PERFORMANCE OF UPFLOW ANAEROBIC SLUDGE FIXED FILM BIOREACTOR FOR THE TREATMENT OF HIGH ORGANIC LOAD AND BIOGAS PRODUCTION OF CHEESE WHEY WASTEWATER

Article Highlights
• Dairy wastewater was successfully treated in UASFF with HRT of 48 h
• The amount of collected biogas was 2.4 L/d. The obtained biomethane had purity of 61%
• COD removal rate of 80% for organic loading rate (OLR) of 25.85 g COD/Ld
• Among the fitted models, Riccati model fitted in good agreement with the experimental data

Abstract
Among various wastewater treatment technologies, biological wastewater treatment appears to be the most promising method. A pilot scale of hybrid anaerobic bioreactor was fabricated and used for whey wastewater treatment. The top and bottom of the hybrid bioreactor known as upflow anaerobic sludge fixed film (UASFF); was a combination of upflow anaerobic sludge blanket (UASB) and upflow anaerobic fixed film reactor (UAFF), respectively. The effects of operating parameters such as temperature and hydraulic retention time (HRT) on chemical oxygen demand (COD) removal and biogas production in the hybrid bioreactor were investigated. Treatability of the samples at various HRTs of 12, 24, 36 and 48 h was evaluated in the fabricated bioreactor. The desired conditions for COD removal such as HRT of 48 h and operation temperature of 40 °C were obtained. The maximum COD removal and biogas production were 80% and 2.40 (L/d), respectively. Kinetic models of Riccati, Monod and Verhalst were also evaluated for the living microorganisms in the treatment process. Among the above models, Riccati model was the best growth model fitted with the experimental data with R² of about 0.99.

Keywords: COD, Riccati model, UASFF bioreactor, high organic load, cheese whey wastewater.

In cheese processing plants, large quantities of wastewater are generated. The wastewater consists of high biological oxygen demand (BOD) and chemical oxygen demand (COD) with concentrations of 40 and 50-70 g/L, respectively [1]. Dairy wastewaters can have different characteristics, depending on the product obtained (yogurt, cheese, butter, milk, ice cream, etc.). Some other parameters such as wastewater management, operating conditions and also types of process cleaning may influence the dairy effluents characterization [2,3].

In process of cheese production, whey is generated from the liquid residue when casein and fat are separated through coagulation of milk. It is a by-product of cheese or casein, and has several commercial uses, such as concentrated whey protein for food additives and nutritional diets [4]. Whey contains lactose (70-75%) and soluble proteins (10-15%) which results in a high COD (50-70 g/L) [5]. High concentration of organic matter in whey wastewater such as whey causes serious pollution problems to surroundings [6,7]. If cheese effluent is discharged to environ-
ment causes depletion of the dissolved oxygen in the stream that may jeopardize the aquatic life. In addition the contaminated water body in irrigation gradually reduces the land fertilities [8-10].

World annual production of whey is around 115 million tons; approximately 47% of the produced whey is disposed into the environment [11]. Since whey naturally contains lactose and biodegradable organic matter, biological treatment is a practical process [12]. Among biological treatment processes, treatment in ponds, activated sludge plants and anaerobic treatment are commonly employed [13]. Anaerobic method for the treatment of whey wastewater is attractive and often catches the attention of researchers because of the presence of high organic content in the waste, low energy requirement of the process, less sludge production and generation of fuel in form of methane [14]. Dairy wastewater has been extensively treated by coagulation/flocculation and sedimentation processes [3,15]. The main disadvantages of these processes are high coagulant costs, high sludge production, and poor removal of COD [16]. Therefore, biological treatment is usually preferred for dairy wastewater treatment [17,18].

Recently, an upflow anaerobic reactor has been successfully employed for treatment of dairy wastewater in full-scale applications [21]. The use of a laboratory-scale bioreactor for the treatment of dairy wastewater at an operational temperature of 30 °C was previously investigated [22]. It was found that COD removal varied between 85 and 88% at a hydraulic retention time (HRT) of 5.0 h and organic loading rates (OLRs) of 8.5 g COD/Ld. Another laboratory-scale investigation resulted in over 97% of COD removal using cheese whey wastewater in an anaerobic treatment process [23]. At a high OLR of 31 g COD/Ld, the UASB reactor treating cheese production wastewater provided removal of 90% for COD [24]. There is no sufficient information available in the literature regarding the COD removal from cheese whey wastewater in UASFF hybrid bioreactors. The main objective of this work is to enhance more thoroughly the treatment of cheese whey wastewater in a hybrid bioreactor. The effects of operating parameters such as temperature and HRT on COD removal and biogas production in the hybrid bioreactor were investigated.

**MATERIALS AND METHODS**

**Wastewater characterization**

The cheese whey wastewater used in present work was collected from a local cheddar cheese processing industry. The characteristics of cheese whey wastewater samples are presented in Table 1; the data were compared with Normex (Mexico) [25] and cheese processing wastewater [26]. The collected wastewater from Gela factor was obtained from the plant discharge known as cheese whey wastewater, which was homogeneously mixed and divided into small portions and kept at 4 °C for further use. Cheese whey wastewater contains most of the essential nutrients for microbial growth without additional nutrients to fresh substrate.

**Hybrid bioreactor setup and start up**

The hybrid reactor was constructed from a cylindrical pyrex column with internal diameter of 2.76 cm and total height of 160 cm. The total volume of the reactor was 960 ml. The density and specific surface of the packing media were 400 kg/m3 and 500 m2/m3, respectively. The shape of packing used in UASFF is similar to honeycomb shape of polyethylene material with high surface area. A funnel shaped gas separator was used to liberate the generated biogas from the effluent, and then the gas was led to a gas collector tank. The gas tank was a cylindrical glass pipe with an internal diameter of 80 mm and height of 1 m. The liberated gas was frequently measured at a def-

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Gela, mg/L</th>
<th>Normex, mg/L [25]</th>
<th>Cheese processing wastewater [26]</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD</td>
<td>50000-70000</td>
<td>54000-77300</td>
<td>60000</td>
</tr>
<tr>
<td>BOD</td>
<td>27000-36000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>TS</td>
<td>55000-65000</td>
<td>3900-58900</td>
<td>-</td>
</tr>
<tr>
<td>TSS</td>
<td>10000-15000</td>
<td>-</td>
<td>2500</td>
</tr>
<tr>
<td>TKN</td>
<td>10-20</td>
<td>500-5600</td>
<td>830</td>
</tr>
<tr>
<td>P-PO4</td>
<td>40-60</td>
<td>-</td>
<td>280</td>
</tr>
<tr>
<td>Lactose</td>
<td>50000-60000</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Alkalinity</td>
<td>200-400</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>pH</td>
<td>6-6.5</td>
<td>4.3-8.7</td>
<td>-</td>
</tr>
</tbody>
</table>
ined HRT and the gas volume was recorded with respect to time. The gas tank was initially filled with water which was saturated with methane. The volume of liberated gas was determined by the displacement of water in the gas tank. Figure 1a and b show a schematic diagram and actual of the experimental setup, respectively.

The system was operated at fixed temperatures, 24 and 40 °C with the aid of temperature-controller. The cheese whey wastewater sample was often collected from Gela factory (Amol, Iran). A sufficient large storage tank (1 m³) was installed in the settlement to collect the cheese whey wastewater from the inlet sewer pipe of the first septic tank. The collected samples of wastewater were stored in the refrigerator at 4 °C to minimize substrate decomposition before any primary treatment. The inoculums for seeding were obtained from anaerobic sludge pound in Gela dairy industrial plant (Amol, Iran).

Bioreactor operation and monitoring

COD and BOD of the raw and treated wastewater were analyzed according to methodology developed by American Public Health Association (APHA) and American Water and Wastewater Association (AWWA) standards [27]. The withdrawn duplicated samples were analyzed and average value was recorded. Biogas production was volumetrically measured by displacement of water in a cylindrical column. Methane content was determined by gas chromatography, using a GC model 7890A (Agilent Technologies, USA) equipped with TCD detector and packed column Carboxen 1000 was used for the gas analysis.

Kinetic models

Biological models are used to determine the relationships between variables. These models were used to control and optimize the treatment in laboratory scale UASFF bioreactor. In this study, an unstructured model describing the exponential cell growth was used. In addition, other models were used to obtain the desired model for the microorganism’s growth. Riccati, Monod and Verhalst models were evaluated to define the related kinetic parameters (Table 2).
Table 2. Evaluated kinetic models

<table>
<thead>
<tr>
<th>Kinetic model</th>
<th>Non-linear model</th>
<th>Linear model</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riccati</td>
<td>$X = \frac{X_0 e^{\mu_m t}}{1 - \frac{X_0}{X_m} (1 - e^{\mu_m t})}$</td>
<td>-</td>
</tr>
<tr>
<td>Monod</td>
<td>$\mu = \frac{\mu_m S}{K_S + S}$</td>
<td>$\frac{1}{\mu} = \frac{K_S}{\mu_m S} + \frac{1}{\mu_m}$</td>
</tr>
<tr>
<td>Verhalst</td>
<td>$\mu = \mu_m - \frac{\mu_m}{X_m} X$</td>
<td>$\mu = \mu_m - \frac{\mu_m}{X_m} X$</td>
</tr>
</tbody>
</table>

RESULTS AND DISCUSSION

Reactor performance

The COD and pH of the cheese whey wastewater were daily measured for the duration of 34 days. The bioreactor operation and variation of pH during the startup of the system was monitored. Figure 2a shows variations in influent and effluent COD over the period of 34 days. Initial COD has increased in five steps and gradually shifted from 4000 to 14000 mg/L. The COD removal has increased from 15 to 62%. The effluent pH altered when feed was added due to initial pH of the feed; however most of the time, the effluent pH was relatively constant nearly recorded at 6.5. The pH of bioreactor varied from 5.5 to 7 throughout the experiment; there was slight variation in pH which was unnecessary to make any adjustment; this range is suitable for anaerobic microbes. Outlet pH was almost neutral (approximately 6.5), and the performance of bioreactor was high and stable. Steady state conditions were achieved at stable conditions with high COD removal. Fluctuations of pH are shown in Figure 2b.

Effect of HRT and OLR in COD removal

After start-up period, COD removal efficiency was examined at different OLR with fixed HRT at 24 °C. OLR was gradually increased with specific HRT from 12 to 48 h. Figure 3a also shows that an increase in HRT and OLR, improved COD removal. Maximum COD removal efficiency was 59% at HRT of 48 h and OLR of 25.61 (g COD/Ld). An increase in HRT resulted in an increase in contact time between wastewater and granular sludge, and enhanced COD removal. Figure 3b shows the COD removal at various organic loading rate (OLR) and constant tempe-

![Figure 2. Examination of the wastewater system: a) COD variation and b) pH variation. Experimental conditions at start up: stepwise input of COD from 4248 to 13845 mg/L at room temperature.](image)
The COD removal was also increased with increase in HRT and OLR. Maximum obtained COD removal efficiency was 80% at an HRT of 48 h, OLR of 25.85 (g COD/L.d) at 40 °C. It can be seen that an increase in microbial activity at high temperature, removal efficiency was improved from 59 to 80% when the bioreactor temperature was increased from 24 to 40 °C. As Figure 3 illustrates, for each HRT there was a maximum in OLR removal. Beyond the maximum value at desired HRT, the OLR removal decreased due to overloading rate of OLR.

The present data were compared with other work for the biological treatment of cheese whey wastewater. It was found that Frigon et al. [5] have treated cheese whey wastewater by sequential anaerobic and aerobic steps in a single digester for duration of 4 days; they removed about that 88% of biodegradable COD. Gavala et al. [26] treated dairy wastewater in UASB for HRT of about 20 days; their reported COD removal was 81-85%. Monroy et al. [28] have also used dairy wastewater treatment in anaerobic filtration; a 70% COD removal was reported for HRT of 4 days. In present work an 80% of total COD at HRT of 48 h was removed.

**Effect of initial COD on effluent pH**

The effluent pH varied due to additional fresh feed and its initial pH. Figure 4a and b shows the effluent pH with initial COD at two different temperatures. The pH of effluent increased at high initial COD. For constant initial COD, effluent pH and COD removal increased with an increase in HRT. It showed that for anaerobic bacteria at high HRT, an expected and desired range of pH would be 6.7-7.0. Therefore, COD removal was increased. Because COD removal had an increasing trend at high temperature, the effluent pH was slightly lowered. This was due to high biological activities and increasing trend of COD removal, which resulted in activity of acid formers in the anaerobic process.

**Biogas production**

The effect of OLR and HRT on the biogas production rate is shown in Figure 5a and b. Accord-
Figure 4. Evaluation of pH effluent in different OLR at: a) 24 and b) 40 °C.

Figure 5. Evaluation of biogas production in different OLR: a) at 24 °C, effluent pH 5.5-5.8, influent COD 8430-63240 mg/L; b) at 40 °C, effluent pH 5.8-6.9, influent COD 9247-65211 mg/L.
According to Figure 5a at 24 °C, amount of biogas was increased by increasing OLR at a constant HRT. Maximum biogas (1.02 L/d) was produced at OLR of 25.61 (g COD/Ld) and constant HRT 48 h. Figure 5b shows effect of OLR on biogas production rate at 40 °C. Biogas production rate was also increased with increasing OLR. With an increase in OLR, biodegradability of the substrate and sufficient microbial community was increased and then the biogas production will increase [29,30]. It is very important that because of removal efficiency was improved at high temperature (40 °C), the amount of biogas production was also slightly increased at low temperature (24 °C). The amount of 2.40 L/d was obtained at high HRT (48 h), OLR (32.60 (g COD/Ld)) and temperature (40 °C). GC analysis of the biogas at optimum condition showed that almost 61% of biogas was methane and the remaining gas was CO2. The obtained results showed that there is a suitable condition for UASFF hybrid bioreactor to operate for maximum COD removal.

Evaluation of kinetic models

The utilization of organic matters and sludge productions are predicted by various microbial kinetic growth models. Monod and Verhalst models are simple models for anaerobic degradation of organic compounds in cheese whey wastewater. Table 3 summarizes the kinetic parameters for all 3 presented models with non-linear and linear equations. The kinetic data for all of the stated models showed that experimental data may be corresponded with theoretical concepts and the models were appropriate for predicting the performance of fabricated UASFF bioreactor. The regression coefficients and the kinetic coefficients were compared. Figure 6a-c indicates the relationship between the cell dry weight with respect to time, the linear model known as Lineweaver-Burke plot and growth rate versus cell dry weight, respectively. The experimental data fitted with the Riccati model had $R^2$ of 0.99. The Riccati model was derived from the Riccati equation with the concept of second order inhibition. Therefore, the Riccati model predicts any possible inhibition may exist in the bioreactor [31]. Figures 6b and 6c show the linearized Monod and Verhalst models, respectively. The results of the analysis indicated that the experimental values were in good agreement with the predicted values. The predicted and observed results indicated that there is a high regression coefficient in the three models but in the other hand, predicted $\mu_{\text{max}}$ obtained with the Riccati model had a high accordance with observed results, so the Riccati model was selected as suggested kinetic models for the hybrid bioreactor. The kinetic constants and regression coefficients resulted in three models are presented in Table 3. Based on the reported value for $\mu_{\text{max}}$, the values for Riccati and Verhalst models were very close, while the maximum specific growth rates were higher than the value in the Monod model. Thus, the Riccati and Verhalst models should project faster rate compared to the Monod model.

Table 3. Kinetic parameters in hybrid bioreactor treating cheese whey wastewater

<table>
<thead>
<tr>
<th>Model</th>
<th>$\mu_{\text{max}}$ / h$^{-1}$</th>
<th>$K_s$ / mg L$^{-1}$</th>
<th>$R^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Riccati</td>
<td>1.31</td>
<td>-</td>
<td>0.995</td>
</tr>
<tr>
<td>Monod</td>
<td>0.807</td>
<td>7.398</td>
<td>0.977</td>
</tr>
<tr>
<td>Verhalst</td>
<td>1.23</td>
<td>-</td>
<td>0.983</td>
</tr>
</tbody>
</table>

CONCLUSION

The hybrid bioreactor was found to be a successful biological treatment system, achieving a high COD removal efficiency for the treatment of cheese whey wastewater. The results are summarized as follows:

1. It was concluded that the hybrid system is much faster than the conventional digester and even with upflow anaerobic sludge bed (UASB). The remaining untreated COD may need further treatment before disposed to environment.

2. COD removal was examined at two different temperatures (24 and 40 °C). Maximum COD removal (80%) was obtained at HRT of 48 h, OLR of 25.85 g COD/Ld and constant temperature of 40 °C.

3. Maximum biogas production was 2.40 (L/d) that was obtained at HRT of 48 h, OLR of 32.60 (g COD/Ld) and constant temperature of 40 °C.

4. Effluent pH was increased when HRT increased from 12 to 48 h. The optimum pH for anaerobic microbes was obtained at HRT of 48 h that it is shown a decreasing organic acid and increasing of COD removal. The pH variation shows shift of bioconversion of organic acid to methane occurred while COD removal increased.

5. When bioreactor temperature increased from 24 to 40 °C the COD removal and biogas production significantly increased.

6. Among the project growth kinetic models, the Riccati model was the best growth model fitted with experimental data.

7. A combination of air flotation and then membrane separation processes are suggested for the post treatment and the removal of remaining contaminants.
Acknowledgements

The authors acknowledged Noshirnavi University of Technology, Biotechnology Research Lab for providing all necessary facilities to conduct present research.

REFERENCES


Figure 6. The plotted kinetic models with initial COD = 51714 mg/L, HRT = 48 h: a) Riccati model; b) linearized Monod model; c) Verhalst model.
NAZILA SAMIMI TEHRANI1
GHASEM D. NAJAFPOUR2
MOSTAFA RAHIMNEJAD2
HOSSEIN ATTAR1

1Department of Chemical Engineering, Science and Research Branch, Islamic Azad University, Tehran, Iran
2Biotechnology Research Lab, Faculty of Chemical Engineering, Noshirvani University of Technology, Babol, Iran

NAUČNI RAD

KARAKTERISTIKE BIOREAKTORA SA NEPOKRETNIM FILMOM ANAEROBNOG MULJA
NAVIŠE ZA OBRADU VELIKOG ORGANSKOG OPTEREĆENJA I PROIZVODNJU BIOGASA IZ
OTPADNE SURUTKE

Među različitim tehnologijama za obradu otpadnih voda, izgleda da biološko prečišćavanje najviše obećava. Poluindistrijski hibridni anaerobni bioreaktor je korišćen za obradu otpadne surutke. Vrh i dno hibridnog bioreaktora poznatog kao nepokretni film anaerobnog mulja koji se kreće naviše (UASFF) je kombinacija prekrivača anaerobnog mulja (UASB) i bioreaktora sa nepokretnim filmom anaerobnog mulja (UAFF) koji se kreće naviše, redom. Ispitivani su efekti radnih uslova, kao što su temperatura i hidrauličko retenciono vreme (HRT), na uklanjanje hemijske potrošnje kiseonika (HPK) i proizvodnju biogasa u hibridnom bioreaktoru. Obradivost uzoraka pri različitim HRT od 12, 24, 36 i 48 h je ocenjena u hibridnom bioreaktoru. Dobijeni su zeljeni uslovi za uklanjanje HPK od HRT = 48 h i radna temperatura od 40°C. Maksimalno uklanjanje HPK i produkcija biogasa su 80% i 2,40 dm3/dan, redom. Kinetički modeli Riccati, Monod i Verhalst su primenjeni za rast mikroorganizama u procesu obrade. Među navedenim modelima, Riccati model je najbolji model rasta koeficijentom determinacije R² = 0,99.

Ključne reči: HPK, Riccati model, UASFF bioreaktor, visoko organsko opterećenje, otpadna surutka.