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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.

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

(Koller *et al.,* 2013). These biopolymer mimic the properties petrochemical based plastics and recyclable to CO_2 and H_2O 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).

Table.1 Quantitative and qualitative analysis of PHAs produced by *Bacillus* species

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^{o}C	PHB	Mohapatra *et al.,* 2017
Lysinibacillus sp. 3HHX	Sodium hypochlorite and Multi-solvent	Glucose	4.006	-	112^{o}C	P(3HB-co-3HDD-co-3HTD)	Mohapatra *et at.,* 2016
Bacillus subtilis (KP172548)	Sodium hypochlorite and Multi-solvent	Glucose	3.090	-	99^{o}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.,*

RKD-12	and Mono-solvent						2015
Bacillus sp. P3	Sodium hypochlorite and Multi-solvent	Glucose	0.948	-	-	PHB	Mohapatra *et al.*, 2015
Geobacillus sp. AY946034	Sonication and Multi-solvent	Glucose	1.30	-	168.8^{o}C	PHB	Giedraityte and Kalediene, 2015
Bacillus OU73T	Multi-solvent extraction	Rice bran	57.76%	-	-	PHB-co-HV	Nagamani *et al.*, 2015
Bacillus sp. KSN5	Sodium hypochlorite and Chloroform	Glucose	95%	-	-	PHAs	Kalaivani and Sukumaran,2015
Bacillus thuringienesis KSADL127	Chloroform	Glucose	0.13	-	283^{o}C	PHB	Alarfaj *et al.*, 2015
Bacillus subtilis	Sodium hypochlorite and Chloroform	Cane molasses	5.30	-	-	PHB	Gomma, 2014
Bacillus licheniformis	Sodium hypochlorite and Di-solvent	Glucose	0.437	-	-	PHB	Dash *et al.*, 2014
Bacillus subtilis G1S1	Sodium hypochlorite and Multi-solvent	Glucose	0.20	-	-	PHB	Shah, 2014
Bacillus thuringienesis KJ206079	Sodium hypochlorite and Multi-solvent	Cane molasses	4.10	-	-	PHAs	Desouky *et al.*, 2014
Bacillus sp. S1 2013b	Sodium hypochlorite and Multi-solvent	Glucose	4.00	-	-	PHB	Mohapatra *et al.*, 2014
Bacillus megaterium	Sodium hypochlorite and Multi-solvent	Glucose	1.60	-	-	PHAs	Israni and Shivakumar, 2013
Bacillus megaterium uyuniS29	Chloroform and Methanol	Glucose	2.35	-	161^{o}C	PHB	Contreras *et al.*, 2013
Bacillus subtilis NG220	Sodium hypochlorite and Multi-solvent	Maltose	5.29	-	132.54^{o} C	PHB	Singh *et al.*, 2013
Bacillus flexus	Sodium hypochlorite and Chloroform	Sucrose	1.00	-	-	PHB	Divyashree and Shamala, 2009
Bacillus flexus	Sodium hypochlorite and Aqueous two phase system	Sucrose	1.30	80%	-	PHB-co-HV	Divyashree *et al.*, 2009
Bacillus sphaericus NCIM5149	Sodium hypochlorite and Multi-solvent	Agro-industrial residues	0.69	-	-	PHB	Ramadas *et al.*, 2009
Bacillus cereus SPV	Sodium hypochlorite and Multi-solvent	Glucose	3.0	95%	-	PHB	Valappil *et al.*, 2007

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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