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Polyhydroxyalkanoate Production from Municipal Waste Material

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Abstract

The utilization of waste material as a resource to produce polyhydroxyalkanoate (PHA) through anaerobic digestion has been identified as a cost-effective approach to the production of bioplastic. This approach involves the conversion of waste material as cost effective substate for PHA production through anaerobic digestion, which is a process that occurs in the absence of oxygen. The resulting bioplastic can be used as a sustainable alternative to traditional petroleum-based plastics. The present research investigates the feasibility of utilizing volatile fatty acids (VFAs) containing leachate, a significant component generated from municipal solid waste through anaerobic digestion, as a feedstock to produce polyhydroxyalkanoate (PHA). However, leachate contains variable concentrations of carbon and nitrogen. To evaluate the effect of enrichment history on PHA producer and production the various carbon and nitrogen levels were utilized during the accumulation phase.In this study, the possibility of mixed culture polyhydroxy alkynoate (PHA) production from waste material was assessed by studying the effects of various carbon and nitrogen levels. For this assessment, Polyhydroxyalkanoate (PHA) producing bacteria was cultured from a mixed microbial culture (MMC) using feast famine (FF) process and acetate and ammonium sulphate as nutrient source. The maximum PHA production of the enriched cultures under nutrient starvation (41%) was evaluated in batch assays. Excess substrate feeding during nutrient excess condition maximizes the PHA production. The influence of the C/N ratio on PHA accumulation capability was evaluated by supplementing the leachates. Leachate can be a promising and sustainable source for PHA production, with a C/N ratio of 3:1 resulting in a yield of 0.1g-PHA/g substrate.

Keywords: Waste Material; Biopolymer; Leachates; Volatile fatty acid; Polyhydroxyalkanoates; Anaerobic digestion.

1. Introduction

Each year, the world consumes over 150 million metric tons of synthetic plastics and plastic-derived products and over a billion metric tons of plastic made from petroleum were dumped, and it might take more than a century for them to be mineralized (Kumar et al., 2020).The resistance to biodegradation of these compounds in soil seem to be caused by their excessive molecular size which causes accumulation of enormous quantities of plastic in environments (Reddy et al., 2003).A significant increase, from between 4.8 and 12.7 million tons of plastic debris, reaches the seas from land each year, and a major increase is expected by 2025 if waste management is not improved (Sabapathy et al., 2020).Aquatic ecosystem toxicity and environmental pollution were brought on by the widespread usage of synthetic plastics made from petrochemical- derived substances.In response to these environmental concerns, eco-friendly bioplastics made from polyhydroxyalkanoates (PHA) have received considerable interest as a potentially viable alternative to plastics. Polyhydroxyalkanoates is a macro-molecular bio polyester which synthesized by bacteria and accumulated in their cellular

structure as carbon and energy source when other essential elements like nitrogen, phosphorus, or oxygen are in short supply (S. Y. Lee & Choi, 1998; Marudkla et al., 2018). PHA has gained economic attention as an acceptable alternative to traditional plastic materials due to its better thermal processability, high biodegradability, biocompatibility, and recyclable nature.

Nowadays, organic waste management has become a great concern due to unavailability of land area for disposal and gap in the disposal technologies. This leads to nutrient imbalances, which can lead to poor soil quality and water pollution (Westerman and Bicudo, 2005) and the decomposition of these organic compounds contributes to greenhouse gas emissions. Converting organic waste into a value-added product can help to alleviate the problem regarding environmental concerns. Organic residues are primarily composed of carbohydrates, proteins, lipids, lignin, and minerals (ash), despite their diverse origins (Fu et al., 2019). Through biological pretreatment, organic waste may be utilized as a substrate for the fermentation of polyhydroxyalkanoate (PHA) and this might be a new strategy for lowering PHA manufacturing cost. Because, using pure carbon sources as substrate raises the cost of PHA manufacturing, which accounts for 40% of the entire cost. As a result, research is concentrated on the converting organic waste as substrates for microbiological PHA synthesis as well as eliminating pollution producing petroleum-based plastic(al Battashi et al., 2021).

The research focuses on a biotechnological approach to waste management that involves incorporating organic waste into PHA manufacturing. However, anaerobic digestion was widely employed to convert complex carbohydrates into directly metabolizing substrates like volatile fatty acids (VFAs) which can be used as a carbon source for PHA synthesis (al Battashi et al., 2021). The goal of this study is to estimate the potentiality of substrates (VFA containing leachate) produced from organic waste through pretreatment methods anaerobic digestion in PHA production by mixed microbial culture. In this experiment, acetate will be used as reference and VFA containing leachate will be used as carbon source and Ammonium sulphate[(NH4)2SO4] will be used as nutrients.

2. Materials and methods

2.1 Anaerobic Digestion

In batch mode, anaerobic digestion of municipal solid waste was carried out in 5L bottles at a mesophilic temperature and firmly sealed to prevent oxygen from entering the reactor. After a two-month digesting phase, the volatile acid-containing leachate separated from the waste residue by gravity filtration method. Afterwards, the supernatant was used to produce PHA after being centrifuged.

2.2 Micro-organism Source

The bacteria were obtained as sludge from the last settling tank of a Sequential Batch Reactor (SBR) at the Shahjalal Fertilizer Company Limited (SFCL) wastewater treatment facility in Fenchugonj, Sylhet, Bangladesh.

2.3 Growth Medium and PHA Accumulation Process

The microorganism was grown in two circular laboratory scale 2L grade glass reactors containing mineral medium KNO3- 1.805 gm/L, 7.0 g/L K2HPO4, 3.0 g/L KH2PO4,

0.5 g/L sodium citrate, 0.1 MgSO4·7H2O, 0.05 g/L FeSO4·7H2O and varying amount of (NH4)2SO4 in different experiments. The two reactors differed in terms of whether the bacteria's principal carbon source was acetate or volatile fatty acid. A 500-rpm magnetic stirrer was used to help the suspended bacteria in each of the circular reactors at 30 degree and pH will be maintained at 7. For aeration, aquarium pumps (RS 248A) were used. Two Experiment was conducted using varying amounts of (NH4)2SO4 and acetate keeping the other constituents same. Two additional tests were conducted by using varying amounts of substrate and nitrogen concentration containing leachate as the primary carbon and nitrogen source. The feast-famine cycle was followed in the creation of mixed culture PHA and done according with the methodology described by Mozumder et al., (2020).While using acetate or leachate as substrate, concentration of acetate was kept in 5g/L after feast-famine cycle. In this experiment, the amount of NH4-N (in mg/L) was measured in time intervals. Measurement of COD (in mg/L) was also conducted to measure the acetate concentration and VFA concentration. MLSS (in gm/L) of the sample was also measured determine dry cell weight which would be used further to measure total biomass present in different time intervals. The enriched PHA accumulated bacteria sludge was then extracted by centrifuging the settled sludge taken from the reactor and was stored for use in our experiment.

2.4 Analytical Method

Measurement of the COD concentration was used to determine the concentration of volatile fatty acids. This method involves adding 4 mL combined reagent consisting of K2Cr2O7 and a catalyst, Ag2SO4, dissolved in concentrated H2SO4, with a ratio of 1:3 to 4 mL of sample. Afterwards, the sample were kept in digester for 4 hour and cooled down to room temperature. Finally, the absorbance will be recorded at 600 nm against a reagent blank. Measurements were performed with a UV-Spectrophotometer. Ammonium concentrations will be measured using the usual Nessler method off-line using a UV spectrophotometer. The concentration of dissolved oxygen (DO) was measured using a DO electrode, and the pH was determined using a pH electrode.

2.5 Quantification of PHA

Active biomass was calculated based on nitrogen consumption and biomass yield over nitrogen is 8.9 g biomass/g-N (Mozumder, Goormachtigh, Garcia-Gonzalez, & Wever, 2014).

Active Biomass=(A-B) *8.9/1000 (g/L) Where, A= Total amount of supplied N in mg/L; B= Amount of nitrogen presence in any moment in mg/L

Total Biomass = DCW+ Optical Density(400nm)

PHA Calculation: Amount of PHA was calculated by the weight difference between Total Biomass and Active Biomass: PHA Accumulation = Total Biomass - Active biomass (g/L)

PHA Content (g-PHA/g-Total Biomass) = (PHA Accumulation/Total Biomass) *100

3. Results and Discussion

3.1 Mixed microbial culture enrichment and PHA production while using Acetate as substrate.

3.1.1 Effect of different nitrogen concentration on PHA production

The experiments were performed under pulse feeding of 1000ppm and 350ppm NH4- N concentration and maintaining acetate concentration 5g/L after feast-famine cycle. At pulse

feeding of 1000ppm NH4-N concentration, the Total biomass concentration in the culture increased exponentially with increasing NH4-N consumption after feast- famine cycle. (Fig 3.1). The bacteria consumed only 535ppm of the nutrients before ceasing further consumption, preventing the maintaining of stress conditions necessary for PHA production which leads to lower PHA accumulation. Total PHA content was found 17%. In the case of 350ppm of NH4-N pulse feeding, a linear consumption of nitrogen was observed that leads to nutrient stress condition (Fig 3.2). Under these nutrient stress conditions (132-155hour), the maximum content of PHA was achieved, resulting in a total PHA content of 41%.

3.1.2 Effect of substrate on PHA production

The aim was to evaluate the impact of 1) A three cycle feast-famine on with a total nutrient concentration of 1g/L. and 2) Addition of excess carbon during nutrient stress condition using a total nutrient concentration of 350ppm.The PHA content and biomass concentration rose with fluctuation during 3 cycle feast-famine phase (Fig 1). In the case of adding 10g/L acetate under nutrient stress condition, a total PHA content of 41% was achieved (Fig 2). The findings of our study suggest that under nitrogen stress conditions, a surplus of available carbon can be maximized PHA production.

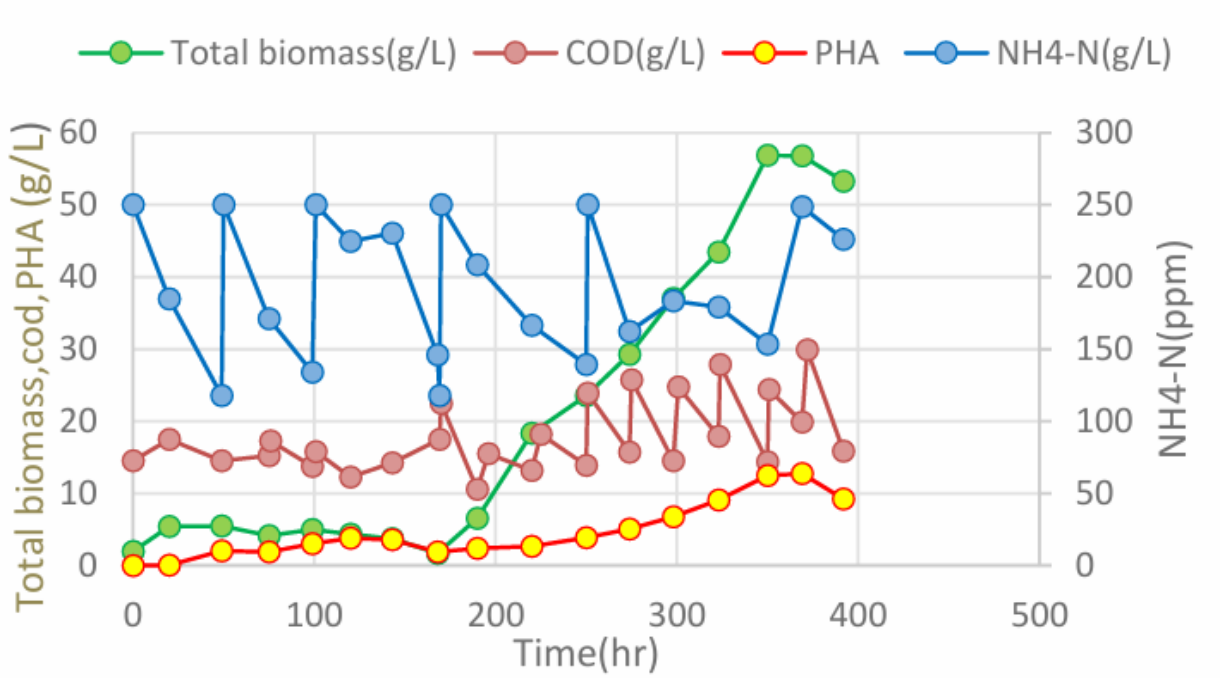

Figure 1: Total biomass growth, PHB accumulation and concentration profile of NH4- N, and COD while using acetate as substrate.

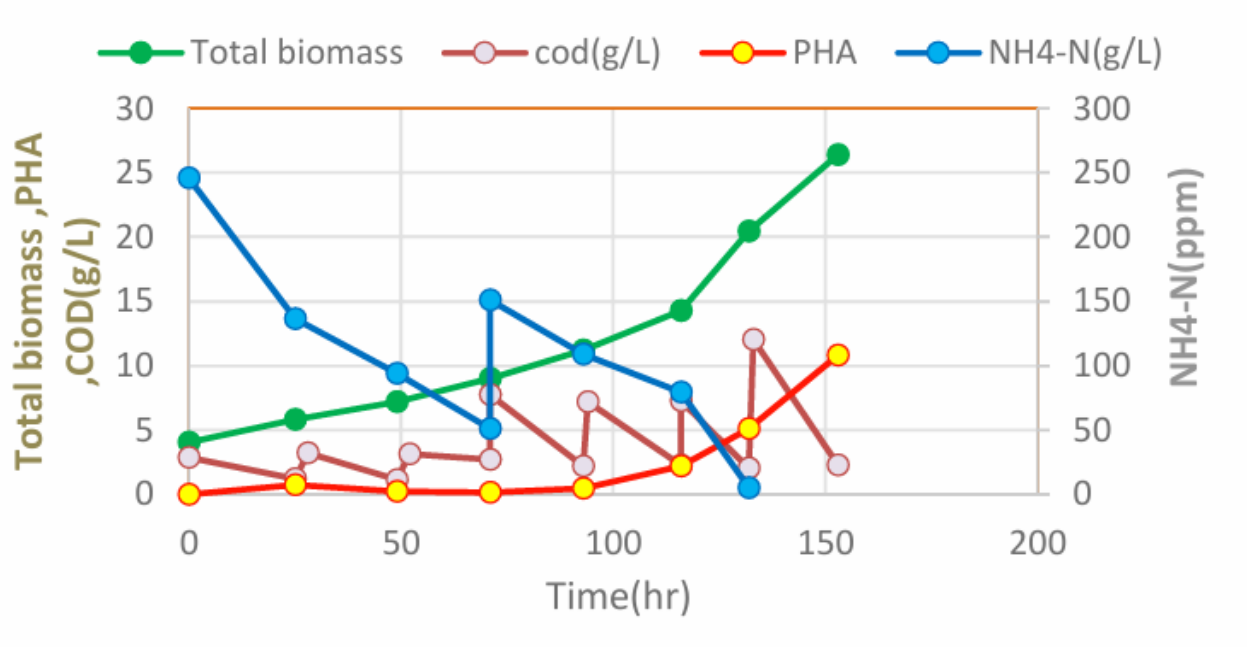

Figure 2: Total biomass growth, PHB accumulation and concentration profile of NH4-N, and COD of while using acetate as substrate.

3.2 Effect of waste material derived carbon and nitrogen level on PHA production

In this study, two additional tests were conducted to assess the PHA-producing capabilities of the mixed culture utilizing leachate as the primary carbon and nitrogen source. Two experiments were performed, one using high carbon and nitrogen concentrations another using low concentration while maintaining a constant C/N ratio. To maintain low carbon and nutrient concentration, a total 6g/L substrate and 150ppm nutrient containing leachate was supplied in the SBR. Nitrogen consumption was found to be increasing and maintained nutrient stress condition. It was observed that the substrate concentration was 0.74g/L during 23.5 to 47hour (Fig:3). The available substrate concentration was lower than the uptake rate which leads to decrease in PHA content. The decrease in the PHA content can be explained by the typical consumption of PHAs as carbon and energy stores to extend the survival of the microorganism after the depletion of carbon substrates. PHA yield was found out to be 0.1g-PHA/g substrate. In case of High carbon and nutrient concentration, the biomass concentration slightly decreased to 9.97g/L from 10.67g/L (from 72 to 120hour) and nitrogen and substrate consumption was lower during this period (Fig:4). This results in a significantly lower amount of PHA. PHB yield was found out to be 0.1g-PHB/g substrate.

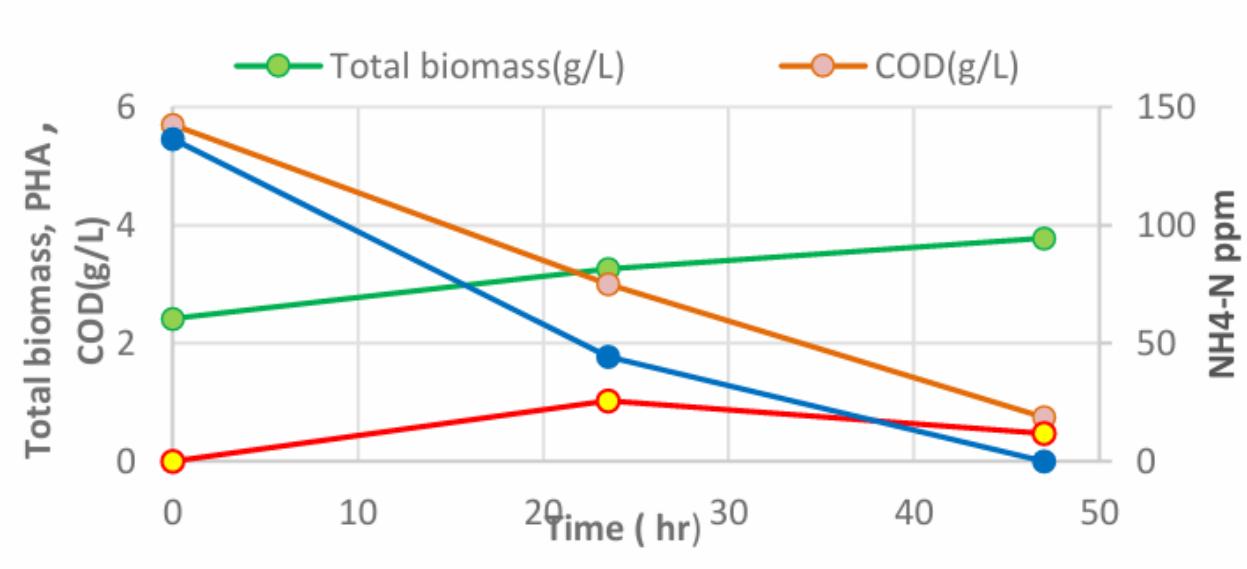

Figure 3: Total biomass growth, PHB accumulation and concentration profile of NH4- N, and COD while using low concentration carbon and nitrogen rich-leachate.

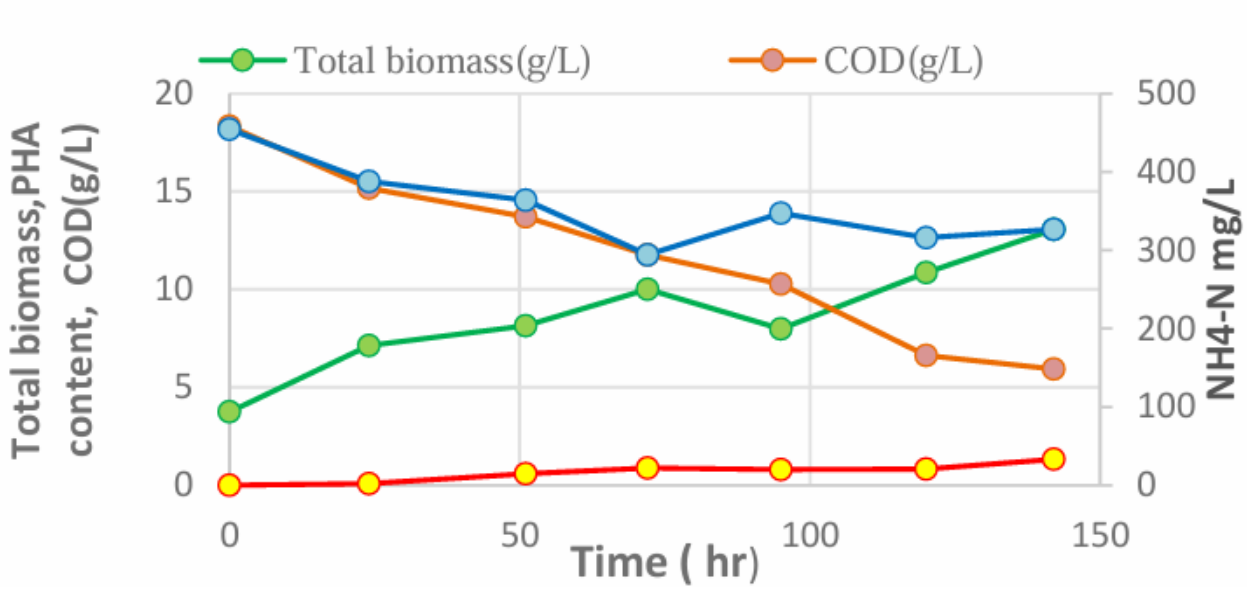

Figure 4: Total biomass growth, PHB accumulation and concentration profile of NH4-N, and COD while using high concentration carbon and nitrogen rich-leachate.

4. Conclusions

In this study, 3-cycle feast famine strategy led to the enrichment of PHA producing bacteria. After the feast-famine phase, adding excess substrate enhance significant amount of PHA, resulting in a maximum content of 41% during nitrogen stress conditions. In contrast, when substrate level is low in the production medium during nutrient stress condition that leads to consume PHA content. The operation of a SBR with leachate as a substrate shows that supplementation of high concentration of nutrient-rich leachate is not effective for maximizing PHA production. Higher substrate load and lower nitrogen showed higher PHA accumulation. Form our experiment it can be concluded that optimum C/N ration can lead to high PHA production.

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