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Research Article

Considering an Up-Flow Anaerobic Sludge Blanket for the Treatment of Spearmint Essential Oil Wastewater and Biogas Production

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ABSTRACT

The aromatic and dark-colored spearmint essential oil wastewater (SEOW) generally contains a large amount of organic matter, including chemical oxygen demand (COD), phenolic compounds, and inorganic contents. In this study, the pollutant removal performance and biogas production rate of an up-flow anaerobic sludge blanket (UASB) reactor used for the treatment of SEOW were investigated. During the 102 days UASB operation at hydraulic retention time of 60 hours, the organic loading rate (OLR) was increased from 0.14 to 2.69 kg COD/m^3.d by increasing the influent SEOW concentration. With increasing OLR from 0.14 to 2.69 kg COD/m^3.d, the concentrations of COD and phenol in the influent of the UASB reactor increased to 6720±383 mg/L and 383±88 mg/L, respectively. At OLR equal to 2.69 kg COD/m^3.d, the steady-state average removal efficiencies of COD and phenol were 72.0±1.4 and 63.1±6.7 %, respectively. The stability of the anaerobic system was confirmed by the average steady-state ratios of the volatile fatty acid/alkalinity and pH in the UASB reactor, which were less than 0.4 and 7.5±0.1, respectively, at different OLRs. The optimum OLR was found to be 2.69 kg COD/m^3.d, where 26.9±1.7 L/d production of biogas containing 63.0±5.2 % and 22.4±4.2 % methane and carbon dioxide, respectively, was obtained. Moreover, at OLR equal to 2.69 kg COD/m^3.d, the biogas yield and net heating value were 462.2±46.9 L/kg COD$_{removed}$ and 24.7±5.2 MJ/m^3, respectively. The results of the current study reveal the substantial potential of the UASB reactor in terms of pollutant removal performance and biogas production for the treatment of SEOW.

1. INTRODUCTION

Consumers' increasing interest in natural resources and the increasing concern about harmful synthetic additives have led to an increase in essential oil consumption. Spearmint oil is an essential oil that is extracted from the flowering tops of *Mentha* spicata and is widely used in the production of chewing gum, toothpaste, mouthwash, and certain pharmaceutical products. The main chemical components of the spearmint essential oil are carvone and limonene [1]. When spearmint essential oil is extracted from spearmint through the process of extraction and steam distillation, a large amount of wastewater is produced and, subsequently, released into rivers and streams. The aromatic and dark-colored spearmint essential oil wastewater (SEOW) generally contains a large amount of organic matter, including large quantities of oil and grease, chemical oxygen demand (COD), phenolic compounds, and inorganic contents such as including sulfates and phosphates. Recently, the improper disposal of SEOW has led to the pollution of soil, waterways, and underground water resources. In this respect, the disposal of SEOW is usually subject to strict standards. Therefore, the development of appropriate physiological or biological methods for the treatment of SEOW is essential.

Anaerobic digestion is considered to be an attractive sustainable environmental technology for the simultaneous treatment of industrial wastewater and renewable energy production. Anaerobic systems provide a valuable possibility for the safe treatment of high-strength wastewater principally because of their advantages, such as lower energy demand, less sludge generation, and economically valuable end-product (methane/biogas) [2]. Furthermore, biogas, the final product of microbial fermentation (product of methanogens via archaea metabolism), can be produced by an anaerobic process as an energy source. Biogas consists of CH_4 and CO_2 whose calorific value is determined by the quantity of CH_4 in biogas [2,3,4]. The up-flow anaerobic sludge blanket (UASB) reactors are widely used for the treatment of industrial wastewater [5,6,7,8]. In this respect, numerous studies have been conducted on biogas production from various types of industrial wastewater using UASB reactors [2,3,9]. Oktem et al. [6] studied the treatment of chemical, synthetic-based pharmaceutical wastewater in a hybrid UASB and obtained a COD removal efficiency of 72 % with an organic loading rate (OLR) of 8 kg COD/m^3 d. Artsupho et al. investigated the removal rate of volatile fatty acid (VFA) and COD from sugar industrial wastewater in an UASB reactor. The results showed that the VFA and COD removal efficiency was about 92 % with a temperature between 29 and 40 ºC [7]. Shi et al. [10] in treating pharmaceutical wastewater with high total dissolved solids (TDSs) and COD content at OLR of 4.11 to 11.26 kg COD/m^3.d in an UASB reactor reported a COD removal efficiency of 41.3 % at OLR of 8.11 kg COD/m^3.d. Li et al. [5] also studied the treatment of high-strength pharmaceutical wastewater in the presence of rich organic sulfur compounds and sulfate in an UASB reactor and reported a COD removal efficiency of about 70 % and biogas production containing 63 % methane at an optimized OLR of 8 kg COD/m^3.d.

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However, to the best knowledge of the authors, there is no reported study on the treatment of SEOW, especially by using UASB reactor. The aim of this study is to investigate the possibility of treating SEOW using an UASB reactor during 102 days of reactor operation at varying OLRs in the range of 0.14-2.69 kg COD/m^3.d. Biogas production and composition (CH_4, CO_2, and H_2S) in different operational conditions are also examined.

2. MATERIALS AND METHODS

2.1. Wastewater characterization

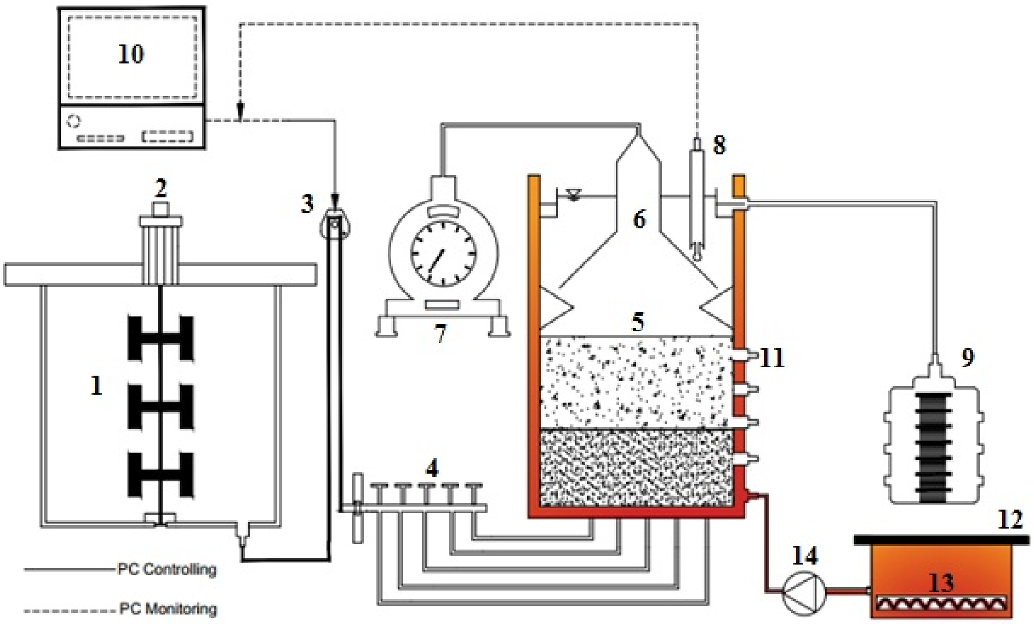
The simulated SEOW used in this study took into account the process of extraction and steam distillation used to produce it at the local plant (Golkaran Agro-Industry Company, Kashan, Iran). The simulated SEOW was prepared weekly and stored at 4 °C. All samples were equilibrated to room temperature before feeding. The analysis of the influent SEOW was carried out in triplicate, and the average composition is shown in Table 1.

Table 1. Physicochemical characteristics of spearmint essential oil wastewater.

Parameter	Concentration
pH (-)	6.5±0.6
Turbidity (NTU)	336 ±23
Conductivity (μS cm^{-1})	3758±223
Salinity (mg L^{-1})	2000±100
TDS (mg L^{-1})	1936±90
COD (mg L^{-1})	6720±383
Phenol (mg L^{-1})	383±88

2.2. Experimental setup and operating conditions

A laboratory-scale, 30 L UASB reactor (Figure 1) was used in this study with 28 L working volume. The UASB reactor was designed and constructed using Plexiglas, with a 0.20 × 0.20 m^2 cross-section and 0.70 m height in the reaction chamber. There was a gas-liquid-solid separator device in the upper part of the reactor that collects the biogas generated within the UASB under all operation conditions. The temperature was electrically controlled and the UASB operated under mesophilic conditions (33±2 °C). The UASB was seeded to 30 % (v/v) of its working volume with an anaerobic granular sludge (40000 mg MLSS/L) obtained from a full-scale UASB reactor treating high-strength milk processing wastewater (Pegah Hamedan Dairy Company, Iran). The SEOW was fed into the influent distribution line of the reactor at its lower zone by a peristaltic pump to ensure the homogeneous distribution of flow into the reactor (Cased 200 Series Stepper Motor Pump, Williamson Manufacturing Company, United Kingdom). Finally, the gravity discharged the effluent from the effluent port on the top of the reactor. No recirculation of effluent was practiced. In order to adapt the granules to SEOW, the concentration of wastewater increased stepwise. To prepare the appropriate concentration of COD, raw wastewater was diluted by tap water. The UASB system operated in 4 phases over a period of 102 days at high sludge retention time and hydraulic retention time of 60 h. During this period, OLR was increased by increasing the feed COD, as described in Table 2. The COD/N/P ratio of the medium was adjusted to approximately 250/5/1 by incorporating appropriate concentrations of NH_4Cl and KH_2PO_4 in the simulated SEOW. The initial pH of the solution was adjusted to values between 7.0 and 7.5 with 1 M NaOH.



1	Feed tank	8	pH meter
2	Mixer	9	Effluent tank
3	Peristaltic pump	10	Computer
4	Flow divider	11	Sampling port
5	UASB reactor	12	Water heating tank
6	GLS	13	Heater with thermostat
7	Wet gas meter	14	Heating water circulation pump

Figure 1. Schematic diagram of the UASB experiment setup.

Table 2. The value of operating parameters during the UASB reactor operation.

Phase	1	2	3	4
Time (d)	1-15	16-40	41-58	59-102
COD (mg/L)	341±155	859±347	3756±727	6720±383
Phenol (mg/L)	9±7	19±4	487±81	383±88
OLR (kg COD/m^3.d)	0.14	0.34	1.50	2.69

2.3. Chemicals and analytical methods

All chemicals were the products of Merck Company (Merck, Germany) with analytical grades. The COD of the samples of the influent and effluent was determined according to closed reflux, colorimetric method (5220D) of APHA Standard Methods [11]. Mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) were determined according to 2540D and 2540E of APHA Standard Methods, respectively [11]. PH, TDS, conductivity, and salinity were measured with a Hach apparatus (Hach, HQ 40D). The turbidity of the feed and effluent samples was measured by a portable turbidity meter (Hach model 2100N). The total phenolic content was determined by Folin-Ciocalteu reagent according to the method of Slinkard and Singleton [12]. All analysis was performed in triplicate. A wet-test gas meter was also used to measure biogas volume. The biogas composition analysis (i.e., measuring CH_4, CO_2, and H_2S) was done by the gas analyzer (OPTIMA 7 Biogas analyzer, MRU Instrument, U.S.A, 2015).

2.4. Statistical analysis

One-way ANOVA analysis (at $p<0.05$) was used to determine the steady-state conditions based on the COD removal efficiency and biogas production rate during each phase of the UASB operation. The statistically significant linear correlation ($p<0.05$) between the changes of different parameters during the UASB operation was identified using univariate linear correlation analysis [13]. All analysis was performed using Minitab version 16 (Minitab Inc., State College, PA, USA).

3. RESULTS AND DISCUSSION

3.1. MLSS and MLVSS

In the start-up phase of the UASB reactor, maintaining an appropriate balance among several groups of microorganisms is essential. The microorganisms are very diverse depending on their favorable growth environment [14]. In order to shorten the start-up phase in the present study, the granular sludge collected from the UASB reactor of the wastewater treatment plant of Pegah Hamedan dairy factory was used for inoculation in the UASB reactor. The variations of MLSS, MLVSS, and the MLVSS/MLSS ratios during the 102 days of the UASB reactor operation at different OLRs are presented in Figure 2. The results for phase 1 to 4 of the UASB reactor operation show that with an increase in OLR from 0.14 to 2.69 kg COD/m^3.d, the average concentrations of MLSS and MLVSS in the bed portion of the UASB reactor increased slightly from 42100±1320 mg/L and 22400±3064 mg/L to 50065±4250 mg/L, and 24899±3599 mg/L, respectively. The increasing trend of MLSS and MLVSS with operation time reveals that the SEOW understudy has no toxic effect on the microbial growth. Nevertheless, the relatively decreasing trend of MLVSS/MLSS ratio during UASB operation from 0.53±0.06 to 0.50±0.04 (Figure 2) demonstrates that the relatively low accumulation of inorganic matter inside the UASB reactor coming from the influent SEOW may occur.

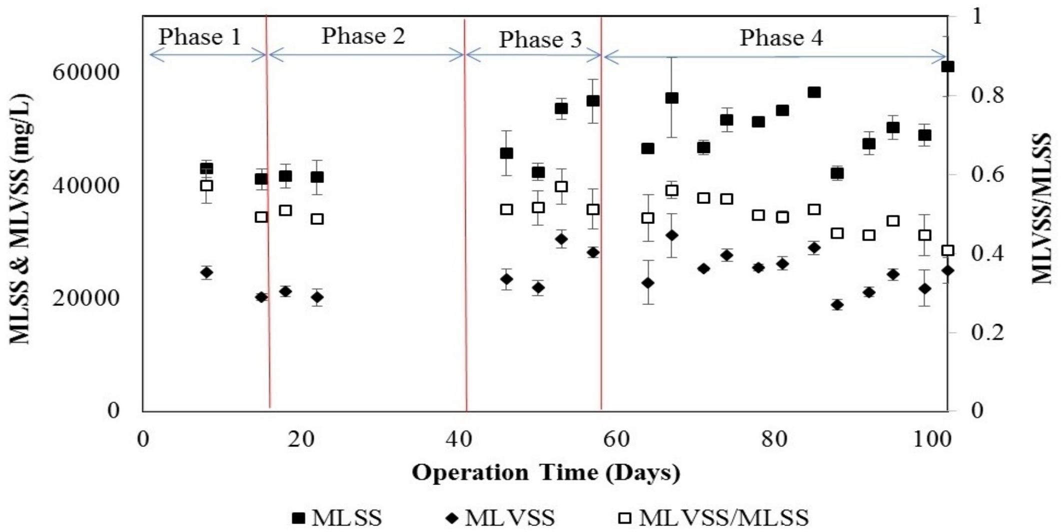


Figure 2. Variations of MLSS and MLVSS concentration and MLVSS/MLSS ratio during the 102 days of the UASB operation at different OLRs.

3.2. Removal performance of COD and phenolic compounds

The variations of COD and phenol concentrations in the influent and effluent of the UASB reactor and their removal efficiencies are presented in Figures 3 and 4, respectively. The corresponding average values and the COD and phenol removal efficiency during the steady-state operation of the UASB are presented in Table 3. As shown in Figure 3., with increasing OLR from 0.14 to 2.69 kg COD/m^3.d, an initial increase and a subsequent decrease in the COD removal efficiency through all 4 phases of the UASB reactor operation are observed. This transient decline in COD removal could be attributed to the effect of shock loads [15] as a result of the increasing concentration of the refractory and toxic compounds in the SEOW at the starting point of each phase, which can affect the performance of the anaerobic system [10]. At each OLR, the UASB was operated to reach a steady-state condition, whereby a constant COD removal efficiency (±5 %) were achieved. With increasing OLR from 0.14 kg COD/m^3.d in phase 1 to 1.50 kg COD/m^3.d in phase 3, the steady state average value of COD and phenol removal efficiency decreased from 71.6±4.8 % and 93.1±1.0 % to 59.5±2.1 % and 47.6±1.5 %, respectively. However, a further increase in OLR to 2.69 kg COD/m^3.d during phase 4 resulted in a significant increase in the steady-state average value of COD and phenol removal efficiency to 72.0±1.4 % and 63.1±6.7 %, respectively. The residual COD content in the effluent of UASB can be attributed to the presence of hardly biodegradable compounds in the effluent and, also, to the release of soluble microbial products resulting from the growth and death of biomass.

In this study, as the OLR increased from 0.14 kg COD/m^3.d in phase 1 to 1.50 kg COD/m^3.d in phase 3, the concentration of phenol in the influent SEOW increased from 9±2 to 487±81 mg/L. However, with a further increase in OLR to 2.69 kg COD/m^3.d in phase 4 due to a change in nature and composition of phenolic compounds of the spearmint plant used for the preparation of the SEOW in the present study, there was a slight decrease in the phenol concentration of the influent SEOW to 383±88 mg/L (Table 2). The results showed the shock effect of increasing the phenolic compounds in phase 3 of the UASB reactor operation on the reactor performance. According to the previous reports, when the anaerobic consortium adapted to phenol, the reduction of phenol to benzoate was the rate-limiting step for phenol decomposition into methane. The high phenolic compounds would easily be inhibited, causing the accumulation of phenol concentration that surpasses the threshold toxicity level of phenol and, therefore had a more direct toxic effect on the methanogens and phenols degraders [16, 17]. The phenol removal efficiency achieved in this study is comparable to an average of 75 % obtained in a comparative laboratory-scale UASB study on the treatment of winery distillery (vinasse) wastewater by Petta et al. [18].

According to the results presented in Table 3, the steady-state effluent concentrations of COD and phenol achieved by the UASB could not meet Iran's Environmental Protection Organization standards (COD and phenol lower than 200 mg L^{-1} and 1 mg L^{-1}, respectively) for the agricultural reuse.

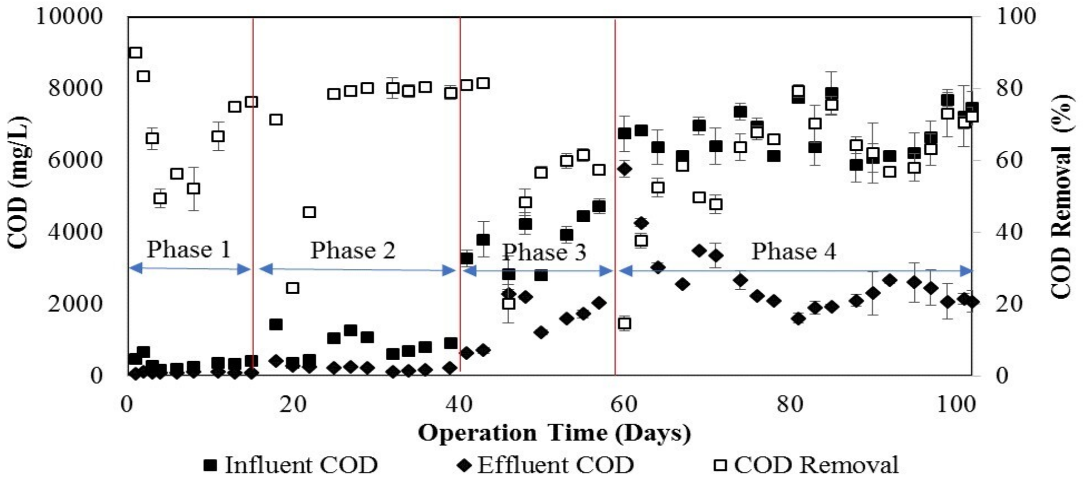


Figure 3. Variations of COD removal efficiency and influent and effluent concentrations during 102 days of the UASB operation at different OLRs.

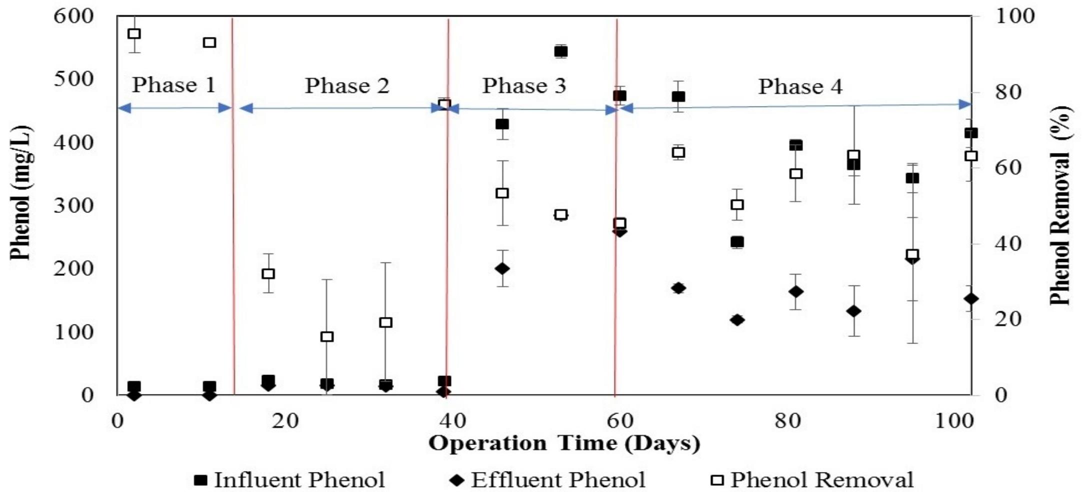


Figure 4. Variations of phenol removal efficiency and influent and effluent concentrations during 102 days of the UASB operation at different OLRs.

Table 3. The average values of COD and phenol efficiency, as well as the COD and phenol concentration in the effluent of the reactor at different OLRs during the steady-state operation of the UASB.

Phase	1	2	3	4
Effluent COD (mg/L)	93±22	223±83	1538±639	2695±983
COD removal (%)	71.6±4.8	79.6±0.8	59.5±2.1	72.0±1.4
Effluent phenol (mg/L)	0.8±0.3	12.9±5.1	242.8±59.7	177.2±52.1
Phenol removal (%)	93.1±1.0	76.8±1.7	47.6±1.5	63.1±6.7

3.3. pH and VFA

It has been reported that several factors including pH, VFAs accumulation, and the imbalance between acidogenes and methanogens could affect the pollutant removal efficiency in the UASB reactor [15]. The pH of an anaerobic reactor is particularly significant because the rate of methanogenesis process is high only when the pH is kept in the range of 6.3-7.8 [19]. Undesirable environmental conditions, such as reduced pH, due to the lack of sufficient alkalinity, VFA accumulation, and toxicity of intermetabolites could inactivate methanogens [20]. By controlling VFAs accumulation in the UASB reactor, the stability of the entire process could be ascertained. A common strategy to control VFA accumulation is alkalinity supplementation, which makes ideal pH conditions for methanogenesis whilst consuming plenty of bicarbonates ($NaHCO_3$) [21]. The results (not presented) showed that the average pH value of the UASB reactor during phase 1 to 4 of operation was 7.5±0.1.

The total VFA and alkalinity concentration in the UASB effluent is shown in Figure 5. Increasing OLR from 0.14 kg COD/m^3.d in phase 1 to 1.50 kg COD/m^3.d in phase 3, followed by increasing COD and phenol concentration as well as toxic compounds in the influent SEOW, resulted in an increase in VFA concentration from 58.2±4.0 mg/L to 624.0±105.1 mg/L. These results are in agreement with those obtained by Rico et al. [22] who emphasized that the minimum presence of VFA in the effluent is indicative of the high performance of the anaerobic process in the degradation of the biodegradable organic substance. With a further increase in OLR to 2.69 kg COD/m^3.d during phase 4, VFA concentration reduced and reached to 451.6±22.5 mg/L, resulting in an increase in COD and phenol removal efficiency during this phase (please see Section 3.2). This result is in agreement with the experiment undertaken by Yi et al. [23], who showed that the reactor's performance increased when VFA concentration decreased and its pH increased. The result also showed that, irrespective of the OLR applied, the production of organic acids depends primarily on the nature and chemical composition of organic matters in wastewater [3].

As shown in Figure 5, with increasing OLR from 0.14 to 2.69 kg COD/m^3.d, the average steady-state concentration of the alkalinity in the effluent of the UASB reactor increased from 452.5±24.7 to 2526.8±146.9 mg/L. In the anaerobic process, sufficient buffering capacity in neutralizing VFA formed is achieved by maintaining alkalinity which, consequently, results in higher removal efficiency of COD and phenolic compounds. The anaerobic system stability is additionally checked using the VFA/alkalinity ratio, which should be kept lower than 0.4 to avoid process instabilities [24]. In the present study, the average steady-state ratio of the VFA/alkalinity in the effluent of the UASB reactor through phase 1 to 4 of operation was less than 0.4 (Figure 5).

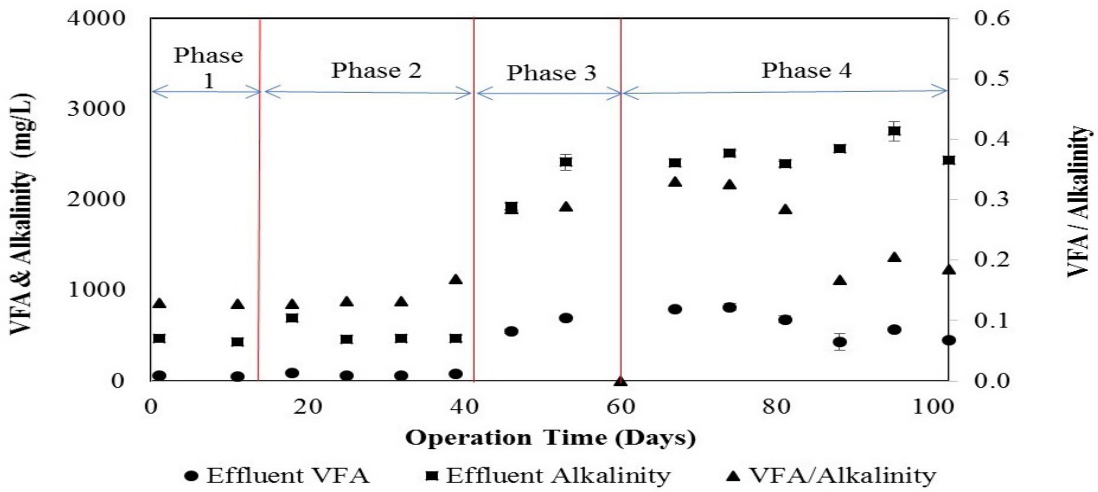


Figure 5. Variations of VFA and alkalinity concentration and VFA/alkalinity ratio in the effluent of the UASB during the 102 days operation at different OLRs.

3.4. Biogas production

Throughout the reactor operation, biogas production volume was measured and the biogas yield was calculated (Figure 6). The biogas yield (liters of biogas produced per kg COD removed) can be a useful parameter to evaluate the anaerobic reactor performance. In the present study, by increasing OLR from 0.14 kg COD/m^3.d in phase 1 to 2.69 in phase 4, the steady-state average value of the biogas production volume and the biogas yield increased from 0.388±0.250 L/d and

138.9±93.1 L/kg $COD_{removed}$ to 26.882±1.738 L/d and 462.2±46.9 L/kg $COD_{removed}$, respectively (Table 4). One-way ANOVA analysis showed a presence of a statistically significant linear correlation between OLR with both biogas production volume and biogas yield (r_P = 0.914, p-value = 0.05; r_P = 0.927, p-value = 0.05, respectively) throughout the UASB operation, indicating that the biogas production and yield tend to increase steadily with increasing OLR, which was also in agreement with the results of the previous work [25]. The values of biogas production yield were similar to those reported by Petta et al., who treated winery distillery wastewater using UASB [18], and those by Cruz-Salomón et al., who treated native beverage vinasse using UASB [2].

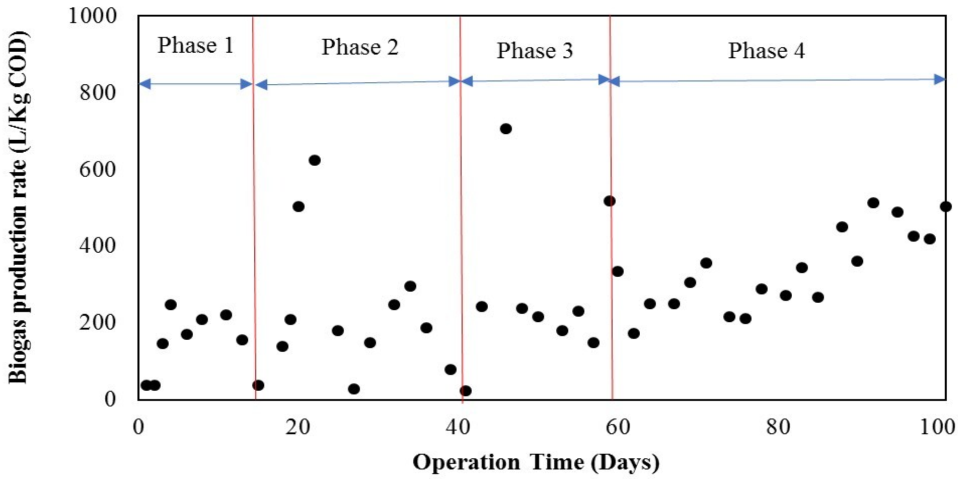


Figure 6. Biogas yield (L/kg $COD_{removed}$) during 102 days of the UASB operation at different OLRs.

The percentage of methane and carbon dioxide in the mixture of biogas produced during the anaerobic digestion of SEOW using UASB was monitored. The methane and carbon dioxide percentage in the biogas, as well as the hydrogen sulfide and net heating values in the UASB reactor, at different OLRs during the steady-state operation is shown in Table 4. At the OLR equal to 2.69 kg COD/m^3.d, the produced biogas contained 63.0±5.2 % and 22.4±4.2 % methane and carbon dioxide, respectively (Table 4). These values are consistent with those of other studies that used UASB [2,3]. It is noteworthy that the high percentage of methane in the biogas in the UASB reactor in treating SEOW suggests that the produced biogas can be considered as fuel. According to the previous reports, biogas with a concentration more than 45 % methane is flammable [2]. With an increase in OLRs to 2.69 kg COD/m^3.d in phase 4, the net heating values of the produced biogas increased to 24.7±5.2 MJ/m^3 (Table 4).

Regression analysis showed that r_P was equal to 0.797, while the linear regression indicated that, for phase 1 to 4, by increasing OLR from 0.14 to 2.69 kg COD/m^3.d, the percentage of methane in biogas increased. This result is compared favorably with the obtained results by Del Nery et al. [3], who achieved statistically significant linear correlation between OLR (ranging from 0.5 to 32.4 kg COD/m^3.d) and methane production (r_P=0.84) for sugar cane vinasse using a pilot-scale UASB reactor during a long-term operation.

Table 4. The average values of biogas production, biogas yield, and methane and carbon dioxide percentage in biogas, as well as the hydrogen sulfide and net heating values in the UASB reactor at different OLRs during steady-state operation.

Phase	1	2	3	4
Biogas production (L/d)	0.388±0.250	1.051±0.362	3.790±0.736	26.882±1.738
Biogas yield (L/kgCOD)	138.9±93.1	133.2±53.7	186.7±33.7	462.2±46.9
CH_4 (%)	45.0±615.4	50.8±14.2	46.4±14.1	63.0±5.2
CO_2 (%)	26.3±4.9	19.4±20.1	19.6±12.1	22.4±4.2
H_2S (mg/L)	3.7±2.5	50.3±33.5	82.7±35.9	195.3±129.0
Net heating values (MJ/m^3)	16.5±5.3	19.9±5.6	18.2±5.5	24.7±5.2

As shown in Table 4, as OLRs increase to 2.69 kg COD/m^3.d in phase 4, the amounts of H_2S concentration in the produced biogas increased to 195.3±129.0 mg/L. It should be stated that due to serious corrosion in pipelines and that within the engine itself, even in limited quantities of H_2S, which is typically found in biogas from anaerobic digestion, the biogas must be clean before its provision for combustion engines. However, there are clean-up technologies that need low energy requirements and cheap capital prices, as might be utilized in such cases [3].

4. CONCLUSIONS

SEOW contains a high concentration of COD, suspended solids, dissolved solids, and phenolic compounds that limit the maximum treatable COD concentration and biogas production. In the present study, an UASB reactor operated at different OLRs in the range of 0.14 to 2.69 kg COD/m^3.d was considered for the treatment of SEOW. The results of this research showed that the used UASB reactor provided efficient COD and phenol removal efficiencies, even when it operated at high OLR of 2.69 kg COD/m^3.d. The stability of the anaerobic system was confirmed by the average steady-state ratio of the VFA/alkalinity and pH in the UASB reactor, which was less than 0.4 and 7.5±0.1, respectively, at different OLRs. At the highest OLR employed, biogas production and biogas yield were 26.9±1.7 L/d and 462.2±46.9 L/kg $COD_{removed}$, respectively, with a net heating value of 23.8 MJ/m^3. In addition, at the highest OLR employed, the mixture of produced biogas contained 63.0±5.2 % and 22.4±4.2 % methane and carbon dioxide, respectively. The results of the present study indicated that the UASB reactor had substantial potential in terms of pollutant removal performance and biogas production for the treatment of SEOW. Nevertheless, the characterization of the effluent achieved by the UASB reactor showed that this wastewater required post-treatment processes to meet Iran's Environmental Protection Organization standards to be used for irrigation and, probably, agriculture purposes.

5. ACKNOWLEDGEMENT

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