Production of polyhydroxyalkanoates (PHAs) utilizing different biological wastes

InzerGul Afghan[ab], Anupama Shrivastav[b*], Arun Kumar[b]

a : Parul Institute of Applied sciences, Microbiology department, Parul University, Limda, Waghodiya, Vadodara.

b: Education Faculty, Chemistry department, Paktia university, Gardiz, Afghanistan

Abstract

Polyhydroxyalkanoates (PHAs) are intracellular storage granules that accumulate by different microorganisms as energy and carbon store materials. They are environmentally friendly, biodegradable and also biocompatible bio plastics, unlike synthetic plastics that take several decades to degrade, PHAs can be fully degraded between one and two years by different microorganism into CO2 and water, in this study, we aim to isolate and screening of PHAs producing bacteria utilizing various agro-industrial wastes, dairy wastes, pulp, and paper mill wastes, activated sludges of treatment plants, rhizosphere, various microorganism are industrially important candidates for the reason that they can use waste substances of various origins as substrate to produce bio products containing PHA. The system to separate their bio products can be economical in regard to production, In conclusion, exploring of capabilities like of dual production through microorganisms, and uses of wastes as renewable source under suitable cultural condition in medium or continuous process can cause reduction in present cost biopolymer production from intracellular granules of PHA.

Keyword: Polyhydroxyalkanoates (PHAs), microorganism, rhizosphere, bio products

Introduction

History of plastic synthesis

The first man who invented the plastic in the year of 1862 was Alexander Parkes, he presented at a great exhibition in London, in which he used Parkesine as a raw material obtained from cellulose after heating once then cooled down to create desired shape, (M Bellis, 2011), Mostly things that cover the world by plastic made from fossil fuel. According to this study production of plastic increased from mid-20th century (Červenková; 2007), according to the estimation in 1930 the plastic production in the world reached to 23000 tones, in the year after world war the production of plastic reached minimum 1.3 million tons, In 1993 it was estimated that production of plastic annual reached to 100 million tones, western countries used 430 kg per years, (Braunegg et al., 2004b).

1.3 Application and properties

Study showed that application and properties of plastic have many criteria for usage including medical applications, packaging materials, different components in cars, computer equipment and in many industries and involving the use of sport equipment’s etc. (Zinn et al., 2001). Today people are using a great amount of plastic according to their chemical and mechanical properties including tensile strength, low density, imperviousness to water, and high resistance to natural degradation processes, non-rusting in different field. (Keshavarz and Roy, 2010).

1.4 Chemical background of conventional plastics

In order to know how to recycle plastics, one has to realize what actually these synthetic polymers are on a chemical basis, In general, synthetic polymers are chemical basis which made from monomer of hydrocarbon, (Červenková; 2007), these hydrocarbon monomers which are obtained from crude oil and natural gas, many examples of monomer that take place in creating synthetic polymer, certain example contain styrene, ethylene glycol, propylene, vinyl chloride, ethylene and many others, these monomers can be linked together through a process called polymerization like chemical synthesis of polystyrene, Polyethylene, polypropylene, polyvinylchloride etc.
(http://www.americanchemistry.com/s_plastics/doc.asp?CID=1571andDID=5972, Buchta; 2010) According to this study properties of polymer depend on the starting monomer, length and final structure of polymer shows the characteristics.

1.5 Incineration

According to the properties of plastic incineration, In this process synthetic plastic could be burned in a particular combustion space, burnt of plastic producing water, carbon dioxide and heat energy, This heat energy could be used for electricity and steam generation purpose, (Braunegg et al.,2004b). The estimation of heat energy from plastic waste approximately (42MJ/kg-1) compare to stone coal (28 MJ/kg-fuel oil (ca. 41 MJ/kg-1), natural gas (ca. 45 MJ/kg-1), although incineration increased the concentration of carbon dioxide in atmosphere, and also release others unhealthy chemical molecules. Like carbon mono oxide, nitrogen mono oxide, hydrogen chloride, hydrogen cyanide, sulpher di oxide, and polycyclic aromatic hydrocarbons, dioxins, phthalates, benzene, ethane, etc. that makes air potentially harmful to health, (Simoneit et al., 2005, Koller et al., 2010b, Reddy et al., 2003). Although combustion chamber design to reach high combustion Temperature, as to reduce the formation of potentioally harmful compound. (http://www.plasticsindustry.org/AboutPlastics/content.cfm?ItemNumber=793andnavItemNumber =1124).

1.6 Biodegradable polymers

According to the last and most undertaking scenario that plastic waste management invented most fascinating scenario for production of biodegradable plastic, many biodegradable polymers that are similar to the petroleum plastic known already by researchers, which is available in the market including polyethylene (PE), poly(p-phenylene) (PPP), polyhydroxyalkanoates (PHA), poly(butylene succinate) (PBS), poly(trimethylene terephthalate) (PTT), poly(lactic acid) (PLA), application of biodegradable polymer due to their certain chemical and mechanical properties allows to be used in a great range of application, although, only polyhydroxyalkanoates can have valuable structural variation along with being monomer made in vivo unlike the other polymers being chemically synthesized by in vitro polymerize. (Chen 2010b, Verlinden et al., 2007). However, study showed that producer of polyhydroxyalkanoates found at various parts of nature and/or living with high organic matter or growth condition such as hydrocarbon contaminated sites, paper mill wastes, dairy wastes, pulp, agricultural wastes, rhizosphere, activated sludges, industrial eluents and few of them produce some extracellular byproducts contain rhamnolipids, polymeric matter, bio hydrogen gas, many various microbes which was present in industrial wastes were so important, these microbes were used the waste materials as meals to produce byproduct contain PHA.

Table 5. Various type of waste for maximum PHA production

Substrate	Treatment applied	Possible constituents	Microorganisms	Maximum PHA produced
Sugarcane molasses	Acidogenic fermentation	VFAs	Glycogen accumulating organisms	0.47–0.66 C-mol PHA/C-mol of total carbon 56–70 mol-% 3HB, 13–43 mol-% 3HV, 1–23 mol-% 3HHx, 0–2 mol-% 3H2MB, and 3H2MV
Cassava starch wastewater	Acidogenic fermentation	Acetate, butyrate acids, propionate, pH-5.3, and high COD value.	*Cupriavidus* sp. KKU38	PHB; 85.53% of CDW
Sugar refinery waste (cane molasses)	Acid treatment and activated charcoal	Carbohydrate (4%)	*Pseudomonas aeruginosa*	PHB; 62.44% of CDW
Seed oil of *Jatropha curcas*	Saponification	Oleic acid, linoleic acid, and palmitic acid	*Pseudomonas oleovorans* ATCC 29347	P(3HB-*co*-3HV); 26.06% CDW
Hemicellulosic feedstock (sugarcane bagasse)	Acid hydrolysis, boiling, precipitation, and activated charcoal	Xylose, arabinose, glucose, and acetic acids	*Burkholderia cepacia* IPT 048 and *Bacillus sacchari* IPT 101	IPT 048 PHB; 62% of CDW IPT 101 PHB; 53% of CDW
25% and 50% of vinasse (ethanol industry waste)	Activated charcoal	Sucrose, oxalate, lactate, malate, and pyruvate	*Haloferax mediterranei*	PHB; 70% of CDW
Paper mill wastewater	Acidogenic fermentation	VFAs	Activated sludge	PHB and PHV; 48% of SDW
Cynodon dactylon grass and *syzygium cumini* seed;	Pulverization followed by hydrolysis	Glucose	SP-Y1 and *Ralstonia eutropha*	SP-Y1 (PHB) H.S 26.76% of CDW H.G 41.7% of CDW *R. eutropha* (PHB) H.S 42.2% of CDW H.G 28.97% of CDW
Milk and icecream wastewater	Acidogenic fermentation	VFAs	Activated sludge	0.25 kg PHA/kg of COD
Palm oil mill effluents	Acidogenic fermentations	Acetate, propionate, and butyrate	*Ralstonia eutropha* JMP 134	At 20th and 40th hr 0.75 g PHA/g CDW At 40th and 60th hr 0.41 g PHA/g CDW
Rice straw	Acidic treatment	Pentose sugars and hemicelluloses	*Bacillus firmus* NII 0830	PHB; 89% of CDW

.(Baljeet Singh Saharan et al,2014)

The dividing of byproducts from whole integrated system contain extracellular and intracellular, economically these two-way was more important related to production, this review showed that usage of various microorganism in different condition, these microorganisms stimulate the dense carbon as a granule of polyhydroxyalkanoates (PHAs) and some factor which regulate it constituents and production, the metabolites which was produce with the production of PHAs has been showed, so the two part of production by microbes showed their ability and usage of industrial waste for a renew purpose under suitable condition and also the continuous production process can effect on the cost of biodegradable polymers to store the granules of PHAs compound.

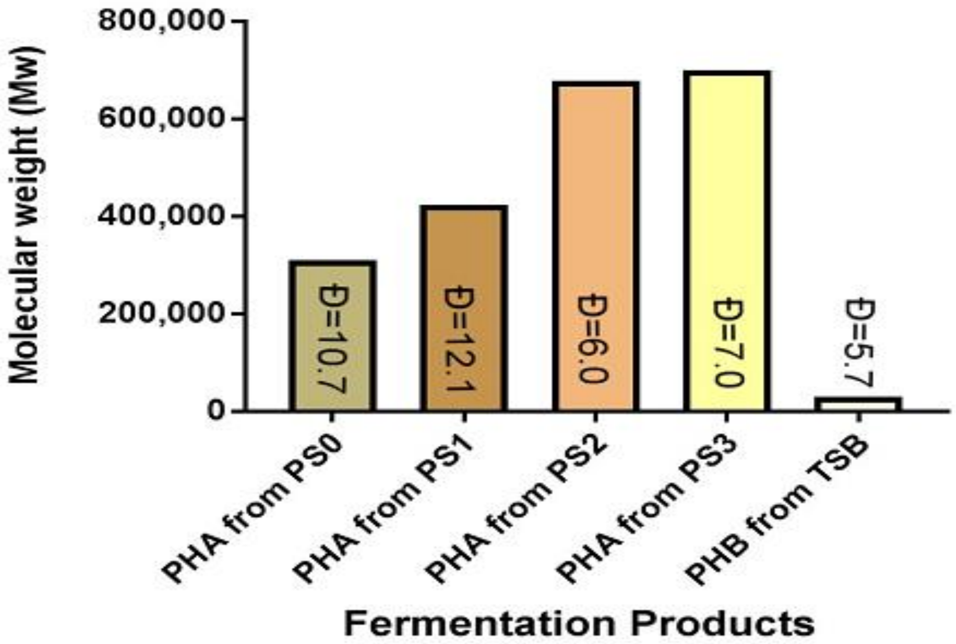
(Baljeet Singh Saharan et al,2014)

Study showed that a great amount of plastic waste into the ocean and also synthetic plastic landfill showed that plastic wastes were a great sources of carbon, so economically with huge traditionally value being neglected, particular biological fermentation processed by the usage of these plastic wastes, after that can get changed to conventional petrol base plastic, the production of polyhydroxyalkanoates plastic family through some various types of bacteria, and identified polyester as a new generation which are biocompatible,

biodegradable and can be discarded to the environment that is not toxic materials, this study explored the usage of pro degradable dense and common polystyrene (PS0), generally polystyrene used for once useable tableware contain packaging, CD cases, meal dish, water dish, etc. certain form of polystyrene stay financially and environmentally so much expensive to send to dump. Waste plastics of prodegraded PS0 were used to break down at different high temperature whenever expose to ozone, the production of different sheet of polystyrene PS0–3 and crashed polystyrene like powder (PS4) with a particular amount of acid.

Various types of production of biomass and PHA obtain after fermentation, the bacteria which are selected for this study was Cupriavidus necator H16 because it has complete genetic profile, robustness, stability and can produce PHA at low temperature, the production of PHA was various from 39% pro degraded up to having nitrogen rich media 48% (w/w) of treated with biomass, Extraction of biopolymer from biomass could be analysis by the nuclear magnetic resonance (NMR) and electrospray ionization mass spectroscopy (ESI-MS/MS) to identify their molecular structure and properties., (Brian Johnston et al,2018)

Figure: 12 Gel permeation chromatography (GPC) result showed that biopolymer produce by the fermentation process through the usage of standard poly styrene, usage of Bifidus selective medium (BSM) media for PSO-4 no PHA produce in flask did not have carbon sources like as a control, but PSO-1 showed the great amount of PHA production,



According to the result PS03 specimens were shown to be the most carbon sources for PHA bio production, with other polymers contain 3-hydroxybutyrate, 12mole % of 3-hydroxyvalerate, 3-hydroxyhexanoate co-monomeric part created. (Brian Johnston et al., 2018). Study showed that biosynthetic polymer PHA that could be degradable after use much important for biodegradable plastic, according to study the effect of environmental factor and structure of PHAs associated with PHA synthesis by the intracellular microorganisms fermentation related to their raw materials used to utilize energy when extraction process occur,

and restricting their possibility for trade, however, this study explains the usage of industries waste water for valorization of PHA extraction, According to the result from laboratory and some pilot scale examination, the design of conceptual process, analysis of technological economics and evaluation of life cycle are development for the large scale synthesis to the most famous general type of PHAs and PHB, polyhydroxybutyrate obtain through bacterial fermentation found in the waste water of industries process under downstream chemical digestion, hypochlorite and surfactant, commercially possibility and impact of environment give us the result to recognize the narrow section and the excellent chances to measure the industrial performance,

Finally, result showed that the industrial waste water was cheapest to produce PHB compare to polyhydroxyalkanoates (PHAs) which was synthesized from sugar because the null environmental burdens and price of raw material for production purpose of bio plastic, meanwhile, for maximum production of bio plastic still required some challenges with other plastics.

(Cora Fernandez Dacosta et al, 2015).

Nevertheless, study showed that biopolymer of Polyhyroxyalkanoates synthesized by bacteria and their different usage Forbidden due to their high cost of Synthesis, to reduce the cost of biopolymer production bring changes by Pseudomonas strains from crude sludge palm oil (SPO) examining of renewable inexpensive raw materials when the Pseudomonas Putida S12 was identified to produce great amount of 41% of high dense and medium length chain PHA from bacteria,

Table 6. Quantity of mlc-PHA synthesis by some Pseudomonas putida S12

Strains	**DCW (g/l)[a]**	**MCL-PHAs (% DCW)**
P. fluorescents	0.65 ± 0.03	3.14 ± 0.06
P. chlororaphis	0.67 ± 0.08	5.02 ± 1.67
P. putida	1.41 ± 0.15	15.73 ± 2.17
P. aeruginosa	0.99 ± 0.07	3.94 ± 0.96
Pseudomonas sp.	0.43 ± 0.01	2.65 ± 0.33
P. gessardii	0.65 ± 0.11	5.13 ± 0.93
P. moorei	0.90 ± 0.08	20.19 ± 4.87
P. mohnii	1.05 ± 0.31	21.55 ± 4.53
*P. putida*KT2440	1.16 ± 0.20	20.80 ± 2.73
*P.putida*S12	1.01 ± 0.16	24.87 ± 3.90

Analysis of mlc-PHA characteristics through instrumentation contain gas chromatography,differential scanning calorimetry,mass spectroscopy, and gel permeation chromatography, these finding of analysis may contribute to more widespread techniques to produce PHAs by reducing the costs of PHAs production. (Du-Kyeong Kang et al, 2017).

Table 7.Sludge palm oil (SPO) contain fatty acid, analysis by GC

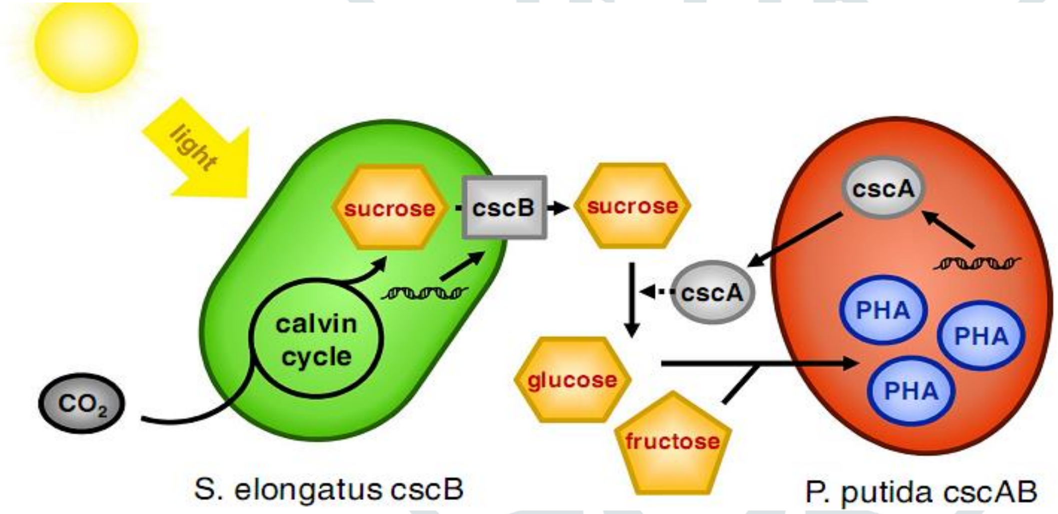
Fatty acids	**Structure**	**Composition (wt %)**
Palmitic acid	C16:0	45.9 ± 1.90
Stearic acid	C18:0	3.35 ± 0.41
Oleic acid	C18:1	38.1 ± 1.08
Linoleic acid	C18:2	9.1 ± 0.13
Others		3.6 ± 0.90

(Du-Kyeong Kang et al, 2017).

Study showed that production of biodegradable compound was one of the greats challenges for future and present generation is to find out the degradable materials from fossils resources, various bacterial strain used to produce biodegradable materials like Cyanobacteria strain and used as a renewable sources in industrials biotechnology for synthesis of different chemicals, fuels that showed the promising synthesis rats when compared to crop related feedstock, However, the actual capacities of cyanobacteria to synthesis biotechnological molecules are less, However, this study describe the present an approach to involve these problems by the co culture of bacteria to produce natural sources of carbon like polyhydroxyalkanoates from carbon dioxide, the co-culture consist of two bio modules, bio modules 1, this bio module in which the cyanobacteria Synechococcus elongatus cscBmake carbon dioxide and further used to produce, sucrose and transfer it to culture supernatant, bio modules 2, the synthesized sucrose use as a carbon sources for Pseudomonas putida cscAB and convert to polyhydroxyalkanoates that is present in the cytoplasm of bacteria, according to the research work obtained of the maximal PHA synthesis rate of 23.8 mg/(L day) and maximum titer of 156 mg/L,

Figure: 13

Finally result showed the possibility of co-culture of *S. Elongatus*cscB and *P. putida*cscABtosynthesis PHA from carbon dioxide and light.



The prediction for synthesis making space for the cornucopia of possible products that are explained for *P. putida*. However, the production of high quality of sucrose molecules using *P. putida* phenotypesand the optimization of process condition will increase Yield and close the current space in the contemporary process, (HannesLöwe et al, 2017).

Conclusion

Polyhydroxyalkanoates (PHAs) are intracellular granules of microorganisms, is similar to synthetic plastics, A technology needs great attention to develop an integrated system of isolation of high-valued bacterial synthesized, certain microorganisms reportedly produce PHA intracellularly with dual production of other metabolites, these significantly important biologically produced bio-products demand severe confirmation of industrial process contributing as key element towards high-cost production, the advent research on dual production of bio polymeric materials (extracellularly and intracellularly). To have this high-valued exopolymers and endopolymers duel synthesized using the same microorganisms under suitable conditions utilizing biological wastes may help us to combat the issues' relevance to cost production, environmental pollutions and their commercialization into the market.

Reference

1. M Bellis,(2011)," The History of Plastics", Timeline of Plastics

2. Braunegg G., Bona R., Schellauf F. and Wallner E. (2004b). "Solid Waste Management and Plastic Recycling in Austria and Europe. Polymer-plastics technology and engineering; 43, 1–13.

3. Zinn M., Witholtb B. And Eglia T. (2001) "Occurrence, synthesis and medical application of bacterial Polyhydroxyalkanoate. Advanced Drug Delivery Reviews; 53 5-21.

4.Keshavarz T. and Roy I. (2010). "Polyhydroxyalkanoates: bioplastics with a green agenda. Current opinion in microbiology"; 13, 321–326.

5.Simoneit B.R.T., Medeiros P.M. and Didyk B.M. (2005). "Combustion Products of Plastics as Indicators for Refuse Burning in the Atmosphere. Environ. Sci. Technol; 39, 6961–6970.

6. Reddy C.S.K., Rashmi R. G. andKalia V.C. (2003). "Polyhydroxyalkanoates: an overview. Bioresource Technology; 87, 137–146.

7. Chen G.Q. (2010b). "Introduction of Bacterial Plastics PHA, PLA, PBS, PE, PTT, and PPP. Plastics from Bacteria: natural functions and applications". Microbiology Monographs 14; 1-16. Alexander Steinbüchel (Series Editor); George Guo-Qiang Chen (Editor), Springer (Berlin, Heidelberg)

8. Verlinden R.A.J., Hill D.J., Kenward M.A., Williams C.D. and Radecka I. (2007)," Bacterial synthesis of biodegradable polyhydroxyalkanoates". Journal of Applied Microbiology; 102, 1437-1449.

9. Braunegg G., Lefebvre G. and Tender K.F. (1998)."Polyhydroxyalkanoates, biopolyesters from renewable resources: Physiological and engineering aspects". Journal of Biotechnology; 65, 127-161.

10. Keshavarz T. and Roy I. (2010)."Polyhydroxyalkanoates: bioplastics with a green agenda. Current opinion in microbiology; 13, 321–326.

11. Pederson E.N., McChalicher C.W. J. and Srienc F. (2006). Bacterial Synthesis of PHA Block Copolymers. Biomacromolecules; 7, 1904-1911.

12. Zinn M. (2003). Tailor-made synthesis of polyhydroxyalkanoates. European Cells and Materials; 5, 38-39.

13. Koller M., Salerno A., Dias M., Reiterer A. and Braunegg G. (2010b). "Modern Biotechnological Polymer Synthesis: A Review. Food Technol. Biotechnol.; 48 (3), 255–269.

14.Zinn M. (2003). Tailor-made synthesis of polyhydroxyalkanoates. European Cells and Materials; 5, 38-39.

15. Baljeet Singh Saharan,AnitaGrewal,andPardeep Kumar,(2014),"Biotechnological Production of Polyhydroxyalkanoates:A Review on Trends and Latest Developments",Hindawi Publishing CorporationChinese Journal of Biology,Volume 2014, Article ID 802984, 18 pages.

16. Cora Fernandez Dacosta, John A. Posada, Andrea Ramirez,(2015),"Large Scale Production of Polyhydroxyalkanoates (PHAs) from Wastewater: A Study of Techno-Economics, Energy Use and Greenhouse Gas Emissions",nternational Journal of Mechanical and Industrial Engineering Vol:9, No:5, .

17. Du-Kyeong Kang, Cho-Ryong Lee, Sun Hee Lee, Jung-HoonBae, Young-Kwon Park, Young Ha Rhee,Bong Hyun Sung, and Jung-HoonSohn,(2017),"Production of Polyhydroxyalkanoates from Sludge Palm Oil UsingPseudomonas putida S12,J. Microbiol. Biotechnol., 27(5), 990–994.

18. Hannes Löwe, Karina Hobmeier, Manuel Moos, Andreas Kremling and Katharina Pfüger‐Grau, (2017),"Photoautotrophic production of polyhydroxyalkanoates in a synthetic mixed culture of SynechococcuselongatuscscB and Pseudomonas putidacscAB",Biotechnol Biofuels 10:190 ,DOI 10.1186/s13068-017-0875-0.