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$	0.1
$\text{CuSO}_4 \cdot 2\text{H}_2\text{O}$	0.01
$\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$	1
$\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$	0.88
$\text{MnCl}_2 \cdot 4\text{H}_2\text{O}$	0.5
$\text{H}_3\text{BO}_3$	0.1

#### Experimental procedure and analytical methods

Phenol concentration was determined by a direct photometric method using 4-amino antipyrine [22]. Before quantifying phenol, samples were centrifuged at 6000g for 10 min. Biomass concentration in the samples was monitored by measuring its absorbance at 600 nm wavelength using a spectrophotometer (Unico, 2100 series, USA). According to the standard calibration curve, the cell dry weight concentration was also determined based on turbidity of the media by light absorbance as a function of cell dry weight. Based on standard methods for the examination of water and wastewater, a colorimetric method using closed reflux system was used for COD measurements [22]. The absorbance of COD samples was measured using the spectrophotometer at a wavelength of 600 nm. A gas tank for collecting lib-

erated biogas was fabricated and connected to the bioreactor. Daily gas production was measured and recorded. The liberated gas was able to replace water; then displacement of water volume was recorded with respect to time. The pH of the effluent was measured by a pH meter (Hanna Model 21, Italy). The temperature in all experiments was maintained at  $22 \pm 1$  °C.

## RESULT AND DISCUSSION

### Start-up and ACSTR operation

By gradual increasing the volume of culture in the reactor, the bacterial activity was assessed. Three concentrations of phenol (100, 200 and 400 mg/L) were examined. The HRT was set at 5 days. The variations of phenol and COD are shown in Figure 2. The first sample analysis after start-up showed that on the 5<sup>th</sup> day, with influent phenol concentration of 100 mg/L, only 67% of phenol was removed. The removal reached 100% on the 7<sup>th</sup> day of operation. The next sample was withdrawn on the 13<sup>th</sup> day at phenol concentration of 200 mg/L, which corresponds to COD of 1753 mg/L. Here, the phenol removal initially was 38% and then reached 76.5%. By increasing the phenol concentration to 400 mg/L, the removal efficiency sharply increased from 26.25% on the 22<sup>th</sup> day to 69.25% on the 27<sup>th</sup> day. This improvement in removal efficiency showed that the biomass in the reactor was active and the next set of experiments could be proceeded with. In Figure 2a, at any concentration of COD, the effluent COD initially increases, then decreases. Also, as the COD concentration in influent at the beginning increased, the effluent COD showed an increasing trend that was due to the dilution rate before the reactor reached a steady state condition. The system reached steady state after 5 days of continuous operation, after which the microorganisms gradually acclimates with the new set of phenol concentration and more phenol was consumed, which means that more COD was utilized.

### Biodegradation characteristics under various hydraulic loading rates

After completion of the acclimation period, the ACSTR was ready for the treatment of phenolic wastewater in a continuous mode operation. The experiments were operated with three different hydraulic retention times for phenol concentrations ranging from 100 to 1000 mg/L. When the system reached steady state condition at any designated HRT, the samples were withdrawn. The experimental results at each HRT are shown in Figures 3-5.

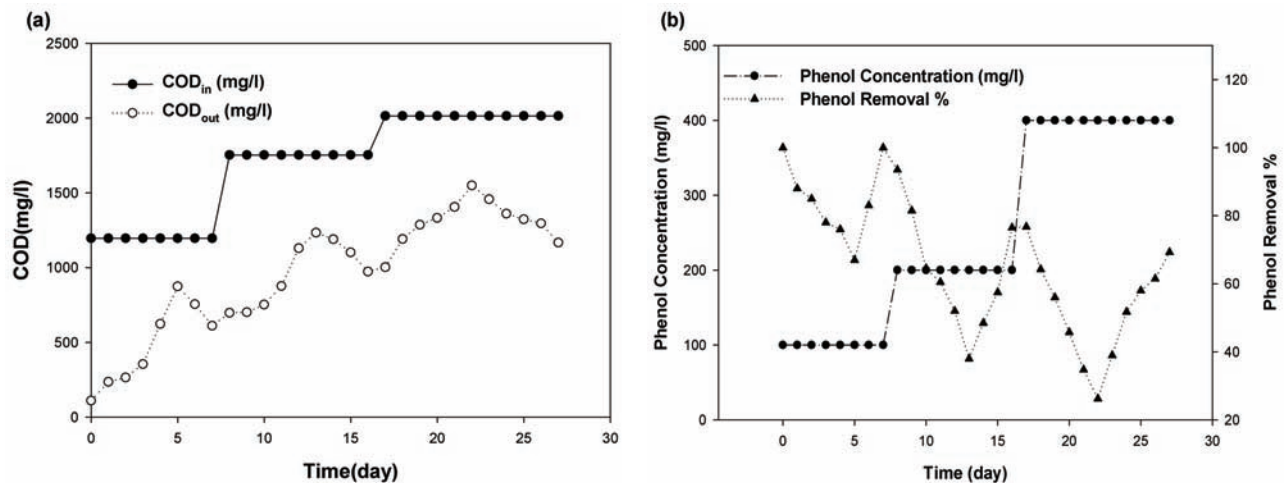


Figure 2. a) Variations of COD in the start-up period and b) variations of phenol removal efficiency in the start-up period.

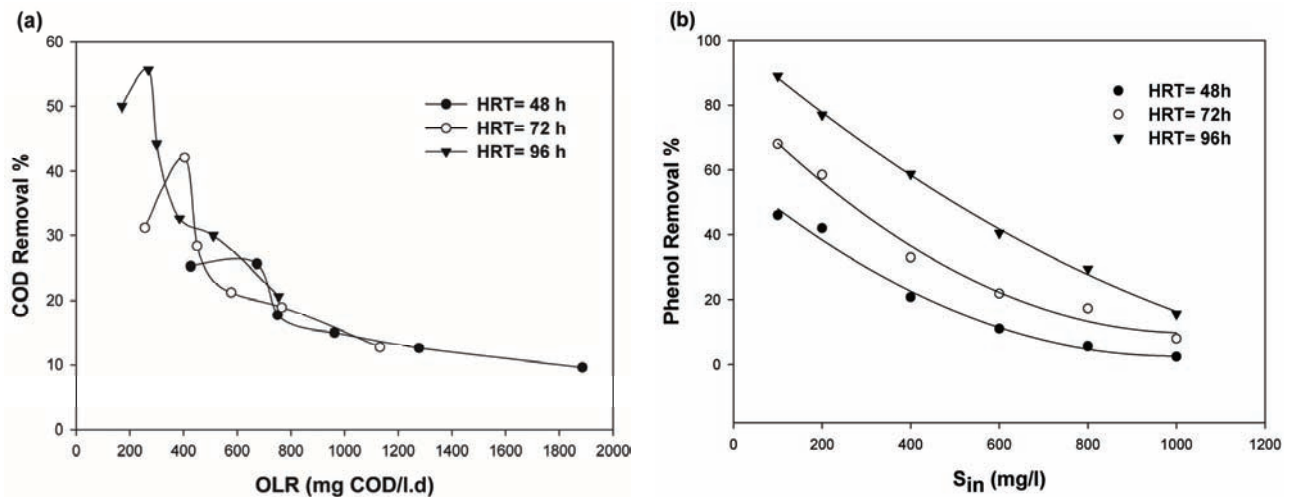


Figure 3. a) COD removal efficiency with respect to OLR at several HRTs and b) phenol removal efficiency with respect to different initial concentration of phenol at several HRTs.

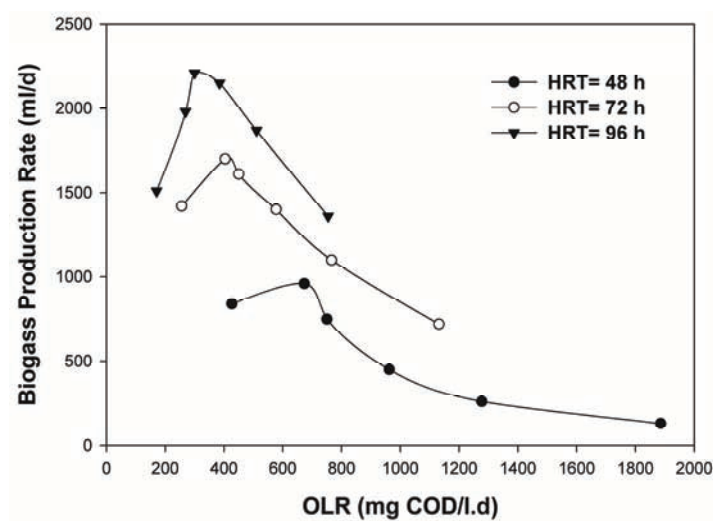


Figure 4. Biogas production rate with respect to OLR at several HRTs.

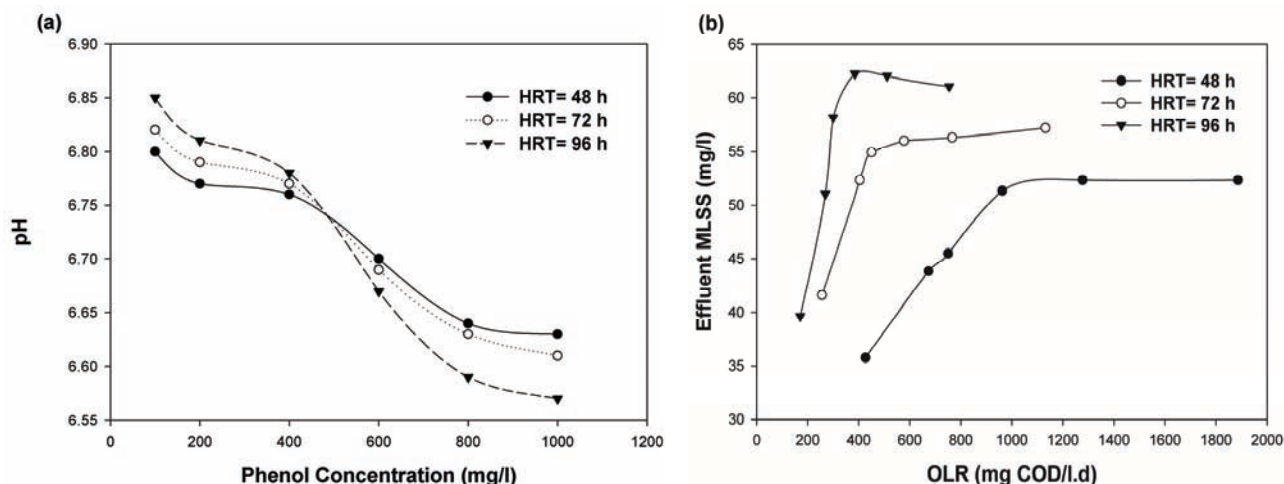


Figure 5. a) Variations of pH with respect to different initial concentration of phenol at several HRTs and b) effluent MLSS with respect to OLR at several HRTs.

Figure 3a depicts the COD removal efficiency with respect to OLR. It was observed that COD removal efficiency first increased with increasing OLR and then reached maximum value. A decrease trend appeared at a point when further increase in OLR was applied. Therefore, an optimum OLR was obtained for the selected HRT. When HRT was reduced from 96 to 48 h, the maximum COD removal efficiency dropped drastically from 55.6 to 25.6%.

Figure 3b shows the phenol removal efficiency with respect to influent phenol concentration. It was found that at any HRT an increase in influent phenol concentration resulted in decrease in phenol removal efficiency, and with increasing HRT, the phenol removal efficiency was increased. Therefore, maximum phenol removal efficiency occurred at HRT of 96 h for low inlet phenol concentration ( $S_{in}$ ) of 100 mg/L. Since the phenol concentration was low and HRT was long enough, then the culture utilized 89% of phenol in the media. Similar results were reported by Fang *et al.* [1] during the degradation of phenolic wastewater.

Phenol bioconversion under anaerobic condition is defined by the following equation [21]:



According to the above equation, some portion of the substrate is converted to biogas in anaerobic processes. In the experiments, the amount of biogas was measured by a biogas collecting column. The biogas production rates from phenol with respect to OLR are presented in Figure 4. It was understood that by increasing OLR, the biogas production rates were initially increased to defined values, and then a decrease appeared for further increase in OLR. An in-

crease in biogas production rates caused increase in HRT for all concentrations of phenol. For phenol concentration of 100 mg/L, in HRTs of 48 and 96 h, the obtained biogas production rates were 840 and 1510 mL/d, respectively. However, at high HRT, the maximum biogas production rate occurred at high concentration. The maximum biogas production rate at HRT of 72 and 96 h happened with phenol concentration of 200 and 400 mg/L, respectively. The experimental results reveal that the maximum biogas production rate obtained for phenol concentration at HRTs of 96, 72 and 48 h were 2210, 1700 and 960 mL/d, respectively.

With phenol concentrations of 100 to 400 mg/L, the biogas production rates reached the highest value. According to Figure 5a, variation of pH with respect to initial phenol concentrations were insignificant. The variations of pH remained in favorable working range ( $7 \pm 0.43$ ) for the anaerobic conditions. The obtained data showed that the pH increased with an increase of HRT at low concentration of phenol (100 to 400 mg/L). These slight changes were confirmed when the running system reached steady state. These results indicated that the concentration of phenol enhanced the anaerobic process. Therefore, an improvement of the methanogenic stage occurred when carbon source was induced. The obtained data could be explained by neutralization of hydrogen ions released from volatile fatty acid together with carbonates dissociated from carbonic acid and bicarbonate alkalinity inside the reactor [23]. When the concentration of phenol was increased to a value greater than 600 mg/L, the pH of the system slightly decreased. Use of high phenol concentration at low HRT in an anaerobic process resulted in upsetting the

balance between acid formation and methane production.

Variations of MLSS in the CSTR effluent with respect to OLR are shown in Figure 5b. As HRT increased from 48 to 96 h, MLSS initially had increasing trends; as OLR gradually increased, the concentration of MLSS reached a steady level with fixed value. At phenol concentration of 100 mg/L, an increase of HRTs from 48 to 72 h, MLSS initially increased and then decreased for HRT of 96 h. But at high HRT, the system was able to easily utilize OLR, as MLSS had increasing trends. This trend was observed at low concentration of phenol. However, at high concentration of phenol, MLSS had increasing trends. At low concentration of phenol, the maximum removal of COD was obtained at HRT of 48 h, which was due to sufficient MLSS; but, at high concentration of phenol, the maximum COD removal occurred only at high HRT.

Table 2 summarizes specific substrate removal rate ( $q_{dn}$ ) decreased with an increase in HRT at low

## CONCLUSION

Phenol was effectively removed in the presented adapted ACSTR. The mixed culture, adapted to high phenol concentrations for a long duration, was quite effective in the elimination of phenol at continuous operation. At low phenol concentrations (100 mg/L), the maximum removal efficiency at HRT of 96 h was achieved. The removal efficiency of phenol at HRT of 96 h was 89%. As HRT decreased, the COD removal efficiency dropped. The highest COD removal obtained at three hydraulic retention times was 55.6% at OLR = 269.4 mg COD/L.d. Other parameters, such as pH, MLSS and biogas production rate of the effluents were measured. The experimental results depict that the mixed culture was able to remove phenol at high concentration. Also, the ACSTR was demonstrated to be a robust and promising process for effectively treatment of wastewaters containing inhibitors or recalcitrant compounds in industrial effluents.

Table 2. Performance of ACSTR with different phenol concentrations and HRT

$S_0$ / mg L <sup>-1</sup>	$S_e$ / mg L <sup>-1</sup>	HRT = 2 days		$S_e$ / mg L <sup>-1</sup>	HRT = 3 days		$S_e$ / mg L <sup>-1</sup>	HRT = 4 days	
		$X$ / mg L <sup>-1</sup>	$q$ / h <sup>-1</sup>		$X$ / mg L <sup>-1</sup>	$q$ / h <sup>-1</sup>		$X$ / mg L <sup>-1</sup>	$q_{dn}$ / h <sup>-1</sup>
100	54	35.74	0.0268	32	41.59	0.0227	11	39.64	0.0233
200	116	43.83	0.0399	83	52.31	0.0310	46	51.04	0.0314
400	317	45.49	0.038	268	54.94	0.0333	165	58.15	0.0420
600	534	51.33	0.0267	469	56.01	0.0324	357	62.25	0.0406
800	755	52.31	0.0179	662	56.30	0.034	564	62.05	0.0396
1000	976	52.31	0.0095	921	57.18	0.0191	844	61.08	0.0266

concentrations of phenol, ranging from 100 to 400 mg/L. Increasing the phenol concentration above 600 mg/L resulted in an increase in the specific substrate removal rate. In anaerobic process, high phenol concentration may have required longer period of time for biodegradation. Therefore, the specific substrate removal rate increased. In anaerobic conditions, the specific substrate removal rate was in the range of reported values. Similar work was conducted by Husain and Lin [24,25]. However, their reported values were much lower than the reported values in aerobic processes [21,26]. It has been reported in literature that the factors affecting phenol biodegradation may be related to the bioreactor, adaptation of predominant microorganisms as inoculums, environment and growth condition tolerance of culture to high organic loading rate [27].

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NAUČNI RAD

## BIOLOŠKA OBRADA FENOLNIH OTPADNIH VODA U ANAEROBNOM PROTOČNOM REAKTORU SA MEŠANJEM

*U sprovedenom istraživanju je anaerobni protočni reaktor sa mešanjem sa konzorcijom mešane culture radio neprekidno 110 dana. Eksperimenti su izvedeni sa tri različita hidraulička vremena zadržavanja i variranjem početne koncentracije fenola u opsegu od 100 do 1000 mg/dm<sup>3</sup>. Maksimalno izdvajanje fenola od 89% je postignuto pri hidrauličkom vremenu zadržavanja od 4 dana i sa organskim opterećenjem od 170,86 mg/dm<sup>3</sup>d. Smanjenje hemijske potrošnje kiseonika odgovara izdvajanju fenola. Mereni su i drugi operativni parametri procesirane vode (pH, suspendovane čvrste čestice i brzina produkcije biogasa). Ovo istraživanje daje korisne informacije za projektovanje anaerobnog protočnog reaktora sa mešanjem za biorazgradnju fenolne otpadne vode.*

*Ključne reči: fenol; biodegradacija; anaerobni; mešana kultura; anaerobni protočni reaktor sa mešanjem*