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The anaerobic treatment of pharmaceutical industry wastewater in an anaerobic batch and upflow packed-bed reactor

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ABSTRACT

The pharmaceutical industry constitutes an important place in terms of health and environment, both in our country and throughout the world. Various complex organic chemicals are present in the wastewater generated by pharmaceutical industries. The accumulation of toxic and persistent organic substances in wastewater is a serious problem for the environment. Therefore, it is important that pharmaceutical industry wastewater is treated before being discharged into receiving waters. Today, anaerobic treatment systems are commonly used for wastewater-containing high organic matter. The first stage of this study statistically investigates the optimization of anaerobic treatment conditions of pharmaceutical industry wastewater in a batch study. In the second stage, continuous treatment processes were planned using data obtained as a result of the batch study. For processing, an upflow anaerobic packed-bed reactor was used for treating substrate mixtures containing 10–100% pharmaceutical industry wastewater. The effects of operating parameters on the chemical oxygen demand (COD) removal efficiency and the methane production rate were evaluated. COD removal efficiencies of 93–97% were obtained for the pharmaceutical industry wastewater using a 2.5–4 d hydraulic retention time and a 0.6–2.2 g COD d^{-1} organic loading rate. The overall results suggested that the mixed bacterial and archaeal biomass was able to efficiently treat pharmaceutical industry wastewater under determined anaerobic conditions.

Keywords: Pharmaceutical industry wastewater; Anaerobic batch treatment; Full factorial design; Upflow anaerobic packed-bed reactor

1. Introduction

The pharmaceutical production industry manufactures a wide range of products for healthy living. Production can be characterized by five main stages: fermentation, extraction, chemical synthesis, formulation, and packaging. All five stages may produce air emissions, liquid effluents, and solid wastes. Liquid effluents resulting from equipment cleaning after batch operation include toxic organic wastes. The

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composition of the effluents varies depending on production, the materials used in the process, and other production details. Generally, pharmaceutical wastewater (PW) is characterized by the presence of toxic chemicals and high chemical oxygen demand (COD) concentration [1].

In parallel with the growth of the health sector, the pharmaceutical industry and its wastewater has been increasing, making it one of the main sources of serious pollution problems worldwide. In particular, this wastewater is potentially risky to the aquatic environment. Consequently, it is considerably important to treat PW before discharging it into the environment.

Wastewater from the pharmaceutical industry is generally treated using physical, chemical, and biological treatments (anaerobic and aerobic processes). Although PW may include resistant organic substances that cannot be easily degraded, biological methods are still an unavailable choice for treatment. Due to the high strength of the organic substances, it is impossible to treat some PW using aerobic biological processes. Instead, an anaerobic process is preferred to degrade high-strength organic substances.

Anaerobic treatment methods are suitable for high-strength wastewater, while the aerobic oxidation of organic matter would result in high energy consumption and the generation of huge quantities of sludge. The most important advantages of anaerobic processes are the ability to degrade high-strength wastes, low nutrient requirement, low-energy input, low sludge output, low-operating expense, low-area demand, and net benefit of energy (biogas) production [2–4]. Recently, anaerobic treatment methods have been used for PW treatment including strong waste effluents [5,6].

This study first statistically investigates the optimization of pharmaceutical industry wastewater treatment in a batch study under anaerobic conditions. The continuous treatment study was started according to the batch reactor results. In the continuous process, both the anaerobic treatability and methane production potential of pharmaceutical industry wastewater were researched in an upflow anaerobic packed-bed (UAPB) reactor.

2. Materials and methods

2.1. Pharmaceutical wastewater

PW was acquired from a drug production plant located near the city of Istanbul, Turkey. The collected PW was stored at −20 ℃ during the research. The plant produces different drug groups. According to the information provided by the plant officials, the wastewater contains ethyl alcohol, components of vitamin B, active ingredients of the cephalosporin group, propylene glycol, carbopol, microcrystalline cellulose, magnesium stearate, talc, and starch. The COD of the wastewater sample from the plant was 6,100 mg ml^{-1} and the pH was 6.9.

2.2. Anaerobic seed sludge

A mixed mesophilic nongranular sludge provided by the department of anaerobic treatment at the Eskisehir Municipal Wastewater Treatment Plant in Turkey was used as the inoculum in the batch and UAPB reactors. The important parameters of the sludge were measured, including pH (7.3), total solids (TS) (46 g l^{-1}), suspended solids (SS) (34 g l^{-1}), and volatile suspended solids (VSS) (14 g l^{-1}).

2.3. Basal medium

The composition of the basal medium (BM) used in the experiments was as follows (concentrations of the constituents are given in parentheses as mg l^{-1}): NH_4Cl (1,200), $MgSO_4.7H_2O$ (400), KCl (400), $Na_2S.9H_2O$ (300), $CaCl_2.2H_2O$ (50), $(NH_4)_2HPO_4$ (80), $FeCl_2.4H_2O$ (40), $CoCl_2.6H_2O$ (10), KI (10), $MnCl_2.4H_2O$ (0.5), $CuCl_2.2H_2O$(0.5), $ZnCl_2$ (0.5), $AlCl_3.6H_2O$ (0.5), $NaMoO_4.2H_2O$ (0.5), H_3BO_3 (0.5), $NiCl_2.6H_2O$ (0.5), $NaWO_4.2H_2O$ (0.5), Na_2SeO_3 (0.5), and cysteine (10) [7,8]. The BM was necessary for anaerobic microbial growth because it contains micro and macronutrients.

2.4. Experimental design

Factorial designs are usually used in experiments containing several factors where it is necessary to study the joint effect of the factors on a response [9]. The 3^3 factorial design was used to minimize the number of experiments. The matching of three factors and their three different levels produced 27 different experiments. In this study, the effects of three factors, BM rates, wastewater concentrations, and types of co-substrate, on COD removal were investigated. For this aim, three levels of each BM, three concentrations of wastewater, and three types of co-substrate were used. All experiments were performed in duplicate. The variable levels noted in the experiments are given in Table 1.

The model for a 3^3 factorial (three factors each at three levels) is as follows:

$$y = \beta_0 + \beta_1 x_1 + \beta_2 x_2 + \beta_3 x_3 + \beta_{12} x_1 x_2 + \beta_{13} x_1 x_3 + \beta_{23} x_2 x_3 + \beta_{123} x_1 x_2 x_3 + \varepsilon$$

Table 1
The independent variables and their levels for the experimental design

	Levels		
Variables	1	0	−1
X_1_Basal medium (%)	15	10	5
X_2_Wastewater (%)	75	50	25
X_3_Cosubstrat type	Acid mix	Glucose	Propionic acid

Because each factor occurs at only three levels, we can include only linear terms in the main effects ($ß_1x_1$, $ß_2x_2$, and $ß_3x_3$ are called the main effects of each factor). The terms $ß_{12}x_1x_2$, $ß_{13}x_1x_2$, $ß_{13}x_1x_3$, and $ß_{23}x_2x_3$ give a measure of interactions of the variables taken in pairs. The term $ß_{123}x_1x_2x_3$ is a three-way interaction [10,11]. Experimental results were analyzed using the SPSS package software.

2.5. The batch reactor experiments (biochemical methane potential; BMP)

The batch reactor experiments for optimization were performed in a 250 ml glass vessel with 200 ml (OxiTop® Control AN12, WTW, Weilheim, Germany) and designed according to Table 2. The effect of operating conditions, such as BM (5–10–15%), wastewater concentrations (25–50–75%), co-substrates (acetic-propionic-butyric acid mixture (ABP) (respectively 1,500, 350, 350 mg l^{-1}), glucose (2000 mg l^{-1}), and propionic acid (1,000 mg l^{-1})) on treatment efficiency was determined by applying an experimental design. The combined effects of these factors were optimized using a factorial design of experiments for COD removal. To ensure pH stability and to remove dissolved oxygen, $NaHCO_3$ and $Na_2S·9H_2O$ were added, respectively. The final pH was adjusted to 7 ± 0.2. Before start up, the glass vessel was purged with N_2 gas for 4–5 min to provide the suitable anaerobic conditions. The glasses were incubated at 35 ± 2 ℃ for a period of 13 d.

Table 2
Full factorial (3^3) experimental design and results of COD removal

Experiments	Basal medium (%)	Wastewater (%)	Co-substrate	COD removal (%)
1	15	75	ABP	29.44
2	15	75	Glucose	14.07
3	15	75	Propionicacid	26.52
4	15	50	ABP	30.53
5	15	50	Glucose	16.00
6	15	50	Propionicacid	80.69
7	15	25	ABP	93.49
8	15	25	Glucose	37.38
9	15	25	Propionicacid	90.20
10	10	75	ABP	28.11
11	10	75	Glucose	15.15
12	10	75	Propionicacid	37.38
13	10	50	ABP	83.46
14	10	50	Glucose	20.76
15	10	50	Propionicacid	30.20
16	10	25	ABP	89.94
17	10	25	Glucose	13.02
18	10	25	Propionicacid	72.00
19	5	75	ABP	27.08
20	5	75	Glucose	15.78
21	5	75	Propionicacid	26.20
22	5	50	ABP	80.29
23	5	50	Glucose	32.55
24	5	50	Propionicacid	44.62
25	5	25	ABP	94.38
26	5	25	Glucose	17.72
27	5	25	Propionicacid	80.67

2.6. The continuous reactor

The continuous reactor process was performed using the UAPB reactor (Fig. 1). Both of the reactors had a total liquid volume of 5.0 l and were filled up to 4.3 l of their volume with plastic balls. A jagged surface was achieved to maintain the bacterial biomass. The temperature of the reactors was maintained at mesophilic conditions through the use of electrical heating mats and feeding reactors were provided with peristaltic pump. The fixed amount of sludge (inoculum) was added to the reactor.

2.7. Operational conditions

In previous experiments, batch reactor experiments were studied to determine the anaerobic biodegradability of the PW and the continuous reactor studies, which were planned using data obtained as a result of a batch study, were started. Two and a half liters of nongranular anaerobic sludge were put into the reactor and were fed with the feed solution. The biofilm formation in the reactor was achieved within the 35-d period. Varying concentrations of pharmaceutical industry wastewater (between 10 and 100%) were used in this continuous reactor. The organic loading rate (OLR) performed on the reactor ranged from 0.61 to 2.244 g COD l^{-1} d^{-1}, while the hydraulic retention time (HRT) was studied at 96–60 h.

2.8. Analytical methods

COD, TS, SS, VSS, alkalinity, and volatile fatty acids (VFA) were determined by standard methods [12]. The volume of methane produced was measured daily by the liquid displacement method after removing CO_2 by adsorption into the KOH solution [13]. The toxicity level was also measured with a Microtox analyzer (Microtox® Model 500 analyser).

3. Results and discussion

3.1. Batch reactor

This statistical study was preferred based on a factorial experimental design that would allow us to infer the effect of the variables with a relatively few number of experiments. The independent variables of the experimental design are shown in Table 1. BM and wastewater concentrations received three values: a high value (shown by the plus sign), a medium value (shown by the zero sign), and a low value (shown by the minus sign). The co-substrates had three types: ABP (shown by the plus sign), glucose (shown by the zero sign), and propionic acid (shown by the minus sign).

A full 3^3 experimental setup, which required 27 different experiments, was used in this experimental design. In this experimental factorial design (3^3), the

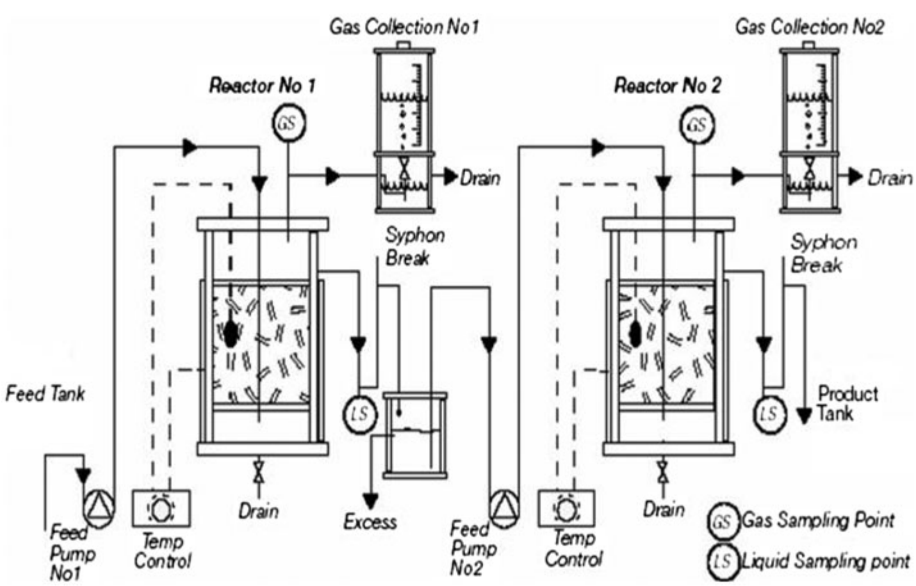

Fig. 1. Design of UAPB reactor (Armfield W8).

effects of BM, wastewater concentration, and types of co-substrate were investigated with an anaerobic batch reactor. In addition, Table 2 shows the results obtained in terms of % COD removal. The maximum COD removal was found in experiment 25, where low BM, wastewater concentration, and the acid mixture co-substrate were used.

The estimation of the average effect and the main effects (the effect of each individual variable) on the response and the two higher order interactions were calculated using statistical package SPSS. The results are indicated in Tables 3 and 4 for COD removal.

The results show that wastewater concentration, co-substrate type, and the three-way interaction have a significant effect on COD removal, while BM concentration was determined to be statistically insignificant (Table 3). According to the variance test analysis, the interaction between the BM concentration and co-substrate types and the interaction between the wastewater concentration and co-substrate types also had significant effects on COD removal ($p < 0.05$).

A general F test was performed to determine significant differences of each experiment. However, the test did not give information about which tests caused the differences. A two- and three-way comparison should be made among the levels of each independent variable that are statistically significant. The Tukey test was used for this aim.

Table 3
Variance analysis for COD removal

Source of variation	Type III sum of squares	df	Mean square	F	Sig.
Corrected model	45969.788[a]	26	1768.069	13.629	.000*
Intercept	111623.395	1	111623.395	860.416	.000*
Basal	122.962	2	61.481	.474	.628
Wastewater	15165.682	2	7582.841	58.450	.000*
Co-substrate	17659.762	2	8829.881	68.063	.000*
Basal–wastewater	985.515	4	246.379	1.899	.140
Basal–co-substrate	2293.123	4	573.281	4.419	.007*
Wastewater–co-substrate	5432.023	4	1358.006	10.468	.000*
Basal–wastewater–-co-substrate	4310.719	8	538.840	4.153	.002*
Error	3502.760	27	129.732		
Total	161095.943	54			
Corrected total	49472.548	53			

[a]R Squared = .929 (Adjusted R Squared = .861)
*p < 0.05.

Table 4
Multiple comparison results for COD removal

					95% Confidence interval	
(*I*) Wastewater	(*J*) Wastewater	Mean difference (I−J)	Std. error	Sig.	Lower bound	Upper bound
%25	%50	18.8550*	3.79666	.000	9.4415	28.2685
	%75	41.0056*	3.79666	.000	31.5920	50.4191
%50	%25	−18.8550*	3.79666	.000	−28.2685	−9.4415
	%75	22.1506*	3.79666	.000	12.7370	31.5641
%75	%25	−41.0056*	3.79666	.000	−50.4191	−31.5920
	%50	−22.1506*	3.79666	.000	−31.5641	−12.7370
(*I*) Co-substrate	(*J*) Co-substrate					
Propionic acid	Glucose	34.0033*	3.79666	.000	24.5898	43,4168
	ABP	−7.5844	3.79666	.132	−16.9980	1.8291
Glucose	Propionic acid	−34.0033*	3.79666	.000	−43.4168	−24.5898
	ABP	−41.5878*	3.79666	.000	−51.0013	−32.1743
ABP	Propionic acid	7.5844	3.79666	.132	−1.8291	16.9980
	Glucose	41.5878*	3.79666	.000	32.1743	51.0013

*Significant.

First, the interactions between different levels of BM, wastewater, and co-substrate types, which had a significant effect on COD removal, were researched. Multiple comparison results for COD removal are indicated in Table 4. According to the results, COD removal was enhanced by 22.15%, on average, when the wastewater concentration decreased from 75% (high level) to 50% (middle level). Decreasing the wastewater concentration from 75 to 25% (low level) enhanced COD removal by 41%, on average. All of these differences were statistically significant. Consequently, the highest COD removal was found when the lowest concentration of wastewater was used.

The presence of a co-substrate is important for enhanced COD removal. In this study, we used three types of co-substrate (an ABP, glucose, and propionic acid). Table 4 shows the multiple comparisons for COD removal; when we used propionic acid instead of glucose as a co-substrate, the COD removal increased by 34%, on average. Similarly, using the ABP instead of glucose enhanced COD removal by 41.58%, on average. As a result, the highest COD removal was found when the acid mixture was used as a co-substrate, and this result was statistically significant.

Finally, the two- and three-way interaction effects, which are significant, need to be studied. The two-way interactions of the BM with the co-substrate and wastewater with the co-substrate, which have a significant effect on COD removal, are indicated in Figs. 2 and 3. The profile diagram for the BM and co-substrate (Fig. 2) shows that the best COD removal occurred when using 5 or 10% BM and the ABP. Similarly, in Fig. 3 (a profile diagram of wastewater and co-substrate), the best COD removal occurred when using 25% wastewater and the ABP.

The three-way interaction, which is also significant, is shown in Fig. 4. According to the profile diagram, COD removal was best when using 25% wastewater and the ABP. The difference between the levels of BM was determined to be statistically insignificant. As a result, adding an acid mixture as a co-substrate was important in the batch reactor. The continuous reactor studies were designed according to the batch study results.

3.2. *Upflow anaerobic packed-bed reactor*

After the batch reactor finished, the continuous reactor study was started. The UAPB reactor was used as the continuous reactor and was operated for 280 d. In the reactor, an acid mixture was used as the co-substrate in accordance with the batch reactor results. The reactor was fed with feed solution for anaerobic micro-organisms for 35 d before the pharmaceutical industry wastewater was added to the reactor. After activation of the micro-organisms, the reactor was put into operation with 10% wastewater. The wastewater concentration was increased by 10% at each stage of operation as the experiment continued. The redox potential of wastewater was measured after 70% concentration and resulted in observations between −350

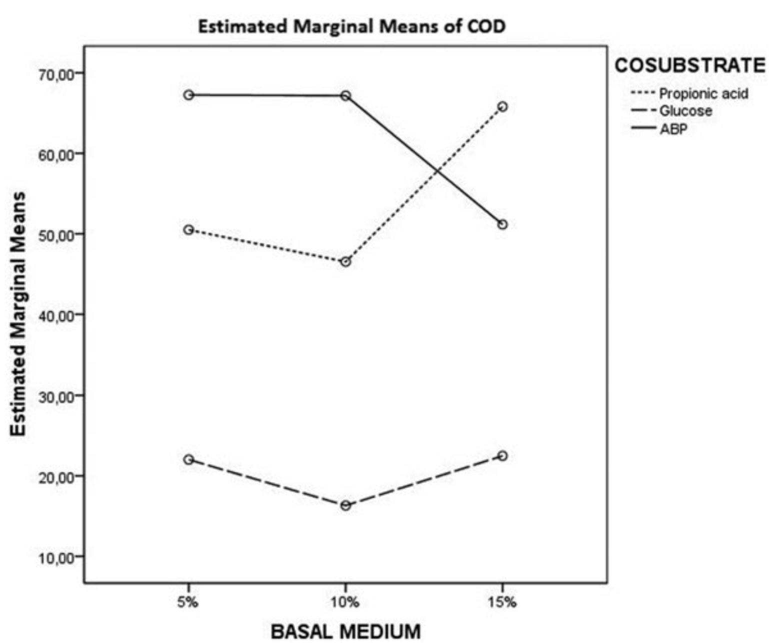

Fig. 2. Profile diagram for co-substrate and basal medium.

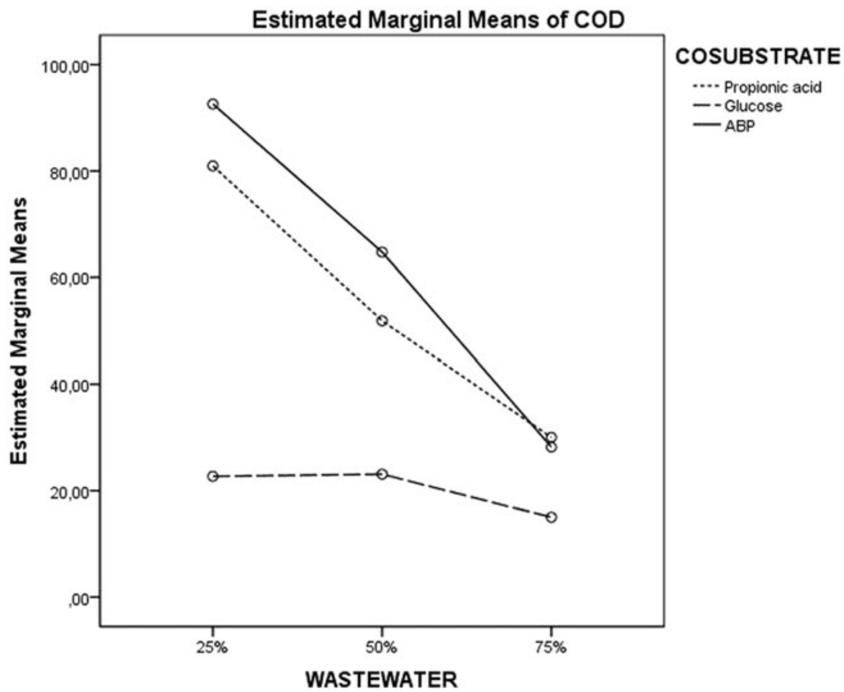

Fig. 3. Profile diagram for co-substrate and wastewater interaction.

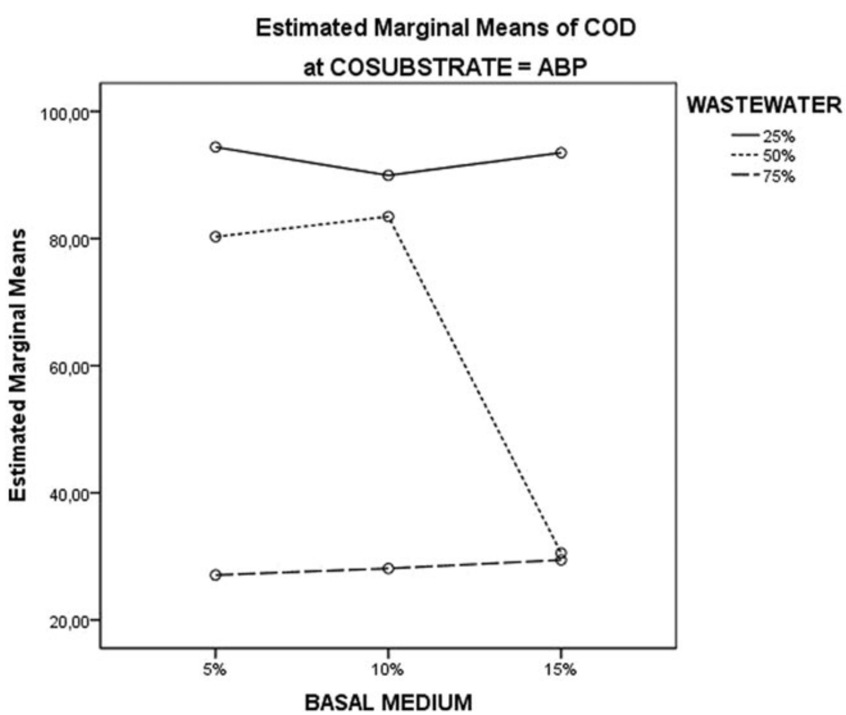

Fig. 4. Profile diagram for the three-way interaction.

Table 5
The results of UABP reactor under different operation conditions

Influent parameters							Effluent parameters							
Day	Wastewater concentration (%)	HRT	COD	pH	OLR	Alkalinity	COD	pH	Gas production	Alkalinity	VFA	COD removal (%)	VFA– alkalinity
1–35	–	96	2,500	7.1	0.625	4,000	160	8.0	0.75	1,500	50	93.60	0.033
36–48	10	96	2,450	7.1	0.612	4,000	135	8.1	0.77	1,480	46	94.50	0.031
49–72	20	96	3,050	7.1	0.763	4,000	132	8.2	0.70	1,480	53	95.70	0.036
73–92	30	96	3,550	7.1	0.888	3,000	125	8.0	0.65	1,550	51	96.50	0.033
93–108	40	96	4,040	7.1	1.010	3,000	130	8.1	0.85	1,575	44	96.80	0.028
109–124	50	96	4,420	7.1	1.105	3,000	132	8.0	0.95	1,590	42	97.00	0.026
125–140	60	96	4,700	7.1	1.175	3,000	120	8.0	1.00	1,550	46	97.40	0.030
141–156	70	96	4,960	7.1	1.240	3,000	130	8.0	1.00	1,600	59	97.38	0.037
157–172	80	96	5,165	7.1	1.291	3,000	138	7.9	1.00	1,640	61	97.33	0.037
173–192	90	96	5,370	7.0	1.342	3,000	141	7.9	1.10	1,570	57	97.37	0.036
193–212	100	96	5,610	7.0	1.402	3,000	132	7.9	1.10	1,510	50	97.65	0.033
213–242	100	84	5,610	7.0	1.603	3,000	140	8.0	0.90	1,530	52	97.50	0.034
243–257	100	72	5,610	7.1	1.870	3,000	142	8.0	0.80	1,510	50	97.47	0.033
257–280	100	60	5,610	7.1	2.244	3,000	225	8.0	0.60	1,510	53	95.99	0.035

Note: HRT (h); COD (mg l^{-1}); OLR (g COD l^{-1} d^{-1}); Alkalinity (mg $CaCO_3$ l^{-1}); VFA (mg l^{-1}); Gas Production l CH_4 l^{-1} d^{-1}.

and −415 mV. Bernet et al. studied the combined anaerobic–aerobic SBR for the treatment of piggery wastewater. According to their results, the redox potential (from −350 to −112 mV) was measured during filling with nitrified effluent [14].

All reactor operating conditions and the input–output values are shown in Table 5. As shown in Table 5, COD removal increased from 93.60 to 97.65% during the reactor stage, while the OLR increased from 0.6 to 1.4 g COD per day; wastewater concentration increased from 10% to 100% and HRT was 96 h. When the OLR increased from 1.4 to 2.2 g COD per day and the HRT decreased from 96 to 60 h, COD removal decreased to 95%. Buitron et al. researched the performance of a sequencing batch biofilter combining anaerobic–aerobic conditions in one tank to treat PW. According to the combined system results, 95–97% COD removal was determined at a HRT of 8–24 h and an OLR of 4.6–5.7 kg COD $m^{-3} d^{-1}$ [15]. In the other study, Ince et al. researched the performance of an upflow anaerobic filter-treating chemical synthesis-based PW and found 65% COD removal [16]. Other researchers studied the treatment of PW containing macrolide antibiotics in an upflow anaerobic stage

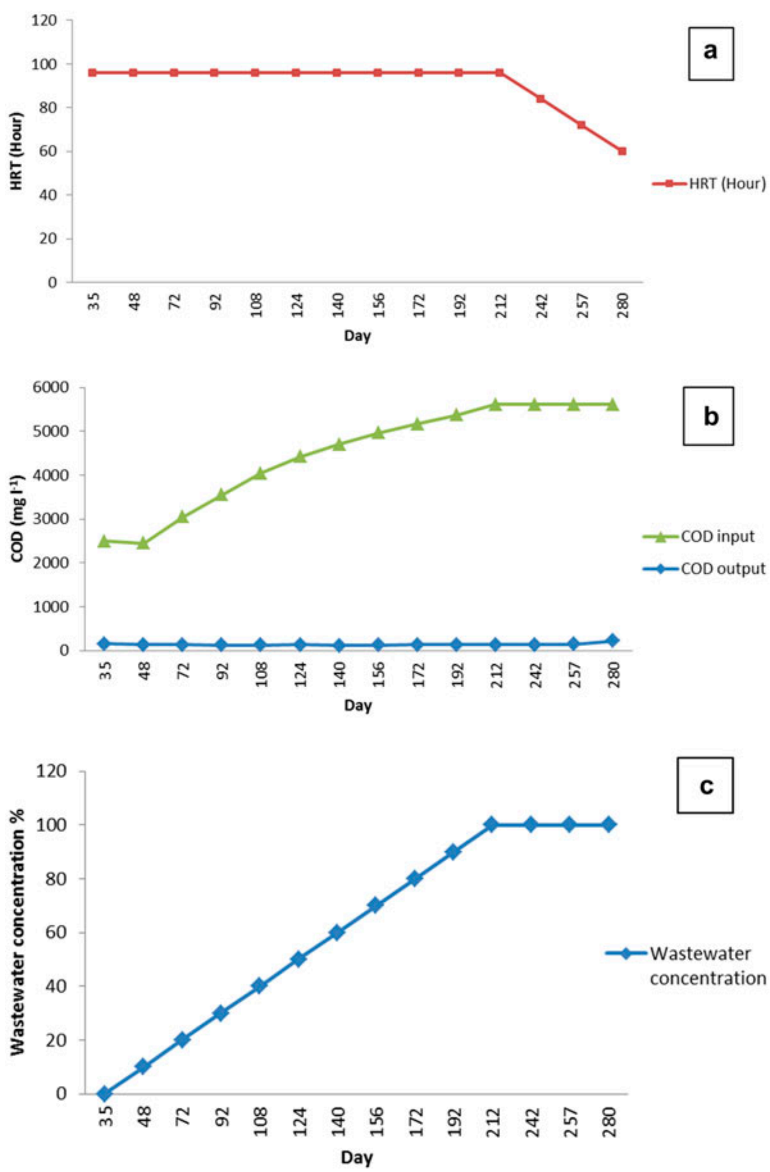

Fig. 5. The exchange of certain parameters in the UAPB reactor process: (a) HRT; (b) input and output COD concentrations; and (c) wastewater concentration.

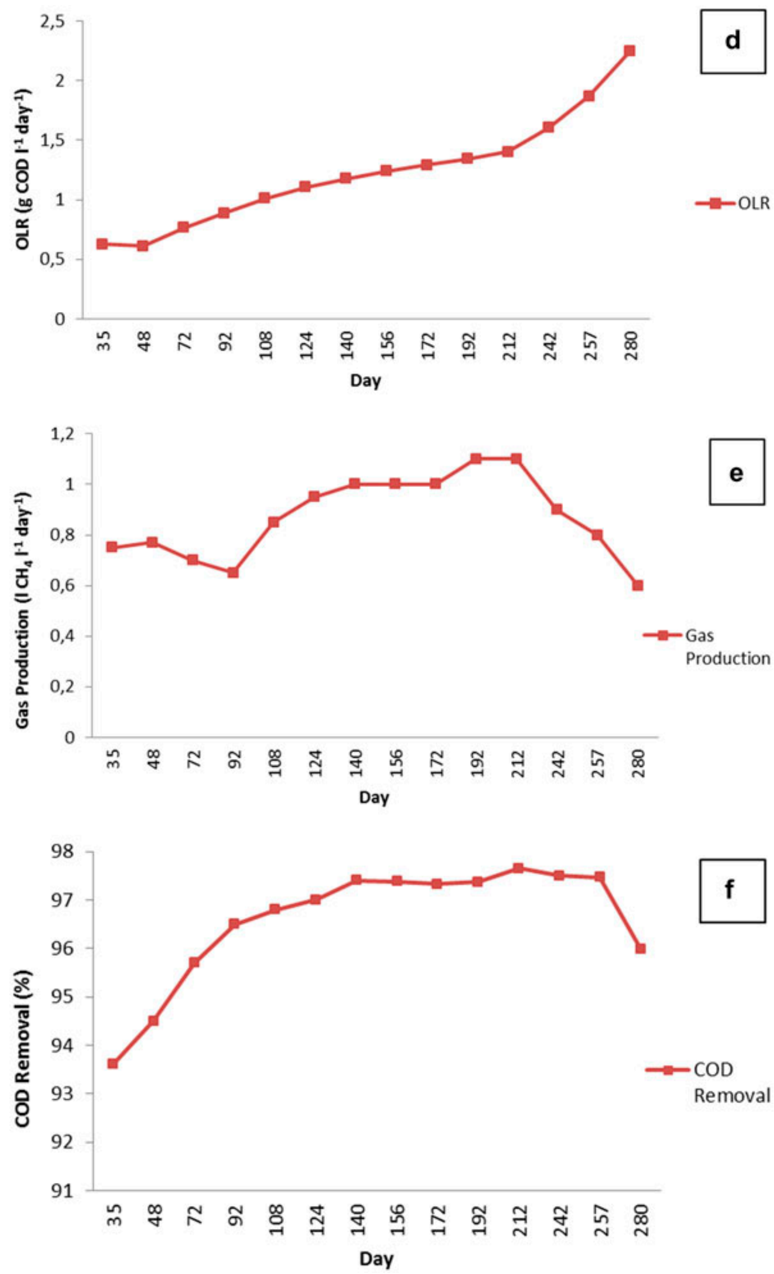

Fig. 6. The exchange of certain parameters in the UAPB reactor process: (d) OLR; (e) gas production; and (f) COD removal.

reactor. According to the results, the upflow anaerobic stage reactor consistently achieved a COD removal of 70–75% that would be a pretreatment system for this wastewater [17].

Figs. 5 and 6 outline the HRT, wastewater concentrations, performed OLRs, input and output COD concentrations, methane production rates, and COD removal. The pH values were measured as almost constant (between 7.9 and 8.2) during the reactor operation time (Table 5). The stable value could be ascribed to carbonate–bicarbonate buffering. When the amount of alkalinity is above 1,000 mg l^{-1}, the reactor system has sufficient buffer capacity. The alkalinity of the input wastewater was very low, and, for this reason, 3 g l^{-1} $NaHCO_3$ was added to the wastewater. The addition of $NaHCO_3$ protects against potential acidification of the reactor, giving a pH that is equally optimal for methanogens. The high pH level and the

Table 6
Toxicity results

	5 min results (% concentration)	15 min results (% concentration)
Influent wastewater	20	18
Effluent (After treatment)	Nontoxic	Nontoxic

buffering capacity are a warranty against an acidification of the reactor that could be caused by an abrupt overloading.

The VFA–alkalinity ratio can be used as a measure of process stability; when this ratio is less than 0.3–0.4, the operation is considered to be operating appropriately without the risk of acidification [18]. As shown in Table 5, the ratio values were lower than the recommended limit value in all operating processes. The toxicity level was also measured with the Microtox analyzer. According to the toxicity analysis of the treated water, it was determined to be nontoxic. The result is shown in Table 6. In a similar study, a Microtox model 500 was used. According to the results of this study, continuous anaerobic wastewater treatment operation could very well indicate a higher methanogenic activity and COD reduction (%), despite their inability to degrade the refractory organic fraction—in the case of a toxic compound—in anti-osmotic pharmaceutical effluents [19]. The amount of volumetric methane gas generation rates for each OLR studied in the UAPB reactor is also given in Table 5. The methane performance in the UAPB reactor was 0.6–1.1 $CH_4\ l^{-1}\ d^{-1}$. The efficiency of methane gas in the UAPB reactor increased linearly with increasing COD removal.

4. Conclusion

According to the results, the COD removal efficiency was observed at 93–97%, and the effluent COD level was below the discharge limits. Alkalinity, nutrient, and BM additions are important for the anaerobic removal of pharmaceutical industry wastewater. According to the performance of the UAPB reactor, archaeal and bacterial biomass were able to efficiently treat a pharmaceutical industry wastewater under anaerobic conditions.

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