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ISOLATION, PRODUCTION AND EXTRACTION OF BACTERIAL POLYHYDROXYALKANOATE ENZYME

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ABSTRACT

Polyhydroxyalkanoate is an enzyme. It is produced from microorganisms and plants. The most efficient production of PHA enzyme through microorganism. Because, it is naturally produced by bacterial species. It is also a subclass of polyhydroxybutyrate. PHA is a polyester compound and have the ability of biodegradable property. It is also biocompatible compound. PHA present in bacteria as inclusion bodies. The Sudan B Black stain used to screen the inclusion body of bacteria under 100x microscope. Phasins protein present in lipid membrane of bacteria that provide the stability and biosynthesis of PHA. PHA is monomeric compound and it help to increase the identification of naturally occurring PHA through physical and chemical properties. PHA is hydrophobic in nature and in crystal form. A number of bacterial include for the production of PHA such as *Protomonas extorguens, P. aeruginosa, P. putida, P. oleovorans, Ralstonia* eutropha, *E. coli, Bacillus cereus, Bacillus subtilis.* Substrate and polyester synthase formation spherical form of granules in the bacterial cells. The in-vitro production of PHA through the cell by using precursor substrate to synthesis of PHA. The In-vivo production of PHA from the cell by the action of amphipathic PHA synthase to synthesis PHA granules. The recovery of PHA from bacterial cells by using different techniques such as chemical, mechanical and enzymatic digestion to isolate and extraction of PHA enzyme. PHA has big advantage to use in industrial and medical application. The use of recombinant strain could be more effective because of renewable carbon sources and cheap. At industrial level, PHA replace synthetic plastic by degrade plastic and also for waste water treatment. In medical application, drug delivery system has great efficient to target area by coating of PHA with drug. The economical cost of PHA is reduce as to synthetic polyester materials.

KEYWORDS: Isolation of PHA, Chemical and physical nature of PHA, In-vitro and In-vivo synthesis of PHA, Role of enzymatic Phac, Recovery techniques for PHA, Application.

INTRODUCTION

History of polyhydroxyalkanoate (PHA)

In advance technology in the global population, plastic is the potentially widest application in everyday life as well as in industries. The use of most conventional plastic is polystyrene, polypropylene, polyethylene and polyvinylchloride. But these types of plastic are non-biodegradable and it increasing the accumulation of toxic compounds into the environment and create pollution. One of the most first and very important strategy is biodegradable plastic. The first biopolymers or biodegradable plastic was initiated in 1862. This type of plastic wad introduced by Parkesin and celluloid. The British company make bioplastic in 1990 from Imperial college London (Mossman, 1994). The formation of biopolymers by two ways. The first way for the formation of biopolymers through microbes and plants. The commercially available of bioplastic are polylactic acid, polyhydroxybutyrate (PHB), polyhydroxyalkanoate (PHA), polycaprolactone (PCL), thermoplastic, starch, bio-polyamides (nylon), bio-polyols, cellulosic. Polyhydroxyalkanoate (PHA) is the highest demand from the other polymers.

Polyhydroxyalkanoate (PHA)

PHA is an enzyme and it is produced by bacterial species. It is used for the degradation of plastic or used for biodegradable agents. PHA is the highest potential of availability and also used as bioplastic or to degrade the plastic (Liu et al., 2006) and (Chen et al., 2009). Polyhydroxyalkanoate (PHA), poluhydroxybutyrate (PHB) and petroleum based polycaprolactone (PCL) are bio-based plastic and degraded by microorganisms such as bacteria and fungi. Basically, PHA enzyme is a subclass of PHB enzyme. PHA and PHB enzyme has ability and more efficient degradability (Hayden and Russel, 2013). Polyhydroxyalkanoate enzyme is a bio-polyester and it is naturally present in many bacterial

species. The indication of bio-Polymer was first discovered by the scientist Lemoigne (Anderson and Dawes, 1990). PHA is an enzyme used for biodegradable agents. PHA has the ability to biochemically degrade the plastic. PHA naturally produced by microorganisms. Approximately 150 type of monomers of PHA have been identified and this increasing number of PHA monomers helps to identify new different types of naturally accruing PHA through the physical and chemical modification (Zinn and Hony, 2005). The specialized function of PHA has been identified by producing of GMOs (Escapa et al., 2011).

Isolation of PHA through bacteria

Polyhydroxyalkanoate (PHA) is produced by many bacterial species. Basically, PHA present in naturally form in bacteria. Many bacterial species have PHA enzyme in the form of inclusion bodies in the bacterial cell wall. Many bacterial species produced PHA enzyme such as *Ralstonia eutropha, P. oleovorans, P. aeruginosa, P. putida*, and *Protomonas extorquens*. *Alcaligenes latus*, *Azotobacter vinelandii* and *E. coli* (Steinbuchel *et al*., 1996). *Pseudomonas* species has great potential to degrade the plastic. The advantage of choosing *Pseudomonas* species has the increase production or highest rate of biosynthesis of PHA and *E. coli* also produce free toxin PHA (Steinbüchel, 1996).

Screening of PHA producing bacteria

The *Pseudomonas* spp. including *P. aeruginosa* has the ability to produce 50% of PHA synthesis. *Pseudomonas Putida* has the ability to produce 36% of PHA synthesis (Shankar et al., 2015). *Pseudomonas* spp. present in contaminated soil and also present in activated sludge. *Pseudomonas* sp. easily extracted in the microbiology laboratory by using specific media. The media used for this species called Cetrimide agar medium (CA). *Pseudomonas* species specifically grow in cetrimide agar medium 37 $^{\circ}$C and show greenish colour pattern on petri-plate. *Pseudomonas flourescens* also produce PHA granules which present as carbon and nitrogen sources. E. coli strain also produce PHA enzyme when expression of PHA synthases gene in recombinant *E. coli* strain. The bacterial species *E. coli* accumulated PHA when grown in medium contain glucose. It also consists of 3 hydroxy-butyrate and also 3 hydroxy-acetone detected when grow in medium solution containing decanoate compound. Polyhydroxyalkanotae (PHA) is also produce by propylene oxide saponification of wastewater contaminated sludge. The bacterial species identified for producing PHA from this activated sludge is *Brevundimonas vescularis* when tested through 16S rDNA sequencing method. The *Brevundimonas vescularis* is also called as *Pseudomonas vescularis*. Because this bacterial species was identified when grow in Cetrimide agar and observe by microscopic analysis and biochemical characterization. Various detection technique for identified PHA granules such as Chromatography, Mass spectroscopy and NMR technique used to identify PHA molecule and structural related molecule of PHA. The PHA granules also further identifies at molecular level by using 16SrRNA sequencing (Aditi et al., 2015).

Biochemical structure of PHA

The structure of polyhydroxyalkanoate (PHA) consists of 3 hydroxy fatty acids. The monomers are different due to differences of pendant R group varying from carbon number 1 (methyl) to carbon number 13 (tridecyl). The ester bond is form by one monomer due to carboxyl group. This carboxyl group form bond with the hydroxyl group of another nearest monomer (Lemoigne, 1926) and (Zinn et al., 2001). The carbon atoms of R – configuration is substituted by hydroxy group in PHA structure. This substitution is due to stereo specificity of the biosynthesis of PHA enzyme (Anderson et al., 1990) and (Sudesh et al., 2000). The synthesis of chemical PHA is slightly difficult because of R – group present in the centre of backbone (Lee et al., 1990). The alkyl group at the carbon number 3 is vary from the position of methyl group to tridecyl group.

Polyhydroxyalkanoate (PHA) producing bacteria divided into two groups. These group are divided on the basis of monomer units of carbon atoms. 3 – 5 carbon atoms consisting PHA are short length and 6 – 14 carbon atoms consisting PHA are long length chain. The carbon atoms in this structure of PHA have different varieties because of saturated, unsaturated and branched or straight chain that containing aromatic or aliphatic side groups. Because PHA is hydrophobic n nature and in crystal form. Many bacterial specie for the production of PHA. PHA further divided into two main major groups (Khanna and Srivastava, 2005). In the first group, bacterial species also have different characteristics from the other second group of bacteria. The essential elements or nutrients such as nitrogen, sulphur, magnesium and phosphorus required for the first group of bacteria. Because, in which present an excess amount of nitrogen and carbon sourced which help to the production of PHA. Some bacterial species included in this group such as *Protomonas extorguens, P. aeruginosa, P. putida, P. oleovorans, Ralstonia* eutropha etc. But the second group of bacterial species does not require essential elements because it directly produce PHA biopolymers as naturally accumulated carbon and nitrogen sources. Many bacterial species included in this group such as A*zotobacter vinelandii, Alcaligenes latus* and *E. coli* (Steinbuchel et al., 1996).

Structure of PHA granules inside cell

PHA present inside the bacteria in granule form as light refracting discrete. Sudan B Black stain and Nile blue stain used for PHA screening. These types of screening method are very specific for PHA granules identification under 100 x microscope. This microscopic analysis understands the microbial cell lipids which contains PHA granules (Schlegel et al., 1970) and (Bhuwal et al., 2013). The separation of polymers in the accumulation of granules from the lumen cell and not affect the osmotic

pressure of the cell. (Anderson and Dawes, 1990). The size and number of granules in each cell is varies on different bacterial species. The diameter of granules in each cell have range about 0.2 – 0.5 µm in bacterial species such as *A. eutrophus* (Byrom., 1994) and (Ballard et al., 1987). Two large granules were analysed in *P. oleovorans* (Kilinke et al., 2000). Basically, the structure of PHA granules are spherical in nature and its surrounded by phospholipids membrane (Stuart et al., 1998) It is composed of two crystalline protein layers. These two layers are separated and it also consist of PHA polymerases, PHA depolymerases intracellular and phasing of amphipathic proteins (Stuart et al., 1998). The coating of membrane of granules is about 2 nm thick in nature and it containing liquid is 0.5% and 2 of protein. These compositions indicate the granules weight (Lundgren et al., 1964). The granules of PHA present in the cytosol of cell. So PHA polymerase is only active when it localizes on the granules surface (Gerngross et al., 1993) and (Pieper-Fürst et al., 1995).

Some activating genes and enzymes involved in the synthesis of PHA enzyme (Mezzina et al., 2104). Phasins is a protein that promote the biosynthesis of PHA enzyme. Basically, phasins is also called granules associated protein and present outer surface covering of polyhydroxyalkanoate (PHA). This phasin protein present in layer form and stabilizes the granules. Basically, this protein phasins helps to inhibiting single granules from angulating and coalescing with the other granules and act as barrier agent between polymer and cellular components (Fuller, 1999) and (Poster et al., 1994). The accumulated cells less PHB in phasin protein of R. eutropha and it contains single large granules. Because, the separated granules coagulate due to hydrophobic PHA in contact and the surface become naked (Wieczorek et al., 1995) and (Potter et al., 2000). It is also observed that phasins protein also have protective function because it reduces the other attachment of cytosolic type proteins.

Physical properties

PHA granules present in cell as water insoluble form of inclusion and they also occur as mobile phase in the cell. PHA exist in the cell in the form of water insoluble inclusion bodies (Ciesielski et al., 2006). PHA exist as mobile phase within the cell and amorphous in nature (Sudesh and Abe, 2000). PHA granules becomes crystalline form after extraction from the cell treated with organic solvent. After extraction of PHA, the characteristics of PHA is similar to that of the conventional plastics like polyethylene, polypropylene and polystyrene (Chang et al., 2014). In-vivo, some structural protein such as phasins attached with PHA by non-covalently to stabilize the PHA granules in the cell. Because, it prevents the PHA to attach with other granules to form agglutination or coalescing (Foster et al., 1994). The role of water in the cell is very important to describe the structure of different molecules in the cell. So, the minor component of PHA granules is water and play a role of plasticizer in PHA granules (Harrison et al., 1992). Approximately 5 – 10% of water present in PHA inclusion bodies in the cell. The hydrogen bond formation with carbonyl groups of polyester backbone by water molecules. This bond formation to be called as pseudo-cross-links and located between the polymeric chain. This phenomenon is known as deformation of plastic and also revealing of PHA as amorphous in nature (Dunlop and Robards, 1973). The apparent crystallization structure of PHA is form when PHA inclusion is treated with centrifugation machine (Sanders, 1993). In the amorphous phase and melting temperature phase, glass to rubber transition phase (Tg) is normally express their mechanical and thermal properties. The length of PHA and distance between ester linkages in polymer depend upon the PHA rand from brittle to elastic and flexible. These ranges describe the mechanical properties of PHA. The short chain length of PHA shows the crystalline in hardest form while longest chain of PHA shows elastomeric in nature. (Table 1) (Williams et al., 1999). The polypropylene phase is not more brittle as than to short length of PHA molecule (Holmes, 1985). PHA not show stress resistance because of its brittleness in nature. In case of PHB, the melting temperature is high around 170°C. This temperature is close to the decomposes of polymer thermally and reduce or limits ability of homopolymer process. The mechanical properties of HB and HV P(HB-HV) copolymeric is better than of PHB homopolymer. But PHB has more brittle and stiffer than H B and HH consisting copolymers. So, due to highly brittle and stiff nature of PHB improve or increase the mechanical properties as compare to polystyrene, polypropylene, polyethylene etc (Lauzier et al., 1992). The properties of crystalline and Tm is decrease when formation copolymer with HB and HV monomer units. Therefore, increase in toughness and decrease in stiffness production of polymers is very potentially important for commercial application. There are many HA monomers that effect the rate of PHA degradation. Polyhydroxy-butyrate and Polyhydroxy-alkanoate has highest potential of degradation as compared to homopolymer PHB. The medium chain length of PHA are rubbery and flexibility in nature and have low crystallinity. Therefore, it is suitable for the application at wide range. But PHB and scl-PHAs not fulfil a wide range of application (koning and Lemstra, 1992). PHA polymer of medium length chain is more potential for application due to increase elasticity and decrease crystallinity as compared to PHB and P(HB – HV). The monomer chain length of PHA have 1 – 12 carbon atoms are more elasticity and mobile phase (liquid). So, the PHA polymers are thermostable biodegradable and elastomer in all bacterial PHAs (Lauzier et al., 1992).

Role of Enzymatic PhaC Type I and II during Biosynthesis of PHA

PhaC is an enzyme that is involve during the biosynthesis of PHA contents. PHA enzyme have 4 classification on the basis of kinetics and mechanism of

reaction in the biosynthesis of PHA. Four classes of PhaC depend upon the specific substrate. Class I, III and IV produce short chain length (scl) but class II PhaC enzyme synthesis of medium chain length (mcl). Short chain length (scl) depends upon different precursors such as valerate, butyrate, propionate and hexanoate. Medium chain length depends upon the alkane precursors on the basis of carbon atoms (C6 – C14).

PHA synthases of class I and II

The formation of PHA synthases of class I and II by single protein (PhaC). The protein size has about 60 kDa. The class I PHA synthases is characterized from *Ralstonia eutropha* and class II from *Pseudomonas* sp. The PhaC enzyme of class I is a dimer and facing to catalytic domain (CAT). The catalytic domain is occupied due to secondary structure. The conformational structure changes due to open of catalytic domain by activation factors and 3HB – CoA is allocated to its binding site (Check et al., 2017).

PHA synthases of class III and IV

There are two subunits of class III of PHA synthases such as PhaC and PhaE. The size of PhaC has about 40 – 52 kDa and PhaE has about 20 – 40 kDa and this PhaE form the PhaEC complex. Because the subunit of PhaE is important for the polymerization of PHA. The structure of PhaC of class III are tetrameric in structure (Zhang et al., 2015). The class III type of enzyme has been isolated and characterize through bacterial species such as *Thiocapa pfennigii* (Liebergesell et al., 2000). This bacterial species belongs to *Archaea* and good perspectives for the production of polymer. PHA synthases of class IV from Bacillus spp. They have catalytic subunit called PhaC and PhaR subunit (Kihara et al., 2017). PHA synthases of class IV is classified into different bacterial species such as *Bacillus magaterium*, *Bacillus cerus* and *Bacillus bataviensis*. This PHA synthase class IV is 33% homology to PhaC sequence (Tsuge et al., 2015).

Table 1: Four classes of PHA synthases.

Sr. No.	Types	Subunits(s)	representative species	Substrate specificity
1	I	PhaC	*Ralstonia eutropha*	C3 – C5
2	II	PhaC	*Pseudomonas aeruginosa*	C6 – C14
3	III	PhaC – PhaE	*Allochromatium vinosum*	C3 – C5
4	IV	PhaC – PhaR	*Bacillus megaterium*	C3 – C5

Biosynthesis of PHA by enzymatic pathway

There are many enzymatic reactions involved from acetyl-CoAs for the synthesis of PHA and the specific substrate used act as catalyse for PHA synthesis. The accumulation of PHA is synthesis in the cytosol of cell (Ojumu and Solomon, 2004). Condensation of Acetyl CO-A molecule in the first reaction and converted into Acetoacetyle-CoA and catalysis by β-ketoacetyleCoA thiolase enzyme. The reduction of Acetyle-CoA in the second reaction and converted into 3-hydroxybutyryl-CoA by the catalysis of an enzyme dehydrogenase/reductase and NADH also dependent. In the PHA, the synthesis of mcl-(R)-3-hydroxyfatty acid convert fatty acid to 3-hydroxyacyle-CoA. There are two strategies of reaction involve in the pathway. If the oxidation of carbon sources with Acetyl-CoA and β-oxidation of fatty acid pathway excluded than intermediated of de novo biosynthesis of fatty acid directly the synthesis of PHA biomass by catalysis of trans-acylase. If the oxidation of carbon sources with β-oxidation pathway of fatty acid rather than Acetyl-CoA then enoyl-CoA is converted into 3-hydroxyacyl-CoA by the catalysis of enoyl-CoA hydratase enzyme. So, in this pathway PHA biosynthesis is directly produce by the substrate of 3-hydroxyacyl-CoA.

Formation of PHA granules through In-vitro

The invitro synthesis of Polyhydroxybutyrate were first produced by Martin and Gerngross. They described the spherical form of granules formation by using purification agents such as substrate and polyester synthase. (Gerngross and Martin, 1995). All features in the prosses of PHA synthase that required for self-assemble into spherical particles. The formation of PHA synthesis by using PHA synthase enzyme from bacteria species. These bacterial species include *Allochrmatioum vinosum*, *Cupriavidus nector*, *Pseudomonas aeruginosa* and *P. oleovorans* (Jossek and Steinbuchel, 1998) and (Rehm et al., 2001). PHA synthases of class II recently identified to purification of 3 hydroxydecanoate – CoA. This substrate sufficient for the production of 3 polyhydroxyalkanoate.

Formation of PHA granules through In-vivo

The formation of PHA granules has two models. The first model is micelle and second is budding. These models describe the exact location of synthases polyester and also some phasin protein. This protein present on the granule surface. The invitro formation of PHA granule supported micelle model in the absence of membrane (Grage et al., 2009). Membrane bounded material observed surrounded on the PHA granules. This membrane like material identified by electron microscope (Dunlop and Robard, 1973). The early stages of granules showing that the distribution of granules close to inner cell membrane but not distributed in the cytoplasm. The location of the granules arises at unknown mediation centre from the cell showing a new model of PHA granule (Tian et al., 2005). The PHB granules from C. nectar cells observed a large structure by using force microscopy. The PHA granules formation at centre of cell also called synthesis degradation centre

(Dennis et al., 2003). Recently PHA synthase by green fluorescent protein (GFP) observed by microscope and fused with PHA synthases of class I and II N-terminus. The monitoring of in vivo formation of PHA granules and localized of subcellular without effect of granules particles (Peters and Rehm, 2005). The localization of cell poles is found at early stages of granules according to the model of budding. The granules formation depends upon the structure of nucleus and this is remained unclear because the synthesis of PHA also depend and required for subcellular of granules localization. The rapidly oscillating of small granules between cell poles was observed by this In-vivo PHA granules synthesis. Basically, synthase labelled GFP polyester support the model of budding by cytoplasmic membrane which localize granules formation at the cell pole.

Techniques for the recovery of isolation and purification of PHA from bacterial cells

Methods of Digestion

In this technique, the surrounding cellular material of the PHA granules are solubilize. This method is used as an alternative technique to the solvent extraction method and also classify into two digestion method such as chemical and enzymatic digestion.

Chemical digestion

Many chemical methods used for the extraction or recovery of PHA granules from the biomass. This method is based on the solubilization of non PHA cellular biomass. The strong chemical agent used such as sodium hypochlorite are strong oxidizing agent and Its recovery of PHA by manipulating to NPCM digestion (Yu and Chen, 2006). The surfactant chemical also used such as dodecyl sulphate, betain as well as SDS because of good performance. Single use of surfactants or sodium hypochlorite is not good enough for the recovery of PHA granules. Both combination of sodium hypochlorite and surfactants used in this digestion method. The cheaper chemical used to lower cost of recover PHA and increase the efficiency. The PHA granules recover about 50 % of molecular weight.

Solvent extraction

PHA recover from the biomass, solvent extraction is the most effective method. Because, this solvent extraction method is very effectively used in the laboratory and extract PHA from the cell (Table 1). This is a rapid and simple method for extraction. There are two steps of solvent extraction method. The first method is cell membrane permeability modification and second step of PHA solubilization. The solvent mostly used such as chlorinated hydrocarbons. The chlorinated hydrocarbons include chloroform, 1,2 dichloroethane and some also used cyclic carbons such as ethylene carbonate and propylene carbonate (Ramsay et al., 1994).

Enzymatic digestion

The enzymatic digestion method used for recovery of PHA is very complex procedure. The cell components of other than PHA granules are wash with surfactants and treatment with heating as well as hydrolysis (Holmes and Lim, 1990). Fermented broth used like S. melloti in which protease secreted microbial species was used such as Microbispora sp. and treated with thermally 80°C for 10 minutes. This species has the ability to induce hydrolysis of PHA. After 72h incubated and thermally heated, intracellular components contact with PHA granules were release (Lakshman and Shamala, 2006). The lysed cell include in culture was purified by filtration and used chloroform mixture to recover 94% PHA granules was purified (Divyashree et al., 2009).

Disruption by Mechanical method

The intracellular protein is extracted by mechanical disruption of cells. There are many methods for mechanical disruption (Harrison, 1991). In which high-pressure homogenization and Bead mill methods used for large scale disruption of cell in biotechnology and pharmaceutical industries (Bury et al., 2001). This method is economically very effective because of less damage of products (Tamer et al., 1998).

Bead Mill

Bead mill is basically sharing action that release energy and transfer this energy from beads to cells. The diameter of bead is 512µm and the agitation speed about 2800 rpm. This agitation speed to complete disruption of cells. *Alkali genus latus* cells are Completed disrupted by passing eight beads when samples were loading about 85%. The loading sample 75% required more than 16 beads passing to release proteins of the cells. The disruption of cells depends upon residence time disruption (RID), types of microorganisms, concentration of cell, suspension feed rate, agitator speed, grinding chamber, stir design and shear forces (Doucha and Livansks, 2008). This beads mill is very effective for PHA recovery because of low power supply and diameter of bead mills is also not affected the disruption process or rate of disruption.

High pressure homogenization

The cell disruption is done by under high-pressure chromatography method. The parameters processing such as temperature, number of passes, operator pressure and valve of homogenizer must be design carefully for disruption (Kelly and Muske, 2004). The efficiency of recovery of PHA granules from *Alkali latus* by homogenizer is less as compared to beads mill disruption method. The parameters processing is not only useful factors but also physiological parameters of microbes and its types and also growth phases of microbes with cell concentration (Ghatnekar et al., 2002). But the drawbacks of this methods are also degrading the product of interest by thermally and cellular debris formation further interfere in downstream processing.

Supercritical fluid (SCF)

SCF method is used for the extraction of protein for PHA recovery. This method is very efficient in which used

superficial carbon dioxide (SC – CO2). This chemical is preferably used due to its low toxicity, pressure (73 atm and temperature (at 31°C) and 89% recovery of PHA by *C. necator* bacteria (Ghatnekar et al., 2002).

Cell fragility

The osmotic level increases during the accumulation of PHA granules by documentation of bacterial species such as recombinant *E. coli* and *Azotobacter vinelandii*. Cell fragility is the mechanism to restricted both Gram-positive and Gram-negative bacteria (Divyashree et al., 2009). The high molecular weight of PHA can be enhanced by the addition of fish peptone in culture medium of *A. vinelandii* (Page and Cornish, 1993). About 92% of PHA is extracted through the fragile cells by using aqueous NH3 at 45°C temperate for 10 minutes.

Floatation

In this floatation method, chloroform is used and mixed with cells at 30°C. This solution placed at room temperature. About 85% PHA is recover after 72h. But this extraction method is costly due to avoided of addition techniques such as centrifugation and polymer wastage during the recovery of PHA granules (Ibrahim and Steinbuchel, 2009). The enhancement of downstream processing, use of green solvent with floatation technique. Air floatation also used for the extraction of PHA granules from the cell's components.

Aqueous two-phase system (ATPS)

This system is formed when two polymers (one is polymer and second is inorganic salts) at low concentration. This system is used when two polymers are present in coexist form (yang et al., 2008). The bacterial species such as Bacillus flexus containing PHA granules and treated with enzymatic hydrolysis. This enzyme extracted from the Microbispora sp. cells and then introduced into ATPS system. About 97% recovery of PHA granules through ATPS system when adjusting the parameters such as pH, temperature etc. The advantage of using Microbispora sp. include protease that also be extracted with PHA. This technique is more effective for good resolution, high yield capacity, low energy consuming, low cost and less time required.

Gamma Irradiation

Gamma irradiation effect on the cell wall of bacteria and disruption of cell to the recovery of PHA. PHA recover about 54% of molecular weight with used of irradiated cells (10kGy). But 18 – 20% of molecular weight of PHA recover with the use of unirradiated cells. Gamma irradiation technique is independent to any chemical agents and the process is contamination free (Divyashree and Shamala, 2009).

Application

The plastic manufacturing industries use more effectively use apparatus against PHAs because of more degradability in nature. Now a days, PHA has great potentially use because, it is insoluble in water, non-toxic compound, crystalline in nature, optically more active, size of range from 105 – 107 Da almost, polymerization increase, more biocompatibility, commercially pure chemical, high degree of piezoelectric properties (Poirier, 2001). So, it is highly competitive feature as compare to synthetic plastic such as polystyrene base and petrochemical based plastic (Loo et al., 2007).

Packaging

PHA are natural thermoplastic polyester product and it replace organic polymeric compound to use for coating and packaging purposes. They employed in a large kind of merchandise as well as luggage, also used for golf tress, cups, bottles, cosmetics, containers, female hygienic merchandise, diaper, paper, pens, razors and also for food packaging. The accustomed turn out a water resistant for paper and cardboard by the latex of PHAs (Renard et al., 2007).

Denitrification of wastewater treatment

The new properties of plastic may be achieved using the mixing of alternative polymers with PHAs and its activity increase during this field. The optimistic application of PHAs because of solid substrate for denitrification of water and also denitrification of waste material. This class of denitrification is also termed as solid-phase denitrification and also has blessings over the standard system increase with liquid organic substrate. The constant source of reducing power for denitrification by distribute of PHAs. In addition, in contrast to standard process, the use of PHAs has no prospect to the risk of organic carbon through the resultant deterioration of effluent water quality. The removal of lipid soluble organic pollutant from water is a successful test for oil absorption capacities (Zhang et al., 2012).

Industrial production of PHA

In the industrial scale, the PHA production, the purifies of PHA and extracted from the bacteria by fermentation and optimum condition provide to the bacteria in fermentation of glucose or sugar. The raw material of fermentation used such as carbohydrates (glucose and sucrose) but some other vegetable oil and glycerine used for the production of biodiesel. The properties of PHA has potentially greatest advantage to degrade plastic. So, many researchers use cyanobacteria grow in olive mill waste water and also genetically modified bacteria to the efficient or increase the production of PHA. PHA also potentially important in medical application such as drug delivery. The potential degradability of PHA on marine effect as compared to other polymers. Biodegradation of PHA is faster as to another polymeric compound (Chen, 2009).

Economical aspect

One of the most issues preventing in the pilot scale production or commercial application and also a wide use of PHA as artefact plastic is its high cost has hindered the utilization of PHAs, since their final value is significantly a lot of petrochemical based artefact

plastic materials. Factors concerned embody the merchandised yield, complexness of the technology and thus the cost of capital of the plant and therefore the ease or difficulty of product separation. Consequently, choice of the organism and substrate will critically influence prices. Further, there is a diversity among microorganism from completely different ecological habitats in their PHA production, modes of growth, types of microorganisms and media ingredients. But the foremost of standardization studies on PHA producing microorganisms, as well as gram – negative and gram – positive species have according the optimum hydrogen ion concentration seven and incubation at 30 – 37°C for optimum yield (MuthEzhilan et al., 2014). The cost of PHA mistreatment the natural producer *A. eutrophus* is US $15 – 30 per kilo that is eighteen times dearer than plastic. The use of recombinant E. coli as producer of PHA, cost will be decrease to US $4 per kilo, that is near different perishable plastic materials such as PLA and aliphatic polyesters. The commercially available worth ought to return to US $3 – 5 per kilo. There are presently economic disadvantages and limited use of bacterial plastic, there is presently a highly quantity of analysis dedicated to improve productivity to scale back production process and a lot of significantly to supply specific functionalize PHAs (Amacher et al., 2013).

CONCLUSION

Overall, the review literature highlights PHA could be a terribly promising polymeric compound for large range of applications. Many PHA may be potential renewable biopolymer with properties closely resembling some common organic compound (petrochemical) plastics. Due to huge range of structurally completely different monomers that may be polymerizes by microbes. The PHA production can be exploited in large scale by metabolic engineering and high-density cell culture technologies. Based on information within the literature, it is not possible to grow microorganism cells up-to a density of 150g/l with a PHA content of over 80% of the cell dry weight. However, the extraction as well as purification of PHA granules from the cell biomass may be a difficult task particularly once one considers the employment of environmentally high-risk chemicals as an unacceptable choice within the production of eco-friendly materials. PHA polymer should be free from endotoxin or contaminating chemicals and solvents in medical application. The recombinant bacterial species mostly used to increase the production of PHA. Some fermentation strategies use for the recovery of PHA techniques. The use of recombinant strain could be more effective because of renewable carbon sources and cheap. This would be very benefit for the commercialization of PHA and exploring more high value in the application of agriculture, industrial and also in medical. The cost of PHA product is reduce at economical level as to synthetic polyester product.

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