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Process Performance of a Granular Single Bioreactor with Continuous Feeding and Intermittent Discharge Regime Treating Dairy Wastewater

M. Jamshidi, A. A. Zinatizadeh*, S. Rezaee, A. Asadi

Environmental research Center (ERC), Department of Applied Chemistry, Faculty of Chemistry, Razi University, Kermanshah, Iran

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ABSTRACT

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NOMENCLATURE

In this study, granulated sludge synthesized in a sequence batch reactor (SBR) and then granulated sludge was transferred to continuous feeding and intermittent discharge (CFID) bioreactor. Two independent variables (air flow rate and hydraulic retention time (HRT)) were considered to optimize the process. Subsequently, long term performance (150 days) of the bioreactor was assessed by monitoring nine responses. The process optimization in CFID regime was performed using a central composite face-centered design (CCFD) and analyzed using response surface methodology (RSM). Based on obtained data, the optimum conditions were achieved at HRT of 7-8 h and air flow rate of 2-3.5 L/min. The CFID with granular sludge acted as a high rate bioreactor as the granular sludge could provide high MLSS concentrations (around 10000 mg/L) in a bioreactor.

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NUMBINGL	IIUKE		
AFR	Anaerobic filter reactor	OLR	Organic loading rate
AS	Activated sludge	PAOs	Phosphorus accumulating organisms
BOD	Biochemical oxygen demand	pCOD	Particulate COD
CFID	Continuous feed and intermittent discharge	PHP	Poly hydroxy butyrate
CFR	continuous-flow reactor	SBR	Sequential batch reactor
CNP	Carbon, nitrogen and phosphorous	sCOD	Soluble COD
COD	Chemical oxygen demand	TN	Total nitrogen
DO	Dissolved oxygen	TKN	Total kjeldahl nitrogen
F/M	Food to microorganism ratio	TP	Total phosphorus
HRT	Hydraulic retention time	UASB	Up-flow anaerobic sludge blanket
MLSS	Mixed liquor suspended solid		

1. INTRODUCTION

Dairy industrial wastewater is one of the serious pollution sources for the water environment [1]. The adverse impact of milk processing effluents on the environment has been proved owing to the very large flows of

*Corresponding Author Email: *zinatizadeh@razi.ac.ir* (A. A. Zinatizadeh)

wastewaters with a high volume of organic matter and nutrients. In order to reduce the environmental problems and health hazards, nutrient content of dairy wastewater must be removed before releasing to environment [2]. As all compounds in dairy wastewater are biodegradable, the effluent of dairy industries are mostly treated using

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From the literature, aerobic granular sludge is known as an intersting technology to achieve high rate biological wastewater treatment systems. Aerobic granular sludge has been attracted by researchers due to its high metabolic activity, significant settle ability, supreme biomass maintenance without any support media and particularly, the excellent simultaneous nitrification and denitrification valency [5, 6]. Aerobic granular sludge has been effectively cultivated in a sequencing batch reactor (SBR) in order to treat different wastewaters for example brewery wastewater [7], abattoir wastewater [8], saline wastewater [9], and palm oil mill effluent (POME) [10]. Besides, dairy wastewater has been treated by aerobic granular sludge in several research works. For instance, higher than 90% of TCOD and 80% TN removal efficiency at a volumetric exchange ratio of 50% and a cycle time of 8 h was reported by Schwarzenbeck et al. [11]. In another study, nitrogen and COD removal efficiencies were reported 80 and 70%, respectively, at organic and nitrogen loading rates of 7 g COD/L.d and 0.7 g NH₄⁺- N/L.d [12].

It was noticed that the most of studies concerning aerobic granule have been focused on cultivated granule and operation for a short time. As a fact, the instability of aerobic granules coused a limitation for pilot- or fullscale applications of the aerobic granulation system [13-15]. Therefore, long term operation of aerobic granular sludge is required to develop this technology. Steadystate continuous-flow bioreactors were favored rather than SBRs as continuous-flow bioreactors are more easier to operate and control. Chen et al. [16] demonstrated that granule stability in both an SBR and a continuous-flow reactor (CFR) could only be maintained for a short period of time. Continuous feed and intermittent discharge (CFID) regime has been introduced in some studies as a new generation of SBR conducted by Asadi et al. [17] both advantages of continuous and batch regimes. Recently, CFID regime has been coupled with high frequency ultrasonic irradiation for treating milk processing wastewater [18-20]. Also, from the literature, an airlift bioreactor was operated based on CFID regime for treating soft drink and milk processing wastewaters [21, 22].

In this research work, biological carbon and nutrients removal in an aerobic granular sludge CFID bioreactor was assessed to treat milk processing wastewater. This means that, the effect of two operating independent variables (Hydraulic retention time (HRT) and air flow rate) on the CFID bioreactor performance was studied to obtain optimum condition. 9 responses were measured to evaluate the bioreactor performance including COD, TN, TKN, TP removal efficiency, SVI, effluent turbidity, and effluent concentrations of NH4⁺-N, NO₂-N, and NO₃-N as a function of two independent variables including air flow rate and HRT.

2. MATERIALS AND METHODS

2.1. Wastewater Characteristics The wastewater was taken from a working dairy plant, Bistoon Co., Kermanshah, Iran. The samples were stored in a refrigerator at 4 $^{\circ}$ C to prevent any changes in the matrix of samples. The wastewater characteristics are shown in Table 1. It should be noted the real phosphorus concentration in the samples was about 5-6 mg/L. To investigate the performance of the system in terms of removing phosphorus, the phosphorus concentration was justified around 50 mg/L by adding KH₂PO₄, so that the ratio of COD: N: P was about 100: 18: 5.

2. 2. Granule Cultivation The activated sludge collected from Bistoon's dairy factory wastewater treatment plant was in the form of conventional flocs. A bubble column type reactor with a volume of 2L was applied as a SBR for granule cultivation. The SBR reactor was operated with the activated sludge. Cycle time was adjusted at 4-h with 30 min settling time and 210 min aeration times. The settling time was stepwise reduced to 10 min during the granule cultivation. At the beginning each cycle, the bioreactor was filled with around 1 liter of wastewater, and the treated wastwater was drained out from the bioreactor at the end of each cycle. The volumetric exchange ratio increased from 50 to 80 % during the granule cultivation. In order to enhance granule formation, some additives including MgSO₄.7H₂O, 200 mg/L; CaCl₂.2H₂O, 10 mg/L were used in this stage. The cultivation phase lasted for 30 days.

 TABLE 1. Characteristics of Bistoon's dairy industrial wastewater

Parameters	Unit	Amount
sCOD	(mg/L)	900-1200
BOD5	(mg/L)	640-850
TN	(mg/L)	160-180
TP	(mg/L)	48-52
TSS	(mg/L)	40-50
pH	-	6.8-7.2

2. 3. Bioreactor Configuration and Operation Figure 1 shows the schematic diagram of the used CFID bioreactor in this study. Internal diameter, liquid height and total volume of the bioreactor was 8 cm, 50 cm, and 2.5 L, respectively. An automatic control valve was fixed on the half-height of the column to obtain an intermittent discharge. HRT was calculated based on 1.25 L of feeding volume (the volume which is filled and discharged). The bioreactor was aerated with an air bubble diffuser connected with an air blower. The CFID bioreactor was operated with granular sludge produced at SBR. The bioreactor was operated under room temperature (20 \pm 2°C). The bioreactor was operated according to experimental runs which were designed by Design Expert software. In this study, two independent effective variables i.e. HRT (4, 6, 8 h), air flow rate (1, 3, 5 L/min) were studied. In the second stage, the system was operated under optimum condition obtained from first stage (HRT: 8 h and air flow rate: 3 L/min) for 60 days as a long-term study. Besides, the HRT values of 10, 11, and 12 h for a long-term operation were examined and the bioreactor was operated at each level of HRT for 30 days.

2. 4. Experimental Design and Mathematical Modeling Statistical design of experiments and data analysis was carried out by Design Expert Software (version 7.0). Two independent effective variables, HRT (4, 6, and 8 h) and air flow rate (1, 3, and 5 L/min) were selected in the experiments design to evaluate the performance of CFID bioreactor with granular sludge. In the basis of the factorial design, 13 experiments (including 4 factorial points, 4 axial points, 1 center point and 4 replications of the center point) were designed.



Figure 1. Schematic diagram of the experimental setup

sCOD removal, TN removal, TKN removal, denitrification rate, effluent nitrate, effluent nitrite, TP removal, effluent turbidity, SVI, and height of sludge were measured or calculated as response in the bioreactors. Table 2 presents the experimental conditions and obtained results. The experimental data obtained was used to determine the coefficients of the polynomial model based on the following equation, Khuri and Cornell [23].

$$Y = \beta_0 + \beta i X i + \beta j X j + \beta i i X i^2 + \beta j j X j^2 + \beta i j X j + \dots$$
(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 assess the effectiveness of the model terms.

2. 5. Analytical Methods The concentrations of COD, TN, TKN, nitrate and nitrite, NH4+-N, TP, MLSS, SVI were determined by using standard methods [24]. Organic nitrogen was measured by deducing the ammonia nitrogen from TN. For sCOD, 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. Biological oxygen demand (BOD) was measured with BOD meter model (OxiTop IS 6). TKN and NH4+-N were determined by TKN meter Gerhardt model (Vapodest 10, Germany). The DO concentration in wastewater was determined using a DO probe. DO meter was supplied by WTW DO Cell OX 330, electro DO probe, Germany. Turbidity was measured by a turbidity meter model 2100 P (HachCo.,USA). Morphology of the granules formed was investigated by taking scanning microscopy (SEM) image.

3. RESULTS AND DISCUSSION

3. 1. Cultivation of Aerobic Granule in SBR (Granule Formation) The granular sludge was cultivated in an SBR and the biomass appearance after 60 days is presented in Figure 2a. Also, scanning electron microscopy (SEM) images of the granules grown after 60 days are presented in Figure 3. From the figure porous and integrated structure with cocci and filament microbial community is approved. It is noted that the cocci population is not as remarkable as the filamentous. The maturated granules presented a good micro pore structure with an average pore width.

3.2. Bioreactor Performance

3.2.1.COD Removal High COD concentration is one of the main problems with many industrial wastewaters like dairy industry wastewater. COD content consists of both soluble COD (sCOD) and particle COD (pCOD). Milk

processing industry wastewater contains pCOD could be removed by physical methods such as flotation and screening and sCOD is mostly removed through a bioprocess [25]. Granular sludge is sensitive and not stable for treating wastewaters with high pCOD. Therefore, in this study, pCOD content of the wastewater was separated by filtration before entering to the bioreactors. The performance of the bioreactor was evaluated by calculating sCOD removal as a response. The experimental data and are ANOVA values of sCOD removal are reported in Tables 2 and 3, respectively. The significance of each coefficient was determined by F-value and P-value. From Table 3, A (HRT) and B (air flow rate), A², B² and AB were significant model terms for the CFID bioreactor with granular sludge. Other model terms were not significant. Figure 3 illustrates the effects of the variables on sCOD removal efficiency. From the figure, an increase in air flow rate from 1 to 3 L/min caused an increase in the response due to higher oxidation potential; however, further increasing in air flow rate to 5 L/min indicated a negative trend in removing SCOD at all HRTs. The reason could be attributed to high hydrodynamic shear force which led to instability of the granules in the system and granule wash out [26]. Also, the results presented in Figure 3 showed that sCOD removal efficiency was increased due to an increase in HRT at all the air flow rates. It might be because of a reduction of F/M from 0.75 to 0.375 g COD/g VSS d as HRT was increased from 4 to 8 h. The maximum predicted sCOD removal efficiency at HRT and air flow rate of 8h and 3 L/min, respectively, was 102% (corresponding to F/M ratio of 0.375 g COD/g VSS. d).



Figure 2. SEM of the granules formed after 60 days.(a) full granule (Mgg- \times 200), (b) granule surface (Mgg- \times 1.7k), (c) granule surface (Mgg- \times 8k)



Figure 3. sCOD removal as a function of HRT and air flow rate

							K	esponses						
Run	Factor 1 A:HRT, (h)	Factor 2 B:airflow rate, (L/min)	F/M	sCOD removal, (%)	TN removal, (%)	TKN removal, (%)	Effluent N-NO ₃ , (mg/L)	Effluent N-NO ₂ , (mg/L)	Denitrification rate, (g/L.d)	TP removal, (%)	Effluent Turbidity, (NTU)	SVI, (mL/g)	Height of sludge, (m)	DO, (mg/L)
1	4	1	0.75	63	46.27	37	6	0.2	0.23	10	25	33.75	0.135	1.3
2	6	1	0.5	66	56	47	5	0.12	0.24	22	17	31.25	0.125	1.1
3	8	1	0.375	97	70.60	64	2.43	0.148	0.26	18	12	18.75	0.075	1.1
4	4	3	0.75	85	63.46	60	3	0.1068	0.29	-6	18	17.5	0.07	2.1
5	6	3	0.5	92.6	72.11	66	1.5	0.1031	0.30	-2	15	16.25	0.065	2.3
6	8	3	0.375	96.94	83.02	78	0	0.23	0.35	8.7	5	12.5	0.05	2.2
7	4	5	0.75	59.79	19.20	2.38	8.7	0.030	0.01	-10	40	35	0.125	3.7
8	6	5	0.5	56.36	36.67	20	7.5	0.137	0.18	-1	32	31.25	0.14	3.9
9	8	5	0.375	55	45	32.6	5.23	0.114	0.17	-3	30	31.25	0.14	3.8
10	6	3	0.5	94	70	65	2.3	0.122	0.28	0	16	16	0.065	2.4
11	6	3	0.5	91.5	71	64.22	1.5	0.126	0.31	-2	14	15	0.065	2.3
12	6	3	0.5	90	69	65	1.8	0.095	0.29	-3	15	17	0.064	2.5
13	6	3	0.5	92	72	66	1.21	0.044	0.28	-4	16	16	0.065	2.4

TABLE 2. Experimental conditions and result

Tribble of fit to the equations of the Design Expert field in studied responses											
Response	Modified Equations with significant terms	probability	\mathbb{R}^2	Adj.R ²	Adeq precision	S.D	CV	PRESS	Probability for lack of fit		
SCOD removal	+90.44 +6.86 A-9.14 B-9.70 A B+3.32 A ² -26.46 B ²	33.13	0.9431	0.9146	14.425	4.92	6.17	995.78	0.2255		
TN removal	+72.02+11.62 A-12 B-26.40 B^2	261.59	0.9887	0.9849	49.680	2.24	3.74	105.04	0.2430		
Denitrification rate	+0.29 +0.042 A- 0.026 B+0.032 AB+0.045 A ² -0.17 B ²	4.03	0.7421	0.5580	6.486	0.071	31.09	0.34	0.0053		
Effluent turbidity	$+14.00 - 6.00A + 8.00 B + 12.00 B^2$	76.09	0.9621	0.9494	26.705	2.16	11.06	84.38	0.3347		
SVI	+15.56 - 3.96 A+ 2.29B + 2.81 A B- 0.96A ² + 15.29 B ²	37.79	0.9643	0.9388	16.190	2.16	9.73	245.63	0.1431		

TABLE 3. ANOVA results for the equations of the Design Expert 7.0. for studied responses

As a point, working wastewater treatment plant (WWTP), Bistoon's dairy factory, Kermanshah-Iran reported around 95% of sCOD removal under HRT values of 72h. The WWTP includes an anaerobic fixed bed followed by an activated sludge system. This comparation verified that granular sludge CFID is a high rate bioreactor with high MLSS concentration. Also, perticular regime of CFID provides different dilution factor over a HRT.

3. 2. 2. Nitrogen Removal As nitrogen removal is performed by autotrophic nitrifier and heterotrophic denitrifier bacteria, aerobic and anoxic bioreactors are required to provide different conditions for nitrogen removal process in activated sludge systems. Recently, single bioreactors have been modified in different ways to remove nitrogen compounds which one of them is to use aerobic granular sludge. Consequently, in this study, nitrogen removal has been selected as a response. In the aerobic granule structure, heterotrophic, nitrifying, and denitrifying bacteria are existed together to remove carbon and nitrogen simultanously [27, 28]. The ANOVA results for TN removal efficiency in this study presented in Table 3. A, B and B^2 were significant model terms for the CFID bioreactor with granular sludge. Figure 4a illustrates the interactive effects of the variables on the response. The figure depicts an increase in the response when HRT increased from 4 to 8 h at all air flow rates, as a results of providing enough time for nitrogen removal processes [29]. From Figure 4a, the maximum TN removal efficiency (85 %) was achieved at 8 h of HRT and 3 L/min of air flow rate. This result proves a good balance between aerobic and anoxic phases for nitrogen removal at air flow rate of 3 L/min. In a comparable study, the maximum TN removal efficiency (94 %) was obtained at 6.3 h, 7500 mg/L and 1000/80/5 of HRT, MLSS concentration and COD:N:P ratio to treat dairy wastewater [30]. Figure 4b, presents denitrification rates at different experimantal conditions. As shown in the Figures 4a and 4b, TN removal and denitrification rate have similar trends with changing the variables. From the figure, the maximum denitrification rate (0.38 g N/L. d was obtained at the HRT and air flow rate of 8h and 3 L/min, respectively.



Figure 4. Nitrogen removal. (a) TN removal and (b) denitrification rate as a function of HRT and air flow rate, (c) nitrogen fractionation in influent and effluent under different operational conditions.

3. 2. 3. Fate of Nitrogen Compounds In the biological nitrogen removal process, N-organic is converted to ammonia, nitrite, and then to nitrate (nitrification) in the aerobic condition [31]. In the anoxic condition, nitrate is

reduced to nitrogen gas (denitrification) [32]. Therefore, in this study, the concentrations of N-organic, NH_4^+ -N, NO_2^- and NO_3^- were determined throughout the experiments. The columns diagrams of nitrogen constitute are shown in Figure 4c. As it is clear in the figure, the effluent nitrite concentration was not detectable at all the experiments, indicating a complete nitrification process. Moreover, the effluent nitrate concentrations were also low at all runs, which is an evidence for a good performance of aerobic granular sludge to provide anoxic zones in contrast to an extended aeration condition.

From Figure 4c, the effluent nitrate concentrations in 5 L/min of air flow rates (runs no. 7, 8 and 9) were higher than other runs because of the penetration of dissolved oxygen into the inner layers of granules led to a restricted anoxic zone at high air flow rates (DO: 3.7-3.9 mg/L) [33]. On the other side, in this condition as a result of high hydrodynamic shear force and low granules stability in the system, the size of granules was decreased gradually caused a decrease in Norganic removal and subsequence TN removal. In the literature mentioned that TN removal efficiency in the granular systems is strongly depended on the granules size [34]. Another result can be seen from Figure 4c, is the low effluent nitrate concentrations in the air flow rate 3 L/min rather than other experimental conditions through the development anoxic zone inside the granules resulted from limitation in oxygen transfer to the biomass aggregations [12] which is in agreement with high TN removal.

3. 2. 4. Phosphorus Removal In order to have a biological phosphorus removal process, polyphosphate accumulating organisms (PAOs) are essential, which take up excess phosphorus in the aerobic zone as long as they have previously accumulated enough poly hydroxy butyrate (PHB) in the anaerobic zone [35]. TP removal was reported as a response (Table 3) in order to investigate the performance of the granular sludge for phosphorus removal. It can be concluded from the results, the granular sludge had a low performance in removing phosphorus compounds. This result can be explained by accumulating phosphorus in the system as the sludge was not discharged and over time PAOs was saturated by phosphorus.

As an aceptable rule, PAOs store phosphorus in their body, so to have an adequate biological phosphorus removal, the excess sludge should discharge from time to time. In this project, granular sludge was not discharged from the system, thus after a certain time, the phisphous could not be absorbed by PAOs. Also, phosphorus stored in PAOs was released over micro anaerobic phase. Therefore, negtive phosphorus removal was observed in some conditions.

3. 2. 5. Effluent Turbidity Effluent turbidity is measured at different experimental conditions to evaluate the process performance (see Figure 5 and Table 3). From Table 3, A, B and B^2 were significant model terms. The

effluent turbidity was obtained in the range of 6 to 41 NTU. From the figure, the response was decreased with increasing HRT which the minimum effluent turbidity was reported at HRT of 8 h and air flow rate of 2.5 L/min, respectively. The maximum effluent turbidity was reported at the highest OLR (corresponding to the highest F/M ratio 0.75 g COD/g VSS) and air flow rate where the sludge disintegration was happened resulted from high OLR and hydrodynamic shear force. From the literature, high shear stress has been proved as main reason for the biomass washout and disintegrated aerobic granules [36]. From the Figure 6, at the similar HRT, with an increase in the air flow rate from 1 to 2.5, the effluent turbidity was decreased from 14 to 6 NTU due to an enhanced oxidation potential, whereas, with further increasing air flow rates from 2.5 to 5, the response was increased from 6 to 41 NTU as a result of detachments of microorganisms from granular structures in high shear stress created by high air flow rate.



Figure 5. Effluent turbidity as a function of HRT and air flow rate.



Figure 6. Sludge characteristics. (a) SVI as a function of HRT and air flow rate, (b) height of sludge under different operating conditions.

As a conclusion, to have a stable granular sludge, the value of OLR and intensity of aeration rate should be monitored and adjusted at the proper ranges.

3. 2. 6. Process Optimization Figure 7 shows the graphical optimization for the CFID bioreactor with granular sludge, which displays the areas of feasible response values (shaded portion) in the factors space. The optimum regions were identified in the figure, based on four critical responses (sCOD removal, TN removal, effluent turbidity and SVI). The shaded areas in Figure 8 show the experimental conditions with presented criteria in Table 4. From Figure 7, the air flow rates of 2 to 3.5 L/min demonstrated the optimal region due to a good balance between anoxic (for denitrification) and aerobic (for nitrification and COD removal) phases. As a result, the optimum conditions were found to be at HRT of 7-8 h and air flow rate of 2-3.5 L/min.



Figure 7. Overlay plot for the optimal region a function of HRT and air flow rate

TABLE 4. The optimization criteria for the responses studied

Response	Limit	Unit
sCOD removal	≥ 95	%
TN removal	≥ 80	%
Effluent turbidity	≤10	NTU
SVI	≤20	ml/g

4. CONCLUSION

In this study, a granular continuous feeding and intermittent discharge (CFID) was operated for removing carbon and nutrients from milk processing wastewater. The performance of the bioreactor was evaluated due to two operating independent variables including HRT and air flow rate. Also, DOE software was applied to design and optimize the experimental conditions. Based on the graphical optimization, the optimum conditions were obtained at HRT of 7-8 h and air flow rate of 2-3.5 L/min to achieve 95, 80%, 10 NTU and 20 mL/g of COD and TN removal efficiency, effluent turbidity and SVI, respectively.

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Process Performance of a Granular Single Bioreactor with Continuous Feeding and Intermittent Discharge Regime Treating Dairy Wastewater

M. Jamshidi, A. A. Zinatizadeh, S. Rezaee, A. Asadi

Environmental research Center (ERC), Department of Applied Chemistry, Faculty of Chemistry, Razi University, Kermanshah, Iran

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Keywords: Carbon and Nutrients Removal Dairy Wastewater Granular Sludge Continuous Feeding and Intermittent Discharge Bioreactor در این مطالعه، لجن گرانول در یک راکتور سری (SBR) سنتز شده و پس از آن لجن گرانولیت به تغذیه مداوم و تخلیه متناوب (CFID) منتقل میشود. دو متغیر مستقل (سرعت جریان هوای و زمان رفع هیدرولیکی) برای بهینهسازی این فرایند مورد توجه قرار گرفتند. پس از آن، عملکرد طولانی مدت (150 روز) بیوراکتور توسط نظارت بر 9 پاسخ بررسی شد. بهینهسازی فرآیند در رژیم CFID با استفاده از یک مرکز مرکزی مرکب مرکزی (CCFD) انجام شد و با استفاده از روش سطح پاسخ (RSM) مورد تجزیه و تحلیل قرار گرفت. بر اساس دادههای به دست آمده، شرایط بهینه در 8 HRT تا 8 ساعت و سرعت جریان هوا 3–5–3 لیتر در دقیقه بود. CFID با لجن گرانروی به عنوان یک بیوراکتور با سرعت بالا عمل میکند و لجن گرانول میتواند غلظت MLSS بالا (حدود 1000 میلیگرم در لیتر) در بیوراکتور فراهم کند.

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