Recent developments in bioreactor scale production of bacterial polyhydroxyalkanoates

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Abstract

Polyhydroxyalkanoates (PHAs) are biological plastics that are sustainable alternative to synthetic ones. Numerous microorganisms have been identified as PHAs producers they store PHAs as cellular inclusions to use as an energy source backup. They can be produced in shake flasks and in bioreactors under defined fermentation and physiological culture conditions using suitable nutrients. Their production at bioreactor scale depends on various factors such as carbon source, nutrients supply, temperature, dissolved oxygen level, pH and processes. Once produced, PHAs find diverse applications in multiple fields of science and technology particularly in the medical sector. The present review covers some recent developments in sustainable bioreactor scale production of PHAs and identifies some areas in which future research in this field might be focused.

Keywords: Bioplastic, Biopolyester, Bioreactor, Fermentation, PHAs

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1 History

Polyhydroxyalkanoates (PHAs) comprise a range of different biodegradable biopolymers - bioplastics or bio-polyesters synthesized by various microorganisms [1]. They were discovered, in 1888, by Beijerinck as cellular inclusions of bacteria and other microorganisms [2]. In 1926 Maurice Lemoigne, a French scientist, was the first to synthesize a PHA (named poly-3-

hydroxybutyric acid) using a bacterial strain of *Bacillus megaterium* [3]. Later on, in 1958, Macrae and Wilkinson claimed that the bacterial cells play a role as cellular factories for PHAs synthesis and storage [4]. Imperial Chemical Industries (ICI), UK, initiated commercial production of PHAs in 1961; whereas, Wallen and Rohwedder (1974) reported synthesizing PHAs other than poly-3-hydroxybutyric acid. In 1980, ICI synthesized a copolymer of PHA via fermentation, while in 1983, [5] reported synthesizing a medium chain length polyhydroxyalkanoate (mcl-PHAs) which in turn became a milestone in the synthesis of PHAs. Also in 1980, the researchers started investigations on the physiochemical properties of PHAs [6] and later on in 1990, scientists used them for the first time in the biomedical field. By 2010, the PHAs market had reached up to 10^{10} US$ with a growth of 170-180 Kt, annually [7].

2 Introduction

Plants have always been used as a major source of polymers such as starch, cellulose, flax and rubber for thousands of years. During the past few decades, plant polymers had extensively been replaced by petrochemical based polymers on mass scale production [8]. At the end of the 20th century, it was realized that these synthetic polymers are non-biodegradable, persistent and continually accumulate in the environment which ultimately represents a threat to life on Earth. During the past two decades environmental protection agencies had started awareness programs for both the public, in general, and manufacturers, in specific, on the importance of preserving and protecting nature from toxic products and hazardous wastes [9]. These concerns had compelled scientists and researchers to further explore new approaches to synthesis and use eco-friendly bioplastics such as PHAs as alternative materials to plastics [10].

PHAs are biodegradable linear thermoplastic polyesters, which can be used as alternative polymers to synthetic ones. They are synthesized by different bacterial strains (both Gram positive and Gram negative) cultivated on different carbon sources like sugars, alkanoic acids, alkanes, alkenes and other renewable carbon sources [11]. During the last two decades, PHAs have attained much attention because of their diverse features such as hydrophobicity, elastomeric nature and biodegradability under a wide range of environmental conditions. They also constitute a natural part of renewable carbon cycle and are used as alternatives to synthetic polyesters. Nevertheless, there are still some limitations in their bulk scale production including low yields and high production costs [12].

Some 150 different types of PHAs congeners are known with different structures (varying side chains or functional groups) and properties had been synthesized using different microbial strains. PHAs can be synthesized though microbial fermentations both at laboratory and pilot plant scales followed by appropriate downstream processing [13]. They are classified according to their chemical unit structure. The polymers containing repeating units with 3-5 carbon atoms are known as short chain length PHA (scl-PHA) and a polymer with repeating units of 6-13 carbon atoms are known as medium chain length PHA (mcl-PHA), while those with more than 13 carbon atoms are known as long chain length PHA (lcl-PHA). A copolymer like poly(3-hydroxybutyrate-co-4-hydroxyhexanoate) could be produced by simply mixing poly(3-hydroxybutyrate), a scl-PHAs, and poly(4-hydroxyhexanoate), a mcl-PHAs monomers under desirable fermentation conditions [14]. A general structure of PHAs is shown in Figure 1.

PHAs find a large number of applications in different fields of science and technology. For instance, a blend of poly(hydroxybutyrate) P(HB) and poly(hydroxyoctanoate) P(HO) is used as food additive which is approved by the US food and drug authority (FDA) [15]. PHB has also been used as a component in making plastic accessories, coating paper, biodegradable bottles, electronic accessories, pharmaceuticals, garments, upholstery and packaging materials [16]. In the field of medicine, PHAs allow us to control drug release and targeted drug action whereas PHB had been used to produce different medical devices [17]. A drug delivery system can be a sustainable way to administrate certain drugs by minimizing their toxic effects. An mcl-PHA had been used to administrate drugs through a transdermal drug delivery system. There are three basic components of drug delivery systems; i.e. a carrier, a target moiety and an active part of the drug. Genetically, engineered bacterial strains produce PHAs and allow us to utilize them in vascular grafting, blood vessel and heart valve development. P(3-HAs) has also been suggested for use as a component of biofuels to reduce global warming [18]. The wide range of applications of PHAs in different fields of life is listed in Table 1.

In this review, we present recent developments in the bacterial production of PHAs on various carbon sources. We focused on the challenges in PHAs production in different types of bioreactors and operational regime using various bacterial strains. A particular emphasis is dedicated to current limitations and advantages of different bioreactor scale setup while concentrating on the efforts devoted to upgrade these processes to industrial scales. Finally, we endeavour to identify the most suitable downstream processing technology.

3 Financial obstacles in PHAs commercialization

Commercialization of a product mainly depends on its production cost, potential applications and market requirements. In the past decades, magnificent efforts had been made to improve PHAs yield both at laboratory and pilot plant scales [7]. The main challenges in the commercialization of PHAs had been their high production cost and low product yields. PHAs produced from different sources require different tools like fermenter, autoclave etc. with different fermentation approaches which increase the production cost ultimately limiting their commercialization. The inability to achieve optimal bacterial growth conditions and production yields for PHAs had been a major disadvantage in this context [19]. In addition, PHAs have some disadvantages in their mechanical properties including; incompatibility with conventional thermal processing, limited functionalities, susceptibility to thermal degradation and production cost which limits their competitiveness and application as an ideal biomaterial when compared with synthetic plastics. The possibility of commercialization of PHAs might be enhanced using mixed microbial culture (MMC) with cheap carbon source like waste frying oil and crude oils. The main obstacle in the commercialization of PHAs had been their production cost which could be reduced using edible oil as carbon source with non-sterilized fermentation conditions [11, 20].

4 Sustainable solutions for PHAs production

The economics of PHA production mainly depends upon substrate cost and compatibility to use product in similar to those produced using expensive growth substrate, efficiency of production and downstream processing [21]. Recent studies on PHAs production not only consider the sustainability of polymer but also its cost-effectiveness. Nitrogen and carbon sources get significant share in the production cost. Using cheap nitrogen sources such as urea and sodium nitrate reduces the cost of production significantly [10]. The utilization of industrial wastes as carbon source to produce a competitive cost effective PHA has also been reported as a promising approach for decreasing overall costs [2]. The economics of whole process is based on three approaches; large scale aerobic batch fermentation, use of cheap carbon source soybean and other waste frying oils, and simplification of the downstream recovery process to achieved an economical PHAs production approach [22]. PHAs bacterial sources and their chemical structure are listed in Table 2.

Other ways to meet financial barrier is though value addition by inserting new functionalities in PHAs to overcome their inherent limitations, improve their desirable properties and enhance potential applications [20]. This is usually achieved by blending bio-polyesters with other natural biodegradable polymers, including cellulose derivatives, poly lactic acid (PLA), starch, lignin and poly(caprolactone). The introduction of different functional groups via chemical routes has been described with regard to the two main synthesis approaches, graft co-polymerization and block co-polymerization [23]. Grafting of different functional groups on side chains of linear bio-polyesters introduces many additional properties, which makes them sustainable with desired properties and controlled polymer structure. For instance, grafting of chitosan on PHA produces a copolymer P(H-co-chitosan) which is a sustainable biopolymer with some important applications in medical field. A cost effective production has been reported, carried out using MMC, under unsterilized conditions for example[20, 24].

5 PHA production in shake flasks

Shake flask fermentations are normally carried out in laboratories mainly to explore feasibility. Here, the information is often vogue and the experimental expenditures are high with large number of trails typically required to identify a set of optimum fermentation conditions. Such fermentations are usually carried out in Erlenmeyer flasks provided with minimal media, carbon source and any precursors at specific pH levels and incubated at certain temperature and shaking speed for a certain period of time. During shake flask fermentations, the process is followed up based on certain parameters such as carbon source or any other nutrients utilization, biomass formation or fermentation kinetics. In the previous studies, bacterial production of PHAs had mostly been carried out using glucose as carbon source. Later on *Pseudomonas sp.* grows on MMC had been employed with volatile fatty acids (VFAs) as carbon source and also used as precursor for PHAs production with higher productivity [25]. The optimized fermentation conditions had been used to produce PHAs with efficient recovery. Different ratios of carbon and nitrogen sources had been used to optimize product yields. An ammonia free fermentation media were used to accumulate PHAs with VFAs. During the first phase of fermentation, glucose was used as carbon source after inoculating the media in within an Erlenmeyer flask and cell biomass harvested by centrifugation at 6000 rpm and 4^{o}C with 20-40% g/g of product yield. The shake flask study was used to determine the feasibility of fermentation process with different

parameters. The main disadvantage of shake flask investigations is the wide variation in the quality of produced product [26].

6 Kinetics of PHA production

The fermentation kinetics were previously investigated by determining the product yields with respect to substrate consumption. The kinetics of production determines how much PHAs cell dry mass (CDM) are produced with respect to time and expressed as g $L^{-1}h^{-1}$. A linear increase in PHAs yield was observed following a zero order reaction kinetics until the carbon source growth substrate was depleted [27]. The kinetics of PHAs production for different bacterial strains with large number of substrates i.e. *Azotobacter beijerinckii* with glucose as growth substrate produced 0.09 g $L^{-1}h^{-1}$ [28]. *Burkholderia cepacia* bacterial strain was grown on glycerol as energy substrate producing 0.103 g $L^{-1}h^{-1}$ [29], *Ralstonia eutropha* with butyrate, propanoic, lactic and acetate acid as growth substrates produced 0.001-0.037 g $L^{-1}h^{-1}$ [30]. *Alcaligenes eutrophus* was grown on potato starch and saccharified wastes as growth substrates producing 1.5 g $L^{-1}h^{-1}$ [31], *Hydrogenophaga pseudoflava* with sucrose and lactose as growth substrates produced 0.02-0.12 g $L^{-1}h^{-1}$ [32]. *Pseudomonas frederiksbergensis* used terephthalic acid as growth substrate and produced 0.004 g $L^{-1}h^{-1}$ [33], *Pseudomonas putida KT 224* produced 0.006 g $L^{-1}h^{-1}$ of PHAs using glucose as growth substrate [34]. *Haloferax mediterranei* produced 0.05-0.2 g $L^{-1}h^{-1}$ PHAs using vinasse as growth substrate [35] and the same bacterial strain with glycerol produced 0.12 g $L^{-1}h^{-1}$ [36]. Further kinetics of PHAs production with different bacterial sources, substrates and production scales are presented in Table 3.

7 Bioreactor scale production of PHAs

Bioreactor scale production of biopolymers is the most suitable production technique on industrial scale using large working volume and avoiding many restrictions encountered under shake flask conditions. Because, PHAs biosynthesis constitutes a multiple phase process, both the feeding strategy and bioreactor operation mode needs to be adapted via optimization of operation conditions. Industrial bioreactor scale production of PHAs can also be operated under both fed batch and continuous fed batch feeding strategies.

7.1 Single batch production

During batch production, desirable amounts of different nutrients are added in the fermentation media. The single batch production process is simple but gives low productivity. The maximum allowed concentration of both carbon and nitrogen sources are added at the beginning of batch fermentation processes which would restrict nutrient addition during PHAs production. In batch productions of PHAs, the amount of nitrogen source are typically added at 0.2-5 g/L and carbon source at 1-30 g/L at the beginning of fermentation process [37]. Moreover, the excess amount of carbon source added in the presence of other growth limiting nutrients such as N, P, S and K. The single batch fermentation process is analogous to other PHAs production processes with minimum conversion toward biomass, CO_2, PHAs and other metabolites: thus resulting in low overall conversion of growth substrate carbon source to PHAs cell biomass yield. Considering hypothetical calculations of carbon to PHAs, when conversion yields are below 0.4 g/g indicates that such processes are not economically feasible. The single batch fermentation scale production of PHAs therefore suffers limitations which need to be addressed. The main set back of batch scale production is that the nutrients are not further supplemented or provided while inhibitors or limitations continue to accumulate restricting production of PHAs. If the fermentation process for PHAs is was carried out over seven days and the maximum amount of PHAs produced was achieved within a shorter period the bacteria start decay and product may be partially degraded. A list of single batch PHAs production using different bacterial strains is shown in Table 4.

7.2 Sequence batch fermentation production processes for PHAs

In sequence batch bioreactor experiments, two or more reactors are connected in series to perform different functions simultaneously to produce PHAs. Albuquerque and co-workers (2010) reported a study of two-configurations system used to produce PHAs from organic activated sludge waste as substrate. In the first configuration, nitrogen (as ammonium) was limited by simple conversion to nitrate and bacteria start to respond these to these conditions and produce PHAs. Both aerobic feasting stage (ammonia conversion to nitrate) and anoxic famine stage (Denitrifying) conditions drive internally stored PHAs as carbon source [38].

First configuration: In the first configuration, continuous feeding with fix interval of 5 min had been carried throughout the fermentation process until desired cell mass was achieved. The fermentation medium was allowed to settle down for 15 min and then fermentation process was

further run under aerobic conditions for an hour which was followed by anaerobic fermentation condition to produce PHAs [38].

Second configuration: Here, the fermentation process had been carried in two bioreactors which are connected in a series using connecting tubing and each performs different functions. In one of the bioreactors, nitration was took place and in second bioreactor, the desired COD/N for selection of PHAs cell mass from fermentation medium occurred. The whole process of nitration was carried out under aerobic fermentation conditions as mentioned above. After nitration in the first bioreactor, the fermentation medium was further processed in the second reactor under aerobic feast and anoxic famine reaction conditions to accumulate high cell mass of PHAs [38].

7.3 Fed batch production of PHAs

During fed batch cultivation, the substrate is added through a pulse feeding when its concentration drops below its optimum value without removing any culture media. In the case of PHAs production, both carbon and nitrogen sources are added at periodic intervals according to their consumption by the microorganisms. In fed batch fermentation process, these substrates are added to avoid any depletion during the production process until the desired cell biomass yield is achieved. Some researchers used fed batch process to produce 0.41 g/g of PHAs using *Pseudomonas putida KT 2440* through feeding octanoic acid as a carbon source [39].

The fed batch production had also been used to examine the effect of different carbon to nitrogen (C/N) ratios on product yield. For that purpose, nutrient enrich media had been prepared using the following recipe: $MgCl_2.6H_2O$, KCl, Fe^{2+}, $NaHCO_3$, $C_6H_{12}O_6$, NaCl, $CaCl_2$, $MgSO_4.7H_2O$, NH_4Cl, KH_2PO_4, NaBr and 1 mL of trace elements solution. An aliquot amount of 60 mL of inoculum was added in the nutrient enrich media of sequence batch reactor after 48 h of cultivation. The pH of fermentation media was maintained at 7.5-7.8 using 1 M NaOH and 1 M HCl solutions. The amount of dissolved oxygen in the nutrients enriched media was 100% at 40^oC for 72 h. The maximal substrate consumption, CDM and PHAs yield decreased with increase in C/N ratio. The yields of 0.970, 0.955 and 0.890 g/L of CDM were reported respectively using 0.47, 1.95 and 7.64 g/L of nitrogen concentrations [13].

7.4 Continuous production process

The fermentative production processes are typically carried out in continuous stirred tank bioreactors (CSTR) where process parameters like agitation speed, air flow, pH, substrate, product concentration and temperature are controlled to investigate PHAs yield. The PHAs production using MMC under continuous feeding strategies had been established. Such a process produced PHAs with improved composition on monomeric level and results in a more sustainable process. Continuous PHAs production process is clearly better than batch production in terms of composition, flexibility and productivity of bio-polyesters [38]. *Pseudomonas putida* had also been reported to produce 80 % (g/g) PHAs, at both shake flask and bioreactor scale fermentations. The pH of fermentation media at lab scale were kept neutral using 2M NaOH and 4M H_2SO_4 solutions at 30^oC, 250 rpm and 20% of dissolved oxygen (DO) throughout fermentation process. At pilot scale, CSTR fermentation with different working volumes has been carried out to produce PHAs. In CSTR, the fermentation parameters were kept at pH 7 (using 30% $H_2SO_4$and 2 M NaOH solutions), temperature 30^oC, rate of agitation 100-1000 rpm and aeration 0-30 L min^{-1} to produce 0.53 g/g of PHAs. The continuous production of PHAs had been carried out at single, double and multiple stages to facilitate the bacteria during fermentation process. In continuous production, each and every aspect regarding the fermentation process proceeded more accurately than fed batch mode. In fed batch production nutrients were added on depletion inside fermented media to produce maximum PHAs [40].

7.4.1 One stage continuous stirred tank reactor

The efficiency of a single stage continuous stirred tank reactor in terms of flexibility in polyester composition and productivity. The schematic diagram used is shown in Figure 2 a. Single-stage continuous stirred tank reactors (single SCSTRs) are not productive for PHAs cell biomass production, because intercellular products of secondary metabolism only boost PHAs production under limited nutrients growth conditions. Hence, it is not possible to continuously supply these nutrients throughout the fermentation process, that's why optimization is needed for both intracellular PHAs mass and high biomass formation. Ramsay et al, (1990) reported on the validity of single SCSTR using propionic acid as growth substrate, *Cupriavidus necator* bacterial strain and glucose as carbon source producing 0.33 g/g P(3-HB-co-3-HV) co-polyesters. The amount of cell biomass and cell dry mass produced in single SCSTR was not competitive with fed batch process when using the same bacterial production strain [41].

7.4.2 Two-stage continuous stirred tank reactor

The two-stage continuous stirred tank reactor fermentation process is used to produce PHAs with maximum yield which is not possible under shake flask conditions and with single SCSTR. A schematic diagram for this is shown in Figure 2 b. During two SCSTR fermentation process, different functions are carried out in two bioreactors, simultaneously. Regarding the cell biomass accumulation for the bacterial strain and substrate ratio, it had been observed that autocatalytic growth was much higher than the PHAs accumulation process. Due to this reason, it is impossible to carry out such process with sufficient productivity at single SCSTR scale. Therefore, it was consequently demonstrated that two-stage continuous stirred tank reactor (two SCSTR) process is better for such type of production. Here, in the first CSTR, a higher amount of cell biomass could be produced with higher density and continuously transferred to second CSTR, where large amount of carbon source are provided continuously, under limiting nitrogen substrate to stimulate PHA production [42].

Ramsay et al. (1990) were first to demonstrate the efficiency of the two SCSTR. The bacterial strain of *Alcaligenes lata* had been used to produce 0.38 g/g of P(3-HB) and 0.55 g/g of P(3-HB-co-3-HV) a copolymer using propionic acid (as precursor), residual sucrose (as carbon source) in the minimal nutrients media. In the first CSTR, the nitrogen source and precursor were completely utilized by the bacterial cells. The fermented broth containing residual sucrose was continuously transferred to the second CSTR, but no additional nutrients were added to facilitate the conversion of sucrose as growth substrate to increase PHAs content in the bacterial cell biomass [43]. Hence, in this phase, bacterial cell was allowed to complete the fermentation process to maximize PHAs yield while depleting the sucrose (i.e. as carbon source). In the context of two SCSTR, optimized bacterial strain and substrates were used to produce different scl-PHA, mcl-PHA as homo and hetero polymers. Some researchers cultivated the bacterial strain of *P. putida* GPo1 in two SCSTR with gaseous substrate *n*-octane as carbon source and produced a blend of two different polymers with block structures. The block copolymer of poly(3-hydroxy-10-undecenoate-co-3-hydroxy-8-nonenoate-co-3-hydroxy-6-heptenoate) was produced at 0.63 g/g. This observation is in contrast to other reports, which claimed that block PHAs copolymers were only produced when adding sufficient amount of growth substrate during the intracellular accumulation of PHAs [44].

7.4.3 Multi stage continuous stirred tank reactor

In the case of multi stage CSTR, three or more bioreactors are connected with each other using plastic tubing. The schematic diagram of continuous stirred tank bioreactor is shown in Figure 2 c. PHAs can be produced with exponential growth rate under continuous growth conditions as used in two SCSTR. The culture condition can be used throughout the process until the desired cell biomass is achieved. The concentrated substrate was loaded to avoid any dilution of nutrients during continuous production condition in three SCSTR [45].

8 Factors affecting the production of PHAs

The bacterial production of PHAs through fermentation is influenced by different factors such as, strain used, growth substrate, C/N ratio, pH, DO and sodium chloride which are further elaborated in the following subsections.

8.1 Bacterial strains for PHAs production

More than 300 types of different Gram positive, Gram negative and archaea bacteria had been reported to produce PHAs. Different carbon source and CDM, PHAs yields and production kinetics are shown have been reported in Table 3.

8.2 Growth substrates

The carbon source is the most crucial and major factor during for bacterial production of cellular metabolites like PHAs. Several types of carbon sources such as alkanes, polysaccharides, glycerides, acids, edible oils, gases, industrial waste, agricultural waste, carbohydrates, alcohols, petroleum products, animal waste, benzene and its derivatives had been investigated for PHAs production. The carbon source is used as basic food component to fulfil energy requirements and to act as biogenetic substrate for microbial growth and precursor for PHAs biosynthesis. There are three basic functions for the carbon source within bacterial cells such as cell maintenance, cell biomass synthesis and polymerization of PHAs molecules [46]. The small amount of growth substrate was used to restrict the molecular size of polymer during fermentation. Because bacteria have thick and rigid cell wall, it does not allow large polymeric molecule to be transported into cells. Different concentrations of glucose (as carbon source) had been used to optimize cellular growth and product yield. The results demonstrated a proportional increase up

to a point after which glucose exhibited an inverse effect on microbial growth. The effect of growth substrates on PHAs yield is entirely based on nutrients media [47].

The selectivity of growth substrate not only depends upon their cost but also on their feasibility in the fermentation process. The selectivity of carbon source as growth substrate depends also on the bacterial strain used. The bacterial strain of *P. aeruginosa* shows compatibility for PHAs production with large number of growth substrates like oils, polysaccharide's and acids [48].

Carbon sources as growth substrates generally belong to three main groups of triglycerol, sugars and hydrocarbons. The growth substrate used include carbohydrates such as sucrose [49], lactose [50], starch [51] and lignocellulose [52]. Other substrates such as ethyl alcohol [11], methanol [53], methane [54] and triglycerol containing compounds such as animal fat [55], plant oils [11], fatty acid [56], glycerol [57] and waste frying oil [58] had also been reported. Hydrocarbons had shown the highest affinity to produce PHAs including alkane, alkene, alkyne and a host of other hydrocarbons [59]. The use of fats, salts, ashes, whey as carbon sources had also been reported to have a large impact on PHAs yield hence decreasing production costs up to 50%. Both culture conditions and the substrate also shown significant effects on both quality and quantity of the PHAs produced [60]. A number of different carbon sources with specific bacterial strains used in PHAs production are listed in Table 3.

8.3 Nitrogen sources and limitation

Several organic and inorganic nitrogen sources had been investigated for suitability and improvement of PHAs yield including different nitrogen sources such as; amides [61], ammonium sulphate [26, 40], ammonium nitrate and ammonia nitrogen [40, 62], ammonium bicarbonate [63], ammonium carbonate, ammonium chloride [13], polyamide poly-γ-glutamate (PGA) [64], urea, nitrates [65] and sodium nitrate [27]. The amount of nitrogen sources like organic nitrogen and inorganic nitrogen sources had also shown linear relation with the bacterial growth i.e. the number of bacterial cells increased with increasing nitrogen contents in the culture media. The PHAs stored inside bacterial cell and nitrogen source concentration had an inverse effect on cell biomass yield [66]. Lower concentration of nitrogen source showed better PHAs accumulation inside the bacterial cell but higher values increased the biocatalyst activity to increase the number of bacterial cell in the fermentation media [38]. The effect of organic and inorganic nitrogen sources on PHAs production are listed in Table 5.

8.4 Carbon to nitrogen ratio

The effect of C/N ratio on PHA yield had also been reported elsewhere [68]. The concept of C/N ratio originated from the biological law according to which limitation of carbon and nitrogen control the molecular size of PHAs and number of bacterial cells. A continuous increase in C/N ratio promotes the accumulation of bacterial cell mass with inverse effect on growth kinetics (yield with respect to time) of PHAs [11]. The depletion of nitrogen in nutrient media promotes the accumulation of PHAs. The highest percentage of PHAs within cell dry mass was produced as 47.22% at 35 C/N ratio with *Haloferax mediterranei* [13]. In another study using *Cupriavidus necator*, it was reported that the C/N ratio caused a prominent effect on PHAs composition and accumulation where most of the cell mass formation occurred during first 12 h of incubation [69]. The effect of C/N ratio on PHAs is determined from production kinetics of PHAs cell mass [68]. Increasing the nitrogen concentration had a positive effect on growth rate however had negative effects on PHA yield. During fermentative production of PHAs, the excess amount of carbon source is typically used with limited nitrogen source. When investigating growth associated production of PHAs using *Alcaligenes lata*, it was observed that PHAs cell biomass yield weren't enough until sufficient amount of both carbon and nitrogen sources were provided. This C/N ratio generates high active biomass productivity with a sufficient PHAs yield [13].

8.5 Effect of pH

The pH value of extracellular environment greatly affects the bacterial enzymatic activity. A fermentation medium with neutral pH produces more PHAs than both under acidic and basic environments, because enzymes are active through higher accumulation rate. In case of pure culture media any fluctuation in neutral pH slows down the fermentative process, with a reduction in cellular activities of enzyme i.e. Pha C and Pha Z, thereby affects the growth rate, and ultimately survival of bacteria. The overall effect of pH on PHAs production was totally depended on the composition of media. A general trend of increase or decrease in pH is usually observed for neutral fermentation media after growth occurs. After loading with a substrate, the pH of a fermentation medium continuously increased in the early stages of incubation, and then followed by a period of decrease in pH to slightly acidic conditions [70]. The increase in pH of fermentation medium was mainly due to the bacterial activity and loading of growth substrate

which was also responsible for the PHAs production. The same effect of pH had also been observed in both continuous and fed batch culture fermentation conditions [71].

8.6 Dissolved oxygen demand

The gaseous requirement in most of the cells is atmospheric oxygen, which is essential for the bio-oxidative respiration process. Oxygen plays a vital role in adenosine triphosphate (ATP) formation and produced energy which is utilizable in cellular activities. Dissolved oxygen acts as carrier gas in the fermentation medium during PHAs production [72]. Under anaerobic conditions bacterial cells which lack the enzyme (Co A) for the respiration in the presence of oxygen grow under anaerobic fermentation to produce PHAs. Both metabolic activity and amount of DO were reported to increase during continuous feeding and pulse feeding fermentation as process proceeded to PHAs production. In an MMC media, the amount of DO the reverse effect on metabolic activity of bacterial cell occured [73]. The reversal effect of both pH and DO was observed in MMC when pH of the fermentative media was less than 8 [74].

8.7 Concentration of phosphorus

Phosphorus in the fermentation media plays an important role in the synthesis of proteins, carbohydrate and fats with some additional roles in cell repair and maintenance of growth rate. The amount of phosphorus present in the nutrients enrich media also acts as buffer to resist the change in neutral pH throughout the fermentation process. Various concentrations of phosphorus had been used to optimize the fermentation condition with respect to product yield. The optimized concentration of phosphorus should be used because at higher concentration a reverse effect on product yield has been reported [75, 76].

8.8 Production with PHAs precursor addition

During the production of co-polyester, different monomers are often used as precursor to produce such co-polymers. Over 150 different types of precursors have been reported used, however most of them were expensive, which limit their utilization at industrial scale. Precursors are the substances which produce a number of different copolymers from simple linear polymer via metabolic reactions. The chemical nature and concentration of these precursors have to be controlled carefully to avoid any inhibitions, toxicity and to ensure the formation desired product. Butyrate, citrate and acetate can act as precursors for PHAs producers. For example,

Ralstonia eutropha showed compatibility with the precursors like propionate (poly-3-hydroxybutyrate-co-3-hydroxyvalerate), gamma butyrolactone, 1,4-butanediol and 4-hydroxybutyrate (poly-3-hydroxybutyrate-co-4-hydroxybutyrate). Fatty acids with odd number carbon atoms have also been used as precursors in PHAs co-polymer production [77]. Different organic acids such as propionic acid and alcohols like methanol had also been used as precursors in the biosynthesis of PHAs copolymers. For example, the late addition of propionic acid to the fermentation media while using glucose as main substrate produced poly-3-hydroxybutyrate-co-3-hydroxyvalerate as co-polyester. Lignocellulosic carbon sources mainly present in liquefied wood have also been used as precursor in PHAs production [78]. The effect of these nutrients on PHA production was studied individually through optimization. The addition of these precursors increased the PHAs yield when using butyrate, citrate and acetate from 30-35% [8]. The addition of precursor facilitates the formation of co-polymer PHAs with remarkable properties which allowing using them in different field of life. A co-polymer of PHAs showed different properties from the parent polymer in different aspect like drug delivery, durability, and many others [79].

8.9 Addition of sodium chloride

The external stress factors such as increased temperature or ionic species (NaCl) cause a stress response leading to enhance PHAs production. The amount of 9 g/L NaCl was added additionally into nutrient enriches media. The NaCl had been used as a cheap, non-toxic, sustainable, simple and nonreactive external stress factor which had been reported to increase PHAs productivity by 30%(w/w) with *Cupriavidus necator*. The results also demonstrated that the nutrients media supplemented with NaCl produced 6% (w/w) more cell biomass as compare to negative control. The product yield increased at a rate of 65-77 % (w/w) in the presence of NaCl. This might be attributed to lower DO in the media at higher salinities [80].

8.10 Production of PHAs using gases

In recent years, PHAs had been produced from industrial by-product gases such as methane CH_4 or CO_2. These greenhouse gases can be fixed and used as carbon sources with additional advantages of cost effectiveness and sustainability [81]. A pre-treatment method has been assisted to utilize these gases as carbon source to produce PHAs. Continuous fed batch production process also accounts for PHAs production using gaseous carbon sources, CH_4 (by a methanotrophic bacterial strain) and CO_2 (autotrophs bacteria) [82]. In the case of *Cyanobacteria*

sp, the availability of substrate to bacteria is limited by the solubility of these greenhouse gases, in the aqueous fermentation media. Both solubility and availability of substrate under these conditions are influenced by parameters such as pH value, size of gas bubble and temperature. A sufficient supply of these gaseous growth substrates to cell biomass is possible through a continuous supply of these substrates [83]. The plants are natural sources to fix these anthropogenic gases like CO_2. In recent days different approaches have been considered to fix them using metal organic framework (MOF) and now PHAs are also produced using them as growth substrates.

9 Downstream recovery processes

The downstream recovery process (DsRP) should be an economical eco-friendly process in which toxic solvents are not used [11]. In DsRP, organic solvent such as methylene, chloroform, carbonate, propylene and dichloroethane are used for ultimately degrading the cell wall and cell membrane to recover the PHAs [67]. Polymers recovery methods also contribute to production costs of polymers produced. The main purpose of DsRP is to minimize production cost, increase the purity of product, obtain greater yield, avoid cell disruption and minimize the use of toxic solvent. In the case of DsRP, different parameters like purity of product, substrate used and properties required must be taken into consideration before a PHAs recovery [84]. A solvent free recovery method (like sodium hypochlorite recovery method) could also be used to recover cell biomass by simple dissolving the non-cellular mass with reduced molecular mass. The polymers used in medical field must be of high purity with no impurities [85, 86]. Some downstream recovery processes and their advantage in purity and recovery of polymer are listed in Table 6.

10 Future trends

In the early stages, PHAs production had been limited due to high costs which restricted their production. Researchers therefore had always been looking for low cost substrates to reduce overall production cost. In the recent years, large number of cheaper carbon sources such as waste frying oil, vegetable oil, whey and organic substrate etc., have been investigated to minimize the production cost. A mutant strain of *P. aeruginosa* produced high yields of PHAs using cheap carbon sources. The strain showed good synthesis of PHAs in preliminary investigation. The future development in PHAs production would be based on two factors; (a) lower production cost with higher yield (b) wider applications in different fields. Synthetic

biology and genetic engineering techniques are expected to produce higher yield PHAs strain with high growing density under optimizing fermentation conditions in short period of time. The purification techniques with controllable lysis can be used to accumulate large size granules and reduce the cost of production. Continuous fermentation with MMC can be used to minimize the production cost operated in two SCSTR and three SCSTR configurations using cheap substrates to obtain higher yield. Different functional groups are attached to the side chain of basic structure of PHAs to give them different properties. The molecular evolution technique should be used to produce the expected structure product of short chain length or medium chain length PHAs. Downstream processing is needed to obtain the desired purity of product for specific application in the mechanical sector and many other fields of science and engineering. The mutant bacterial strain *P. aeruginosa* was used with soybean oil always produces mcl-PHA like P(3-HB). *P. aeruginosa* synthase has a good ability to produce mcl-PHA monomers efficiently utilizing vegetable oil as growth substrate to produce PHA with low production of by-products. Moreover, there is need to work on the production of desirable congeners of PHAs under controlled fermentation and to elucidate the physico-chemical and biological properties of each individual congener.

Conclusions

The present review exemplifies a number of different bioreactors (fed batch, continuous fed batch and multiple stages continuous stirring reactors) systems and bacterial strains with large number of feeding regimes to produce PHAs of different molecular mass. It became clear that a number of different combinations of bacterial strain and substrates demand different fermentation schemes, bioreactor facilities and feeding regimes. The cultivation time and process design have to be accommodated to the physiological kinetics of the system to optimize the final cell biomass productivity and product quality. As a concluding result of recent studies or experiments, it could be expected that future PHAs production would be combined with continuous stirring method. Robust extremophilic bacterial strain would be used to produce PHAs with minimum energy input. The utilization of cheap carbon enriched feed stocks should be applied to minimize substrate cost and develop sustainable DsRP method for recovery and purification of PHAs. Only the combination of these different techniques would allow for economic and sustainable production of PHAs biopolyesters. A downstream recovery process allows us to obtain PHAs with high purity to be used in for medical application.

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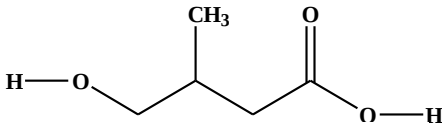
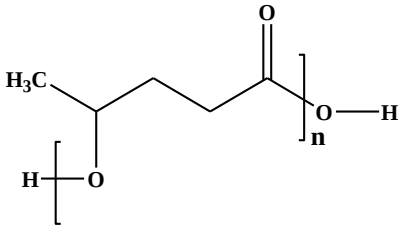
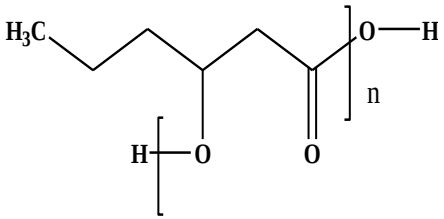
Table 1. Applications of PHAs in various fields and their example

Application	Example
Packaging	A polymer used to pack material for short time period which includes daily consumable, films, and electronic appliances
Printing	PHAs used as coloring agent in painting industry [87].
Bulk Chemical	PHA used as smart gels, heat sensitive adhesive, latex and nonwoven matrices to remove facial oils.
Block co-polymerization	A PHA with two hydroxyl group used as block co-polymer.
Plastic processing	PHAs used to process plastic.
Textile industry	PHA used in textile industry to produce nylon fibers through grafting polymerization of polyamide groups on side chain of linear polymer.
Fine chemical industry	PHAs isomer with rectus (R) configuration were used to synthesize antibiotics [88].
Medical implants	PHA are used in medical implants due to their biodegradable nature [18].
Medical	The rectus (R) PHB used in treatment of Parkinson's, Alzheimer's, osteoporosis and

	improvement in memory [89].
Food additives	PHA oligomer with ketone moiety used as food additives approved by food and drug testing authority (FDA)
Industrial microbiology	Operon produce from PHAs was used to either enhance the metabolic activity or resist process to improve industrial microbial strain [90].
Biofuels	PHA are hydrolyzed to produce hydroxyalkanoate, methyl ester which combustible in nature and used them as biofuels.
Protein purification	PHA are binding protein to purifying them.
Specific drug delivery	The co-expression of PHaP with specific ligands to achieve such properties like targeted drug delivery [91].

Table 2: Polyhydroxyalkanoates bacterial source and their chemical structure

Polymer name	Bacterial source	Chemical structure	Reference
			[56]
Poly-3-hydroxybutyrate P(3-HB)	*P. pseudomallei, P. putida, Azotobacterchrooccum, A. sp, R. eutropha*, and *Zoogloearamigera*		
Polyhydroxyvalerate P(HV)	*P. oleovorans*		[92]
Polyhydroxyhexanoate P(HHx)	*P. putida*		[38]

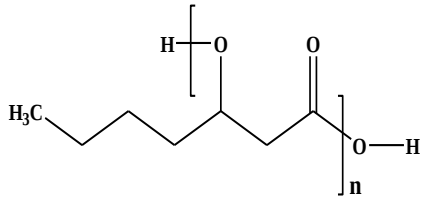
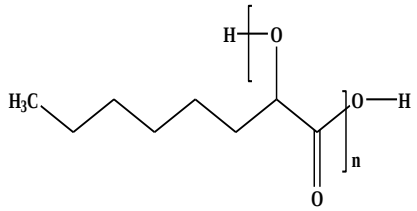
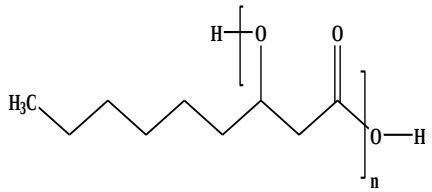
Polyhydroxyheptanoate P(HHp)	*P. putida*		[93]
Polyhydroxyocantoate P(HO)	*Streptmoyceslividans*		[94]
Polyhydroxynanoate P(HN)	*Alcaligenes sp.*		[95]

Table 3. PHAs producing microbial strains, growth substrates and their kinetics

Microorganism	Growth substrate	PHA/monomer	Cell dry mass (%, w/w)	Kinetics of PHA production (g L^{-1} h^{-1})	Reference
Gram negative bacteria					
Azohydromonas australica	Malt waste as carbon source	P(3-HB)	72	0.4-0.445 g	[96]
Azohydromonas lata	Sucrose as carbon source	P(3-HB)	55-88	0.05-4.9 g	[97]
Azotobacter vinelandii UWD	Sugar beet molasses	PHB	52-55		[98]
Azohydromonas lata	Glucose and Fructose as carbon source	P(3-HB)	76-79	0.121-0.128 g	[99]
Azotobacter beijerinckii	Glucose as sole carbon source	P(3-HB)	25	0.09 g	[28]

Burkholderia cepacia	Use large number of carbon sources xylose, glycerol, fructose, sucrose and glucose	P(3-HB)	Xylose 58.4	Not measure	[100]
			Glycerol 31.3	0.103 g	[29]
			50-59	Not measure	[99]
Burkholderia sp.	Uses oleic acid, myristic acid, lauric acid, palmitic acid and stearic acid as carbon source	P(3-HB)	1-69	Not measured	[101]
Caulobacter vibrioides	Uses Glucose as sole carbon source.	P(3-HB)	18.5	Produces 0.008 g	[102]
Cupriavidus necator, Alcaligenes eutrophus, Wautersia eutropha and *Ralstonia eutropha*	Glucose and fructose as carbon source.	P(3-HB)	67-71	0.052-0.067 g	[99]
	4-Hydroxyhexanoic acid as carbon	P(3-HB)	76.3-78.5	Not measured	[103]

	source.				
	Corn, olive and palm oil as carbon source.	P(3-HB)	79-82	0.041-0.047 g	[95]
	Butyrate, propanoic, lactic and acetate acid.	P(3-HB) and P(3-HV)	4-40.7	0.001-0.037 g	[30]
	Carbondioxide.	P(3-HB)	89	0.230 g	[104]
Alcaligenes eutrophus N9A, *Cupriavidus necator* and *Ralstonia eutropha N9A.*	4-Hydroxyhexanoic acid as sole C source	P(3-HB)	65.5-66.2	Not measured	[103]
Alcaligenes eutrophus TF93, *Cupriavidus necator*, *Wautersia eutropha* and , *Ralstonia eutropha* TF93	4-Hydroxyhexanoic acid	P(3-HB)	67.2	Not measured	[103]
	Carbondioxide gas used as carbon source	P(3-HB)	60	0.6 g	[105]
Alcaligenes eutrophus,	Glucose as carbon	P(3-HB)	76	2.4 g	[106]

Cupriavidus necator, *Wautersia eutropha* and , *Ralstonia eutropha*	source				
	Saccharified waste and potato starch as carbon source	P(3-HB)	46	1.5 g	[31]
	When Molasses as carbon source	P(3-HB)	31-44	0.8-1.2 g	[107]
	Food waste	P(3-HB)	87		[14]
	Waste glycerol	P(3-HB)	15-36.1	0.3-4.2 g	[108]
Wautersia eutropha	Canola Oil	P(3-HB-*co*-3-HV-*co*-3-HO-3-HDD)	90	0.46 g	[14]
Halomonas boliviensis LC1	Hydrolyzed starch as sole carbon source.	P(3-HB)	56	Not measured	[109]
Hydrogenophaga pseudoflava	Sucrose and lactose as carbon source	P(3-HB-co-3-HV)	20-62.5	0.02-0.12 g	[32]
	Valerate and	P(3-HB-co-3-	46	s 0.5 g	[110]

	Hydrolyzed whey used as carbon source	HV)			
Methylobacterium extorquens	Methanol used as carbon source	P(3-HB)	40-46	0.25-0.6 g	[111]
Methylocystis sp. GB25	Methane gas used as carbon source	P(3-HB)	51	Not measured	[112]
Novosphingobium nitrogenifigens Y88	Glucose as carbon source	P(3-HB)	81	0.014-0.02 g	[113]
Paracoccus denitrificans	n-pentanol as carbon source	P(3-HV)	22-24	Not measured	[114]
Pseudomonas aeruginosa	fructose, glucose, cane molasses, sucrose and glycerol as carbon source	P(3-HB)	12.4-62	0.01-0.1 g	[115]
Pseudomonas aeruginosa PAO1	Polythene wax and oil as carbon source	mcl-PHA	25	Not measures	[33]
Pseudomonas	Terephthalic acid as	mcl-PHA	24	Produce 0.004 g	[33]

frederiksbergensis GO23	carbon source				
Pseudomonas marginalis	Octanoate and 1,3-butanediol as carbon source	Scl-PHA mcl-PHA	12-31.4	Not measured	[116]
Pseudomonas mendocina	Octanoate and 1,3-butanediol as carbon source	Scl-PHA mcl-PHA	13.5-19.3	Not measured	[116]
Pseudomonas oleovorans	4-Hydroxyhexanoic acid as carbon source.	Scl-PHA mcl-PHA	18.6	Not measured	[103]
Pseudomonas putida CA-3	Styrene as sole carbon source	mcl-PHA	36.4	0.06 g	[117]
Pseudomonas putida GO16	Terephthalic acid as carbon source.	mcl-PHA	27	0.005-0.008 g	[33]
Pseudomonas putida GO19	*n*-Alkanes used as carbon source	mcl-PHA scl-PHA,	23	0.005-0.008 g	[33]

Pseudomonas oleovorans	*n*-Alkane as a sole carbon source	mcl-PHA	6-60	Not measured	[119]
Pseudomonas putida KT2440	Nonanoic acid		26.8-75.4	Produce 0.25-1.1 g	[119]
	4-Hydroxyhexanoic acid	mcl-PHA	25-30	Not measured	[103]
	Glucose		32.1	Produce 0.006 g	[34]
Pseudomonas putida F1	Toluene, benzene and ethyl benzene as C source	mcl-PHA	1-22	Not measured	[120]
Pseudomonas putida mt-2	Pentanoic acid, acetic acid, glycerol, octanoic acid, citric acid, succinic acid and glucose used.	mcl-PHA	4-77	Not measured	[48]
Thermus thermophilus HB8	From whey	scl-mcl-PHA	35.6	0.024 g	[129]

Gram positive bacteria					
Bacillus megaterium	Citric acid, succinic acid, glucose, octanoic acid and glycerol.	P(3-HB) scl-mcl-PHA mcl-PHA	3-48	Not measured	[48]
Bacillus megaterium SRKP-3	Dairy waste	P(3-HB)		0.311	[14]
Corynebacterium glutamicum	Acetic acid, glucose, succinic acid, glycerol and citric acid	P(3-HB) mcl-PHA	4-32	Not measured	[48]
Corynebacterium hydrocarboxydans	Glucose and acetate	P(3-HB) P(3-HV)	8-21	Not measured	[122]
Microlunatus phosphovorus	Glucose as sole C source	P(3-HB) P(3-HV)	20-30	Not measured	[123]
Nocardia lucida	Succinate and acetate	P(3-HB) P(3-HV)	7-20	Not measured	[122]

Rhodococcus sp.	Acetate, 1,4-butanediol, fructose, hexanoate, lactate, succinate, 2-alkenoate, 5-chlorovalerate, glucose, molasses and valerate.	P(3-HB-co-3-HV)	4-53	Not measured	[122]
Archaea					
Haloferax mediterranei	From vinasse	P(3-HB-co-3-HV)	50-73	0.05-0.2 g	[35]
	From hydrolyzed whey		72.8	0.09 g	[124]
	From glycerol		75-76	0.12 g	[36]

Table 4. Batch scale production of PHAs

Bacterial strain	Carbon source	Yield (g/g)	Reference
Chelatococcus daeguensis TAD 1	Glycerol	0.81	[125]
Cupriavidus necator H16	Jatropha oil	0.91	[126]
Halomonas campisalis	Maltose	0.80	[26]
Bacillus firmus NI 0830	Rice straw	0.89	[127]

Table 5. Different organic and inorganic nitrogen sources for PHAs production

Nitrogen source 0.2 g L^{-1}	PHAs produced g L^{-1}	Reference
Inorganic nitrogen sources		
Sodium nitrate	4.52	[113]
Potassium nitrate	2.46	[128]
Diammonium hydrogen orthophosphate	0.32	[129]
Ammonium chloride	2.48	[108]
Ammonium sulphate	2.02	[26]
Ammonium nitrate	0.62	[39]
Ammonium acetate	2.48	[130]
Organic nitrogen sources		
Glycine	1.02	[131]
Peptone	0.80	[132]

Beef extract	5.26	[68]
Yeast extract	3.20	[132]
Tryptone	0.68	[133]
Urea	3.42	[67]

Table 6: PHAs isolation methods and their advantages

Method	Recovery Method	Advantage	Reference
Digestion method	• Dissolve cell mass to conserve PHA granules. • Alkali acid and strong oxidizing agent used to non-selective digestion method. • Protolytic enzyme and anionic surfactant used for selective digestion of non-cell dry mass.	• 98% purity of product. • Increase intracellular polymer content recovery. • Appropriate size of PHA granule • Non cell dry mass converted to sustainable by value added way.	[134]
Heat pretreatment	• To assess the various enzyme (trypsin, lysozyme) for PHA extraction.	• Greater yield with 99% purity. • Reutilization of nutrients released during enzymolysis.	[135]
Digestion by acid	• Sulphuric acid used for degradation of PHA	• Define mechanical strength of PHA.	[82]
Mechanical disruption method	• Bead milling and high pressure homogenization used for disruption. • Purity of product was increased by using surfactant in process of recovery.	• Minimum damage to the environment and polymer.	[136]

Cell fragility	• Gram positive and Gram negative bacteria both are used. • Cell wall strength of bacteria compromised by modifying the composition. • The accumulation of PHA in cell cause fragility. • Inorganic salt media was used which decrease other amino acids.	• Inorganic media was used to recover the polymer with greater yield and ease.	[137]
Supercritical fluid extraction	• Supercritical fluid extraction method used to recover polymer with greater efficiency. • Recovery method totally based on disruption of cell to collect biomass with greater purity.	• 86-98% pure cell biomass were obtained. • Nontoxic, Non flammability and low reactivity • Purity of product • Low maintenance cost	[138]
Solvent extraction	• A solvent based recovery of PHA and collected out by precipitation method • Halogen containing solvent and other solvent also used like (Methanol) • Water can also be used as solvent.	• Purity of product obtained from 95-97% • Solvent extraction agent work at 100^{o}C. • The use of water make process cheaper	[138]

Solvent gelation	• Recovered PHA from mixed microbial culture and accounts for a quality of product in final applications. • Controlled microbial mass with tunable structure from batch to batch. • The rich solvent gelation blends with PHA to produce granules. • The solvent like ketone, alcohol, aliphatic, ester and aromatic hydrocarbon are solvent dissolve non rich cell mass on cooling.	• A strict criterion for polymer quality • The minimized recovery process steps • Optimization of solvent to minimize the production cost • Particle size from 0.77-2 mm	[137]
Ultrasound assisted extraction	• The efficient mass transfer obtained through ultrasound radiation • Cell biomass extracted from solvent and non-solvent base media by ultra sound radiation. • The heptane is used to extract non-solvent based PHA recovery with higher yield. • A non-solvent mixed with an appropriate ratio minimizes the solvate ratio to dissolve solute.	• Product obtained with 96% purity. • Reduce the cost of recovery process • Minimum volume of mild solvent used with reduced recovery time.	[137]

	• The post extraction method with optimal condition to define molecular weight, chemical structure and thermal properties.		
Usage of Ionic liquids	• The biomass containing PHA were disclosed by using ionic liquid. • PHA polymer dissolved in ionic liquid by gentle heating, sonication, agitation and radiation energy.	• Accelerate the dissolution of polymer recovery.	[136]
Insect based recovery of PHA	• Insect have ability to lyophilized cell with polymer granules. • The larvas of Tenebrio molitor and mealworm beetle recover PHA by engulfing them.	• The cell biomass was obtained with 54% of yield.	[139]
PHA recovery by Animal	• The rats consumed freeze dried mass of *Cupriavidus necator* and excreted pellets of PHA pure cell biomass. • The pellets are washed with detergent to increase the purity of product.	• The 82-97% purity of the product obtained from this method. • No further use of solvent and ecofriendly process.	[206]

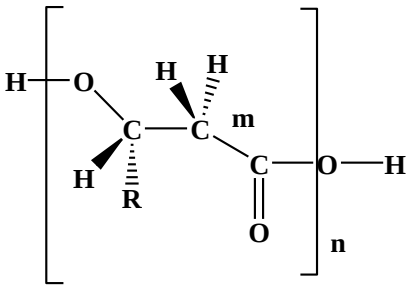


Fig. 1. A general structure of PHAs

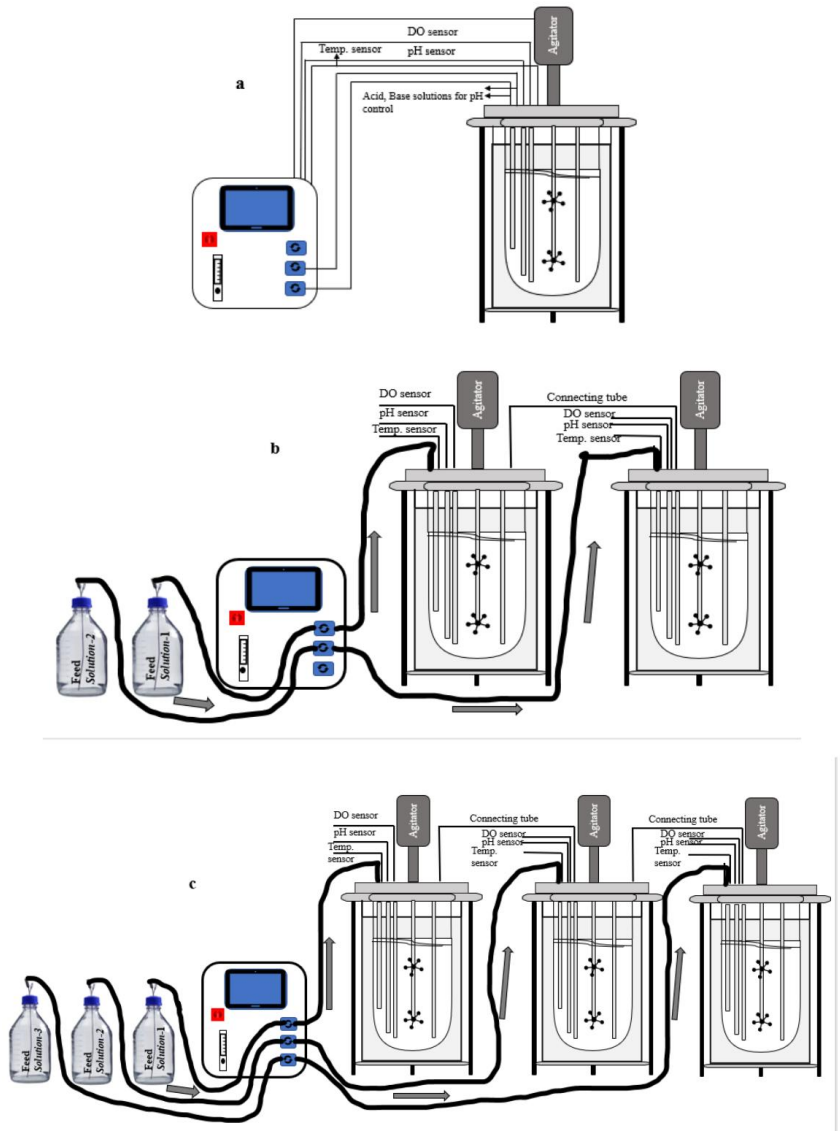


Fig. 2. Schematic diagrams of continuous tank bioreactors (a) single stage (b) two stage and (c) three stage.