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Effect of substrate load and nutrients concentration on the polyhydroxyalkanoates (PHA) production using mixed consortia through wastewater treatment

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ABSTRACT

Production of biodegradable plastics in the form of polyhydroxyalkanoates (PHA) especially from renewable substrates is gaining interest. The present work mainly aims to investigate the influence of substrate load and nutrient concentration (nitrogen and phosphorous) on PHA production using wastewater as substrate and mixed culture as biocatalyst. PHA accumulation was high at higher substrate load [OLR3, 40.3% of dry cell weight (DCW)], low nitrogen (N_1, 45.1% DCW) and low phosphorous (P_1, 54.2% DCW) conditions. With optimized nutrient conditions production efficiency increased by 14%. Fractional composition of PHA showed co-polymer [poly(β-OH) butyrate-*co*-poly(β-OH) valerate, P3(HB-*co*-HV)] contains PHB (88%) in more concentration compared to PHV (8%). Dehydrogenase and phosphatase enzymatic activities were monitored during process operation. Good substrate degradation (as COD) of 75% was registered during PHA production. The phylogenetic profile of 16S rRNA sequencing showed the dominance of *Firmicutes* (71.4%) and *Proteobacteria* (28.6%), which are known to involve in PHA accumulation and waste treatment.

1. Introduction

Now there is a growing concern over the use of conventional plastics like polypropylene and polyethylene. Production of these plastics is dependent on depleting sources of hydrocarbons. These petroleum derived plastics takes several years to decompose and during degradation they produce harmful toxic compounds. Because of these, an alternative is required for future economical and ecologically safe polymers. Polyhydroxyalkanoates (PHA) production through biological source is gaining importance to overcome the adverse effects during conventional plastics degradation (Rehm, 2010). PHA are the class of linear polyester compounds naturally produced by many bacteria with similar properties to polypropylene and polyethylene but completely biodegradable, biocompatible and produced from renewable resources. Upon disposal they are degraded by microorganisms to water and carbon dioxide under aerobic condition and methane under anaerobic conditions. PHA has been industrially produced by pure cultures including *Alcaligenes latus*, *Azotobacter vinelandii*, *Pseudomonas oleovorans*, recombinant *Alcaligenes eutrophus* and *Escherishia coli*. However, one of the largest drawbacks of this method involves the requirement of high operational costs which accounts for nearly 11% of total production costs that includes media sterilization and reactor maintenance. Development of pure culture fermentation and commercialization of PHA increase their cost about 4–9 times higher than that of the conventional plastics (Moita and Lemos, 2011).

Another way to decrease the operational costs would be to design a novel system that do not require sterilization and reactor maintenance. So there is a great interest, using mixed culture and wastewater to produce PHA. Compared to pure culture, the merits of PHA production with mixed culture includes an enhanced economy, a simpler process control, non sterile conditions and an improved use of wastes. Mixed culture utilization can lower the input costs by allowing for large scale fermentations to occur without overhead costs of sterilization. It also allows for a greater variety of substrates to be used due to the presence of several PHA producing organisms. A considerable effort has gone in production of PHA using mixed culture and different wastewaters like, municipal wastewater (Chua et al., 2003), sugar cane molasses (Albuquerque et al., 2010), paper mill wastewater (Bengtsson et al., 2008), tomato cannery wastewater (Liu et al., 2008), olive oil mill effluent (Beccari et al., 2009), biohydrogen reactor effluent (Venkata Mohan et al., 2010) and food waste (Venkateswar Reddy and Venkata Mohan, 2012). Culture selection with a high PHA storage capacity is one of the challenges in PHA production process using mixed culture. Operating the system sequentially under a carbon excess phase (feast) followed by substrate exhaustion (famine), a selective pressure is imposed on the system. Mixed culture operated under feast and famine conditions were subjected to

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