Design and construction of a micro-photo bioreactor in order to dairy wastewater treatment by micro-algae: parametric study

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ABSTRACT
The present study demonstrates a construction procedure of a micro-photo bioreactor which has been used for the treatment of the simulated dairy wastewater (henceforth; SDW) by micro-algae chlorella vulgaris (C. Vulgaris). Moreover, this research is an experimental study in the laboratory scale and has been conducted in specific condition and parameters. Therefore, the effect of determined parameters such as flow rate, length of the micro-photo bio reactor, initial pH, luminous intensity and temperature on micro-algae has been studied. The obtained results from the SDW treatment by using specified micro-algae in the optimum situation illustrated that the removal efficiency of the chemical oxygen demand (henceforth; COD) in 120 working hours for flow rate, length of micro-photo bioreactor and the initial acidity, luminous intensity and the temperature effect is equal to 24.21% and 29.26% and 36.05% and 37.81%and 42.57%, respectively. The maximum percent of COD removal efficiency has been obtained in residence time of \( t = 52 \) hours, and the optimum conditions of COD are equal to 42.57%. The turbidity value and existed TDS in SDW has been measured and compared together. Furthermore, significant differences were obtained from the analysis of each parameter. Consequently, the optimum concentration of micro-algae and the optimum pH for SDW purification was obtained \((m_i = 0.150 \text{ L})\) and \( (pH_i, op = 8.00) \) on \( \text{(COD)} \), respectively. The purpose of this study is to evaluate the effects of micro-algae in COD removal from SDW by using C. Vulgaris micro-algae in the designed micro-photo bioreactor.

Introduction
Food industry production would endanger the environment, to overcome this major problem and particularly to protect the environment; we need to use appropriate biological ways, the dairy wastewater purification is an efficient and essential approach that leads to a healthy and clean environmental ecosystem. The biological oxygen demand (BOD) and chemical oxygen demand (COD) is one of the specifications of highly-concentrated wastewater(Orhon et al. 1993; Skoczko, Struk-Sokolowska, and Ofman 2017; Verma and Suthar 2018). Food processing industries due to the production of sewage containing suspended solids, fats, and oils and nutrients that are characterized by the required biological (BOD) oxygen and chemical oxygen demand (COD), have caused the serious environmental problems(Andrade et al. 2014; Andrade, Motta, and Amaral 2013; Shirmohammadi, Soltanieh, and Romeo 2018). The wastewater of this industry, regarding volume and specification of the produced wastewater for each liter of processed milk, is approximately \((0.2–10)\) liters(Vourch et al. 2008). The wastewater of the dairy industry has an
important organic material such as fats, lactose and proteins (mainly casein) which leads to the demolition of the environment (Fraga et al. 2017; Li et al. 2017; Praneeth et al. 2014; Davarpanah 2018b, 2018c). The industry of dairy products such as the receiving station, the bottle packing unit, cheese, and ice cream unit provides the waste of organic materials. For purification of wastewater in the dairy industry, physic-chemical and biological methods are being used due to the abundance of them and also availability (H. Farizoglu and Uzuner 2011; Gough et al. 2000; Razmjoo et al. 2017; Shirmohammadi et al. 2015). Usage of the approaches as mentioned above leads to indiscriminate use of energy and chemical material which is not economical (Akhundzadeh and Shirazi 2017; Cremiato et al. 2018; Tchobanoglous, Burton, and Stensel 1991). One of the disadvantages of the physic-chemical methods is the safe disposal of the byproducts. The produced byproducts by physic-chemical methods would lead to secondary contamination (Hoffmann 1998). Purification of Wastewater using algae is a suitable and practical method for the removal of these materials with less cost and more safety compared to chemical and physical approaches. Also, other benefits such as improved recycling and better recovery can be mentioned (Greenwell et al. 2009; Oswald 2003, Davarpanah 2018a). The use of micro-algae in comparison with mentioned methods, in addition to the great effect on cost reduction, leads to the removal of COD, reduction of the factors causing illnesses, phosphorus and nitrogen (Chen, Yen, and Chung 2014; Prajapati et al. 2014). It should be mentioned that micro-algae along with photobioreactor has better protection against culture contamination, better mixing, less evaporative loss, and higher cell densities than above mentioned methods (Barros et al. 2015; Farizoglu and Uzuner 2011; Mata, Martins, and Caetano 2010; Skoczko, Struk-Sokołowska, and Ofman 2017; Sudhakar et al. 2017). It can be said that tubular photobioreactors are the only type of closed systems employed at large scale (Chisti 2007). Vertical, horizontal, and helical designs are high usage, although helical designs are perceived the easiest to scale up (Carvalho, Meireles, and Xavier Malcata 2006; Davarpanah 2018a; Davarpanah, Razmjoo, and Mirshekari 2018). Recently, anaerobic digestion of wastewater has grown algal biomass with due to their small size (3–30 µm) in laboratory-scale accumulating-volume bioreactor has been reported in the literature. Significant features of the micro-algae utilization in the production of biofuels especially in the treatment of sewage have been reported due to the many pigments (fat, fatty acids, and carbohydrates) and the ability to absorb bacteria which shows that algae are generally able to remove nutrients through the absorption (absorption) mechanism. This method is considered one of the economic methods in industries (Tam and Wong 2000; Xin et al. 2010; Zhao et al. 2016; Davarpanah et al. 2019). Nutrients in sewage sources act as a platform for the formation of biomass. The main nutrients of chlorine are carbon, nitrogen, and phosphorus, although the role of nutrients Ca, Fe, Zn, Mg is also known. It should be noted that different types of algae are different (Ge and Champagne 2017; Hou et al. 2016). Therefore, the effect of operating conditions (i.e., duration, initial nutrients, the ratio of recovery) and seasonal conditions (e.g., temperature, light period) strongly affects the results (Sindelar et al. 2015).

Although there are numerous studies have been widely reported in the literature to propose the utilization of micro-photo bioreactor, in this comprehensive study, the following concepts were taken into consideration; optimization of mass transfer limitations, optimum lighting strategy, and micro-photo bioreactor geometry to maximize irradiance and increasing surface. Moreover, its industrial integration included scale-up, catalyst separation, and retrofitting to existing systems.

**Methods and materials**

**Wastewater**

The required dairy wastewater for the studies and experiments has been simulated, and this simulated wastewater has been using with a constant combination throughout the study. For a careful study and also in order to obtain a constant concentration, the SDW has been prepared freshly. The SDW was produced by dissolving 4g of milk powder (Nestle Brand, Kazvin-Iran) in 1
liter of distilled water. The specification and analysis of the milk powder and the used SWD are presented in Table 1.

For the experimental study of the SDW, many researchers have been used this method for preparing SWD (Balannec et al. 2005; Kushwaha, Srivastava, and Mall 2010; Leal et al. 2006; Ramasamy et al. 2004).

**Micro-algae cultures, medium, and chemicals**

The micro-algae, Chlorella Vulgaris (C. vulgaris), were acquired from the laboratory Marine micro-algae Culture Center in Tehran, Iran. The size of C. vulgaris was 5–10 μm, having a circular shape (Figure 1). The cells of C. vulgaris were cultured in Jaworski’s Medium in deionized water with light emitting diode lamps at ambient temperature. Every 1 ml in 1 L of deionized water was disinfected in an autoclave (Tehran, Iran) at 15 psi for 30 min. The cultures were incubated at a 25°C at constant temperature and continuous light exposure of 5,000 lux for eight days (Choi and Lee 2012).

**The shape and network of the designed micro-photo bio reactor**

The used micro-photo bioreactor for experiments in this study has been fabricated from two glass plates with the dimensions of (30 × 40). The micro channels with the length of 10–16m and circular cross section with the diameter of 150 micrometers, which is shown in (Figure 2), have been created on these glass plates. In order to seal the microchannels, first, the glass plates of micro-photo bioreactor have been placed into the furnace with the temperature of 700–760°C. To prevent the

| Parameters          | Unit     | Value Range |
|---------------------|----------|-------------|
| pH                  |          | 3.8–6.6     |
| COD                 | mg l⁻¹   | 3900        |
| Total solids        | mg l⁻¹   | 3090        |
| Turbidity           | NTU      | 1744        |
| Conductivity        | μs cm⁻¹  | 220         |
| Chloride            | mg l⁻¹   | 31          |
| Total N             | mg l⁻¹   | 113.81      |

**Figure 1.** Photomicrograph of Chlorella vulgaris in 1000 x on the fifth day.
glass plates from breaking, the temperature of the furnace should increase gradually at the rate of 60° C per half hour and finally in the temperature of 760°C, a proper bond would be created between these plates, and we would have a complete seal. (Figure 3a). Moreover, (Figure 3b) shows a view of micro-photo bioreactor captured as SEM at low and high temperature. In SEM image of the micro-photo bioreactor, the seal between the plates can be observed. The micro-photo bioreactor consists of a large number of micro-channels, which are the same and the distance between the main input and output is 35cm. Each micro-photo bioreactor consists of a main input and an output, and the length of the microchannels are determined to be L = 10-16m for SDW purification based on the defined experiments. The end of the microchannels is formed by a 180-degree semicircular bend and length of them is 25 cm, and the number of these microchannels for a laboratory study has been defined to be 40–64. Also, the effective volume of the microchannel has been obtained 2.4–3.8 cm$^3$, which has been varied with the increase in the length of the micro-photo bioreactor for different experiments and investigating the pollutant removal. The retention time of SDW contains micro-algae for the purification process has been defined ($\tau = 33 – 35$) hr. The linear velocity through a solution containing micro-algae and wastewater in the pipes of micro-photo bio reactor in the optimum flow rate was obtained equal to $u = 0.495$ cm$^3$ min$^{-1}$. The micro-photo bio reactor has been embedded in a chamber in order to study and investigate the defined parameters for the SDW process.

Measurement methods of analytical parameters

All sampling and measurements have been conducted the same day. For the laboratory studies, the initial and final pH of the SDW solution and micro-algae has been measured and recorded by a pH meter. The conductivity and TDS have been measured by a British made conductivity meter (4510 Jenway). To control and regulate the inlet flow rate to the bioreactor with acceptable accuracy, a digital micro flow meter has been used. Fusion Syringe micro pumps constructed by Chemyx Company have been used to control the solution and make it uniform. COD has been measured by
digestion unit (200DRB* HACH* USA), and double-ray UV-V is spectrophotometer (HACH* DR 5000* USA). Also, in order to specify the luminous intensity, a Lux meter has been used and to determine the turbidity, turbidity meter offered by German Aqua lytic was used.

**Manufacturing device**

Ingredients of the user device used to filtrate diary wastewater are shown in the form (Figure 4) and (Figure 5). To manufacture this device, first, the micro-photo bioreactor was determined for laboratory study. Four 2-liter cylinders to keep diary wastewater and four 1-liter cylinders to grow micro-algae were used. To control temperature and measure the temperature which is being studied, were used digital sensor and thermometer Sam Wong Eng. model: SU – 105IP. With using available cooling and the heater with a power of 300 watts, continuously was used throughout the process just to mix better and appropriate temperature distribution for experiments inside the chamber. Controlling the temperature in the chamber was performed with an accuracy ±0.5°C. 48 LED lamp which each lamp is with a light intensity of 330 ± 3 LUX was used to control the light intensity of chamber for growth of micro-algae in the micro-photo bioreactor and the amount of light intensity was identified and determined depending on the tests used throughout the experiments. Also, a digital timer Super Digital Timer Model: SDT- 8M during testing was used to control the amount of brightness and extinction of LED light to study and evaluate experiments.
Figure 4. Schematic representation of a filtration device. (1- Tank containing micro-algae, 2- tank containing simulated dairy wastewater SDW, 3- tube between the lid and the environment of the tank to adjust discharge, flow control, 4- inlet valve to control discharge, 5- magnetic stirrer, 6- microsyringe pump, 7- microflow meter, 8- digital thermometer, 9- heater, 10- the temperature sensor, 11- cooling fan, 12- diode lamp (Ahmed et al.), 13- Super digital Timer, 14- photo bio micro-reactor (micro model), 15- the tank containing of sampling, 16- the chamber (thermal and light insulator)).

Figure 5. Photo of micro-photo bioreactor with micro-algae.
Experimental method

The designed device has performed investigations on the contamination and COD removal for purification of SDW using micro-algae cultivated in different conditions of temperature and luminous intensity on four levels on the parameters of flow rate (Q = 0.0125–0.0500 Cm³min⁻¹), microchannel length (L = 10-16 m), initial pH (pH = 5–10), luminous intensity (Ei = 1000–4000 Lux) and temperature influence (T = 20–35°C) with the accuracy of ±0.5. The influence of these parameters and concentration value of micro-algae (mi = 0.050–0.200 L) in each liter of SDW has been evaluated on the COD removal. Each test has begun by entering a certain amount of micro-algae into a tank containing 1 liter of distilled water and a tank containing 1 liter of SDW, with the certain initial acidity of the (pH) and initial COD concentration (COD = 3900 mg L⁻¹) begins. Then, the output solution of both tanks has mixed with mixing valve, and the flow rate was determined using the syringe pump connected to the micro-photo bioreactor. Both tanks have been stirred for 5 minutes by 200 RPM, and then the stirring speed has decreased to 20 RPM, then the system has been kept in this condition for 120 hours. Then, the output liquid of micro-photo bioreactor has been filtered through a what man filter paper number 1 to analyze the COD. To optimize the pH, the purification of the SDW using micro-algae has been investigated within the range of pH = 5–10 in the micro-algae concentration of mi = 0.150 L in COD = 3900 mg L⁻¹. The pH of SDW has been regulated by H₂SO₄ or NaOH solutions. In order to optimize mi, the purification tests have been conducted by variation in the amount of concentration within the range of mi = 0.050–0.200 L in the initial pH, and COD = 3900 mg L⁻¹. The final pH (pHf) and the remained COD in the pH, and mi have been observed during the time. Then to measure the initial and the optimal flow rate, the manual control of the input to micro-photo bioreactor has been performed, and it has been recorded every moment.

Results and discussion

By repeating the experiment, the results of the analysis are expected at each stage of the process, with the aim of ensuring that the design and innovation of this pilot project are operational. However, the average stagnation, the results dispersion, might be occurred as a random error. For this purpose, by the design and specific geometry of the photo-microbial test reactor, the results of the experiments were recorded, taking into account the existing errors (±) in the experiments in different process sections. An analysis was carried out to evaluate the ability of the photo-micrographic reactor to be used in the treatment of dairy wastewater, as well as analyzes related to the effect of microalgae usage on the percentage of separation and sewage filtration. Then the results of this analysis were interpreted and reported.

Influence of the flow rate (Q)

The influence of input flow rate on the removal efficiency of the COD from SDW in pH = 8.00 has been shown in (Figure 6). It can be observed from Figure 1 that increasing the output flow rate of the tanks containing SDW and Microalgae would lead to a reduction in the removal behavior of the COD. Due to the appropriate retention time of 52 hours, the removal efficiency of the COD would cause an increase in the COD removal by reducing the input value of the Q.

Influence of the length of the microchannels of the micro-photo bioreactor (L)

The influence of variation in the initial length of microchannels L = 10-16 m on the removal efficiency of the COD from SDW in pH = 8.00 and the optimum flow rate of Q = 0.0125 Cm³ min⁻¹ has been presented in the (Figure 7). It is obvious from Figure 7 that by increasing the length of the micro channels up to L = 12 m the removal efficiency of the COD would increase, and then in L = 14 m it
has remained constant considerably and then in $L_i = 16$ m it would be increased again. The reason for these variations observed in the diagram is that by increasing the length of microchannels, the retention time would increase from 34 hours to 52 hours. This increase in the retention time would give an adequate time for the microalgae to digest the contaminations in the wastewater, and so the COD removal efficiency would increase until to reach a constant value.

**Influence of pH**

Figure 8a represents the influence of $pH_i$ on COD removal. It can be observed that by increasing the $pH_i$ of SDW, COD removal would increase up to $pH_i = 8.00$ where the maximum COD removal efficiency equal to 42.57% would be obtained. Increasing the $pH_i$ higher than $pH_i = 8.00$, the COD removal would be decreased. For the $pH_i > 8.00$ decrease in the COD removal would be
approximately 11%. Therefore, the low reduction in the COD removal efficiency when pH ≥ 10 is due to the reduction in the growth rate of micro-algae, and finally, the increase in the growth rate in the region of pH_i = 8.00 would lead to absorption of contamination and decomposition of them by micro-algae. (Figure 8b) Indicates the comparison between pH_i and pH_f for SDW purification in various pH_i. It can be observed that in all the studied range the relation of pH_i>pH_f is established. It is clear that the growth of the micro-algae cells would lead to the protein and other components removed from the milk and though a reduction in the pH can be observed. Therefore, pH_i = 8.00 is considered as an optimum value for COD removal and the other test for determining the optimum concentration, has been performed in this pH.

**Temperature influence**

Studying the temperature influence on the COD_f removal efficiency form the SDW had been conducted in different optimum concentration m_op and = 8.0pH_i,op for the initial COD (COD_o = 3900 mgL^{-1}) and optimum flow rate (Q_i = 0.0125 Cm^3 min^{-1}) and the length of microchannels equal to L_i = 16m. It can be observed form (Figure 9) that the COD removal efficiency of the SDW, would cause an increase in the growth rate of micro-algae in the appropriate temperature condition by increasing the temperature, which leads to Digestion, combination or absorption of the contaminations in wastewater, so the contamination removal from the SDW would be increased considerably.
Influence of luminous intensity

The result of changes in $E_i$ in all four levels of the fifth experiments revealed that the optimal luminous intensity is equal to $E_i = 4000$ Lux. As it can be observed from (Figure 10), by increasing the luminous intensity from 1000 Lux to 2000 Lux, no considerable change in the COD removal would happen. It can be concluded from analyzing the diagram, increasing the luminous intensity from 1000 Lux to 4000 Lux, would increase the COD removal. This increase would cause an increase in the growth rate of micro-algae and consequently a considerable increase in the contamination removal of the wastewater would occur.

Figure 9. Effect primary temperature and obtain optimum temperature in order to remove of wastewater situation. ($Q_i = 0.0017 \text{ cm}^3 \text{ min}^{-1}, L_i = 16 \text{ m}, \text{pH}_i = 8.00, E_i = 1000 \text{ Lux}, M_i = 0.150 \text{ L}, \text{COD}_i = 3900 \text{ mg L}^{-1}$).

Figure 10. Effect of primary luminous intensity and obtain optimum light radiation in order to remove COD of wastewater situation. ($Q_i = 0.0017 \text{ cm}^3 \text{ min}^{-1}, L_i = 16 \text{ m}, \text{pH}_i = 8.00, T_i = 30^\circ\text{C}, M_i = 0.150\text{L}, \text{COD}_i = 3900 \text{ mg L}^{-1}$).
Removing the turbidity and TDS from SDW using micro-algae

In all the laboratory studies, according to Tables 2 and 3 initial and final turbidity of SDW was measured. Turbidity prevents light penetration to SDW which cause a reduction in the growth rate of micro-algae in the SDW. So, the other reason for the slow growth of microalgae is probably the high turbidity of the solution containing the simulated dairy wastewater which limits the light penetration and dominance of heterotrophic bacteria. From the conducted experiments, it is clear that the maximum removal of final turbidity has been occurred in pH$_i$ = 8.00. Removing the turbidity would cause a faster rate of growth in microalgae in SDW.

The turbidity is the result of high concentration of suspended solid particles in the dairy wastewater. Therefore, in Tables 4 and 5, the amount of TDS has been measured and analyzed. A series of experiments have been done in the concentration of 5%, 10%, 20%, and 25% and the limitation of light penetration has been prevented. It can be concluded that the concentration of microalgae has a significant effect on the removing of contaminations in dairy wastewater and particularly cause an increase in the efficiency of turbidity removal.

| Table 2. Simulated dairy effluent analysis and microalgae analysis. |
|----------------------------------------|
| (SDW)$_i$ NTU | Micro-algae(15%) NTU | (SDW)$_f$ NTU |
| 1 | 389 ± 15 | 19.2 ± 5 | 1744 ± 50 |
| 2 | 336 ± 15 | 19.2 ± 5 | 1744 ± 50 |
| 3 | 286 ± 15 | 19.2 ± 5 | 1744 ± 50 |
| 4 | 248 ± 15 | 19.2 ± 5 | 1744 ± 50 |
| 5 | 215 ± 15 | 19.2 ± 5 | 1744 ± 50 |

| Table 3. Simulated dairy effluent analysis and microalgae with different concentrations in optimal conditions. |
|----------------------------------------|
| (SDW)$_i$ NTU | Micro-algae(15%) NTU | (SDW)$_f$ NTU | Error % |
| 1 | 1744 ± 50 | 6.2 ± 2 | 298 ± 10 | 5% |
| 2 | 1744 ± 50 | 14.2 ± 2 | 255 ± 10 | 10% |
| 3 | 1744 ± 50 | 19.2 ± 2 | 215 ± 10 | 15% |
| 4 | 1744 ± 50 | 25.2 ± 2 | 205 ± 10 | 20% |
| 5 | 1744 ± 50 | 31 ± 2 | 198 ± 10 | 25% |

| Table 4. Simulated dairy effluent TDS analysis and microalgae analysis. |
|----------------------------------------|
| (SDW)$_i$ NTU | Micro-algae(15%) NTU | (SDW)$_f$ NTU |
| 1 | 68 ± 5 | 858 ± 12 | 712 ± 15 |
| 2 | 68 ± 5 | 858 ± 12 | 672 ± 15 |
| 3 | 68 ± 5 | 858 ± 12 | 592 ± 15 |
| 4 | 68 ± 5 | 858 ± 12 | 523 ± 15 |
| 5 | 68 ± 5 | 858 ± 12 | 489 ± 15 |

| Table 5. Simulated dairy effluent TDS analysis and microalgae with different concentrations in optimal conditions. |
|----------------------------------------|
| (SDW)$_i$ NTU | Micro-algae(15%) NTU | (SDW)$_f$ NTU | Error % |
| 1 | 68 ± 5 | 422 ± 12 | 742 ± 15 | 5% |
| 2 | 68 ± 5 | 728 ± 12 | 584 ± 15 | 10% |
| 3 | 68 ± 5 | 858 ± 12 | 489 ± 15 | 15% |
| 4 | 68 ± 5 | 1051 ± 12 | 426 ± 15 | 20% |
| 5 | 68 ± 5 | 1241 ± 12 | 398 ± 15 | 25% |
Conclusion

In the conducted parametric study, the COD removal from SDW using micro-algae in the photo-microbioreactor has been investigated. According to the results, the efficiency of the contamination removal has a direct relationship with the increase in the length of the micro channels and flow rate. The concentration of the microalgae has been determined to be \( m_{\text{op}} = 0.150 \text{ L} \) in one liter of SDW. The obtained results for each parameter indicates that the removal efficiency of the required chemical oxygen (COD) in 120 working hours for the optimum parameters such as flow rate \( (Q_{\text{i}}=0.0125 \text{ Cm}^3 \text{ min}^{-1}) \), length of photo-microbioreactor \( (L_{\text{op}} = 16m) \) and the initial acidity \( (pH_{\text{i,op}}=8.00) \), temperature \( (T_{\text{i,op}}=30°C) \) with the accuracy of ±5%, luminous intensity \( (E_{\text{i,op}}=4000 \text{ Lux}) \) is equal to 24.21% and 29.26% and 36.05% and 37.81%and 42.57%, respectively. The biological purification of the SDW in the micro-photo-bioreactor revealed that the maximum COD removal efficiency is equal to 42.57%. Finally, the present study indicates that the ability to control the parameters above for purification of the dairy industry wastewater makes this approach a flexible, reliable, fast, efficient and economical approach due to the high removal efficiency, and therefore, the output wastewater can be discharged to the environment with confidence.

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