Journal on New Biological Reports
JNBR 7(2) 68 – 73 (2018)

ISSN 2319 – 1104 (Online)

Published by
www.researchtrend.net

Polyhydroxyalkanoate (PHA) Production by Genetically Engineered Microalgae: A Review

Jyothi Kaparapu

Department Of Botany, Andhra University, Visakhapatnam, Andhra Pradesh

***Corresponding author**: jyothikaparapu@gmail.com

| **Received**: 20 June 2018 | **Accepted**: 11 August 2018 |

ABSTRACT

Polyhydroxyalkanoates (PHA) are biodegradable and biocompatible green thermoplastics, synthesized by variety of microbes as an intracellular carbon and energy storage intermediate. They are used as an alternative to non renewable petroleum derived plastics. The current interest in these bio polyesters is stimulated by the search for cost-effective capitalized production. Algal bioplastics mainly evolved as a by product of algae biofuel production. Algae based plastics have been a recent trend in the era of bioplastics compared to traditional methods of utilizing feedstocks of corn and potatoes as plastics. This paper attempts to achieve maximized sustainable production rate of bio-plastics from recombinant system using microalgae, an inexpensive substrate and presents some interesting avenues of research that remain to be explored.

Key words: Bio polyesters, Polyhydroxyalkanoates, Microalgae, Recombinant system

INTRODUCTION

Bioplastics are a form of plastics derived from renewable biomass sources such as vegetable oil, corn starch, pea starch (unlike fossil-fuel plastics derived from petroleum). Bioplastics provide the advantages of conservation of fossil resources and reduction in CO_2 emissions, which make them an important innovation of sustainable development. To increase sustainability and reduce dependency of fossil fuels the bio-based economy can be achieved, by developing an industrial platform for sustainable production and recovery of hydrocarbons and carbohydrates from algae. Research has been conducted to find out alternatives to generate energy, chemicals and recyclable materials from biological origin. Culturing algae is extremely sustainable and contain similar raw materials as traditional crops: high-quality oils, proteins, pigments as well as hydrocarbons and sugars.

Culturing microalgae has several advantages over conventional farming. Algae serve as an excellent feedstock for plastic production owing to its many advantages such as high yield and the ability to grow in a range of environments:

- Algae don't need agricultural land, therefore there is no competition for food or farmland.
- Microalgae have much higher yields per hectare and are extremely efficient with water and high lipid accumulation.
- Algae may grow on nutrients from waste resources.
- The use of algae opens up the possibility of utilizing carbon, neutralizing greenhouse gas emissions from factories or power plants.
- Other advantages of these materials over petrochemical plastics are that they are natural, renewable and biocompatible.

Growth of the microalgae can be improved by improving growth conditions such as production of UV-induced mutants and select on quick growth and incorporation of genes from quick growing algae into specific microalgae used in production of microalgae. Algae produce a variety of base materials that can be used for bio-plastics production. Most important are carbohydrates and hydrocarbons. For example, the algae *Botryococcus braunii* has the capacity to produce and excrete these materials into the medium. Using novel technologies, these compounds will be extracted from the medium, leaving algae cultures intact. These chemicals are converted into bio-plastics to replace oil-based plastics.

Bioplastics are manufactured using biopolymers derived from two ways, biopolymers from living organism (typically made from cellulose, soy protein and starch) and polymerizable molecules (typically made from lactic acid and triglycerides, wherein these molecules come from renewable natural resources, and can be polymerized to be used in the manufacture of biodegradable plastics).

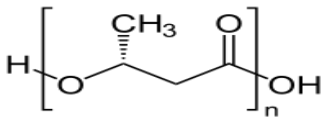
Plastics made from algae feedstock

(a) Cellulose-based Plastics are the oldest forms of bioplastics which were cheap and low quality plastics that are derived from cellulose. In some strains of algae, 30% of the biomass produced after extraction of algal oil contains cellulose. These strains are thus ideally suited to be feedstocks for cellulose-based bioplastics.
(b) Hybrid Plastics are made by adding denatured algae biomass to petroleum based plastics like polyurethane and polyethylene as fillers. It decreases the amount of petroleum used per unit of plastic, and often provides biodegradability. Filamentous green algae of the order Cladophorales are claimed to be well suited for use in hybrids.
(c) Poly-Lactic Acid (PLA) is usually produced by fermentation of feedstocks (by bacterial fermentation of algal biomass) and polymerization to produce polylactic acid.
(d) Bio-Polyethylene can also be derived from bacterial digestion of algal biomass, or directly from algae. The above types of plastics from algae are technically feasible, but their economic feasibility is yet to be worked out.

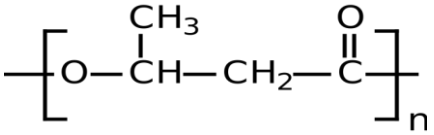
Presently, there are worldwide initiatives to reduce the usage of petrochemically derived plastics as they pose a great risk to the environment especially in marine ecosystems. In order to find alternative materials, researchers have developed fully biodegradable plastics, such as polyhydroxyalkanoates (PHAs). PHAs extracted from microalgal cells show material properties that are similar to polypropylene. Concern over petrochemical plastics in the environment has created a renewed interest in biologically derived polymers. During recent years, intensive research has investigated the algal production of PHAs and a great effort is underway to improve this procedure. Since CO_2 is utilized by microalgae for photosynthesis and converted to various forms in central metabolism, exploring microalgae as a base for bioplastic production could be a means of direct carbon capture by removing CO_2 from the atmosphere (Satyanarayana & Mariano 2011). Atmospheric carbon is directly trapped as a polymer if microalgae biomass is converted to bioplastics.

Production of PHAs under environmental stress conditions

Some Cyanobacteria were screened for the presence of PHA which was reported to be species specific. They are mostly producing PHB, stimulated by phosphorus deficient conditions and presence of excess amount of reducing equivalents (Rahman et al. 2015). *Synechococcus* sp. MA19, *Nostoc muscorum*, *Spirulina platensis* and *Synechocystis* sp. produced PHB under phosphate limited conditions (Sambrook & Russel, 2001; Potvin & Zhang. 2010). *Synechocystis* sp. UNIWG, and *Synechocystis* sp. PCC 6803 accumulated PHB up to 14% under nitrogen limiting conditions, respectively (Blatti & Michaud, 2013; Lu et al. 2011). Sulfur deprived conditions enhanced PHB content by 3.5-fold (Mayfield et al., 2003). Rasala et al. (2010) demonstrated that *Nostoc muscorum* could produce PHB five times higher under mixotrophy, chemoheterotrophy with nitrogen-limiting state than what was produced under photoautotrophic conditions. Accumulation of PHB in *Nostoc muscorum* was found to be regulated by addition of exogenous carbon sources, pH, light-dark cycles, and phosphorus and nitrogen status.



Structure of PHA



Structure of PHB

Production of PHAs in genetically engineered systems

PHAs are also produced in genetically engineered systems such as in bacteria, microalgae and plants (Somleva et al. 2013). There are over 155 unique

PHA monomers produced by a variety of microorganisms (Agnew & Pfleger 2013). PHAs have a melting temperature range of 50-180^{0}C, a crystallinity of 35-65% (Rehm 2010), a molecular weight range of 1.5 x 10^5 to 2.5 x 10^6 Da, and are biodegradable (Akaraonye et al.2010). Due to their diverse range of properties there are many potential applications (Gumel et al. 2013). Of the 155 unique PHAs, one of the most studied are the polyhydroxybutyrates (PHBs). PHBs are classified as short chain length polymers having a methyl as the branched group (Agnew & Pfleger 2013). PHBs have a melting temperature of approximately 180^{0}C and a tensile strength of 40 MPa. Respectively (Khanna & Srivastava 2005). PHBs have comparable properties to their petrochemical counterparts (polypropylene and polystyrene have melting temperatures of 170^{0}C and 110^{0}C and tensile strengths of 35 MPa and 50 MPa). The three enzymes: b-ketothiolase (phaA), acetoacetyl-CoA reductase (phaB), and PHB polymerase (phaC) were used to produce PHB from acetyl-CoA in Microorganisms. Two acetyl-CoAs are condensed with b-ketothiolase and the resulting product is reduced with acetoacetyl- CoA reductase (fig: 1). Then polymerization with PHB polymerase that forms the long chain PHB polymer. PHB polymers have a tendency to form amphipathic granules within the cell, keeping the PHB polymerase covalently attached (Rehm 2003).

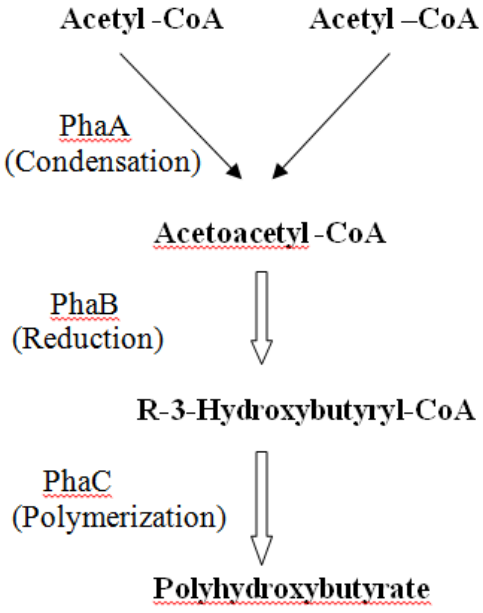


Fig. 1. PHB Synthesis from Acetyl – CoA.

(i) Production of recombinant proteins in microalgae

There is significant research in widespread production of recombinant proteins in microalgae (Potvin & Zhang 2010). Microalgae combining both the high growth rate and ease of cultivation of microorganisms with the ability to perform post-transcriptional and post-translational modifications of plants. The development of economically viable microalgal expression systems is, however, hindered by low recombinant protein yields. Although there are still many obstacles to overcome before microalgae become standard expression systems, considerable progress has been made in recent years in regards to elucidating the causes for these low yields and in the development of strategies to improve them. Transgenes have successfully been expressed in both nuclear and chloroplast microalgal genomes, although at economically viable levels only in the latter. Genetically engineered microalgae systems are being used to make PHAs (Rasala et al. 2010; Specht et al. 2010).

Through increased synthetic biology strategies, microalgal strains will be developed to produce specialty chemicals (Fu et al. 2015). *Chlamydomonas reinhardtii* is the most common strain that have been studied at the genetic level. The single-celled green alga, *Chlamydomonas reinhardtii*, has proven to be an outstanding model organism for studies of mechanisms of photosynthesis, cilia/flagella based motility and, more recently, biochemical pathway for production of lipids and other materials of interest to the renewal biofuels community and specialty chemical companies. *C. reinhardtii* grows rapidly, has a fully sequenced and annotated genome, is easily genetically transformed, is amenable to classical genetic analyses and has associated with it both a wealth of biochemical, molecular, cellular information and numerous experimental tools to expedite research. Because it is a haploid organism, mutant phenotypes are easily created and identified through classical screening techniques or through new, high throughput DNA analysis approaches.

(ii) PHB production through genetic manipulation techniques

The genome of *C. reinhardtii* was fully sequenced and found to have similar phylogenetic properties as plants (Larkum et al. 2012; Merchant et al. 2007). *Cyanidioschyzon merolae, Nannochloropsis sp., Ostreococcus tauri, Phaeodactylum tricornutum*, and *Thalassiosira pseudonana* are some other strains that are suitable for genetic manipulation (Hlavova et al. 2015). The phbB gene was successfully incorporate into *C. reinhardtii* from *R. eutropha* by Chaogang et al. 2010. *Ralstonia eutropha* is a facultatively chemolithoautotrophic bacterium able to grow with organic substrates or H_2 and CO_2 under aerobic conditions. Under conditions of nutrient imbalance, *R.eutropha* produces copious amounts of poly (R)-3-hydroxybutyrate (PHB). Wang et al. (2004) incorporated part of a native bioplastic synthesis pathway into a non native host by this

study. Chaogang et al. (2010) demonstrated successful incorporation of phbB and phbC genes from R. eutropha into *C. reinhardtii*, with the third enzyme phbA, already present in *C. reinhardtii*. This study demonstrated detectable quantities of PHB (6 mg/g dry cell weight) produced by *C. reinhardtii*. Hempel et al. (2011) incorporated the full PHB pathway from R. eutropha H16 into P. tricornutum. It was the first instance of a complete PHA pathway being incorporated into a microalgal strain. The results of this study were impressive as it was reported that approximately 10.6% PHB was achieved in this engineered strain after Seven days of culturing. Further optimization of the system for increased PHB production could be conducted in the future Codon optimization and extensive screening.

The demonstration of PHA production in a microalgae strain allows for the possible biological manufacturing of other PHAs such as scl PHAs and mcl PHAs. Atmospheric CO_2 is essentially a free carbon source in case of *P. tricornutum* and *C. reinhardtii* which makes PHB production economically viable in microalgae (Choi & Lee 1999).Though the cost of the carbon substrate is significant, the other fact that needs to be noticed is that of purification of PHB from microalgae biomass. Generally, cells are lysed and PHB is extracted. This is a major expensive step in biologically produced PHBs and alternative methods need to be developed to make PHB production economically feasible (Jacquel et al.2008). However, with development of genetic toolkits microalgae can able to produce high yields of plastic when compared to genetically engineered *Escherichia coli* grown with glucose as the carbon substrate. Besides, in a biorefinery context PHB-producing microalgae could have a twin purpose, thus providing biomaterials and biofuels from a single algae source.

The advanced technologies developed for genetic manipulation of microalgal system:

Noor-Mohammadi et al. (2014) and co-workers demonstrated pathway engineering in the baker's yeast *Sacharomyces cerevisiae* and then successful transfer of the assembled pathway into the nucleus of *C. reinhardtii*. Such techniques can be used to assemble genes in the chloroplast of *C. reinhardtii* (Noor et al. 2012). There could be potentiality to genetically engineered microalgae strains to produce PHAs in the near future, by using clustered regularly interspaced short palindromic repeats (CRISPR) and Cas9 (an RNA guided cleaving enzyme) cloning systems (Sternberg & Doudna 2015). Studies using the CRISPR-Cas9 system have already been developed for use with the microalgae strain C. reinhardtii (Jinkerson & Jonikas 2015; Jiang et al. 2014). The advent of CRISPR/Cas9 technology for facile gene editing in numerous organisms has heralded rapid progress in creating targeted gene knockouts and gene replacement by homologous recombination. Transcription activator-like effectors and zinc-finger nucleases have also been successfully used in *C. reinhardtii* (Gao et al. 2014; Sizova et al. 2013).

Table 1. Production of Polyhydroxyalkanoate through different mechanisms

Mechanism applied	**Algae used**	**Reference**
Production of PHA under environmental stress conditions	*Synechococcus* sp. MA19, *Nostoc muscorum*, *Spirulina platensis* and *Synechocystis* sp.	Sambrook & Russel, 2001; Potvin & Zhang 2010.
Production of recombinant proteins in microalgae	*Chlamydomonas reinhardtii*	Fu et al. 2015
production of PHB through genetic manipulation techniques	*Cyanidioschyzon merolae*, *Nannochloropsis sp.*, *Ostreococcus tauri*, *Phaeodactylum tricornutum*, and *Thalassiosira pseudonana* *Ralstonia eutropha* and *Chlamydomonas reinhardtii*	Hlavova et al. 2015 Wang et al.2004; Chaogang et al.2010.

Future Outlook

As the demand for plastic products continues to increase, there will be a place for microalgae-derived bioplastics. Even though bioplastics are expensive they are still considered as a viable option to improve environmental sustainability. Coupling modern genetic engineering with innovations in cultivation techniques has the potential to increase bioproduct production in microalgae. Future work could consist of a hybrid of both genetic engineering of microalgae strains to produce PHAs and blending with petrochemical plastics. Algae bioplastics can be commercialized in the future if they can neutralize the technical problems they possess.

In the market the bioplastics made of algae (Cereplast Algae Plastics, a company) contain only 50% algae. Plastics that comprise material derived 100% from algae are still not a reality and require innovative developments. The use of biotechnology techniques can play a key role in conducting the feasibility and sustainability studies in algae bioplastics. Fermentation and genetic engineering

can take the lead in using novel techniques to make algae bioplastics commercially viable. Significant research and development investments are to be made into bioplastics and these efforts are likely to result in significant cost reductions.

REFERENCES

Akaraonye E, Keshavarz T, Roy I. 2010. Production of polyhydroxyalkanoates: the future green materials of choice. J Chem Technol Biotechnol 85: 732-743.

Agnew DE, Pfleger BF. 2013. Synthetic biology strategies for synthesizing polyhydroxyalkanoates from unrelated carbon sources. Chem Eng Sci 103: 58-67.

Blatti JL, Michaud J, Burkart MD. 2013. Engineering fatty acid biosynthesis in microalgae for sustainable biodiesel.Curr Opin Chem Biol 17: 496-505.

Chaogang W, Zhangli H, Anping L, Baohui J, 2010. Biosynthesis of poly-3-hydroxybuytyrate (PHB) in then transgenic green alga *Chlamydomonas reinhardtii*, J. Phycol. 46 : 396-402.

Choi S, Lee Y. 1999. Factors affecting the economics of polyhydroxyalkanoate production by bacterial fermentation.Appl Microbiol Biotechnol 51: 13-21.

Fu W, Wichuk K, Brynjolfsson S. 2015. Developing diatoms for value-added products: challenges and opportunities.New Biotechnol 32: 547-551.

Gao H, Wright DA, Li T, Wang Y, Horken K, Weeks DP. 2014. TALE activation of endogenous genes in *Chlamydomonas reinhardtii.* Algal Res5 :52-60.

Gumel AM, Annuar MSM, Chisti Y. 2013. Recent advances in the production, recovery and applications of polyhydroxyalkanoates. J Polym Environ 21: 580-605

Hlavova M, Turoczy Z, Bisova K. 2015. Improving microalgae for biotechnologydfrom genetics to synthetic biology. Biotechnol Adv 33 :1194-1203.

Hempel F, Bozarth AS, Lindenkamp N, Klingl A, Zauner S, Linne U. 2011. Microalgae as bioreactors for bioplastic production. Microb Cell Fact.10.

Jacquel N, Lo CW, Wei YH, Wu HS, Wang SS. 2008. Isolation and purification of bacterial poly (3- hydroxyalkanoates). Biochem Eng J 39: 15-27.

Jiang W, Brueggeman AJ, Horken KM, Plucinak TM, Weeks DP.2014. Successful transient expression of Cas9 and single guide RNA genes in *Chlamydomonas reinhardtii*. Eukaryot Cell 13 : 1465-1469.

Jinkerson RE, Jonikas MC. 2015. Molecular techniques to interrogate and edit the Chlamydomonas nuclear genome. Plant J 82 :393-412.

Khanna S, Srivastava AK, 2005. Recent advances in microbial polyhydroxyalkanoates. Process Biochem 40 :607-619.

Larkum AWD, Ross IL, Kruse O, Hankamer B. 2012. Selection, breeding and engineering of microalgae for bioenergy and biofuel production. Trends Biotechnol 30 : 198-204.

Lu J, Sheahan C, Fu P. 2011. Metabolic engineering of algae for fourth generation biofuels production.Energy Environ Sci 4 :2451-2466.

Merchant SS, Prochnik SE, Vallon O, Harris EH, Karpowicz SJ, Witman GB. 2007. The Chlamydomonas genome reveals the evolution of key animal and plant functions.Science 318 :245-250.

Noor-Mohammadi S, Pourmir A, Johannes TW. 2014. Method for assembling and expressing multiple genes in the nucleus of microalgae. Biotechnol Lett 36: 561-566.

Noor-Mohammadi S, Pourmir A, Johannes TW, 2012. Method to assemble and integrate biochemical pathways into the chloroplast genome of *Chlamydomonas reinhardtii*. Biotechnol Bioeng 109:2896-2903.

Potvin G, Zhang Z. 2010. Strategies for high-level recombinant protein expression in transgenic microalgae: a review. Biotechnol Adv 28: 910-918.

Rasala BA, Muto M, Lee PA, Jager M, Cardoso RMF, Behnke CA. 2010. Production of therapeutic proteins in algae, analysis of expression of seven human proteins in the chloroplast of *Chlamydomonas reinhardtii* .Plant Biotechnol J 8 : 719-733.

Rehm BHA. 2009. Microbial Production of Biopolymers and Polymer Precursors: Applications and Perspectives. Caister Academic Press, Norfolk, UK.

Rehm BHA. 2010. Bacterial polymers: biosynthesis, modifications and applications. Nat Rev Microbiol 8:578-592.

Rehm BHA. 2003. Polyester synthases: natural catalysts for plastics. Biochem J 376 :15-33.

Rahman A, Putman RJ, Inan K, Sal FA, Sathish A, Smith T. 2015. Polyhydroxybutyrate production using a wastewater microalgae based media. Algal Res 8 : 95-98.

Rosenberg JN, Oyler GA, Wilkinson L, Betenbaugh MJ. 2008. A green light for engineered algae: redirecting metabolism to fuel a biotechnology revolution. Curr OpinBiotechnol 19 : 430-436.

Sambrook J, Russel DW. 2001. Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press. Cold Spring Harbor, NY.

Satyanarayana KG, Mariano AB, Vargas JVC. 2011. A review on microalgae, a versatile

source for sustainable energy and materials. Int J Energy Res 35 :291-311.

Sizova I, Greiner A, Awasthi M, Kateriya S, Hegemann P. 2013. Nuclear gene targeting in Chlamydomonas using engineered zinc-finger nucleases. Plant J 73 : 873-882.

Somleva MN, Peoples OP, Snell KD. 2013. PHA bioplastics, biochemicals, and energy from crops. Plant Biotechnol J 11 : 233-252.

Specht E, Miyake-Stoner S, Mayfield S. 2010. Micro-algae come of age as a platform for recombinant protein production. Biotechnol Lett 32 : 1373-1383.

Sternberg SH, Doudna JA. 2015. Expanding the biologist's toolkit with CRISPR-Cas9. Mol Cell 58 : 568-574.

Wang C, Hu Z, Hu W, Lei A. 2004. Expression and molecular analysis of phbB gene in *Chlamydomonas reinhardtii*. Chin Sci Bull 49: 1713-1717.