High rate simultaneous nutrients removal in a single air lift bioreactor with continuous feed and intermittent discharge regime: process optimization and effect of feed characteristics

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Abstract

In this research, continuous feed and intermittent discharge air lift bioreactor (CFIDAB) was evaluated under two independent variables to treat milk processing wastewater (MPW). The effect of HRT (6-14 h) and air flow rate (1-3 l/min) on the performance of CFIDAB was studied in a laboratory scale of the bioreactor. The optimum values of HRT and air flow rate were 10 h and 3 l/min, reaching SCOD, TN, and TP removal efficiency around 98, 85, and 78%, respectively. The results showed that TP and TN removal were enhanced simultaneously with an increase in HRT and air flow rate. Oxidation-reduction potential (ORP) measurement at the optimal condition showed the successful occurrence of anaerobic, anoxic, and aerobic zones in the CFIDAB. Moreover, the effect of feed characteristics on the process performance for milk processing and soft drink wastewaters was investigated. Based on the obtained data, CFIDAB operated with soft drink wastewater (SDW) showed a better performance in removal of carbon and phosphorus compounds, while nitrogen content was removed more efficient for MPW. As a result, the performance of CFIDAB is sensitive to BOD_5/COD ratio of wastewater. Overall, this single compartment air-lift bioreactor could act as an efficient biological nutrient removal system, providing the conditions for good carbon and nutrient removal. It showed an excellent reactor geometry as well as operating strategy to be used as a membrane bioreactor.

Keywords: Biological nutrient removal, single bioreactor, airlift bioreactor, milk processing wastewater (MPW), soft drink wastewater (SDW)

Nomenclature

A ² /O	Anaerobic/anoxic/aerobic
BAF	Biological aerated filter
BNR	Biological nutrient removal
BOD	Biological oxygen demand
CFID	Continuous feed and intermittent discharge
CFIDAB	Continuous feed and intermittent discharge airlift bioreactor
CNP	Carbon, nitrogen and phosphorus
COD	Chemical oxygen demand
sCOD	Soluble COD
DO	Dissolved oxygen
F/M	Food to microorganism ratio
FBR–MBR	Fluidized bed reactor–membrane bioreactor
HRT	Hydraulic retention time
k	BOD rate constant
MPW	Milk processing wastewater
OLR	Organic loading rate
OPR	Oxidation-reduction potential
PAOs	Phosphorus accumulating organisms
RAAIB	Radial anaerobic-aerobic immobilized biomass bioreactor
SBR	Sequencing batch reactor
SDW	Soft drink wastewater
SND	Simultaneous nitrification–denitrification
SMPs	Soluble microbial products
SVI	Sludge volume index
TN	Total nitrogen
TKN	Total Kjeldahl nitrogen
TP	Total phosphorus
UAASB	Up-flow aerobic anoxic sludge bed
WWTPs	Wastewater treatment plants

1. Introduction

Discharging industrial wastewaters containing excessive nitrogen (N) and phosphorus (P) concentrations into lakes and rivers causes eutrophication, which has become one of the current major environmental issues with regard to the environmental health and save water supply. Biological nutrient removal (BNR) processes have been developed for wastewater treatment plants (WWTPs). These processes generally rely on providing anaerobic, anoxic, and aerobic conditions in three separate compartments [1]. A major problem with conventional BNR systems is their low capacity in terms of reactor volume which has made these methods costly in investment, operation and maintenance [2].

The research to date has tended to focus on designing new systems to increase efficiency and removal rate of the BNR process. Different combinations of anaerobic, anoxic, and aerobic systems have been proposed for nutrient removal from synthetic and real wastewaters. In recent years, there has been an increasing interest in single bioreactors. Single bioreactors are considered to be the most economical, efficient and technological sustainable process for wastewater treatment [3]. Three strategies have been applied to create different zones of dissolved oxygen (DO) concentration in a single bioreactor including: 1) time-based process cycle [4], 2) mass transfer limitation (granular sludge) [5], and 3) physical separation [6]. At the first strategy, aeration pump is on and off periodically and anaerobic, anoxic, and aerobic zones are taken place sequentially. This method is often used in sequencing batch reactor (SBR) and continuous feeding and intermittent discharge SBR (CFID SBR). In a study, an industrial estate wastewater was treated in an upflow aerobic/anoxic sludge bed bioreactor based on CFID SBR regime and intermittent aeration strategy, so that, aeration time with a range of 40- 60 min/h was examined at different hydraulic retention times (HRTs). HRT of 12 h and aeration time of 40

min/h were reported as an optimum condition to achieve 91, 60 and 32% as chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP) removal, respectively [7].

Mansouri et al. reported that intermittent aeration strategy with 50-min aeration time in a 6.5 h cycle provided more than 80, 65, and 60% of COD, TN, and TP removal efficiencies, respectively, in an intermittently aerated SBR [8]. One of the major problems associated with this method for its scale up is complicated operating procedure.

Another approach to accomplish nutrient removal in a single bioreactor is aerobic granular sludge (mass transfer limitation). The nitrification process occurs at the surface aerobic layer of the granule, while, denitrification process takes place in the inner part of granules where the penetration of DO is limited (anoxic zone) [9]. Aerobic granular sludge is not capable for efficient phosphorus removal. Therefore, in the majority of the researches, aerobic granular sludge has been coupled with intermittent aeration strategy to integrate carbon and nutrients removal processes [10].

Physical separation tactic offers the probability to simultaneous CNP removal in single bioreactors. From the literature, baffled bioreactors [11], radial anaerobic-aerobic immobilized biomass (RAAIB) bioreactor [12], and air-lift bioreactors [13] are utilized as single bioreactor with physical separation. Single bioreactors with physical separation strategy are easy to operate and handle compared to time-based processes and diffusion limited operation. Consequently, this group of single bioreactors could consider as a valuable replacement for conventional BNR systems in WWTPs.

Air-lift bioreactors have attracted substantial attention for simultaneous carbon and nutrients removal through the combination of three different zones (anaerobic, anoxic, and aerobic) in a single bioreactor. Haiyan et al. proposed a CNP removal processes based on an airlift

intermittent circulation membrane bioreactor. It was reported that 78.6 g/m³.day and 13.20 g/m³.day of TN and TP were removed [14]. In another study, Airlift loop reactor under a limited filamentous bulking state was operated to treat synthetic wastewater, which the average removal efficiencies for COD, NH₄-N, TN and TP were 91, 92, 86 and 94%, respectively at HRT of 11 h [15].

It should be mentioned that the regime of the reported air-lift bioreactor configurations has been continuous. In an earlier study published [16], an air lift bioreactor with continuous feed and intermittent discharge (CFID) was operated successfully for treating soft drink wastewater (SDW). In the current study, one of the main aims was to study the effect of feed characteristic including organic compounds and nitrogen content on the system performance in terms of N and P removal. Total COD and nitrogen content in the wastewaters selected (soft drink and milk processing wastewaters) in this study were the same but with different nitrogen fractionation. Milk processing wastewater (MPW) with organic nitrogen concentration of 110-120 mg/l and moderate ammonical nitrogen (45-50 mg/l) and soft drink wastewater (SDW) with org-N of 5-10 mg/l and high ammonical nitrogen (140-150 mg/l). Moreover, carbon source in MPW is mainly in the form of lipid, while it is carbohydrate in SDW. Another novel objective was defined based on the significant finding on CNP removal obtained from the CFIDAB at HRT of 12 h as reported in the previous study (116 mg/l of TN and 30 mg/l of TP), indicating a remarkable difference in performance removing CNP relative to other single bioreactors. This result encouraged the authors to monitor the concentration profiles of COD, TP, NO₂⁻, NO₃⁻, and TKN in the riser and downcomer under optimum condition to find the mechanisms of nutrients removal in different zones of the bioreactor.

Besides, in this study, air flow rate as an influential factor in the airlift bioreactor was studied in a wide range (1, 2, and 3 l/min) to assess the capability of the CFIDAB providing anaerobic and anoxic zones (as a function of air flow rate). The effects of two independent variables, HRT and air flow rate, on the process were investigated and the experimental conditions were designed by design expert software. sCOD removal, TN removal, effluent NO_3^- concentration, effluent NO_2^- concentration, TP removal, sludge volume index (SVI), and effluent turbidity were monitored as process responses to analyze and model the process performance. Moreover, a comparative assessment was performed to illustrate the effect of feed characteristics with different BOD_5/COD ratio on the performance of CFIDAB removing CNP are discussed.

2. Materials and methods

2.1 Wastewater characteristics

MPW was collected from a working milk processing production plant, Bisetoon Co., Kermanshah, Iran. COD:N:P ratio of the wastewater was set about 100:20:5 by adding NH_4Cl and KH_2PO_4 . The characteristics of the wastewater is shown in Table 1. Also, at the second stage, SDW was collected from a working soft drink processing production plant, Zamzam Co., Kermanshah, Iran. The characteristics of SDW is presented in supplementary Table S1. The samples were kept at 4 °C to avoid any changes in the wastewater characteristics.

2.2 Bioreactor configuration and process description

The schematic of Plexiglas bench-scale CFIDAB used is depicted in Fig 1. The main column was fabricated with an internal diameter of 10 cm and liquid height of 140 cm. A draft tube was located in the bioreactor with the diameter and height of 5 and 90 cm, respectively. At the height

of 50 cm (50% of the total volume), an automatic control valve was fixed on the reactor body to discharge the effluent. As the effluent level was lower relative to upper edge of the draft tube (about 25 cm), the content of inner and outer of the submerged tube was separated totally at the beginning of each run. In this case, an anaerobic condition is occurred at the beginning of each experiment depending on HRT and air flow rate. Therefore, aerobic zone (into the draft tube) and anaerobic zone (into annulus) were provided at the beginning of each run. Air was supplied into the submerged tube by a blower connected to a fine air bubble diffuser from the bottom of the tube. Also, to achieve an adequate mixing in the anaerobic zone, two fine mixers were mounted on the body of the bioreactor. After a certain time (depending on HRT and air flow rate), the circulation is occurred in the bioreactor and aerobic (into draft tube) and anoxic (annulus) zones are developed. The influent was introduced into the CFIDAB through the middle of annulus. HRT was calculated based on 5.3 L of feeding volume (the volume which is filled and discharged). An air flow-meter and a timer were connected to the blower to control the air flow rate and aeration time, respectively.

Fig. 1. The schematic of CFIDAB used in this study.

2.3 Bioreactor operation and experimental design

The CFIDAB was inoculated with activated sludge obtained from dairy wastewater treatment plant and operated at HRT and MLSS of 6 h, 6000 mg/l, respectively. This condition was sustained to achieve a steady state. Afterwards, the bioreactor was operated according to conditions designed by Design Expert Software (DOE) (Stat-Ease Inc., version 7.0). The experimental runs were designed based on two independent variables including: HRT (6, 10 and 14 h) and air flow rate (1, 2, and 3 l/min). The two operating variables were considered at three levels: low (−1), central (0) and high (1). The design consisted of 2k factorial points augmented by 2k axial points and a center point where k is the number of variables. Accordingly, 13 experiments were conducted with 9 experiments organized in a factorial design (including 4

factorial points, 4 axial points and 1 center point) and the remaining four involving the replication of the central point to get good estimate of experimental error. Repetition experiments were carried out after other experiments followed by order of runs designed by DOE as shown in Table 2. The settling time for all runs was 40 minutes.

Each run was continued about 10 days to get adapted microorganism populations under a steady state condition. In order to start a new run, TP absorbed by PAOs microorganism was released and discharged under an anaerobic condition applied in the bioreactor. To provide the anaerobic condition required for the phosphorous release, the bioreactor was filled with fresh wastewater containing COD concentration of 2000 mg/l and maintained in this condition for about 5h, respectively. After that, the bioreactor operation was conducted based on the designed experimental conditions (Table 1). In the second stage, the bioreactor was operated at the optimum condition for a 60-run (30 days) to monitor the phosphorus removal efficiency over long time operation. It should be mentioned that SRT was kept around 20 days by draining out the surplus sludge over long term operation.

Besides, in the second stage of study, the CFIDAB bioreactor was conducted at three selected experimental conditions comprising air flow rate of 1 l/min and three levels of HRT (6, 10, and 14 h) to investigate the simultaneous carbon and nutrients removal from soft drink.

Table 1. Experimental conditions and results.

Variables			Responses								
Run	Factor1 A:HRT	Factor2 B: Air flow rate	sCOD removal	TN removed	TN removal	Effluent N-NO ₃	TP removal	Effluent turbidity	DO	SVI	Superficial gas velocity
	h	l/min	%	mg/l	%	mg/l	%	NTU	mg/l	ml/g	m/min
1	6	3	97	142	68	1.4	59	4	3.5	119	1.52
2	6	2	96	116	56	1.28	33	7	2.2	126	1.019
3	6	1	81	100	49	0.9	27	24	1	96	0.509
4	10	3	98	186	85	2.32	78	9	7.5	177	1.52
5	10	2	97	166	79	0.82	62	6	6.5	167	1.019
6	10	1	96	158	75	0.54	61	7	5.5	173	0.509
7	14	3	99	178	86	2.16	72	4	8.8	182	1.52
8	14	2	99	175	85	1.58	66	2	8.5	178	1.019
9	14	1	99	179	85	1.4	65	3	8.4	165	0.509
10	10	2	98	163	78	0.85	65	7	6.2	170	1.019
11	10	2	97	164	79	0.7	64	6	6.7	173	1.019
12	10	2	96	166	75	0.84	58	6	6.5	167	1.019
13	10	2	98	166	76	0.9	55	8	6.6	160	1.019

2.4 Mathematical Modeling

The experimental data obtained was used to determine the coefficients of the polynomial model (Eq. 1).

$$Y = \beta_0 + \beta_i X_i + \beta_j X_j + \beta_{ii} X_i^2 + \beta_{jj} X_j^2 + \beta_{ij} X_i X_j + \dots \quad \text{Eq. 1}$$

Where, i and j are the linear and quadratic coefficients, respectively, and β is the regression coefficient. P value with 95% confidence level was considered to evaluate the effectiveness of the model terms. The results were completely analyzed using analysis of variance (ANOVA) by Design Expert software. Three-dimensional plots were obtained based on the effect of the levels of the two factors. From these three-dimensional plots, the simultaneous interaction of the two factors on the responses was studied.

2.5 Analytical methods

Statistical design of experiments and data analysis was carried out by Design Expert Software (version 7.0). The concentrations of COD, biological oxygen demand (BOD), TN, nitrate and nitrite, $\text{NH}_4\text{-N}$, TP, and MLSS were determined by using standard methods [17]. For COD, a colorimetric method with closed reflux method was developed. Spectrophotometer (DR 5000, Hach, Jenway, USA) at 600 nm was used to measure the absorbance of COD samples. Total kjedahl nitrogen (TKN) was determined by TKN meter Gerhardt model (Vapodest 10, Germany). The DO concentration in wastewater was determined using a DO probe supplied by WTW DO Cell OX 330, electro DO probe, Germany. Turbidity was measured by a turbidity meter model 2100 P (Hach Co., USA). Air flow rate was measured by an air flow meter model 101325Pa. Superficial gas velocity was calculated according to Eq. 2:

$$J_G = \frac{Q}{A_r} \quad \text{Eq. 2}$$

where, J_G is gas superficial velocity, Q is air flow rate, and A_r is cross-sectional area of riser zone.

2.6 Statistical analysis

As COD, TN, and TP removal are the main criteria to evaluate the performance of a single bioreactor for CNP removal from wastewaters, these parameters were modeled with Design Expert software (Ver. 7.0) in this study. The obtained regression equation is used to predict the bioreactor performance in the different operating conditions. In order to fit obtained data, different degrees of polynomial models were applied (supplementary Table S2). In the Design Expert Software, the response data was analyzed by default. Polynomial models were selected for each response based on the fitness with actual data, considering R-squared (R^2) as main criterion. The regression equations and ANOVA results for all studied responses after rejection of insignificant model terms have been summarized in supplementary Table S2. The polynomial equations presented in Table S2 are based on coded values (-1, 0, +1) to predict the responses in the design space for each variable. HRT and Air flow rate are represented with A and B, respectively, in the regression equations. From the statistical analysis, very low probability values (<0.0001) verifies high significance of the models. The square of correlation coefficient for each response was computed as the coefficient of determination (R^2). The adequacy of the models was tested through lack-of-fit F-tests [18]. The lack of fit F-statistic was not statistically significant as the P-values were greater than 0.05.

Adequate precision is a measure of the range in predicted response relative to its associated error, in other words, a signal-to-noise ratio. Its desired value is 4 or more. The value was found

desirable for all models. Separately, low values of the coefficient of variation (CV) (2.79 – 136.97 %) indicated good precision and reliability of the experiments as suggested by Kuehl [19]. In order to check the accuracy and precision of the models used in this study, more statistical analysis was done as shown in supplementary Fig. S1.

The predicted vs. actual plot for the four responses for sCOD removal, TN removal, TP removal, and effluent turbidity are shown in supplementary Fig. S1a, c, e, and g, respectively. The experimental and predicted values are in good agreement, which assures the correctness of the generated models [20].

Also, in order to evaluate the sensitivity of the responses as a function of the studied variables, the values of the responses at different levels of variables were calculated using the obtained equations (supplementary Table S2). A linear regression line was fitted to the predicted values of responses versus each variable (keeping the other variables constant at three levels) which the average slope of the lines was used as sensitivity parameter as presented in supplementary Fig. S2. From the figure, all studied responses were more sensitive to air flow rate compared to HRT. As a result, COD removal and effluent turbidity are influenced by air flow rate considerably rather than effluent NO_3 concentration, TN and TP removal.

3. Results and discussion

3.1 CFIDAB performance

3.1.1 sCOD removal

COD removal is a communal and main parameter to assess the performance of a bioreactor, consequently, sCOD removal at different experimental conditions was reported to investigate the performance of this novel single bioreactor and its treatment capacity. The results of ANOVA analysis for sCOD removal are presented in supplementary Table S2. From supplementary Table

S2, a reduced quadratic model was used to model the actual data of sCOD and the significant model terms are A (HRT), B (Air flow rate), AB, A^2 , and B^2 . This data verifies that the selected variables have serious influence on sCOD removal efficiencies in the CFIDAB. The interactive effects of variables on the response is demonstrated in Fig. 2 as three dimensional plot.

It was observed that MPW was treated in the CFIDAB with sCOD removal efficiency between 80% and 100% in the design space studied. Due to the unique feature of air-lift bioreactors that enhance the efficiency of oxygen bubbles, the sCOD removal was high even at the minimum values of HRT and air flow rate. Another reason that can be given is the high MLSS concentration during a HRT which changes from 12000 mg/l at the beginning of run to 6000 mg/l at the end of run.

HRT and air flow rate indicated an increasing effect on the response. However, a reducing trend of sCOD removal was observed at the highest values of HRT (14 h) with increasing air flow rate from 2 to 3 l/min. This might be attributed to high oxidation potential at this condition which causes the release of soluble microbial products (SMP) [21]. Moreover, at the highest air flow rate (3 l/min), the response was not influenced by the variation of HRT as the oxidation potential was high enough in this air flow rate to get the maximum removal efficiency (around 98%) independent to HRT value. The maximum SCOD removal was predicted to be 100% when HRT and air flow rate were 14 h and 2 l/min (superficial gas velocity of 1.019 m/min), respectively.

As a result, the CFIDAB could be operated at air flow rate of 1 l/min and HRT of 10 h (organic loading rate of 1.91 kg BOD/m³.d) to reach more than 90% of sCOD removal. The high sCOD removal rate of this bioreactor allows it to treat wastewaters with higher COD loads compared to conventional systems such as activated sludge systems which have an average organic loading of 0.3 kg BOD/m³.day [22].

Fig. 2. Response surface plot for sCOD removal efficiency as a function of air flow rate and HRT.

3.1.2 TN removal

The capability of CFIDAB to provide efficient aerobic and anoxic zones was evaluated by measuring TN removal throughout the experiments. From supplementary Table S2, the variables influencing the TN removal efficiency in the CFIDAB were A, B, AB, A^2 , B^2 . Fig. 3a shows the impact of HRT and air flow rate on TN removal efficiency as a three dimensional plot. The range of TN removal efficiency was between 48% and 88%. Increasing HRT values from 6 to 12 h promoted TN removal efficiency, however, the response was fairly constant with a further increase of HRT to 14 h. This result is an indication to improve nitrification process in the bioreactor with increasing HRT from 6 to 12 h (corresponding to organic loading rate (OLR) to 4

and 2 kg/m³.d, respectively). It was reported in the literature that nitrification process is developed at low values of OLR [23].

The restricted nitrification process was supported by the positive effect of air flow rate on the response, so that TN removal efficiency was increased from 48% to 68% with varying air flow rate from 1 to 3 l/min at HRT of 6h. Nevertheless, TN removal was less sensitive to air flow rate at the HRT values more than 10 h due to an adequate nitrification process at higher HRTs. The maximum TN removal efficiency achieved was around 88% (180 mg/l) at HRT of 14h and any air flow rate in the range studied. In a research reported by Rezaee et al., the maximum TN removal from MPW was 110 mg/l at HRT of 6h in a single up-flow aerobic/anoxic sludge bed (UAASB) bioreactor with CFID regime [4].

The effluent NO₃⁻ concentration and the amount of nitrogen removal due to cell synthesis and denitrification process are shown in Fig. 3b. The contribution of cell synthesis in TN removal was more significant at the lowest HRT (6h). In contrast, the denitrification process was sharply developed from 100 to 150 mg/l with increasing HRT from 6 to 10 h at air flow rate of 3 l/min (run no: 1,4). This outcome was consistent with the TN removal data, so the main mechanism to remove nitrogen compounds in the CFIDAB was simultaneous nitrification-denitrification process (SND). The effluent NO₃⁻ concentration was in the range of 0.5-2 mg/l, indicating an enhanced anoxic zone occurred in annulus of the CFIDAB. It must be noted that the desirable operating condition to obtain favored TN removal and effluent NO₃ concentration in this bioreactor could be along with HRT and air flow rate of 10 h and 2 l/min, respectively.

Fig. 3. (a) Response surface plot for TN removal efficiency as a function of air flow rate and HRT. (b) Nitrogen under different operating conditions (details on various runs see table 1).

3.1.3 TP removal

Conventional biological treatment systems involve aerobic and anaerobic bioreactors to cultivate phosphate accumulating bacteria (PAOs) for enhancing phosphorus removal. Nowadays, there is an interest to apply advanced bioreactors to offer the required conditions for TP removal process with smaller footprint and lower cost. In the CFIDAB, anaerobic and aerobic zones are provided in sequence during treatment. TP removal was monitored at all different experimental conditions (Table 1). According to supplementary Table S2, similar to sCOD and TN removal, A, B, AB, A^2 , and B^2 were significant model terms to describe TP removal data.

The variation of TP removal as a function of the operating variables is depicted in Fig. 4. HRT showed a reverse impact on TP removal efficiency. Increasing in HRT from 6 to 12 h caused a positive effect on the response by reason of enhancing TP adsorption over longer aerobic condition. Further increase in HRT to 14 h resulted in a negative effect on the response at high range of air flow rate (2-3 l/min), however, at low range of air flow rate (1-2 l/min) the response was fairly independent of the variation of HRT from 12 to 14 h. This might be due to restricted anaerobic zone at the beginning of run befallen by higher superficial gas velocity (1.5 m/min at air flow rate of 3 l/min versus 0.5 m/min at air flow rate of 1 l/min) which triggered a faster mixing of annulus and inside draft tube content at the high range of air flow rate. TP removal efficiency was predicted between 26% (13 mg/l) and 80% (40 mg/l). An operating condition with HRT of 10 h and air flow rate of 3 l/min was the favorable circumstance for achieving the maximum TP removal efficiency.

A combined system of fluidized bed reactor–membrane bioreactor (FBR–MBR) was reported as removing 10 mg/l of phosphorus from sewage wastewater applying intermittent aeration strategy [24]. A combined anaerobic/anoxic/aerobic (A^2/O)-biological aerated filter (BAF) system

treating domestic wastewater was reported to have a maximum phosphorus removal capacity of 5.63 g P/m³.day. [25]. Air-lift bioreactors with a continuous operation regime were not effective to remove phosphorus when compared to the CFIDAB with continuous feed and intermittent discharge regime used in this study. For instance, 12.1 and 6.6 mg/l of phosphorus was eliminated from synthetic wastewater in an airlift intermittent circulation membrane bioreactor and airlift loop reactor under a limited filamentous bulking state, respectively [14, 15]. Hence, compared to other BNR systems, it can be concluded that the proposed single bioreactor is a good alternative to treat industrial wastewaters with high phosphorus concentration.

Fig. 4. Response surface plot for TP removal efficiency as a function of air flow rate and HRT.

3.1.4 Effluent turbidity

The effluent turbidity could be considered as a proper indication for evaluating the effluent quality of a bioreactor. According to actual data presented in Table 1, the effluent turbidity was

in the range of 2 to 9 NTU, although a relatively high turbidity (24 NTU) was an exception in the data which was at a condition with HRT of 6 h and air flow rate of 1 l/min. In this condition, high OLR and low air flow rate caused an expanded sludge, resulting in high effluent turbidity. As a conclusion, the effluent quality of the CFIDAB was satisfactory, considering that the CFIDAB is a single bioreactor with sludge separation integrated into the bioreactor.

3.1.5 Process optimization and verification

To achieve high SCOD, TN, and TP removal efficiencies and low effluent NO_3 and effluent turbidity, an optimization study has been performed. The graphical optimization for the system is shown in Fig. 5. The region which meets the offered criteria (including: SCOD removal $> 95\%$, TN removal $> 72\%$, Effluent $\text{NO}_3 < 5 \text{ mg/l}$, TP removal $> 60\%$, and Effluent turbidity $< 10 \text{ NTU}$) is recognized with shaded area in the figure. The optimum operational region was predicted at the conditions with 7-14 h of HRT and 1-3 l/min of air flow rate.

The bioreactor was subsequently operated at a condition within the optimum region (HRT of 10 h and air flow rate of 2 l/min) to check the accuracy of the models used to define the responses. 5 responses were measured over the selected experimental condition. The actual and predicted data and also the standard deviation for each response are listed in Table 2. The predicted data are in good agreement with the obtained data from the bioreactor, indicating well designed models.

Fig. 5. Overlay plot for the optimal region.

Table 2. Verification experiments at optimum conditions.

Conditions		Response				
		TCOD removal, %	TN removal, %	TP removal, %	Effluent turbidity, NTU	Effluent nitrate, mg/l
HRT= 10 h Air flow rate=2 mg/l	Experimental values	99	80	65	4	1.3
	Model values	96.72	78.26	63.55	6.28	0.8
	Standard deviation	1.61	1.23	1.02	1.61	0.35

3.2 Carbon and nutrient changes in different zones of the reactor under optimum condition

In order to analyze the process in different zones of the CFIDAB, the profiles of ORP and pH values (a), COD and TP concentrations (b), NO_2^- and NO_3^- concentrations (c), and TKN concentration (d) during operation under optimum conditions are presented in Figs. 6a-d. Each

cycle included 2 stages, before and after circulation. Before circulation, two phases comprising aerobic (riser) and anaerobic (downcomer) exist simultaneously in the bioreactor. Fig. 6a shows the changes in ORP and pH values over a HRT. ORP value for downcomer sharply dropped from -50 to -150 mv after half hour, and it continued decreasing until reaching -250 mv. This result indicates the occurrence of anaerobic phase at the beginning of each cycle. After circulation (in mixing stage), the bioreactor composed two main zones, aerobic (riser) and anoxic (downcomer) zones. In this stage, the ORP values were in the range of 50-250 mv and 0-100 mv at riser and downcomer, respectively. As a result, anoxic zone was provided successfully over circulation phase at downcomer as the range of ORP for anoxic zone is reported between -100 and +100 in the literature [26]. pH was also monitored as another indicator for aerobic and anaerobic zones (Fig. 6a). It was about 6.9-7 at anaerobic zone, while a range of 7-7.8 was measured at aerobic zone.

Fig. 6b indicated that COD concentration in downcomer was increased severely from 25 to 300 mg/l and then it varied in the range of 300-600 mg/l. The release of soluble microbial products (SMP) was supposed to happen through high MLSS concentration and absence of oxygen [27]. It is noted that MLSS concentration at the beginning of each cycle is more than 10.0 g/l. To assess the influence of MLSS the system was operated once with MLSS concentration of 2.5 g/l and the results were compared with those at high MLSS concentration in Fig. 6e. From the Fig., at the low MLSS, COD concentration showed a decreasing trend with a level lower than that in high MLSS concentration, indicating SMP release in downcomer zone by high population of microorganism [30].

Fig. 6b showed that TP concentration was increased from 12 to 24 mg/l after 1 h in downcomer phase, considering that only 500 ml of influent with TP content of 50 mg/l was diluted in 4.8 L

(sludge zone) over 1h. This result is in a good agreement with an earlier report [28]. Also, despite of downcomer, TP concentration at riser zone showed fluctuations with reducing trend. This difference between TP concentrations in downcomer and riser confirmed the occurrence of TP-release process in anaerobic zone. It is noted that the level of phosphorus after one hour was nearly constant due to an inhibiting effect of nitrate on the p-release process (Fig. 6c) [29]. TP concentration dropped slightly after circulation in spite of continuous feeding with TP of 50 mg/l, implying a good performance of PAOs in phosphorus uptake.

The NO_3 and NO_2 concentration in different zones are presented in Fig. 6c. NO_2 and NO_3 concentrations had a similar increasing variation in downcomer, so that NO_3 and NO_2 concentrations reached 8 and 0.16 mg/l, respectively. In contrast, in riser zone the concentration of NO_2 was negligible and NO_3 concentration was always below 3 mg/l resulting from a partially anoxic zone. According to Fig. 6a, ORP of riser was in the range of anoxic zone as a result of high MLSS concentration at the beginning of each run (around 12.0 g/l) which caused higher oxygen uptake rate. After circulation phase, NO_3 concentration was declined to lower than 1 mg/l, verifying the incidence of an appropriate anoxic zone in this novel airlift bioreactor.

Fig. 6d represents changes in TKN concentration at different zones under the optimum condition. Before circulation phase, TKN data showed a slight increasing trend in downcomer from 30 to 35 mg/l. The same range was observed in the riser. As mentioned, the influent TKN concentration was 126 mg/l, hence, it can be consumed by microorganisms to produce extracellular polymeric substances (EPS) and SMP in downcomer as it was mentioned earlier in Fig. 6b [27]. After circulation, TKN concentration was reduced gradually and it remained constant around 25 mg/l after 5 h with continuous feeding condition, implying an acceptable balance between aerobic and anoxic zones created by the physical separation

Fig. 6. The variation trends of ORP and pH values (a), sCOD and TP concentrations (b), NO₃⁻ and NO₂⁻ concentrations (c), TKN concentration (d) over optimum condition and the effect of MLSS concentrations on sCOD concentration in anaerobic zone (e).

3.3 The effect of wastewater characteristics on the performance

One of the most important variables for bioreactor performance, particularly BNR systems, is wastewater characteristics in terms of biodegradability of carbon source and nutrient compounds. Therefore, the potential of the CFIDAB for removing carbon and nutrients from two different wastewaters, milk processing and SDW, was evaluated. The characteristics of both wastewaters are presented in supplementary Table S1.

The obtained data of SCOD, TN, and TP removal and effluent turbidity for three conditions with air flow rate of 1 l/min and three HRTs (6, 10, and 12 h) is presented in Fig. 7. It should be noted that 1 l/min of air flow rate was selected because of its lower energy demand which leads to cost effective operation. As shown in Fig. 7a, the rate of SCOD removal efficiency for SDW was higher than MPW due to easier degradation of SDW compared to MPW. The BOD rate constant for SDW and MPW were 0.38 and 0.18 d⁻¹, respectively (. With increasing HRT from 6h to 14 h, the difference between treated wastewaters in terms of SCOD removal efficiency was negligible (>99% of SCOD removal efficiency for both wastewaters) as a result of high oxidation potential. The capability of the bioreactor to remove TN content form two different wastewaters is shown in Fig. 7. At all HRTs, the amount of nitrogen removed from MPW was considerably more than the nitrogen removal for SDW. Higher TN removal efficiency for MPW resulted from a higher nitrogen content of the milk wastewater. The decomposition rate of the protein compounds is slower than carbohydrates in SDW, so required carbon source for the denitrification process will remain in the system with MPW [30]. This assumption was substantiated by increasing TN removal efficiency with increasing HRT from 6 to 14 h for MPW, which indicated a satisfactory denitrification process for MPW. Whereas, TN removal was reduced with varying HRT from 10 to 14 h for SDW.

In contrast to TN removal, TP removal efficiency for SDW was more favorable (Fig. 7c). As a fact the enhanced phosphorus removal process requires readily biodegradable organic substrate in the anaerobic phase to produce PHB inside the cellular of PAOs. As the sugar is more easily to ferment in VFA (the main substrate for PAO) it was expected that indeed P-removal would be better than for milk wastewater.

The effluent turbidity data is shown in Fig. 7d. The effluent turbidity was lower than 10 NTU for both wastewaters at HRT of 10 and 14 h, while a relative high effluent turbidity (20 NTU for soft drink and 24 NTU for MPW) was observed due to high F/M ratio.

Fig. 7. Performance of the reactor fed by two different wastewaters; (a) sCOD removal efficiency, (b) TN removal, (c) TP removal efficiency, (d) effluent turbidity.

3.4 Long term performance at optimum condition

In this bioreactor, TP removal was carried out through PAO bacteria cultivated in sequencing anaerobic and aerobic phases created by physical separation technique. Hence, long term

capability of the CFIDAB to remove phosphorus compounds was evaluated in this section. In this mean, the CFIDAB was operated for about 30 days at optimum condition (HRT of 10 h and air flow rate of 2 l/min) and SCOD, TN, and TP concentrations were monitored over optimum condition as presented in supplementary file 1. As it is observed in supplementary Fig. S3, the SCOD and TN removal were approximately constant over long term operation, however, the effluent TP concentration varied between 20-30 mg/l (40-60% removal efficiency). The maximum capacity for TP removal at the operated condition was 65% during the first run. In general, 20-30 mg/l of influent phosphorus was removed from MPW in CFIDAB under optimum condition. This result was a good indication of the capability of this system to provide a balanced sequencing anaerobic and aerobic phases in a single bioreactor. It should be mentioned that SRT was kept around 20 days by removal of surplus sludge during long term operation.

4. Conclusion

A continuous feed and intermittent discharge air lift bioreactor (CFIDAB) was successfully operated for nutrients removal from MPW. The system performance was also compared for two industrial wastewaters with different BOD_5/COD ratios (0.7 and 0.8). In this bioreactor, anaerobic and anoxic zones were implemented in combination with continuous aeration and feeding but an intermittent discharge regime. For MPW, SCOD removal efficiency was more than 80% at different conditions and 186 and 39 mg/l of TN and TP concentration were removed, respectively, at an optimum condition with HRT of 10 h and air flow rate 2 l/min. It was concluded that the wastewater with higher BOD_5/COD (SDW) triggered a better COD and TP removal efficiencies. While, the bioreactor operated with MPW (BOD_5/COD of 0.7) showed higher TN removal rate.

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