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Influence of cultivation time on the production of polyhydroxyalkanoates by activated sludge

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Abstract
Polyhydroxyalkanoates (PHAs) are polyesters formed and accumulated by various bacterial species under unbalanced growth conditions. Due to the problems resulted from the disposals of plastic wastes and excess sludge of waste water treatment plants, the production of polyhydroxyalkanoates by treating activated sludge and determining the effect of cultivation time was the main issue of this paper. In this study, we tried to optimize the cultivation time to achieve the highest rate of PHA production by activated sludge. Experiment procedure was included adding carbon source (sodium acetate), aeration, sampling in defined times (every 6 hours), measuring of PHA and biochemical oxygen demand (BOD) of samples. Based on the results the amount of PHA increased with increasing cultivation time and the maximum amount of PHA is reached at 30 hours of cultivation which is equivalent to 0.6 $g.L^{-1}$, after that the PHA content dropped. Moreover, the percentage of BOD removal was 68.93 % during this time.

1. Introduction

In recent years, public concern has increased about harmful effects of synthetic plastics in environment. This problem has led on researchers from different countries for development of biodegradable plastics. Production of Eco-friendly plastics such as bio plastics is a reality that can solve pollution problems of irresolvable plastics.

Polyhydroxyalkanoates (PHAs) are biological polyesters produced by a wide variety of microorganisms as a form of intracellular carbon and energy storage material. PHAs have recently attracted the attention of industrial scientists because of their potential use as practical biodegradable and biocompatible plastic materials with properties ranging from thermoplasts to elastomers [1-4]. PHAs accumulate at specific conditions such as limitation of nitrogen, phosphorus and oxygen sources and excess carbon source [5]. The major barriers to the wide application of PHAs are current high cost of biological process, equipment costs, optimizing the conditions required for their synthesis and adding extra carbon source [6]. A good candidate for economical PHAs production would be a mixed culture (such as activated sludge) that can store high PHAs content while growing on an inexpensive substrate and it contains of microorganisms known for PHA production [7-12]. Some useful suggestions for reduction of costs include using a cheap and renewable carbon source, using mixed cultures and inexpensive substrates and optimization of process variables such as cultivation time [13,8,3,14].

Raw sludge produced in waste water treatment plants (WWTP) consist of organic and inorganic solids, water (over 95%), nutrients, small amount of trace elements and pathogens. According to the high cost of treatment and disposal of excess activated sludge, there is a strong need to developing techniques for volume reduction of sludge in WWTP and disposal it, recycling valuable compounds or energy production of excess sludge. Production of PHA from waste water has advantages such as cheap production of bio plastics, reduce the sludge volume and subsequently reduce the cost of waste water treatment (WWT). Based on previous studies, the volume of excess sludge can be reduced to less than 30 percent by extracting of PHA from activated sludge.

Mixed cultures such as activated sludge have been considered in the past two decades. The idea of using mixed culture in PHA production is due to identify PHA's roles as a metabolic intermediate in biological WWT. In most cases, mixed culture generate PHA from organic acids of waste water or organic acids that are added from other industrial waste. During the PHA production process, microorganisms use dissolved organic materials such as volatile fatty acids (VFAs) and convert to PHAs. So, if VFAs are produced during WWT process we can be produced significant amounts of bio plastics by using suitable microorganisms.

The effect of process variables on the production of PHA have attracted the attention of many researchers. A number of studied have been carried out on PHA production by activated sludge and the influence of carbon source on the PHA content [15,13,16,17].

The biochemical oxygen demand (BOD) is an indicator used to evaluate the degree of the contamination of water and sewages with the organic substances. Although it is much faster to indicate the chemical oxygen demand (COD) and the total organic carbon (TOC), it is difficult to resign the indication of BOD and more precisely the five-day BOD (BOD_5) because it informs about the concentration of the organic substances decomposed in the biological way [18]. Concern over the pollution risk to the environment from industrial manufacturing processes and intensive agriculture has highlighted the need for rapid, easy-to operate, low cost screening procedures which can operate in the field [19]. Among all the parameters used to assess the pollutional load of water bodies/waste-waters, the biochemical oxygen demand is one of the most important and widely used parameter in the measurement of organic pollution [20]. Then simultaneous PHA production and BOD removal from activated sludge is an effective process.

In the present research, we have attempted to show the effect of cultivation time on the PHA content and BOD removal. The activated sludge, collected from Kerman municipal waste water plant in Kerman, Iran, was transferred into PHA production reactor (PPR).

2. Method and Material

2.1. Batch Reactor

One batch reactor, with individual working volume of 1 L, was used for this experiment. In this system, excess activated sludge, with sludge retention time (SRT) of 5 days, was transferred into the tank. Activated sludge was collected from municipal waste water plant in Kerman, Iran. Characteristics of sludge are presented in the results. Sewage cannot produce the polymer alone, thereupon activated sludge was fed with sodium acetate as the best substrate for PHA production [10,21], with concentration of 3000 $mg.L^{-1}$ at a level. Reactor was aerated with air compressor for 48 hours and oxygen rate was 1 $L.min^{-1}$. The activated sludge in batch reactor was sampled at regular intervals and PHA, BOD and pH were determined. The reactor was operated without pH control and the temperature was 15˚C. pH of samples was measured by pH meter (Metrohm 654 pH-meter, Metrohm, Herisau, Switzerland).

2.2. Analytical Technique

Measuring of PHA content was performed according to the method described in Ataei's research [16]. 5mL of samples were centrifuged at 6000 rpm and 15 min. Then 2 mL of chloroform and 1 mL of acidified methanol containing benzoic acid as the internal standard was added to the deposit sludge. Simples heated for 2 hours at 100˚C. After cooling, 1mL of distilled water was added and they were shaken for 1 min to separate phases. Then 2 µL of bottom phase was injected into gas chromatograph (model Varian CP 3800) at 180˚C, which was equipped with a flame ionization detector (FID) and column (Capilary cp – sil 8 cp, 30m×1µm). The detector temperature was 150˚C. Helium was used as the carrier gas. Initial oven temperature was 100˚C which was held constant for 1 min. then temperature was increased to 200˚C at a rate of 20˚C/min and retained for 1 min. calibrations of PHA were done with a standard poly (3- hydroxybutyric-co-3-hydroxyvaleric acid) (12 wt% PHV).

Also amount of BOD was measured by standard method [18].

3. Result and Discussion

The production system of PHAs and the schematic of a 1-liter batch reactor that was used in this study, is shown in Figure 1 and 2, respectively. The reactor was operated with excess activated sludge of municipal waste water treatment in Kerman, also some initial characterization of activated sludge are presented in Table 1.

Table 1. *Initial characterization of activated sludge.*

Characterization	Range
C:N:P	24:0.14:1
pH	7.65
COD (ppm)	5485
BOD (ppm)	1286
SRT[1](day)	5
HRT[2](hour)	10
SVI[3]	90-95
VSS[4]/TSS[5]	0.75

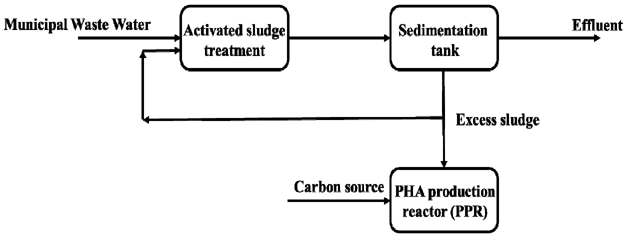

Figure 1. *The production system of PHAs.*

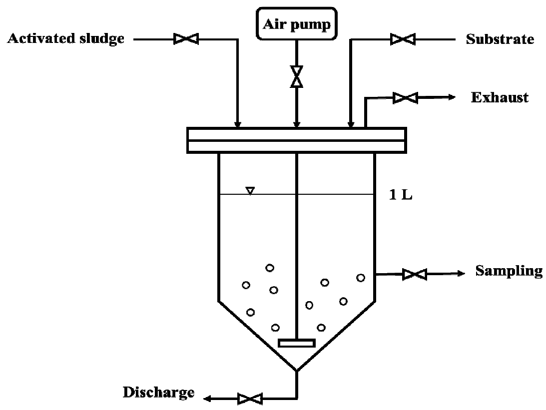

Figure 2. *PHA production reactor (PPR).*

In the process of PHA production, organic material (such as carbon source) is converted into volatile fatty acids (VFAs) at fermentation stage. Then VFAs are consumed by microorganism and pH of culture is increased slowly. pH changes during 48 hours cultivation are shown in Figure 3.

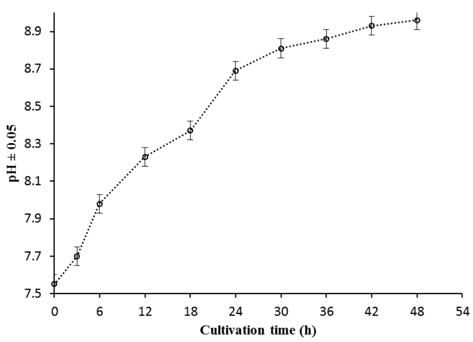

Figure 3. *pH changes during 48 hours cultivation.*

[1] Sludge Retention Time
[2] Hydraulic Retention Time
[3] Sludge Volume Index
[4] Volatile Suspended Solid
[5] Total Suspended Solid

To study the effect of cultivation time of activated sludge on the PHAS production potential, preliminary experiments were carried out at 15°C for periods of 0–48 hours. The relationship between cultivation time and PHAs production, which was positive, is shown in Figure 4. Microorganisms encounter with food restriction during fermentation stage and conditions can provide for PHA production. In this study, the highest amount of PHA produced at 30 hours cultivation which was equivalent to 0.6 g.L^{-1}. We found that PHA production has declined after 30 hours.

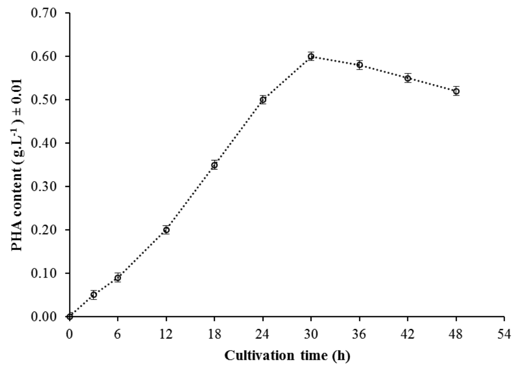

Figure 4. *Effect of cultivation time on the amount of PHAs produced.*

As shown in Figure 4, the PHAs production capability of activated sludge decreased with increasing of aeration time from 30 to 48 hours. The reason why at longer aeration timeless PHAs was produced, is that as aeration time increases the substrate concentration decreases and microorganisms are forced to declining growth and then endogenous phase.

In this phase not only the microbial population is reduced but also microbial communities, which are dominants in the system, may change. Therefore, longer aeration time may select microbial community with lower PHAs production capacity than that selected under shorter aeration times. At lower than 30 hours aeration time, the system may be at log-growth phase. During this period an excess amount of food exists and the population of microorganisms is less than that in stationary phase. As a result, activated sludge processes operating in stationary phase can produce more PHAs compared to that with endogenous or log-growth phases. Based on these results, the optimum aeration time for PHAs production for this system was chosen to be 30 hours.

Probably after 30 hours cultivation, the famine stage started and microorganisms used storage carbon source (PHA) and then PHA content decreased. Therefore, the best phase of growth curve for PHA production is stationary phase. In other words, the activated sludge in stationary phase has the more ability for PHA production compare with the activated sludge in death phase.

According to importance of BOD reduction in waste water treatment, we investigated reduce of BOD during PHA production process.

In the second step of tests, BOD removal was determinate. BOD of activated sludge before and after adding sodium acetate as excess carbon source are given in Table 2.

Table 2. *Biochemical oxygen demand of activated sludge before and after adding sodium acetate.*

	Before adding sodium acetate	After adding sodium acetate
BOD (ppm)	1286	6116

The excess carbon that can be decomposed by microorganisms is increased by adding sodium acetate as carbon source into the activated sludge. In PHA production process, biodegradable organic material and excess carbon source are converted into VFAs. Then VFAs are consumed by microorganisms to produce of PHA. So the mounts of biodegradable organic materials are reduced during PHA. Figure 5 shows BOD changes during 30 hours. Amount of BOD is reduced 68.93 % compared to the initial value (after adding sodium acetate) at 30 hours cultivation.

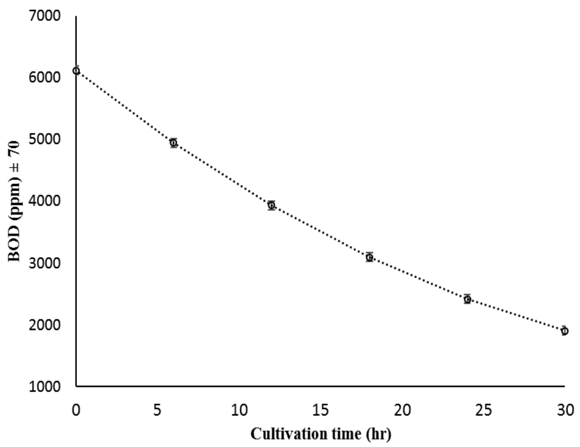

Figure 5. *BOD changes during 30 hours cultivation.*

Simultaneity BOD removal with PHA production is useful process in waste water treatment.

4. Conclusion

The main results can be summarized in the following cases:

- Increasing pH of sludge during PHA production process.
- The highest amount of PHA was 0.6 g.L^{-1} that occurred at 30 hours cultivation. After 30 hours, PHA production rate reduced.
- The best phase to PHA production is stationary phase.
- Aeration time increases the substrate concentration decreases and microorganisms are forced to declining growth and then endogenous phase.
- Reduction in amount of BOD during PHA production process.
- Amount of BOD is reduced 68.93 % compared to the initial value (after adding sodium acetate) at 30 hours cultivation

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