



Short communication

PHA production from wastewater by mixed microbial culture under short-term microbial enrichment

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ABSTRACT

In this research, the possibility of polyhydroxyalkanoates (PHAs) production in a mixed microbial culture fed by industrial soft drink wastewater was assessed. To enrich the microbial culture, an uncoupled carbon and nitrogen feeding strategy were used in sequencing batch bioreactor (SBR). To evaluate the efficiency of the strategy, PHA, substrate, dissolved oxygen, biomass and nitrogen concentration profiles were reported in the 16th cycles of the SBR. From the obtained data, COD and nitrogen removal efficiencies were 89 % and 75.5 %, respectively at the cycle time of 12h. Also, the maximum poly-hydroxybutyric acid (PHB) content and specific PHA production rate (q_p) were achieved as 13.8% (mg-PHB/mg-TSS) and 6.4×10^{-3} (mg COD-PHA/mg COD-X.h), respectively.

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1. Introduction

Polyhydroxyalkanoates (PHAs) are linear polyesters accumulated inside microbial cells as carbon and energy source storage under unsteady conditions. They have resembling properties to petroleum-based plastic besides of being, biodegradable, biocompatible, and renewable. PHA is produced industrially using pure culture which is not economical due to requiring a sterile environment, expensive substrate and high energy consumption. Recently, mixed microbial culture (MMCs) is currently proposed to PHA production by researchers (Valentino et al. 2017). MMC PHA production generally needs a culture selection stage to enrich the activated sludge from PHA-accumulating microorganisms (Van Loosdrecht et al. 1997). The culture selection stage is based on applying the short period of excess external carbon substrate (feast phase) following with a long period of carbon substrate deficiency (famine phase). In the feast phase, biomass growth and PHA storage are carried out simultaneously. With consuming all the external carbon, stored PHA can be used as carbon and energy sources. The PHA accumulation is usually performed in the deficiency of nutrients (nitrogen and phosphorus). In the deficiency conditions, PHA production is reinforced rather than the growth. Serafim et al. (2011) proved that nitrogen deficiency caused an increase of PHA accumulation and productivity by limiting growth condition.

The main problem with applying MMC strategy A bottleneck in the PHA production by MMC is the low volume of products resulted from the low enrichment of PHA-producing microorganism in the culture selection stage (Reis et al. 2011). From the literature, different strategies have been used for increasing the population of PHA-accumulating microorganisms. Serafim et al. (2004) and Lemos et al. (2006) introduced an effective feast and famine strategy (uncoupled carbon and nitrogen feeding), in which nutrient was available only during the feast phase. The present work is aimed to apply uncoupled carbon and nitrogen feeding strategy to PHA production using soft drink wastewater as substrate.

2. Materials and methods

2.1. Culture selection stage

To enrich the activated sludge from the PHA-producing microorganisms a lab-scale SBR with 4.5L working volume was used

in this study as displayed in Fig. 1. SBR was inoculated with activated sludge (5000-6000 mg TSS/L) obtained from the aeration tank of a municipal wastewater treatment plant (Kermanshah, Iran). SBR cycle (12h) consisted of 30 min carbon feeding, 115 min reaction time (aeration feast phase), 10 min settling, 2 min withdrawal, 10 min nutrient feeding, 521 min reaction (aeration, famine phase), 30 min settling and 2 min withdrawal. pH was not controlled during the operation of SBR. The SBR was fed by soft drink wastewater with COD concentration of 3000 mg/L. The applied C/N/P ratio was 100 mg COD-C/4 mg N/0.5 mg P.

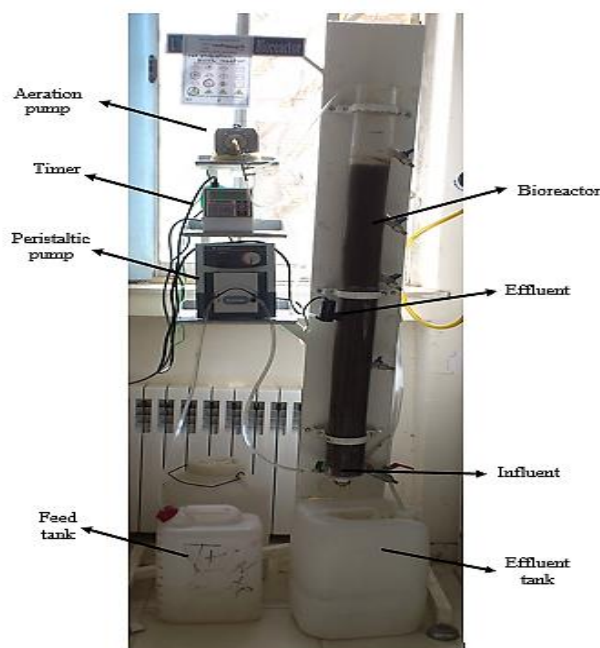


Fig. 1. Laboratory-scale experimental set up used in this study.

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2.2. PHA accumulation stage

PHA accumulation efficiency of the selected culture was evaluated in a fed-batch reactor under continuous aeration (DO concentration was less than 2 mg/L). The test was done in a 2L working volume bioreactor. The enriched biomass (2000 mg TSS/L) was taken at the end of famine phase and inoculated in the accumulating bioreactor fed by soft drink wastewater (5000 mg COD/L) with no adding of nutrients. The accumulation run was operated for 24h.

2.3. Analytical methods

Chemical oxygen demand (COD), total nitrogen (TN), total phosphorus (TP), ammonia, nitrite and nitrate concentration were measured according to the standard methods (P.H. Association et al. 1913). pH of the wastewater was measured using a (Metrohm, Switzerland) model 827. PHA concentration was determined by a gas chromatograph spectrometer (GC, Agilent Technologies model 7890B). About 15 ml of biomass was collected from the bioreactor at a specific time and centrifuged at 3600 rpm for 20 minutes. After removing supernatant, 2 ml methanol (3% v/v, H₂SO₄) and 2 ml chloroform were added to the biomass. The sample tubes were sealed off and heated at 100 °C for 3.5 h and cooled down to room temperature. After digestion, 1 ml of deionized water was added to the sample tubes and shake well for 10 minutes. After 60 minutes, three phases were separated. Then, 1 µL of the dense phase with a specified amount of internal standard was injected into the GC.

2.4. Process parameters studied

The PHA content of the biomass (% PHA, mg-PHA/mg-TSS), specific substrate uptake rate (-q_s, mg COD-S/mg COD-X.h), specific PHA production rate (q_p, mg COD-PHA/mg COD-X.h), activated biomass yield (Y_{X/S}, mg VSS/mg COD-S.h), and yields of PHA (Y_{PHA/S}, mg COD-PHA/mg COD-S) were the process responses studied in this work. The following equations were used to calculate the parameters. Where PHA_e, S_e, MLSS_e describe the amount of this parameters at the end of feast or famine phase and PHA₀, S₀, MLSS₀ define the amount of parameters at the start of feast or famine phase. Activated sludge (X_a) is the average activated biomass concentration.

$$\% \text{ PHA} = \frac{\text{PHA}_e - \text{PHA}_0}{\text{MLSS}} \times 100 \tag{1}$$

$$qp = \frac{\text{PHA}_e - \text{PHA}_0}{X_{a.t}} \tag{2}$$

$$-qs = \frac{S_0 - S_e}{X_{a.t}} \tag{3}$$

$$Y_{X/S} = \frac{\text{MLSS}_e - \text{MLSS}_0}{S_0 - S_e} \tag{4}$$

$$Y_{\text{PHA}/s} = \frac{(\text{PHA}_e - \text{PHA}_0) \times 1.67}{S_0 - S_e} \tag{5}$$

3. Results and discussion

To use an uncoupled carbon and nitrogen feeding strategy, the selection bioreactor was fed by soft drink wastewater (carbon source) at the beginning of each cycle to provide feast phase. Then, with consuming COD content and increasing DO concentration in the bioreactor over the time, the settling process was done. In the next step, the bioreactor fed by nutrients solution without a carbon source, providing a famine phase. It should be mentioned that, biomass growth was limited during the feast phase due to the lack of nutrients, therefore it could be expected that the amount of PHA stored will increase

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compared to the conventional methods (Oliveira et al. 2017). Also, adding nutrients at the beginning of the famine phase provided a good condition to enrich PHA-producing organisms. The selection bioreactor was operated for approximately 16 cycles (8 days). PHB, substrate, DO, biomass and nitrogen concentration profiles were illustrated over the 16th cycle of the SBR in Fig.2. From the results, the feast phase lasted 115 minutes (feast to famine ratio: 0.2). At the end of the feast phase, the highest PHB concentration was 116 mg/L. COD and nitrogen removal efficiencies was 89 % and 75.5 %, respectively. The performance of selected biomass during the 16th cycle of SBR was summarized in Table 1.

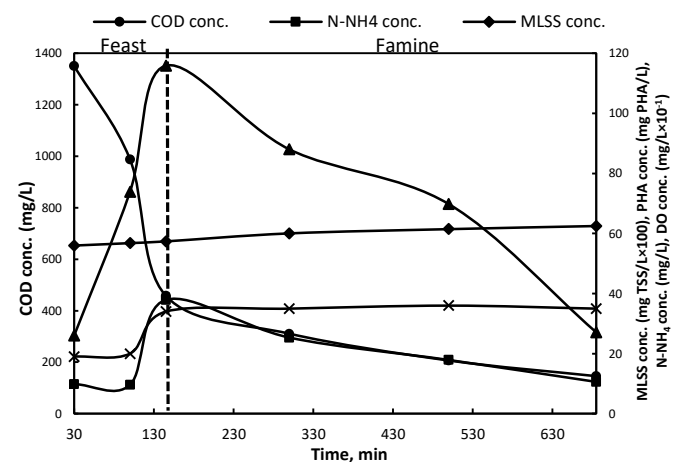


Fig. 2. Profile of the PHB, substrate, DO, biomass and nitrogen concentration over 16th cycles using soft drink wastewater.

Table 1. Performance of selected biomass during the cycle of SBR with uncoupled carbon and nitrogen feeding strategy.

Parameter	Unit	End of feast phase	End of famine phase
Substrate concentration	mg COD/L	457	145
Polymer concentration	mg COD/L	194	45
Substrate consumption rate	mg COD/L.h	104	7.3
Biomass yield (Y _{X/S})	mg-VSS/mgCOD-S	0.15	1.31
PHA yield (Y _{PHA/S})	mg COD-PHA/mg COD-S	0.16	—
Max. PHA content, %	mg-PHA/mg-TSS	8.12	1.9
Specific PHA production rate (q _p)	mg COD-PHA/mg COD-X.h	0.009	—
Specific substrate uptake rate (-q _s)	mg COD-S/mg COD-X.h	0.057	0.003

To evaluate PHA content in the selected biomass, the fed-batch reactor was operated under nutrients deficiency condition for 24 hours (Wen et al. 2010). The biomass (2000 mg/L) was collected at the end of the feast phase. Soft drink wastewater (5000 mg COD/L) was used as the substrate. The maximum PHB content and specific PHA production rate (q_p) were 13.8% (mg-PHB/mg-TSS) and 6.4x10⁻³ (mg COD-PHA/mg COD-X.h), respectively.

4. Conclusions

An uncoupled carbon and nitrogen feeding strategy as a culture selection strategy was successfully carried out for improving PHA production. From the results, the maximum PHB content in the biomass was 13.8% (mg-PHB/mg-TSS). As a conclusion, it would be feasible to produce PHA using industrial soft drink wastewater as substrate.

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