Original Research Article

Bacillus and Biopolymer with Special Reference to Downstream Processing

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A B S T R A C T

Polyhydroxyalkanoates (PHAs) are the most fascinating group of biopolymer emerges to be the potential candidate for substitute of synthetic plastics. However, high cost of both upstream and downstream processing has limited their successful commercialization. Among these two processes, recovery methodology of PHAs significantly affects the overall production economics. Thus, various recovery technologies including chemical digestion, solvent extraction, enzymatic treatment, supercritical fluid disruption, mechanical disruption, flotation techniques, aqueous two-phase system and use of gamma irradiation have been used in different industry and academia. In this review, we summarized the quantity and quality analysis of PHAs produced, particularly by Bacillus species with special reference to downstream processing, which may lead to get high purity and maximum recovery at a low production cost.

Keywords
Polyhydroxyalkanoates, Downstream, Industry, Academia and Bacillus

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Introduction

The global petrochemical based plastic production has been increased from 1.5 million tons in 1950 to 299 million tons in 2013. Rapid exploitation of these synthetic, non-biodegradable plastics has generated large amounts toxic waste as well as a setback on their management (Yang et al., 2015). Thus, it is the need of the hour to replace these synthetic plastics by an alternate biopolymer. Polyhydroxyalkanoates (PHAs) are the most fascinating group of biopolymer, synthesized by a wide range of Gram positive and Gram negative bacteria as carbon and energy storage inclusion in their cytosol (Sudesh et al., 2000). Among different genera, Bacillus species are ideal by numerous industries and academia, as a matter of fact; they are genetically stable, fast growing, consume reasonably priced carbon sources and produce endotoxin free PHAs as evaluated against Gram negative bacteria (Mohapatra et al., 2015; 2017). In general, PHAs synthesis followed by accumulation is one of the responses towards stress experienced by bacteria residing at different ecological niches (Koller et al., 2011). The molecular weight of PHAs varies between 200,000 to 2000,000 dalton depending on bacterial strain, fermentation conditions and substrate used in the bioprocess technology.
These biopolymer mimic the properties petrochemical based plastics and recyclable to CO₂ and H₂O in the natural condition (Khanna and Srivastava, 2005). Hence, PHAs can be used for preparation of plastics materials, medical implants, drug delivery carriers, nutritional supplements, drugs and fine chemicals (Maity et al., 2017). Nevertheless, replacement of conventional plastics is inadequate due to their elevated production cost, which holds back its unbeaten market penetration (Waltz, 2008). As a result, more efforts are needed for making the bioprocess technology economically feasible.

In this regard, maximum attention has been given towards upstream processing (Maity et al., 2017) than downstream processing and quality analysis of PHAs.

Earlier studies also recommended that, cost of production, quantity, molecular weight and purity of PHAs extracted from bacteria depends on various physical, chemical and biological methods used in downstream processing (Koller et al., 2013; Mohapatra et al., 2015; Kunasundari and Sudesh, 2011; Dibyashree and Shamala, 2010). In this review, we summarized the quantity and quality analysis of PHAs produced by Bacillus species with special reference to downstream processing.

**Quality analysis of PHAs extracted from Bacillus species**

Cost affordable PHB production and pharmacological purity is mainly dependent on the microbial strain used and the extraction method employed to separate the biopolymer (Valappil et al., 2007). Majority of the separation processes including sodium hypochlorite multi-solvent, di-solvent and mono-solvent, chloroform-methanol, sodium hypochlorite aqueous two phase system and chloroform have been used for the recovery of PHB from Bacillus species. The data analysis (Table 1) suggested that, higher amount of PHB recovery has been achieved in the sodium hypochlorite multi-solvent extraction method. More specifically, 5.29g/l and 5.30g/l of PHB was extracted from Bacillus subtilis NG220 (Singh et al., 2013) and Bacillus subtilis (Gomma, 2014) by sodium hypochlorite multi-solvent extraction method respectively. In addition, Bacillus cereus SPV was produced 3.0g/l of PHB with 95% purity by the same process (Valappil et al., 2007).

| Bacterial strain          | Downstream process          | Carbon source  | Quantity of PHAs (g/l) | Purity of PHAs | Melting Point (Tm) | Type of PHAs | Reference                  |
|---------------------------|-----------------------------|----------------|------------------------|----------------|--------------------|--------------|---------------------------|
| *Bacillus subtilis* (KP172548) | Sodium hypochlorite and Multi-solvent | Fish solid waste | 1.620               | -              | 120°C              | PHB          | Mohapatra et al., 2017    |
| Lysinibacillus sp. 3HHX   | Sodium hypochlorite and Multi-solvent | Glucose       | 4.006                 | -              | 112°C              | P(3HB-co-3HDD-co-3HTD) | Mohapatra et al., 2016    |
| *Bacillus subtilis* (KP172548) | Sodium hypochlorite and Multi-solvent | Glucose       | 3.090                 | -              | 99°C               | PHB          | Mohapatra et al., 2015    |
| *Bacillus thuringiensis* RKD-12 | Sodium hypochlorite and Multi-solvent | Glucose       | 1.110                 | -              | -                  | PHB          | Mohapatra et al., 2015    |
| *Bacillus thuringiensis* RKD-12 | Sodium hypochlorite and Di-solvent | Glucose       | 1.080                 | -              | -                  | PHB          | Mohapatra et al., 2015    |
| *Bacillus thuringiensis* | Sodium hypochlorite         | Glucose       | 0.450                 | -              | -                  | PHB          | Mohapatra et al., 2015    |
Though, sodium hypochlorite extraction method leading to degradation of PHB as well as reduction of the polymer chain length, however the level of degradation varies from microbes to microbes. Thus, this method is widely used for extraction of PHB as it results in less polymer degradation (Valappil et al., 2007). Moreover, the different extraction techniques analyzed in this review were found to have an effect on the thermal and structural properties of the PHB extracted from Bacillus species.

Different downstream processing strategies have been conducted for recovery of PHAs from Bacillus species depict that, sodium hypochlorite digestion followed by solvent extraction method can lead to high purity and endotoxin free PHAs as compared to other method. Although, this method is not cost affordable and environmental friendly, however improvement of this downstream processing method can lead to an economic recovery of PHAs, with a high purity for its substantial biomedical applications.

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