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Influence of aerobic and anoxic microenvironments on polyhydroxyalkanoates (PHA) production from food waste and acidogenic effluents using aerobic consortia

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ABSTRACT

The functional role of aerobic and anoxic microenvironments on polyhydroxyalkanoates (PHA) production using food waste (UFW) and effluents from acidogenic biohydrogen production process (FFW) were studied employing aerobic mixed culture as biocatalyst. Anoxic microenvironment documented higher PHA production, while aerobic microenvironment showed higher substrate degradation. FFW showed higher PHA accumulation (39.6%) than UFW (35.6%) due to ready availability of precursors (fatty acids). Higher fraction of poly-3-hydroxy butyrate (PHB) was observed compared to poly-3-hydroxy valerate (PHV) in the accumulated PHA in the form of co-polymer [P3(HB-*co*-HV)]. Dehydrogenase, phosphatase and protease enzymatic activities were monitored during process operation. Integration with fermentative biohydrogen production yielded additional substrate degradation under both aerobic (78%) and anoxic (72%) microenvironments apart from PHA production. Microbial community analysis documented the presence of aerobic and facultative organisms capable of producing PHA. Integration strategy showed feasibility of producing hydrogen along with PHA by consuming fatty acids generated during acidogenic process in association with increased treatment efficiency.

1. Introduction

Polyhydroxyalkanoates (PHA) are the polymers of hydroxy fatty acids that are naturally produced by specific group of bacteria as an intracellular carbon and energy rich material (Rehm, 2009). Bioplastics in the form of PHA are stored as granules in the cytoplasm by microorganisms under stress conditions due to limitation of a nutrient, electron donor or acceptor (Gujer et al., 1999). Established industrial processes utilize pure sugars such as glucose, sucrose, or other sugar based compounds such as corn, which have a high market price as principle substrate for PHA production (Reddy et al., 2003). In recent years, there has been a great interest in decreasing the polymer production cost. Utilizing low value substrates such as waste feedstocks (Reddy et al., 2003) and mixed culture (Lemos et al., 2006; Venkata Mohan et al., 2010a) as biocatalyst makes the process economical to certain extent. The combination of these two factors allows saving energy and reduces the fermentation cost. One of the advantages of using mixed culture is the possibility to make use of a wide variety of complex waste substrates which are mostly opposed to many pure strains. Using waste streams and mixed culture evades requirement of aseptic conditions.

Currently research is focusing on the use of waste streams like molasses, paper mill wastewater (Bengtsson et al., 2008, 2010), biodiesel wastewater (Dobroth et al., 2011), olive mill wastewater, synthetic peptone mixture (Cokgor et al., 2011), agricultural wastes (Rhu et al., 2003), food processing industrial wastewater (Kumar et al., 2004) and designed wastewater (Venkata Mohan et al., 2010a) for production of PHA using mixed culture. On the other hand the type of PHA produced in a waste based process depends primarily on the composition of the waste stream and the microenvironment. The organisms present in the mixed culture rely on substrates that can be fermented and store the fermentation products inside the cell rather than excreting them. These organisms can also use internal stored glycogen for energy release through glycolysis and subsequently used to accumulate fermentation products (i.e. volatile fatty acids (VFA)) in the form of PHA.

The main aim of this study was to evaluate the potential of anoxic and aerobic metabolic function on PHA production using two forms of food waste viz., un-fermented (UFW) and fermented (FFW) (generated during biohydrogen (H_2) production process) as substrate and aerobic consortia as biocatalyst. Presence of high organic fraction in food waste makes the process feasible for their conversion into energy in the form of H_2 (Venkateswar Reddy et al., 2011), bioelectricity (Kannaiah Goud and Venkata Mohan, 2011) and other valuable products with simultaneous treatment. Major criteria for the selection of food waste as suitable substrate for PHA production is its easy availability, less cost, feasibility,

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higher carbohydrate, protein content and easily biodegradable nature. However, its origin, composite nature, physicochemical characteristics including particle size, composition, protein and oil content of waste makes the treatment process difficult. Food waste mainly contains the carbohydrate content which is easily degradable and on hydrolysis produces simple sugars like hexoses, pentoses and soluble acid metabolites. Hexoses are used for production of PHA and energy required for the maintenance of normal metabolic activities occur in the organisms (Rehm, 2009). Pentoses release the electron carrying molecules which are involved in PHA production and production of nucleic acids which are used in building of genetic material of the organism. The main challenges encountered with fermentative H_2 production processes are low substrate conversion efficiency and residual acid-rich effluents generated from the acidogenic process (Venkata Mohan, 2008; Venkata Mohan et al., 2008a, b; Mohanakrishna et al., 2010). Even under optimum conditions about 60–70% of the original organic matter in the form of VFA remains as residue in the wastewater. H_2 can be considered as an intermediate towards the production of VFA which is important precursor for further transforming into PHA. Conversion of fermentative products into PHA could also be an interesting option from wastewater treatment point of view, solid materials like PHA are easy to separate from the liquid than soluble fermentation products which are present in low concentrations (Rehm, 2009). Thus PHA production process could generate clean water as well as an attractive product at the same time. Microbial composition and changes in the communities during defined intervals were evaluated along with enzyme activity to understand the process.

2. Methods

2.1. Substrates

Two types of food wastes viz., un-fermented (UFW) and fermented (FFW) with same organic loading rate (OLR, 1.11 kg COD/m^3-day) were used as substrates for PHA production. Food waste (pH, 6.52 ± 1.2; total solids (TS), 36,290 ± 110 mg/l; total suspended solids (TSS), 23,068 ± 74 mg/l; total dissolved solids (TDS), 13,220 ± 75 mg/l; total alkalinity, 1084 ± 20 mg/l; chlorides, 1420 ± 104 mg/l; VFA, 5879 ± 340 mg/l; chemical oxygen demand (COD), 3,30,000 ± 560 mg/l; biochemical oxygen demand (BOD_5), 2,47,500 ± 640 mg/l; total carbohydrates, 95,874 ± 92 mg/l; nitrates, 9.8 ± 0.42 mg/l; proteins, 31,250 ± 35 mg/l; oil and grease, 75,000 ± 130 mg/l) was procured from IICT canteen (Venkateswar Reddy et al., 2011). The waste was composite in nature including uneaten food and food preparation left overs mostly comprising of boiled rice (60 ± 5%; wet weight basis), cooked vegetables (14 ± 4%), un-cooked vegetables (spoiled) (2 ± 1%), cooking oil (6 ± 2%), vegetable peelings (3 ± 2%), cooked meat (5 ± 2%) and others (2.5 ± 1%) with water content of 6% to 10%. The collected food waste was masticated using electrical blender and filtered through stainless steel sieve to remove coarse materials so as to avoid potential clogging problems. Subsequently, the waste was separated using oil separating system by gravity. The resulting oil free filtrate was used as feed by diluting with domestic sewage (pH, 7.5; COD, 420 mg/l; TDS, 460 mg/l) up to the required OLR for both H_2 and PHA production. The characteristics of the UFW prior to load showed good biodegradability (pH, 6.8 ± 0.2; VFA, 489 ± 8 mg/l; COD, 4000 ± 35 mg/l; carbohydrates, 2878 ± 20 mg/l and proteins, 542 ± 10 mg/l). The effluent generated from H_2 producing reactor through the treatment of UFW at OLR of 3.38 kg COD/m^3-day after 72 h of fermentation was acidic in nature with high concentration of VFA (pH, 4.2 ± 0.2; VFA, 2120 ± 14 mg/l; COD, 4000 ± 35 mg/l; carbohydrates, 1585 ± 14 mg/l; proteins, 280 ± 6 mg/l) was used for PHA production after adjusting to the required pH and OLR.

2.2. Biocatalyst

Aerobic consortia acquired from an operating activated sludge process (ASP) treating 10 MLD of composite wastewater from domestic and industrial processes was used as biocatalyst for PHA production. Anaerobic consortia from operating lab scale UASB reactor treating wastewater was used as parent inoculum for H_2 production. Prior to inoculation, respective parent cultures were washed in saline buffer (5000*g*, 20 °C and 10 min) and enriched in the designed synthetic wastewater (DSW), [NH_4Cl – 0.5 g/l, KH_2PO_4 – 0.25 g/l, K_2HPO_4 – 0.25 g/l, $MgCl_2$ – 0.3 g/l, $CoCl_2$ – 25 mg/l, $ZnCl_2$ – 11.5 mg/l, $CuCl_2$ – 10.5 mg/l, $CaCl_2$ – 5 mg/l, $MnCl_2$ – 15 mg/l, $NiSo_4$–16 mg/l, $FeCL_3$ – 25 mg/l] under aerobic/anaerobic microenvironments (120*g*; 28 °C) for 24 h.

2.3. Experimental design

2.3.1. Biohydrogen production

Biohydrogen production from dark-fermentation was studied in bioreactor (total/working volume, 1/0.82 l) with suspended growth configuration having a gas holding capacity of 180 ml. The reactor was fed with 750 ml of UFW with OLR of 3.38 kg COD/m^3-day after inoculating with 70 ml anaerobic consortia. Prior to feeding, pH of the UFW was adjusted to 6.0 using 2 N orthophosphoric acid. Reactor was operated under anaerobic microenvironment in fed batch mode with a retention time of 72 h at ambient temperature (29 ± 2 °C) by continuous stirring (100 rpm).

2.3.2. PHA production

The feasibility of PHA production was evaluated with both FFW and UFW as substrates under aerobic (FFWA, UFWA) and anoxic (FFWAx, UFWAx) microenvironments. Experiments were performed in four separate sequential batch reactors operated in suspended growth mode. AeSBR system was operated under aerobic microenvironment by closing with cotton plugs to ensure the exchange of gases. AxSBR system was closed with septum (butyl rubber) to maintain anoxic metabolic function with intermittent sparging of air (4 min for every one hour time interval using aquarium pump). Four bioreactors were designed to have total/working volume of 250/110 ml and operated in fed batch mode with continuous mixing (100 rpm) at ambient temperature (29 ± 2 °C). Four reactors prior to startup was inoculated with 10 ml of aerobic consortia along with 100 ml of feed after adjusting pH to 7 ± 0.1 using 2 N NaOH at OLR of 1.11 kg COD/m^3-day. Similar operating conditions were used for evaluating PHA production with both substrates.

2.4. Analysis

H_2 gas accumulated in head space of bioreactor was estimated using a microprocessor based pre-calibrated sensor (ATMI GmbH Inc., Germany). The sensor had a measuring range of 0.01–10% H_2 with 5 s response time in a temperature range of 20–80 °C.

2.4.1. PHA

Extraction and estimation of PHA was performed based on the procedure reported elsewhere (Kumar et al., 2004). The biocatalyst was separated from the substrate by centrifugation (3000*g* for 30 min at 10 °C) and the resulting pellet was washed with acetone and ethanol separately to remove unwanted materials. The pellet was suspended in an equal volume of 4% sodium hypochlorite and incubated at room temperature for 3 h. The resulting mixture was centrifuged (3000*g* for 30 min at 10 °C) and the supernatant was discarded. The pellet with lysed cells after