International Journal of Engineering

Journal Homepage: www.ije.ir

Influence of Different Culture Selection Methods on Polyhydroxyalkanoate Production at Short-term Biomass Enrichment

F. Ahmadi[a], A. A. Zinatizadeh*[a], A. Asadi[b], H. Younesi[c]

[a] *Environmental Research Center (ERC), Department of Applied Chemistry, Faculty of Chemistry, Razi University, Kermanshah, Iran*
[b] *Department of Gas and Petroleum, Yasouj University, Gachsaran, Iran*
[c] *Department of Environmental science, Faculty of Natural resources and Marine Science, Tarbiat Modares University, Noor, Iran*

PAPER INFO

Paper history:
Received 16 August 2018
Received in revised form 28 December 2018
Accepted 03 January 2019

Keywords:
Polyhydroxyalkanoate
Short-term Enrichment Time (STE)
Acetate
Soft-drink Industrial Wastewater

ABSTRACT

In this study, the potential of four different culture selection methods under short-term enrichment time (STE) to accumulate PHA-producing bacteria in mixed activated sludge was compared and the most efficient culture selection method was introduced. This means, PHA-producing microbial community was firstly enriched in a sequencing batch bioreactor (SBR) with four different selection methods including an anaerobic-aerobic process (SBR1), a fully aerobic batch process (SBR2), an uncoupled carbon and nitrogen feeding regime (SBR3) and aerobic/anoxic process (SBR4). In the next step, cellular PHA content was maximized in a fed-batch accumulator. From the obtained results, PHA could be accumulated up to 13.2, 10.8, 22.36, and 6 % (mg-PHA/mg-TSS) in SBR1, SBR2, SBR3, and SBR4, respectively. Uncoupled carbon and nitrogen feeding regime (SBR3) showed the best PHA accumulating ability when acetate was used as feed. Also, the SBR3 was fed by soft-drink industrial wastewater to evaluate the capability of the selected strategy for treating real wastewater, which 13.75% of mg-PHA/mg-TSS was achieved.

***doi**: 10.5829/ije.2019.32.02b.01*

NOMENCLATURE

SBR	Sequential batch reactor	DO	Dissolved oxygen
PHA	Polyhydroxyalkanoate	MLSS	Mixed liquor suspended solid
PHB	Polyhydroxybutyrate	SVI	Sludge volume index
TSS	Total suspended solid	TP	Total phosphorus
MMC	Mixed microbial culture	VSS	Volatile suspended solids
ADF	Aerobic dynamic feeding	TN	Total nitrogen
A/O	Anaerobic/aerobic	TKN	Total Kjeldahl nitrogen
FF&unCN	Uncoupled carbon and nitrogen feeding	GC	Gas chromatography
PAOs	Phosphorus accumulating organisms	q_p	Specific PHA production rate
GAOs	Glycogen accumulating organisms	$Y_{X/S}$	Activated biomass yield
SRT	Sludge retention time	$Y_{PHA/S}$	Yield of PHA production
COD	Chemical oxygen demand	$-q_s$	Specific substrate uptake rate

1. INTRODUCTION

Polyhydroxyalkanoates (PHAs) are biodegradable, biocompatible and flexible polyesters which could be synthesized by special bacteria [1]. Nowadays, pure or recombinant cultures are mostly used for PHA industrial production, which requires costs between 4 and 9 times higher than that of conventional plastics production [2,

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

Please cite this article as: F. Ahmadi, A. A. Zinatizadeh, A. Asadi, H. Younesi, Influence of Different Culture Selection Methods on Polyhydroxyalkanoate Production at Short-term Biomass Enrichment, International Journal of Engineering (IJE), IJE TRANSACTIONS B: Applications Vol. 32, No. 2, (February 2019) 184-192

3]. Therefore, PHA production by mixed microbial cultures (MMCs) has been attracted by researchers as this technology is easy to operate and more efficient in terms of feedstock utilization and economic aspects. To increase the efficiency of MMCs for producing PHA, the enrichment of PHA accumulating bacteria should be applied in a step known as culture selection [2, 4-6]. Dionisi et al. [7] and Takabatake et al. [8] proposed the use of an activated sludge process fed by raw organic waste for PHA production. Three different steps were considered in this report including (1) fermentation of organic waste stream, (2) selection of PHA-accumulating cultures, and (3) batch production of PHA. The proper enrichment of PHA-accumulating organisms in MMC is a vital matter in this technology. From the literature, the effect of various culture selection methods (oxygen supply patterns) on poly-β-hydroxybutyrate (PHB) production and the diversity of the bacterial community was assessed [9]. The maximum yield for PHB production in anaerobic/aerobic and aerobic dynamic feeding methods reported 64 and 53 wt%, respectively.

Low-cost wastewaters containing abundant valuable organic matters could be proper resources for the large-scale and cost-effective production of PHA. In a research work done by Satoh et al. [10], the possibility of using activated sludge to produce PHA was examined. The results verified that the PHA content of activated sludge could be reached to 62% by applying a microaerophilic–aerobic activated sludge process. Over last two decades, many researchers have been investigating on culture selection of PHA-producing bacteria using feast/famine (FF), anaerobic/aerobic (A/O), uncoupled carbon and nitrogen feeding (FF&unCN), aerobic/anoxic strategy, etc. Each method provides a different operational condition for the growth of various PHAs accumulating microorganisms. For instance, anaerobic/aerobic strategy irritates the accumulation of the polyphosphate accumulating organisms (PAO) and the glycogen-accumulating organisms (GAO). Mino et al. [11] used the anaerobic-aerobic process to increase biological phosphate removal and PHA production yield. Chua et al. [12] studied the effect of acetate concentration, pH and sludge retention time (SRT) on the capability of activated sludge treating municipal wastewater to produce PHA. The obtained results demonstrated that lower values of SRT (3 days versus of 10 days) and pH range of 8-9 were more favored conditions to produce PHA.

Moreover, from the literature, around 31-89% (g PHA/g dry biomass) was reported for feast/famine feeding regime at different studies applying sequencing batch reactor (SBR) [13-19]. Khumwanich et al. compared the potential of activated sludge of three different wastewater treatment plants of the brewery, canned pineapple, and fruit juice manufacturing factories to produce PHAs through feast/famine feeding regime. From the reported data, up to 7.57, 15.10, and 51.82% (g PHA/g MLSS) were achieved for the brewery, canned pineapple, and fruit juice wastewaters, respectively [20]. Also, the influence of ammonium on PHB production at accumulation stage was investigated by Johnson et al. [19]. It was found that ammonium starvation stimulated activated sludge to accumulate PHB, so that, up to 89% wt PHB was achieved. In another study, the effect of dissolved oxygen concentration (DO) on PHB accumulation in activated sludge was assessed by Third et al. [20]. In this work, oxygen limitation as a vital factor was verified to obtain optimum PHB yields with minimal biomass growth.

Uncoupling carbon and nitrogen supply is another strategy to enrich activated sludge. Uncoupling carbon and nitrogen feeding strategy were compared with the conventional feast and famine strategy in a research work. Form the results, the proposed method was more efficient to grow different PHA-storing microbial communities rather than the conventional ones [21].

In the present work, the performance of different culture selection strategies for PHA production was compared. In this mean, an anaerobic-aerobic, a fully aerobic, an uncoupled carbon and nitrogen feeding, and aerobic/anoxic process were used in SBR fed by acetate. Most of the reports in the literature focused on long-term culture selection which requires plenty of energy and cost. In order to reduce the production cost of PHA. This study was aimed to evaluate how rapidly an activated sludge could acclimate PHA producer species under short-term biomass enrichment.

2. MATERIALS AND METHODS

2. 1. Culture Selection Stage Four identical lab-scale SBRs with a working volume of 4.5 L were used in this study as shown in Figure 1. All SBRs were inoculated with activated sludge harvested from the aeration tank of a municipal wastewater treatment plant (Kermanshah, Iran) to provide MLSS concentration of 5000–6000 mg TSS/L. SBR1, SBR2, SBR3, and SBR4 were operated under alternating anaerobic-aerobic (A/O) conditions, the complete aerobic condition under the feast-famine regime (ADF), uncoupled carbon and nitrogen feeding regime (FF&unCN) and aerobic–anoxic regime, respectively. The systems with batch mode were operated with a cycle time of 12 h. The operational details for all the bioreactors are presented in Figure 2. All the SBRs were fed by synthetic acetate wastewater, however, the performance of SBR3 was evaluated to treat industrial soft-drink wastewater. The characteristics of the used wastewaters are presented in Table 1. Air was supplied using a compressed air pump through ceramic diffusers and had a stable flow rate of about 5 L/min.

2. 2. PHA Accumulation Stage PHA accumulation

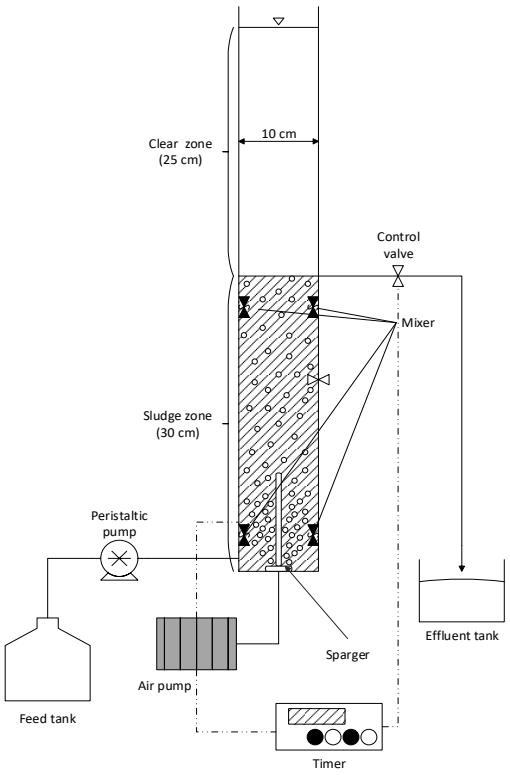

Figure 1. The experimental set-up

assays were accomplished to evaluate the maximum PHA accumulation capacity, storage yield and production rate of the selected culture in each SBR. The accumulation test was conducted in SBR with an initial working volume of 2 L under aerobic conditions at 20–25 °C. Dissolved oxygen (DO) level was maintained around 1–2 mg/L. Air was supplied by an air pump through a ceramic diffuser and the proper mixing was provided by magnetic stirring. The accumulated SBR was operated with the biomass of each stable SBR at the end of feast phase to provide 2000 mg TSS/L of MLSS concentration. To start the PHB production, a pulse of about 5000 mg/L feed solution (acetate) was introduced into the bioreactor. The SBR was operated for at least 48 h. Nitrogen source was not added to the feed to inhibit microbial growth. The progress of the experiments was monitored online (DO, pH) and offline (COD, TSS, PHA, ammonia, and SVI) measurements.

2. 3. Analytical Methods The concentrations of chemical oxygen demand (COD), total nitrogen (TN), nitrate and nitrite, NH_4-N, total phosphorus (TP), and mixed liquor suspended solids (MLSS) were determined by using standard methods [22]. 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

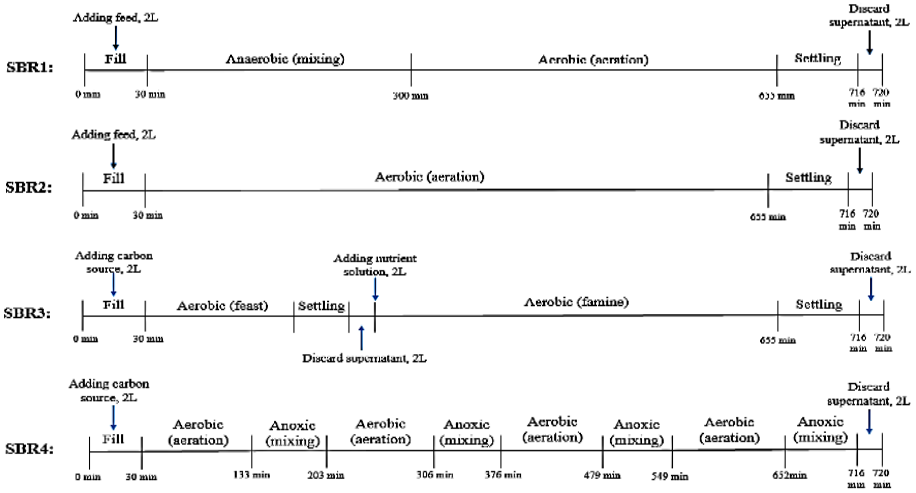

Figure 2. Reaction scheme for four SBRs

TABLE 1. The composition of feed used in this study

Selection reactor	Type of feed	COD Conc.(mg/L)	NH_4Cl (mg/L)	KH_2PO_4 (mg/L)	COD/N/P	pH
SBR1	Acetate	3000	1146.5	131.6	100/10/1	7–8
SBR2	Acetate	3000	1146.5	131.6	100/10/1	7–8
SBR3	Acetate	3000	344	65.8	100/3/0.5	7–8
	Soft-drink industrial wastewater	3000	344	65.8	100/3/0.5	7–8
SBR4	Acetate	3000	1146.5	131.6	100/10/1	7–8

COD samples. N-NH_4 were determined by total Kjeldahl nitrogen (TKN) meter, Gerhardt model (Vapodest 10, Germany). The DO concentration 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). The air flow rate was measured by an air flow meter model 101325Pa. A digital pH meter (Metrohm, Switzerland) model 827, equipped with a glass electrode (Metrohm) was utilized for pH measurements. UV–Vis spectrophotometer model JENWAY 6320D was employed.

The PHA production percent in the biomass was measured using a gas chromatography (GC) method proposed by Braunegg et al. [23]. In this method, 15 mL of biomass samples were collected which centrifuged firstly at 3600 rpm for 20 minutes and the thickened biomass was transferred to 5 mL vials. A mixture of 2 ml methanol acidified with H_2SO_4 (3% v/v) and 2 mL chloroform was added to the thickened biomass. The mixed solution was heated in a screw-capped glass tube at 100 °C for 3.5 h and then cooled to room temperature. 1 mL deionized water was then added and the whole sample was shaken well for 10 min. After separation of the three phases, 1 mL of the dense chloroform phase containing the PHA was transferred to a GC vial and add 3 µL methyl benzoate as internal standard and determined by gas chromatography (Agilent Technologies model 7890B).

2. 4. Process Parameters Studied The PHA content of the sludge (% 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 relationships are the parameters which were determined.

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

$$q_p = \frac{\text{PHA}_e - \text{PHA}_0}{\text{X}_a \, .t} \quad (2)$$

$$-q_s = \frac{\text{S}_0 - \text{S}_e}{\text{X}_a \, .t} \quad (3)$$

$$Y_{x/s} = \frac{(\text{MLSS}_e - \text{MLSS}_0)}{\text{S}_0 - \text{S}_e} \quad (4)$$

$$Y_{\text{PHA}/s} = \frac{(\text{PHA}_e - \text{PHA}_0) \times 1.67}{\text{S}_0 - \text{S}_e} \quad (5)$$

where PHA_e and PHA_0 denote PHA content at the time substrate is consumed and right after feeding, respectively; S_0, $MLSS_0$ is the substrate and biomass concentration after feeding, respectively, S_e and $MLSS_e$ are the residual substrate and biomass concentration when the substrate is consumed in one operating cycle, activated biomass (X_a) is average activated biomass concentration (it is shown the difference between volatile suspended solids (VSS) and PHAs storage); t represents the length of the feast phase in one SBR operating cycle as well as the duration of one batch assay. Substrate consumption and PHA formation data were achieved due to calculating COD units based on the analytical results using oxidation stoichiometry: 1.067 mg COD/mg acetate, 1.067 mg COD/mg glucose, 1.42 mg COD/mg X (active biomass), 1.67 mg COD/mg (3-hydroxybutyric acid) and 1.92 mg COD/mg (3-hydroxyvaleric acid).

3. RESULTS AND DISCUSSION

3. 1. Anaerobic/Aerobic Strategy One of the selection method used in this study was an anaerobic/aerobic strategy. From the literature, the important factor affecting the selection process is anaerobic to the aerobic ratio (min/min). In this stage, this ratio was determined to be 0.68. Figure 3 shows the COD, PHB, and phosphate concentration profiles in a typical cycle of the SBR at 16th cycle. Overall, COD and nitrogen removal efficiencies were about 93.6 and 71.3% after the 16th cycle, respectively. As observed in the figure, at 16th cycle, ~77% phosphorous removal was obtained. The continuous increase of phosphorous release and uptake rates was an indicator of an increase in the PAO population. It should be mentioned that a quick COD removal occurred over the first 275 minutes which led to fast releasing phosphate from PAOs. The phosphate release and uptake rates were 1.23 and 1.65 mg-P/g-VSS.h, respectively. From the figure, over anaerobic phase, along with a sharp decrease in the COD content (from 1350 to 155 mg COD/L), the PHB accumulation was increased in the biomass from 90 to 144 mg-PHB/L. In the duration of the aerobic phase, where COD concentration remained at its minimum level (95 mg COD/L), the PHB consumption, as well as phosphorus uptake, corroborate the growth of PHA producing bacteria.

At the end of the 16th cycle, the biomass was used to operate the accumulation bioreactor. In the accumulation phase, the PHA content after 0, 24 and 48 hours were obtained 7.2, 9.5 and 13.2% (mg-PHA/mg-TSS), respectively. Table 2 exhibits a summary of some researches conducted under various operation conditions using anaerobic/aerobic strategy. Based on the table, the anaerobic to the aerobic ratio in the selection phase has a direct relationship with the PHB content. The reason why the PHB content of this study is relatively low could be related to short-term operation of the bioreactor over selection step and also the usage of high carbon to nitrogen ratio (10).

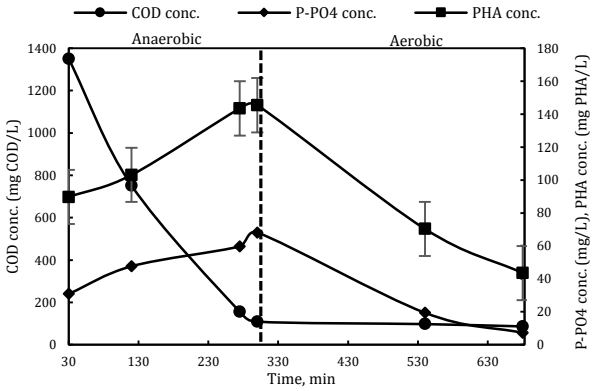

Figure 3. COD, PHB, and phosphate concentration profiles in the SBR over 16th cycles

3. 2. Aerobic Dynamic Feeding (ADF) Strategy

ADF as a culture selection strategy for PHA production was studied in an SBR using acetate. The selector system was unstable in the first two cycles of the operation resulted from microbial accumulation with the new conditions applied. The system was operated under a growth phase for approximately 16 cycles (8 days). Pseudo-steady state was recognized as a circumstance which MLSS and PHA concentration were relatively constant at the whole of cycle time for at least 16 consecutive cycles. Biomass, substrate, PHB, DO and ammonical nitrogen concentration was reported throughout the SBR cycles in Figure 4. The profile of DO during the cycles was monitored to determine the time of substrate exhaustion which it's a sign of beginning famine phase. The microbial activity is significant high over feast phase which led to a decrease in DO concentration, however, it was increased over famine phase resulted from a limitation in microbial activity (Figure 4). From the results, the feast phase lasted about 90 min with the feast to famine ratio of 0.15. Generally, COD removal efficiency of the reactor was about 94% after 16 cycles and then remained nearly constant, so that the effluent COD concentrations were achieved in the range of 80-90 mg/L. Table 3 shows the average data of the measured parameters under the pseudo-steady states.

The batch test is usually preferred to increase the productivity through intermittent feeding which provides favored condition storing more biopolymer with a higher rate. Figure 5 illustrates the profile of COD, MLSS and

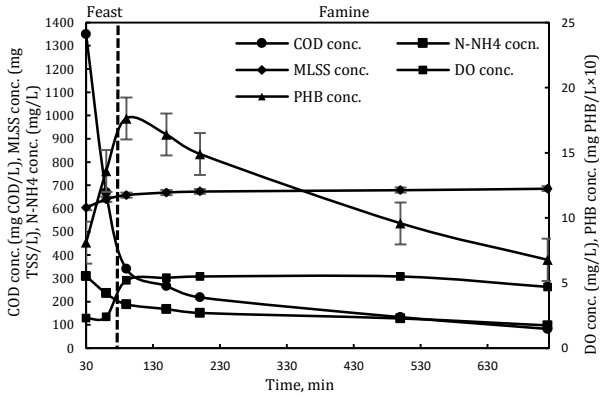

Figure 4. DO, COD, NH_4-N, MLSS and PHA concentration profiles in the SBR over the 16th cycle

TABLE 2. The operational conditions and results obtained from different studies in the culture selection stage, by using anaerobic/aerobic strategy

Type of substrate	Cycle length (h)	Operation time (day)	COD/N/P	Anaerobic/ aerobic ratio	F/M ratio in the batch test	The content of PHB (%)	Reference
Acetate	8	20	100/5/1	0.5	0.66	59	[24]
	8	100	100/4.76/1.17	0.44	0.5	51	[25]
	8	450	100/2.89/0.92	0.72	0.4	60	[26]
	12	8	100/10/1	0.68	2.5	13.2	This study

TABLE 3. Average values of the measured parameters during the cycle of SBR with ADF strategy

Parameter	Unit	End of feast phase	End of famine phase
Substrate concentration	mg COD/L	340	82
Polymer concentration	mg COD/L	295	115
Substrate consumption rate	mg COD/L.h	150	5.7
Biomass yield ($Y_{X/S}$)	mg-VSS/mgCOD-S	0.4	—
PHA yield ($Y_{PHA/S}$)	mg COD-PHA/mg COD-S	0.15	—
Max. PHA content, %	mg-PHA/mg-TSS	8.8	3.4
Specific PHA production rate (q_p)	mg COD-PHA/mg COD-X.h	0.011	—
Specific substrate uptake rate ($-q_S$)	mg COD-S/mg COD-X.h	0.075	0.018

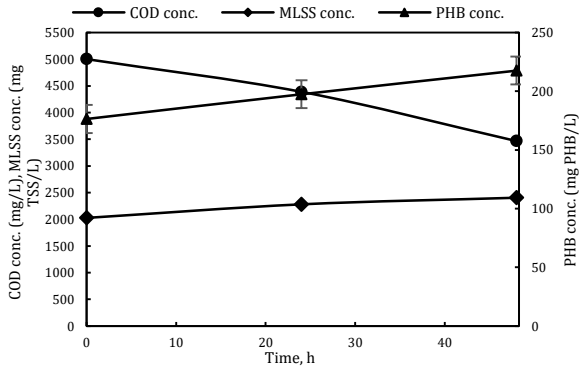

Figure 5. PHA, MLSS, COD concentration profiles in the accumulation reactor

PHB concentrations during a batch experiment for accumulation. From the figure, the PHA content at the end of the experiment obtained 10.8% (mg-PHA/mg-TSS). The initial concentration of COD and PHB were varied over 48 h so that COD concentration was decreased from 5000 to 3465 mg/L and PHB concentration was increased from 176.4 to 217.6 mg/L.

Besides, the biomass concentration significantly increased as sufficient nutrient was available for growing microorganisms. In this situation, PHA storage rate (0.004-0.005 mg COD-PHA/mg COD-X.h) and productivity (0.6-0.7 mg COD-PHA/L.h) were less than that of nutrient limitation [18, 27, 28]. Average results obtained during the accumulation stage are reported in Table 4.

In order to increase the PHA production, the enrichment and accumulation processes require further optimization. Obviously, the previous cultivation condition is the main factor on the performance of the biomass. Table 5 compares the literature with the results obtained in this study. As indicated in Table 5, the amount of PHA storage at the end of feast phase is increased by decreasing F/F ratio. Results obtained from some researches have revealed that the shortage of nitrogen content could improve the capability of biomass to storage polymer.

3. 3. Uncoupled Carbon and Nitrogen Feeding Strategy In the FF&unCN reactor, the carbon source is fed at the beginning of the feast phase which is consumed at the initiation of the famine phase. Consequently, in the feast phase, the microbial growth is prevented since an essential nutrient for growth (nitrogen) was not available and thus only storing organisms can grow in the absence of carbon source in the famine phase. Similar to the literature [21, 29-31], imposing nitrogen limitation favored the metabolism of PHA-storing organisms from the first cycle. It should be noted that using only conventional feast and famine regime requires a number of cycles for accumulating storing-organisms in the biomass [21]. Conceivably, the FF&unCN reactor could reach the culture with PHA-storing species quicker than the FF reactor [32]. Figure 6 reports COD, PHA, ammonical nitrogen and MLSS concentration profiles obtained over a 16th cycle of the selection stage using acetate. As expected, PHA content was degraded as an internal source of carbon and energy for microbial growth throughout the famine phase. In fact, some residual substrate (380 mg COD/L) was present at the end of feast phase, which used over the famine period, thus hindering a real famine. To provide a real famine, the elimination of the supernatant after a settling period is strongly recommended which is not done in this work. From the Figure 6, a slight increase in biomass concentration was observed during the famine phase by adding a gradual increase in the nitrogen source. The decrease in the accumulated PHB concentration was due to the biomass growth. The microbial growth yield ($Y_{X/S}$) and PHA production yield ($Y_{PHA/S}$) were obtained 0.16 mg-VSS/mg COD-S, 0.149 mg COD-PHA/mg COD-S, respectively, at the end of feast phase. Specific PHA production rate (q_p) was also calculated to be 0.01 mg COD-PHA/mg COD-X.

In the accumulation stage, the PHA content after 0, 24 and 48 hours were 5.3, 11.9 and 22.36% (mg-PHA/mg-TSS), respectively. As a result, the FF&unCN strategy showed to be a more efficient PHA-production process rather than the FF strategy. The rewarding data obtained

TABLE 4. The overall performance of selected biomass during accumulation stage

Parameter	Unit	Initial value	Final value
Substrate concentration	mg COD/L	5000	3465
Polymer concentration	mg COD/L	295	363
Substrate consumption rate	mg COD/L.h	149.5	16
Overall biomass yield ($Y_{X/S}$)	mg-VSS/mg COD-S	—	0.24
Overall PHA yield ($Y_{PHA/S}$)	mg COD-PHA/mg COD-S	—	0.02
Max. PHA content, %	mg-PHA/mg-VSS	8.8	10.8
Specific PHA production rate (q_p)	mg COD-PHA/mg COD-X.h	—	4.56×10^{-4}
Specific substrate uptake rate ($-q_s$)	mg COD-S/mg COD-X.h	—	0.01

TABLE 5. The operational conditions and results obtained from different studies using ADF strategy

Type of substrate	Cycle length (h)	Operation time (day)	COD/N/P	Feast/famine ratio (F/F)	F/M ratio in batch test	Content of PHB (%)	Reference
Acetate	12	60	100/5.5/4.48	0.09	4.04	52	[20]
	12	30	100/2.1/2.8	0.07	1.26	66.4	[32]
	12	100	100/2.82/1.68	0.22	0.6	51	[9]
	12	8	100/10/1	0.15	2.5	10.8	This study

from acetate substrate were a motivation to the extend experiments with soft-drink industrial wastewater. Figure 7 reports COD, PHA, ammonical nitrogen and MLSS concentration profiles obtained over a 16th cycle of the selection stage using soft-drink industrial wastewater. Overall, COD and nitrogen removal efficiencies were about 89.2 and 74.6%, respectively. During feast phase in the FF&uCN reactor, storage was clearly more than growth but with less rate ($Y_{X/S}$ 0.15 mg-VSS/mg COD-S, q_{PHA} 0.007 mg COD-PHA /mg COD-X.h, and $Y_{PHA/S}$ 0.16 mg COD-PHA/mg COD-S).

Comparing Figure 7 with the data presented in the Figure 6 shows that the changing trends of the parameters were almost similar but the use of acetate as substrate resulted in a higher PHB production and substrate consumption rates with lower feast and famine ratio. In order to determine the maximum PHA storage capacity of selected MMC, PHA accumulation assay was done when the performance of the bioreactor was somehow stable. In the accumulation stage, the PHA content after 0, 24 and 48 hours were obtained 5.3, 11 and 13.75% (mg-PHA/ mg-TSS), respectively. The maximum PHA contents for acetate and soft-drink industrial wastewaters after 48 h were in a good agreement with the performance of the selector bioreactors.

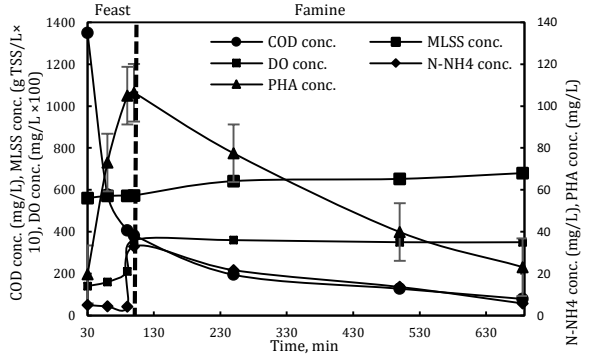

Figure 6. Profile of the COD, PHA, ammonical nitrogen and MLSS concentration in the SBR using acetate as a substrate over 16th cycles

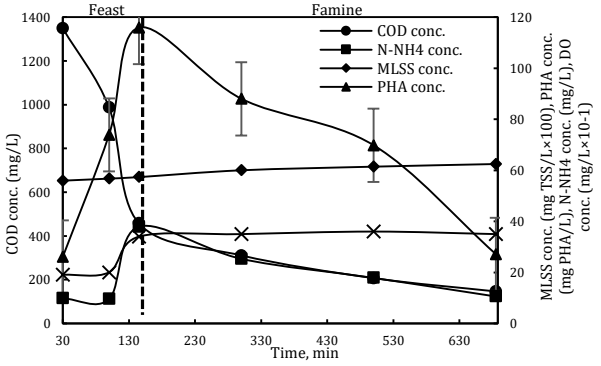

Figure 7. Profile of the COD, PHA, ammonical nitrogen and MLSS concentrations over 16th cycles using soft-drink industrial wastewater

3. 4. Aerobic/Anoxic Strategy The current section was aimed to evaluate the integration of nitrogen removal driven by the stored PHA inside the PHA storing biomass. Acetate was used as the carbon source in the experiment. Acetate was rapidly consumed at the beginning of the SBR cycle (67 min) and stored as PHA and some other compounds such as carbohydrate, etc. Figure 8 reports COD, NO_2^-, NO_3^-, ammonical nitrogen and DO concentration profiles obtained over a 16th cycle of the selection stage. At the end of the feast phase, the PHA content was 4.8% (mg-PHA/mg-TSS) that was consumed subsequently in the anoxic phase. In general, COD and nitrogen removal efficiencies of the bioreactor were about 94 and 88%, respectively. The achieved data verify a development in anoxic zones despite the external COD source shortage. In the accumulation stage, the PHA content after 48 hours was obtained 6% (mg-PHA/mg-TSS).

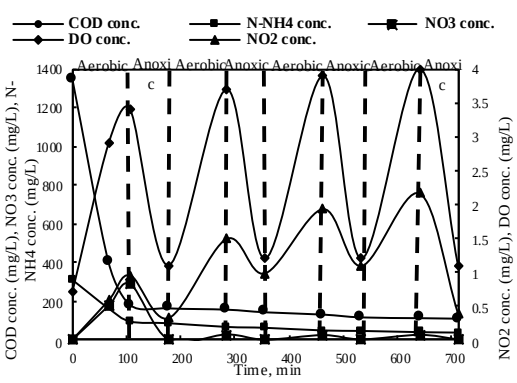

Figure 8. Profile of the COD, NO_2^-, NO_3^-, ammonical nitrogen and DO concentration over the 16th cycle

4. CONCLUSION

The performance of four different strategies for the enrichment of biomass with PHA-producing bacteria was assessed in this research work. The following results could be concluded from this research:

- Uncoupled carbon and nitrogen feeding regime was recognized as the best strategy to increase PHA content so that, 22.36 and 13.75 % of mg- PHA/mg-TSS were obtained with acetate and soft-drink industrial wastewater as a feed, respectively.
- Increasing operational time is an effective factor to enhance PHA content of activated sludge during the selection stage.
- Nitrogen deficiency over the selection stage was also found to be a crucial factor to store more PHA content.

5. ACKNOWLEDGMENT

The authors would like to acknowledge Iran National Science Foundation (INSF) for the full financial support provided for this research work. The authors also wish to thank Razi University and the Ministry of Science and Technology-Iran for their financial support and lab equipment.

6. REFERENCES

1. Morgan-Sagastume, F., Hjort, M., Cirne, D., Gérardin, F., Lacroix, S., Gaval, G., Karabegovic, L., Alexandersson, T., Johansson, P., and Karlsson, A., "Integrated production of polyhydroxyalkanoates (PHAs) with municipal wastewater and sludge treatment at pilot scale", ***Bioresource Technology***, Vol. 181, (2015), 78–89.
2. Mumtaz, T., Yahaya, N.A., Abd-Aziz, S., Yee, P.L., Shirai, Y., and Hassan, M.A., "Turning waste to wealth-biodegradable plastics polyhydroxyalkanoates from palm oil mill effluent – a Malaysian perspective", ***Journal of Cleaner Production***, Vol. 18, No. 14, (2010), 1393–1402.
3. Majone, M., Dircks, K., and Beim, J.J., "Aerobic storage under dynamic conditions in activated sludge processes. The state of the art", ***Water Science and Technology***, Vol. 39, No. 1, (1999), 61–73.
4. Rhu, D.H., Lee, W.H., Kim, J.Y., and Choi, E., "Polyhydroxyalkanoate (PHA) production from waste", ***Water Science and Technology***, Vol. 48, No. 8, (2003), 221–228.
5. Dionisi, D., Carucci, G., Papini, M.P., Riccardi, C., Majone, M., and Carrasco, F., "Olive oil mill effluents as a feedstock for production of biodegradable polymers", ***Water Research***, Vol. 39, No. 10, (2005), 2076–2084.
6. Bengtsson, S., Werker, A., Christensson, M., and Welander, T., "Production of polyhydroxyalkanoates by activated sludge treating a paper mill wastewater", ***Bioresource Technology***, Vol. 99, No. 3, (2008), 509–516.
7. Dionisi, D., Beccari, M., Di Gregorio, S., Majone, M., Papini, M.P., and Vallini, G., "Storage of biodegradable polymers by an enriched microbial community in a sequencing batch reactor operated at high organic load rate", ***Journal of Chemical Technology & Biotechnology***, Vol. 80, No. 11, (2005), 1306–1318.
8. Takabatake, H., Satoh, H., Mino, T., and Matsuo, T., "Recovery of biodegradable plastics from activated sludge process", ***Water Science and Technology***, Vol. 42, No. 3–4, (2000), 351–356.
9. Liu, C., Liu, D., Qi, Y., Zhang, Y., Liu, X., and Zhao, M., "The effect of anaerobic–aerobic and feast–famine cultivation pattern on bacterial diversity during poly-β-hydroxybutyrate production from domestic sewage sludge", ***Environmental Science and Pollution Research***, Vol. 23, No. 13, (2016), 12966–12975.
10. Satoh, H., Iwamoto, Y., Mino, T., and Matsuo, T., "Activated sludge as a possible source of biodegradable plastic", ***Water Science and Technology***, Vol. 38, No. 2, (1998), 103–109.
11. Mino, T., van Loosdrecht, M.C.M., and Heijnen, J.J., "Microbiology and biochemistry of the enhanced biological phosphate removal process", ***Water Research***, Vol. 32, No. 11, (1998), 3193–3207.
12. Chua, A.S., Takabatake, H., Satoh, H., and Mino, T., "Production of polyhydroxyalkanoates (PHA) by activated sludge treating municipal wastewater: effect of pH, sludge retention time (SRT), and acetate concentration in influent", ***Water Research***, Vol. 37, No. 15, (2003), 3602–3611.
13. Salehizadeh, H., and Van Loosdrecht, M.C.M., "Production of polyhydroxyalkanoates by mixed culture: recent trends and biotechnological importance", ***Biotechnology Advances***, Vol. 22, No. 3, (2004), 261–279.
14. Beccari, M., Majone, M., Massanisso, P., and Ramadori, R., "A bulking sludge with high storage response selected under intermittent feeding", ***Water Research***, Vol. 32, No. 11, (1998), 3403–3413.
15. Dionisi, D., Majone, M., Tandoi, V., and Beccari, M., "Sequencing Batch Reactor: Influence of Periodic Operation on Performance of Activated Sludges in Biological Wastewater Treatment", ***Industrial & Engineering Chemistry Research***, Vol. 40, No. 23, (2001), 5110–5119.
16. Kumar, M.S., Mudliar, S., Reddy, K., and Chakrabarti, T, "Production of biodegradable plastics from activated sludge generated from a food processing industrial wastewater treatment plant", ***Bioresource Technology***, Vol. 95, No. 3, (2004), 327–330.
17. Johnson, K., Jiang, Y., Kleerebezem, R., Muyzer, G., and van Loosdrecht, M.C.M., "Enrichment of a Mixed Bacterial Culture with a High Polyhydroxyalkanoate Storage Capacity", ***Biomacromolecules***, Vol. 10, No. 4, (2009), 670–676.
18. Johnson, K., Kleerebezem, R., and van Loosdrecht, M.C.M., "Influence of the C/N ratio on the performance of polyhydroxybutyrate (PHB) producing sequencing batch reactors at short SRTs", ***Water Research***, Vol. 44, No. 7, (2010), 2141–2152.
19. Johnson, K., Kleerebezem, R., and van Loosdrecht, M.C.M., "Influence of ammonium on the accumulation of polyhydroxybutyrate (PHB) in aerobic open mixed cultures", ***Journal of Biotechnology***, Vol. 147, No. 2, (2010), 73–79.
20. Khumwanich, P., and Napathorn, S., "Polyhydroxyalkanoate production with a feast/famine feeding regime using sludge from wastewater treatment plants of the food and beverage industry", ***Journal of Biobased Materials and Bioenergy*** , Vol. 8, No. 6, (2014), 641–647.
21. Oliveira, C.S.S., Silva, C.E., Carvalho, G., and Reis, M.A., "Strategies for efficiently selecting PHA producing mixed microbial cultures using complex feedstocks: Feast and famine regime and uncoupled carbon and nitrogen availabilities", ***New Biotechnology***, Vol. 37, (2017), 69–79.
22. APHA, Standard methods for the examination of water and wastewater, American Public Health Association, and American

Water Works Association, (2005).

23. Braunegg, G., Sonnleitner, B., and Lafferty, R.M., "A rapid gas chromatographic method for the determination of poly-?-hydroxybutyric acid in microbial biomass", ***European Journal of Applied Microbiology and Biotechnology***, Vol. 6, No. 1, (1978), 29–37.
24. Wen, Q., Chen, Z., Tian, T., and Chen, W., "Effects of phosphorus and nitrogen limitation on PHA production in activated sludge", ***Journal of Environmental Sciences***, Vol. 22, No. 10, (2010), 1602–1607.
25. Kasemsap, C., and Wantawin, C., "Batch production of polyhydroxyalkanoate by low-polyphosphate-content activated sludge at varying pH", ***Bioresource Technology***, Vol. 98, No. 5, (2007), 1020–1027.
26. Bengtsson, S., "The utilization of glycogen accumulating organisms for mixed culture production of polyhydroxyalkanoates", ***Biotechnology and Bioengineering***, Vol. 104, No. 4, (2009), 698–708.
27. Dionisi, D., Majone, M., Papa, V., and Beccari, M., "Biodegradable polymers from organic acids by using activated sludge enriched by aerobic periodic feeding", ***Biotechnology and Bioengineering***, Vol. 85, No. 6, (2004), 569–579.
28. Md Din, M.F., Ujang, Z., van Loosdrecht, M.C.M., Ahmad, A., and Sairan, M.F., "Optimization of nitrogen and phosphorus limitation for better biodegradable plastic production and organic removal using single fed-batch mixed cultures and renewable resources", ***Water Science and Technology***, Vol. 53, No. 6, (2006), 15–20.
29. Huang, L., Chen, Z., Wen, Q., and Lee, D.-J., "Enhanced polyhydroxyalkanoate production by mixed microbial culture with extended cultivation strategy", ***Bioresource Technology***, Vol. 241, (2017), 802–811.
30. Campanari, S., Augelletti, F., Rossetti, S., Sciubba, F., Villano, M., and Majone, M., "Enhancing a multi-stage process for olive oil mill wastewater valorization towards polyhydroxyalkanoates and biogas production", ***Chemical Engineering Journal***, Vol. 317, (2017), 280–289.
31. Ahmadi, F., Zinatizadeh, A.A., and Asadi, A., "PHA production from wastewater by mixed microbial culture under short-term microbial enrichment", ***Journal of Applied Research in Water and Wastewater***, Vol. 5, No. 1, (2018), 389–391.
32. Guo, Z., Chen, Z., Wen, Q., Huang, L., Bakke, R., and Du, M., "Strategy to reduce the acclimation period for enrichment of PHA accumulating cultures", ***Desalination and Water Treatment***, Vol. 57, No. 60, (2016), 29286–29294.

Influence of Different Culture Selection Methods on Polyhydroxyalkanoate Production at Short-term Biomass Enrichment

F. Ahmadi[a], A. A. Zinatizadeh[a], A. Asadi[b], H. Younesi[c]

[a] *Environmental Research Center (ERC), Department of Applied Chemistry, Faculty of Chemistry, Razi University, Kermanshah, Iran*
[b] *Department of Gas and Petroleum, Yasouj University, Gachsaran, Iran*
[c] *Department of Environmental science, Faculty of Natural resources and Marine Science, Tarbiat Modares University, Noor, Iran*

P A P E R I N F O

Paper history:
Received 16 August 2018
Received in revised form 28 December 2018
Accepted 03 January 2019

Keywords:
Polyhydroxyalkanoate
Short-term Enrichment Time (STE)
Acetate
Soft-drink Industrial Wastewater

چکیده

در این پژوهش، پتانسیل چهار روش مختلف در مدت زمان غنی‌سازی کوتاه جهت انتخاب میکروارگانیسم‌های تولیدکننده بیوپلاستیک زیستی (PHA) در جمعیت میکروبی مقایسه شد و روش با کارآیی مناسب معرفی شد. در این میان جامعه میکروبی تولیدکننده PHA ابتدا در یک بیوراکتور دسته‌ای متوالی (SBR) با چهار روش مختلف گزینش شامل: یک فرآیند هوازی بی‌هوازی (SBR1)، یک فرآیند دسته‌ای کاملاً هوازی (SBR2)، یک کربن نیتروژن رژیم تغذیه (SBR3) و فرآیند هوازی/آنوکسی (SBR4) انتخاب شد. در مرحله بعد، محتوای سلولی PHA در یک راکتور تجمع PHA، بیشینه شد. از نتایج به دست آمده، مقدار PHA انباشته شده در SBR1، SBR2، SBR3 و SBR4 به ترتیب ۱۳/۲، ۱۰/۸، ۲۲/۳۶ و ۶٪ (mg-PHA / mg-TSS) می‌باشد. رژیم تغذیه کربن و نیتروژن مجزا (SBR3) بهترین تجمع PHA را (زمانی که استات به عنوان خوراک استفاده شد) نشان داد. همچنین SBR3 با استفاده از فاضلاب صنعتی کارخانه نوشابه‌سازی تغذیه شد تا توانایی استراتژی انتخاب‌شده برای تصفیه فاضلاب واقعی ارزیابی شود که ۱۳/۷۵٪ mg-PHA / mg-TSS به دست آمد.

***doi**: 10.5829/ije.2019.32.02b.01*