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POLYHYDROXYALKANOATES FROM MUNICIPALWASTEWATER ACTIVATED SLUDGE

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Abstract *Conventional plastics derived from petroleum has been detrimental to the quality and aesthetics of the natural environment. It causes faster depletion of non renewable resources and widespread damage to human and animal life. So the concept of biodegradable plastics emerged which has garnered huge attention of the scientific community till date. However, the production cost of these bioplastics have limited their broad application. So scientists developed biopolymers from renewable natural resources that are cheaper and readily available such as agricultural wastes, activated sludge. The aim of this study was to produce polyhydroxyalkanoates from the municipal wastewater activated sludge acclimated with cassava starch as the carbon source.*

***Key Words*: Plastics, Petroleum, Detrimental**

1. INTRODUCTION

Plastics are found extensively in all the sectors of society which has posed serious threat to the environment due to the problems associated with the accumulation of plastic residues in the ecosystems causing serious harm to the wildlife and human health (Chae and An, 2018). Conventional plastics are made from petroleum derivatives, a non renewable resource which not only depletes the resource but, leaves the plastic product sin tact for many years of the known 8.3 billion metric tons that has been produced, 6.3 billion metric tons has become plastic waste. Of that, only nine percent has been recycled. The vast majority—79 percent—is accumulating in landfills or sloughing off in the natural environment as litter (Geyer *et al*., 2017). Therefore, many studies have been carried out to meet the conditions of finding an alternative to the non-renewable resources that will maintain the resource pool and also, ensure timely plastic disposal .Polyhydroxyalkanoates (PHA), the intracellular biopolymers derived from various microorganisms as carbon and energy storage units throw light upon their potential use as a precursor for bioplastic production because of its structural similarity to its synthetic counter-part. These are polyesters composed of hydroxyl acids which can be easily degraded in the environment by microbial attacks under favorable conditions

Objective of study

(1) To study the PHA production potential of synthetic cassava starch wastewater (CSW) using the acclimatized activated sludge from the sewage treatment plant (STP) as a seed in a sequencing batch reactor.
(2) To optimize the reactor conditions with different SRTs, pH and Nutrient limited conditions.
(3) To check the PHA production capability in the biomass generated.
(4) To evaluate the PHA yield in ultrasonically pre-treated CSW.
(5) To characterize the intracellular bacterial polyhydroxyalkanoates (PHA)

2. METHODOLOGY

The methodology for the study is divided into two phases. In phase I, the aeration tank wastewater of oxidation ditch medical college Calicut (STP) was used as the inoculum for the generation of activated sludge and thereby, acclimation of the sludge using cassava starch as the carbon source. The sequencing batch process for biomass growth over change in two SRTs were discussed. The MLSS, MLVSS and COD removal efficiency were investigated for SRTs 5 and 10 d each. Subsequently, the PHA production and its FTIR characterization were also studied in this phase. In phase II, a synthetic cassava starch wastewater (CSW) will be prepared and henceforth, characterized. Also ,other parameters like pH and nutrient limitations (nitrogen (N) limitation, phosphorus (P) limitation and both N and P limitations) will be examined on synthetic CSW. The COD removal efficiency will be studied in the next phase. Also, the optimized conditions will be checked for PHA accumulation. Further chacterization studies (FTIR, H^1NMR) will also be done on the extracted PHA. The flowchart for the experimental methodology phase I and phase II are shown in Fig. 3.1 and Fig. 3.2below:

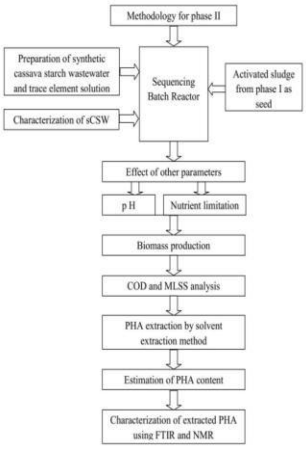


Fig. 3.2 Flowchart of experimental methodology of phase I

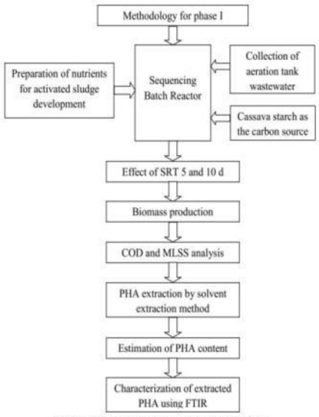


Fig. 3.1 Flowchart for experimental methodology of phase I

3. MATERIALS

The various chemicals, materials and instruments used for phase I

- **Nutrient Solutions Preparation**

The nutrient solutions for the activated sludge development were prepared by mixing different salts in distilled water to obtain the required concentrations. The salts used for nutrient solutions were: $K_2H_2PO_4$, KH_2PO_4, $MgSO_4.7H_2O$, $CaCl_2$, KNO_3, NaCl and cassava starch. All chemicals except cassava starch were purchased from Merck. Cassava starch powder was purchased from the local market in Calicut. The amount of compounds used for nutrient solutions preparation is given in Table 3.1

Table 3.1 Amount of Chemicals for Preparation of Nutrient Solutions

Chemicals	Concentration (mg/L)
$K_2H_2PO_4$	1060
KH_2PO_4	200
$MgSO_4.7H_2O$	500
$CaCl_2$	50
KNO_3	2000
NaCl	1000
Cassava starch	4000

1Sequencing batch reactor

Sequencing Batch Reactor (SBR) comprised of 20 ml of mixed aeration tank wastewater added in a 1 litre reactor vessel with 10 ml of each nutrients and the volume made upto1 litre with distilled water. The daily process included 4 steps: (1) fill (10min), (2) aeration (23 h), (3) settling (45 min) and withdraw (5 min). Fish aerators were used to supply aeration in the reactors. The initial pH of the reactor liquid was adjusted at 7±0.05 by 1 N HCl and 1 N NaOH. COD, MLSS and MLVSS of the samples were measured every 2^{nd}, 4^{th}, 6^{th}, 8^{th}, $10^{th}$and 12^{th}day. Fig 3.3 and Fig 3.4 show the schematic diagram of experimental set up and the actual set up respectively.

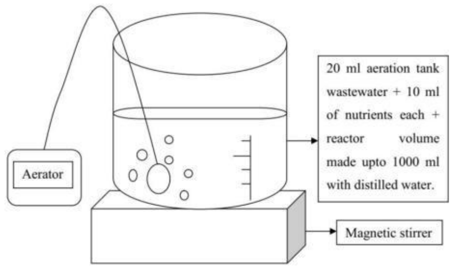


Fig. 3.5 Schematic Diagram of Experimental Set Up

3.1 EXTRACTION AND ESTIMATION OFPHA

Extraction and estimation was performed based on the procedure reported elsewhere (Mohan et al., 2010; Reddy and Mohan, 2012). The biomass was centrifuged at 10000 rpm for 30 min. The photographic image of biomass accumulation at the end of the process of phase I is shown in Fig A1. The resulting pellet was dried at 105°C in hot air oven and cell dry weight (CDW) was measured. Then, it was washed with acetone and ethanol separately (10000 rpm, 5 min) to remove the unwanted materials. The pellet was suspended in an equal volume of 5% sodium hypochlorite (NaClO) solution and incubated at room temperature for 3 h to lyse the cell. The resulting mixture was centrifuged (10000 rpm, 30 min) and the supernatant was discarded. The pellet with lysed cells was again washed with acetone and ethanol separately (10000 rpm, 5 min) and then it was dissolved in hot chloroform and passed through a glass

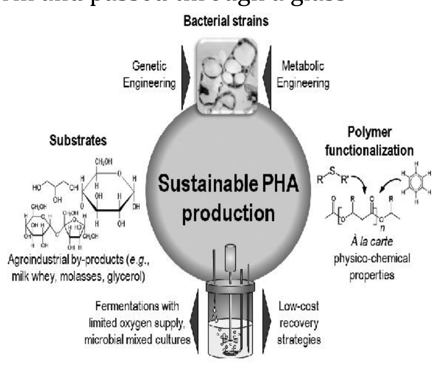


Fig 3.7 Bioprocesses And Downstream Processing

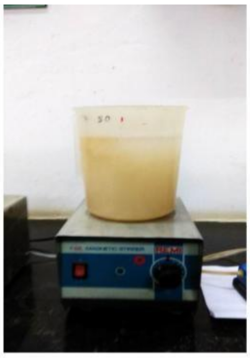
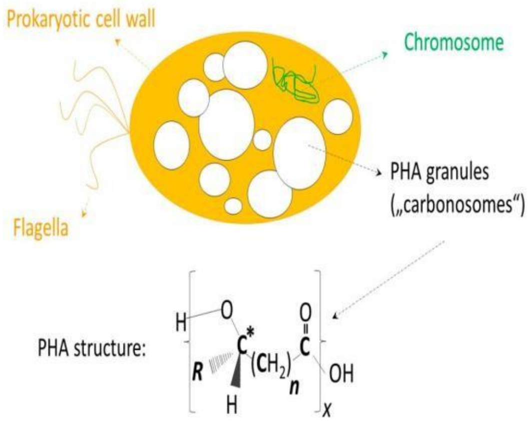
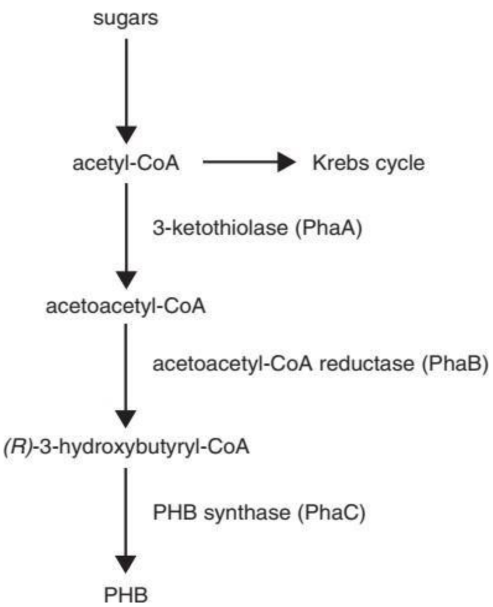


Fig 3.13 Biomass accumalation

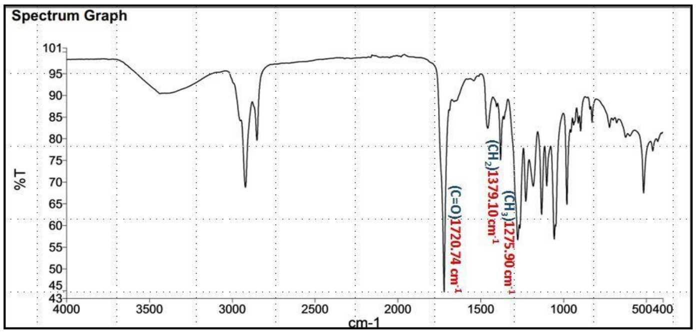




4. RESULTS AND DISCUSSION

PHA Accumulation

Table 4.1 shows the profile of PHA production at different SRTs. The PHA content was measured very low i.e., 1.62% and 0% at SRT= 5 d and SRT= 10 d respectively. There are many factors that could have caused this decrease in PHA production. Firstly, this was not an enrichment phase for selecting PHA accumulating organisms, rather it was an activated sludge development phase to grow the biomass to a sufficient amount. So there was a generous supply of carbon, nitrogen and phosphorus sources among which nitrogen and phosphorus limitation causes PHA accumulation (Lee, 1996). Also starch has a very complex nature which prevents many bacterial strain to hydrolyze it (Raza*et al.*, 2018). Limited presence of such bacterial strains in the activated sludge could be a plausible explanation for low PHA production. Aeration is another important factor for PHA accumulation for aerobic microorganisms since the microbial inoculums was from the aeration tank wastewater. Zhang *et al.*, 2018 reported that there was a gradual decline in the PHA accumulated when the aeration was low. There were times when aeration was very low in the reactors due to the blockage of diffusers of the fish aerators and also the top of the reactors were covered with a tile throughout the process, thus preventing the atmospheric air flow. Johnson *et al.*, 2010 reported that lower SRTs are preferred by microorganisms for PHA accumulation but very low SRTs are not favored as it causes maintenance problems in the reactors which explains PHA accumulation only in SRT= 5 d in this case.



5. CONCLUSIONS

(1) PHB, a homopolymer PHA was produced in Sequencing Batch Reactors with mixed microbial culture under non-sterileconditions.
(2) Activated sludge acts as a good inoculum for PHA production as it reduces the operational cost of PHA production using expensive raw materials and sterilization cost required when pure cultures are used as the inoculums. Moreover, it also reduces the excess sludge produced from wastewater.
(3) Highest biomass production (MLSS= 3000 mg/L, MLVSS= 2134 mg/L) was observed in SRT= 10 d but, highest sludge activity (0.88-0.91)was noticed in SRT= 5 d indicating that longer SRTs favor higher biomass growth.
(4) Highest COD removal efficiency (86%) was measured at higher SRT which is again attributed to the excessive biomass growth that facilitates in COD removal.
(5) In this study, PHA production was found to be very low : 1.68% at SRT= 5 d and no PHA production at SRT= 10 d caused by the limited presence of microorganisms capable of hydrolyzing the complex starch structure, availability of nutrients to be limited in excess and hindrance in the aeration during the process

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BIOGRAPHIES

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