

Review Article

Biotechnological Production of Polyhydroxyalkanoates: A Review on Trends and Latest Developments

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Polyhydroxyalkanoates (PHA) producers have been reported to reside at various ecological niches which are naturally or accidentally exposed to high organic matter or growth limited conditions such as dairy wastes, hydrocarbon contaminated sites, pulp and paper mill wastes, agricultural wastes, activated sludges of treatment plants, rhizosphere, and industrial effluents. Few among them also produce extracellular by-products like rhamnolipids, extracellular polymeric substances, and biohydrogen gas. These sorts of microbes are industrially important candidates for the reason that they can use waste materials of different origin as substrate with simultaneous production of valuable bioproducts including PHA. Implementation of integrated system to separate their by-products (intracellular and extracellular) can be economical in regard to production. In this review, we have discussed various microorganisms dwelling at different environmental conditions which stimulate them to accumulate carbon as polyhydroxyalkanoates granules and factors influencing its production and composition. A brief aspect on metabolites which are produced concomitantly with PHA has also been discussed. In conclusion, exploring of capabilities like of dual production by microbes and use of wastes as renewable substrate under optimized cultural conditions either in batch or continuous process can cause deduction in present cost of bioplastic production from stored PHA granules.

1. Introduction

Ecological niches are positions designated to microbes where they perform individual role and interact all together to constitute ecosystem. Ecosystem is influenced by biotic and abiotic alteration as result of natural and anthropogenic activities. Microorganisms derive their food *via* diverse behavioural adaptation in environment to put together thriving existence. PHA accumulation is among such responses towards stress experienced by microorganisms residing at different ecological niches. Ecological niches like estuarine sediments, marine microbial mats, rhizosphere, groundwater sediments, and engineered ecosystems with fluctuating nutrient contents support the population actively involved in PHA accumulation to meet the metabolic energy requirements during starvation period and this concept can be

implemented industrially to reduce the cost of biopolymers commercially with sustainable production processes [1].

We need to clearly understand ecological conditions prevailing and phenotypic characteristics of PHA producers. Waste disposal creates toxic, growth inhibiting, and unfamiliar environmental conditions [2]. Even though, at such state of physiological stress, PHA producers have been reported to be residing with properties like degrading dyes, aromatic compounds, and left over organic matter discharge from industries [3–7]. This implausible behavioural property can be considered towards industrial production aspects and issues related to removal of pollutants like PAHs, pesticides, dyestuffs, and so forth. In concern to increasing load of industrial waste on soil, researchers are trying all possible alternatives to use different wastes like textile effluent [4, 8], distillery effluent [9], dairy wastes [10–12], oil mill wastes [13],

starchy waste [14, 15], paper mill wastes [16], animal residues [17], biodiesel waste by-product [18], and agricultural wastes [19], for PHA production. PHAs are produced under nutrient limiting conditions with excess of carbon [20] and get stored in polymerized form as inclusion bodies [21].

Last few years have contributed different aspects of bioplastic production where use of activated sludge as PHA producers [22] enriched *via* aerobic dynamic feeding (ADF) [23] and alternating anaerobic and aerobic system (AN/AE) [24] in sequencing batch reactor (SBR) and fermented effluents [25], exhibiting high BOD and COD value, as substrate have put on much importance. It is clearly understood that PHA production from mixed culture, enriched phosphate accumulating organisms (PAOs) [26, 27], enriched glycogen accumulating organisms (GAOs) [28], and Archaea [29] inhabiting low or high salt conditions rule out need of proper modelling of operational parameters to solve the issues associated with sterile conditions, synthetic medium preparation, maintenance of pure cultures, and high product cost. PHA as stored energy source increases survivability in *Bacillus megaterium*, a potential degrader and biocontrol agent, in water [30]. Stored copolyesters PHBHHx in *Aeromonas hydrophila* 4AK4 have been reported to survive off various environmental stresses like nutrient-limited conditions, cold treatment, UV-irradiation, ethanol, H_2O_2 , and osmotic shocks [31]. PHA production enhances their feasibility to survive off the harsh conditions not being favourable for the normal growth of cells. Wastes like seafood cannery, rich in lipids, ammonia, and phosphates, are proved to be toxic to its inhabiting microorganisms. PHB accumulation had an advantage here not being only as stored material but also as alternate to metabolize excess fatty acids in order to cope with stress existed [32]. The property of storing carbon source intracellularly and utilizing the same to support activity of cells at later phase is possibly an advantage and it becomes of significant importance being commercially valuable by-product.

On exhaustion of nutrients and with excess of carbon sources some microbes also produce extracellularly metabolites like rhamnolipids [33, 34], alginate oligosaccharides [35], extracellular polymeric substances [36, 37], and biohydrogen [38, 39] along with intracellular PHA production stored as inclusion bodies. Studies have also reported how substrate composition, concentration, and its uptake modulate the production and composition of PHA polymer formed [40–42]. pH [43] and temperature [44] have also been reported to affect PHA production and its composition. Thus, understanding of PHA production and its associated kinetics in respect to simultaneous production of other bioproducts may provide us with platform to design cost effective and efficient production unit.

2. PHA Producers along with Their Ecological Niches

2.1. Hydrocarbon Degradars as PHA Producers. Environmental stress, like presence of xenobiotic compounds, diverts physiological responses of residing organisms to produce

more PHA [45]. Oil contaminated sites contain higher amount of carbon (84%) and less in nitrogen contents (>1%) creating a real prerequisite for cells to produce PHA [46]. Various bacterial strains capable of producing PHA while degrading oil have been isolated belonging to genera *Pseudomonas*, *Acinetobacter*, *Sphingobacterium*, *Brochothrix*, *Caulobacter*, *Ralstonia*, *Burkholderia*, and *Yokenella* from oil contaminated sites [3]. In an experiment, *Ralstonia eutropha* JMP 134 has been reported to produce PHB up to 50% of cell dry weight (CDW) when provided with growth inhibiting substances like phenol or sodium benzoate as carbon source under nutrient limiting state. Thus, it is of immense interest to prevent intoxication and explore microbial potential for production of valuable bioproducts [47]. *Bacillus cereus* FA11 isolated from trinitrotoluene contaminated soil (creating a necessary stress conditions) has been reported for the production of copolymer (3HB-co-3HV) up to 48.43% at pH 7 and temperature 30°C with glucose as carbon source [48].

In a case, extracellular deposition of PHB by mutant of marine bacterium *Alcanivorax borkumensis* SK2 has been reported grown on aliphatic hydrocarbon [49]. Construction of a bioprocess where waste comprising of toluene as sole carbon source enables the *Rhodococcus aetherivorans* IARI to produce copolymer P(3HB-co-HV) and triacylglycerols (TAGs) resulting in cost reduction of production unit with effectual removal of waste material [50]. Similarly, in another experiment of fed-batch fermentation with toluene as volatile aromatic hydrocarbon (carbon source) at flow rate of $0.42\text{ g L}^{-1}\text{ h}^{-1}$ into a 7-L jar fermentor resulted in accumulation of *mcl*-PHA (58.9% by weight), a copolymer constituting 3HD (55.2%), 3HO (26.8%), 3HH (3.7%), 3HDD (8.2%), and 3HDD (6.1%) by *Pseudomonas fulva* TY16 after 48 hr of incubation, thus proving to be a potential candidate for bioconversion of petrochemical waste into valuable products like PHA [51].

2.2. Halophiles as PHA Producers. Archaea are considered as extremophiles since reported to reside at hot springs, marshlands, oceans, salt lake, and so forth and have been reported to produce PHA. These organisms require salts to sustain their growth. They grow optimally at 5% and at least tolerate 10% of salt NaCl (w/v) [69]. The first case of PHB accumulation by archaea had been reported in 1970 from Dead Sea named as *Halobacterium marismortui* analysed by freeze-fracture technique [70]. Extremely halophilic archaeobacteria (Halobacteriaceae) produce PHB under nutrient-limited conditions and abundant carbon sources [71]. *Haloferax mediterranei*, which grows at 25% (w/v) of salt concentration, produces 60 to 65% PHA of its cell dry weight (CDW) when grown in phosphate limiting conditions and glucose or sucrose as best carbon source [72]. *Halomonas boliviensis* LC1, a moderate Halophile growing at 3–15% (w/v) of salt concentration, produces higher amount of PHB up to 56% of CDW, when grown on starch hydrolysate as substrate. Pretreatment results in conversion of higher complex form of organic substances into easily consumable form (simpler form). *H. boliviensis* LC1 preferably utilizes maltose from starch hydrolysates. It has been found that oxygen limitation

enhances the PHA production [73]. *H. boliviensis* LC1 could produce high amounts of PHB up to 88% of CDW in the presence of excess of butyric acid and sodium acetate as carbon sources under nutrient-limited conditions (yeast extract at 0.1%, w/v) during stationary phase [74]. Similarly, when *Halopiger aswanensis* (strain 56^T) was grown under nutrient-limited conditions (and excess carbon), the cells accumulated large amounts of polyhydroxybutyrate [75].

Study on enzyme, PHA synthase, activity of extremely halophilic archaeon, *Haloarcula marismortui*, has suggested that PHA is constitutively expressed independent of nutrient rich or nutrient-limited conditions [76]. Similarly, another report shows that PHB and P(HB-co-HV) producers like *Halococcus* sp., *Halorubrum* sp., *Halobacterium noricense* DSM 9758, and haloalkaliphiles (*Natronobacterium gregoryi* NCMB 2189^T, *Natronococcus occultus* DSM 3396^T) require alkaline and salt conditions irrespective of complex medium and nutrient-rich or nutrient-limited conditions [77]. Most importantly, extremely halophilic archaeon, *Haloarcula marismortui*, has been reported to produce PHA under shake flask conditions using vinasses (by-product of ethanol industries) as substrate. Utilization of 10% raw vinasses and 100% pretreated vinasses resulted in PHB production up to 24% and 30% of CDW, respectively [29]. In a review [78] described PHA producing halophiles and concluded that there is need of understanding synthetic and regulatory mechanisms controlling polymer synthesis to overcome the economical competition at industrial scale.

2.3. Photosynthetic Bacteria as PHA Producers. Cyanobacteria are photosynthetic prokaryotes with short generation time, reported to produce PHA by oxygenic photosynthesis. Studies have led to conclude that some cyanobacteria have natural capabilities to store PHAs. Some Cyanobacteria were screened for the presence of PHA which was reported to be species specific, mostly producing PHB, stimulated by phosphorus deficient conditions and presence of excess amount of reducing equivalents [79]. *Synechococcus* sp. MA19 (accumulated up to 55% of CDW), *Nostoc muscorum*, and *Spirulina platensis* produced PHB under phosphate limited conditions [80, 81]. Phosphorus deficient conditions and supplementation of 0.4% of acetate resulted in accumulation of PHA up to 29% of CDW by pregrown cells of *Synechocystis* sp. PCC 6803 in glucose [82].

Synechocystis sp. UNIWG, and *Synechocystis* sp. PCC 6803 accumulated PHB up to 14% and 15% of CDW under nitrogen limiting conditions, respectively [83, 84]. *Synechocystis* sp. PCC 6803 when subjected to nitrogen, phosphorus, and gas-exchange limiting conditions PHB production was enhanced [85]. Sulfur deprived conditions enhanced PHB content by 3.5-fold [86]. A study showed that *Nostoc muscorum* could produce PHB five times higher under mixotrophy, chemoheterotrophy with nitrogen-limiting state than what was produced under photoautotrophic conditions [87]. Addition of exogenous carbon sources, pH, light-dark cycles, and phosphorus and nitrogen status were found to regulate accumulation of PHB in *Nostoc muscorum* [87]. Use of Cyanobacteria ability to produce PHB with energy

obtained from sunlight can result in reduction of cost and CO₂ a “greenhouse gas” as well [83].

2.4. Plant Growth Promoting Rhizobia (PGPR) as PHA Producers. Soil adjacent to roots of plants is termed rhizosphere which may harbour microbes enhancing the growth of roots and plants by secreting extracellularly metabolites [88]. Root exudates release nutrients to its surrounding rhizosphere which in turn serve as hot-spot for microbial interaction and thus, recycling of nutrients takes place [89]. It has been clearly elucidated that rhizosphere has hidden or untapped reservoirs for PHA accumulators in addition to PGPR and antagonistic effect. Some microorganisms, *Burkholderia terricola*, *Lysobacter gummosus*, *Pseudomonas extremaustralis*, *Pseudomonas brassicacearum*, and *Pseudomonas orientalis* have been reported as PHA producers based on PCR technique having *phaC* as targeted gene [90].

Azospirillum brasilense is a plant growth promoting rhizobacterium (PGPR) that is being increasingly used in agriculture in a commercial scale. Recent research has elucidated key properties of *A. brasilense* that contribute to its ability to adapt to the rhizosphere habitat and to promote plant growth. They include synthesis of the auxin indole-3-acetic acid, nitric oxide, carotenoids, and a range of cell surface components as well as the ability to undergo phenotypic variation. Storage and utilization of poly- β -hydroxyalkanoate polymers are important for the shelf life of the bacteria in production of bioinoculants, products containing bacterial cells in a suitable carrier for agricultural use. It was reported that mutants of *phaC* and *phaZ* genes in *Azospirillum brasilense* were somehow less tolerant to stress conditions such as UV-radiations, heat, osmotic pressure, osmotic shock, H₂O₂ presence, and desiccation, in comparison to wild type [91, 92]. Production of PHA holds advantageous characteristics towards enhanced root colonization, plant growth promotion, survivability, chemotaxis, motility, and cell multiplication. PHA production is also very effectual in improving reliability, efficiency, and shelf life of inoculants of *A. brasilense* [91]. Based on cultivation-dependent methods, earlier it was reported that rhizosphere has low PHA production in comparison to nonrhizosphere soil [93, 94]. Cultivation-independent and molecular techniques allowed us to conclude that wheat, oilseed rape, and sugar beet rhizosphere have more PHA production [95–97]. Root of oily and carbohydrate producing plants harbours more PHA producers as supported by the fact the root exudates contain abundant source of carbon and inorganic nutrients being in limited state [90].

A relationship between PHB production and survivability during starvation in *Sinorhizobium meliloti* within alfalfa was established, where they produced PHB up to 0.25 pg cell⁻¹ to support multiplication and survival during starvation period. Later, PHB accumulation is found to be quantitative trait as being differentially regulated by different genes among rhizobia [98]. PHA accumulation in nodule gets induced under low oxygen state or decreased redox potential as studied in *Azotobacter beijerinckii*, *Azotobacter insignia*, and *Rhizobium* ORS571 [99–102]. The rhizobia include the genera *Sinorhizobium*, *Rhizobium*, *Bradyrhizobium*, *Mesorhizobium*,

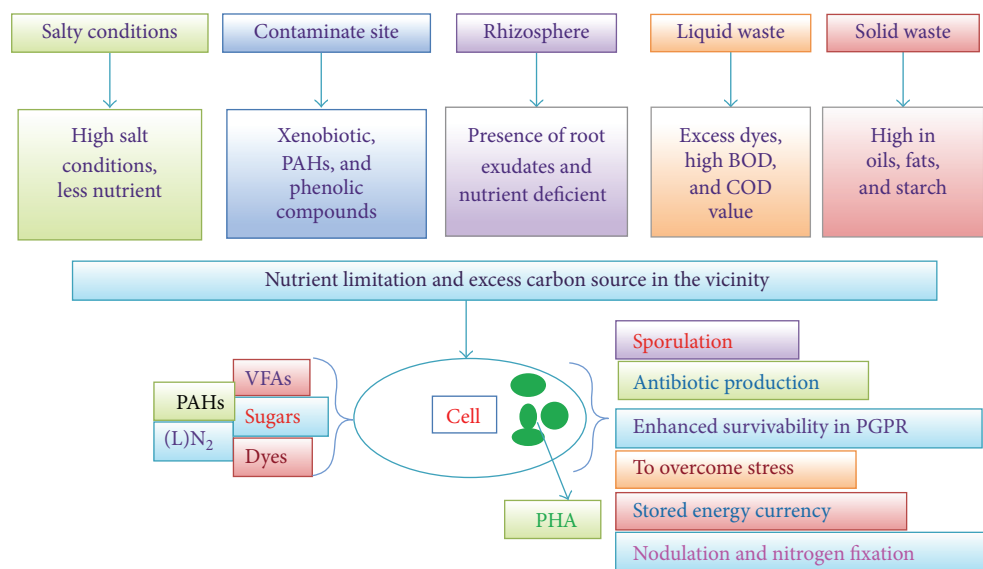


FIGURE 1: A diagram representing different ecological stress conditions which favour PHA accumulation to sustain the metabolic activities at optimal conditions.

and *Azorhizobium* which form nitrogen fixing nodule in legumes. Stored PHA fuels rapid colonization, efficient nodulation, and nitrogen fixation. The accumulation of PHB has been reported in both rhizobia associated with roots actively involved in nodulation (rhizobia-legume symbiosis) or free living nitrogen fixing organisms. Ability to accumulate PHB is directly correlated to type of nodule being formed, that is, determinate or indeterminate differing in possession of persistent meristem [103]. Proteobacteria and Firmicutes were isolated from root nodules of eight leguminous plants reported to be accumulating PHB [104].

2.5. Antibiotic Producers as PHA Producers. *Streptomyces* are aerobic, gram positive filamentous bacteria known for their valuable metabolites production. *Streptomyces* are reported for PHA production intracellularly in granular form working as supplier of carbon units for antibiotic synthesis and sporulation [105]. Nitrogen supplementation and increased carbon source showed positive effect on PHB production in *Streptomyces griseorubiginosus* and produced up to 9.5% of mycelial dry mass at the early stationary phase [106]. PHB production in different strains of *Streptomyces* has been studied and investigated for its role in antibiotic production.

Later, reports showed that production of PHB occurred in *Streptomyces coelicolor* A3 (2) M145 during exponential phase and supplied acetyl-CoA being precursors for the production of three different antibiotics namely actinorhodin, γ -actinorhodin, and undecylprodigiosin at stationary phase [107]. During synthesis of actinomycin A, stored PHB was utilized in *Streptomyces antibioticus* [108]. Similarly, it was reported that PHB served as supplier of acetoacetyl-Co-A and butyryl-Co-A for the production of chloramphenicol in *Streptomyces venezuelae* and macrolide ascomycin FK520 in *Streptomyces hygroscopicus*, respectively [109, 110]. PHB being a primary metabolite serves as building blocks for

the synthesis of metabolites [111]. *Streptomyces aureofaciens* 84/25, *S. griseus*, *S. olivaceous*, *S. fradiae*, *S. parvus*, *S. albus*, and so forth are reported to produce PHB with glucose as carbon source [105, 107]. A diagram representing different ecological stress (suboptimal) conditions which favour PHA accumulation to sustain the metabolic activities at optimal conditions is being depicted as in Figure 1.

2.6. Activated Sludge as PHA Producers. Continuous inflow of industrial wastes from industry is of environmental concern in reference to its toxic pollutant constituents. Wastes are often rich in organic contents and less rich in nutrient contents. This state of unbalanced nutrients supports growth of PHA producers but has minimal consumption rate of organic matter (substrate) and storage rate [112, 113]. These issues were resolved using pretreated waste of several kinds when subjected to different treatments like thermal treatment, anaerobic treatment, enzymatic treatment, mechanical treatment, and so forth. Treated waste results in having simpler forms like volatile fatty acids (VFAs) and sugar moieties which are easy to uptake and store by cells. Three-stage processes have been followed by some researchers [15, 114, 115] where treatment of wastes constitutes first step followed by enrichment of activated sludges and final batch production of PHA using treated waste as substrate and enriched activated sludge as inocula. Activated sludge can be enriched by AN/AE and ADF in SBR [24]. The composition of VFAs influences composition and microstructure of polymer. So, it is of prior importance to consider. PHA production under aerobic microenvironment showed that consortium accumulated more PHA (39.6%) with fermented food waste than with unfermented food waste (35.6%) as substrate. Because the fermented food waste contained ready to take up VFA in comparison to unfermented food waste with complex organic substrates to be degraded before being

used up [116]. Waste of various origins as substrate with the treatment applied and resulting possible constituents of treated waste and percentage of PHA obtained are given in Table 1.

2.6.1. Anaerobic and Aerobic System. Waste treatment by alternating aerobic and anaerobic conditions like enhanced biological phosphate removal (EBPR) can be an effective process in respect to concurrent implementation of PHA production using the enriched activated sludge. Synthesis of PHA by PAOs was first reported by Wallen and Rohwedder [117]. Phosphorus accumulating organisms (PAOs) accumulate PHA with energy derived from polyphosphate breakdown (release of phosphorus) under anaerobic phase and later utilize the stored form (PHA) to restore polyphosphate and sustain normal metabolic activities [118, 119]. Also, having phosphate released into stream could be useful to field cultivating crops as fertilizers [120]. *Microlunatus phosphovorus* from activated sludge was studied for its capabilities to store PHB under AN/AE conditions subjected to different growth conditions and carbon sources. PAOs usually take up short fatty acids which are converted to polymeric form of energy and further they were concluded as potential candidate for PHA production [27].

Different glycogen accumulating organisms (GAOs) like *Candidatus competibacter phosphatis* and *Defluviicoccus vanus* [28, 121–124] within SBR under AN/AE conditions have been reported in literature. GAOs utilize glycogen to derive energy for uptake of volatile fatty acids and subsequently convert them into PHA via acetyl-CoA and propionyl-CoA. Glycogen is also converted to PHA. Under aerobic conditions, stored PHA is utilized to sustain the normal functions of cell and restore glycogen. Stored glycogen is utilized under both anaerobic and aerobic conditions with different stoichiometry. 3HB and 3HV up to 49% of CDW were produced in anaerobic conditions, whereas only 3HB up to 60% of CDW was produced in aerobic conditions suggesting that different metabolic routes were followed by GAOs under alternating anaerobic and aerobic conditions [121]. GAOs have been reported to accumulate PHA using fermented cane molasses [28, 125] and synthetic carbon sources like acetate and propionate as well [126–128].

Bacterial richness and dynamics of PHAs producers from activated sludge enriched in sequencing batch reactor fed with mixture of molasses and acetic acids were explored under AN/AE conditions based on PCR-DGGE technique [129]. It was investigated that excess activated sludge consisting mainly of PAOs and GAOs could be an innovative alternative to typical axenic PHA production in reference to cost and flexibility in operating conditions. PHA productivity of $0.5\text{--}2.19\text{ g PHA L}^{-1}\text{ h}^{-1}$ and intracellular content of 21–51% under defined conditions of pH and anaerobic contact time by PAOs and GAOs, respectively, were reported [130]. Unlike ADF suitability to treat effluents having high organic content and deficient in nutrients, AN/AE system could be of choice with industrial effluent constituting high carbon and high phosphorus contents. Thus, implementing anaerobic conditions followed by aerobic conditions could result in

to higher productivity when compared to under separate anaerobic and aerobic conditions [131].

2.6.2. Aerobic Dynamic Feeding System (ADF). Alternate “feast” and “famine” conditions in SBR are another concept to enrich the mixed culture [132]. Feast condition is period of excess carbon and storage of carbon in the form of polymers and famine condition is period of carbon deficient and consumption of stored polymer to sustain growth. Famine conditions result in less metabolic RNA and enzymatic activity of cells. This retarded metabolic status of cells when subjected to feast conditions results in storage of carbon [133]. With use of diazotrophic mixed cultures using acetic and propionic acids as carbon sources under ADF strategy in SBR resulted in production of PHB and P(3HB-co-HV) [134]. Similarly when activated sludge was enriched by ADF under different nitrogen regimes, it had been notified that PHA accumulated was always higher in activated sludge enriched under nitrogen deficient conditions [135]. These reports were significant in relation to use of nitrogen deficient wastewater but abundant in carbon contents for production of valuable bioproduct like PHA.

Production of PHA up to 89% of CDW was reported within 7.6 hr by open mixed cultures, dominated by γ -proteobacterium, in acetate-fed sequencing batch reactor enriched under feast and famine conditions [136]. They also suggested that engineering of ecological niche rather than the organisms could be an alternative promising approach to explore capabilities like PHA accumulation. Successful production of copolymer by dominating group of β -Proteobacteria constituting 70% of HB monomers and 30% of HV monomers from bio-oil obtained after pyrolysis of chicken-beds by mixed microbial culture had been elucidated under feast-famine conditions [137].

Recently, an efficient method of PHA production was notified where acetate as carbon source and photosynthetic consortium of algae as source of oxygen along with bacteria were cultured under feast and famine conditions [138]. This opens up a new possibility of using sunlight driven PHA accumulation without any external oxygen supply to reduce the cost of production. In a review, the authors clearly detailed about techniques and trends towards PHA accumulation with use of mixed culture from waste material as low cost substrate under transient feast and famine conditions and aerobic-anaerobic conditions in SBR. Further, it has been reported that nonsterile reactors and little process control eliminate traditional bottlenecks of continuous monoseptic fermentations [23].

3. Factors Influencing PHA Production and Composition

3.1. Nutrients Availability and Optimization. Nutrient limitation leads to increase in PHB production whereas high nitrogen state directed increase in biomass growth with no PHB production [139]. Low concentration of phosphorus and nitrogen is favourable to PHB accumulation rather than complete underprovided conditions. PHA production

TABLE 1: Waste of various origins as substrate with the treatment applied and resulting in possible constituents of treated waste and percentage of maximum PHA obtained.

Substrate	Treatment applied	Possible constituents	Microorganisms	Maximum PHA produced	References
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	[28]
Cassava starch wastewater	Acidogenic fermentation	Acetate, butyrate acids, propionate, pH-5.3, and high COD value.	<i>Cupriavidus</i> sp. KKU38	PHB; 85.53% of CDW	[52]
Sugar refinery waste (cane molasses)	Acid treatment and activated charcoal	Carbohydrate (4%)	<i>Pseudomonas aeruginosa</i>	PHB; 62.44% of CDW	[53]
<i>Cynodon dactylon</i> grass and <i>syzygium cumini</i> seed;	Pulverization followed by hydrolysis	Glucose	SP-Y1 and <i>Ralstonia eutropha</i>	SP-Y1 (PHB) H.S 26.76% of CDW H.G 41.7% of CDW <i>R. eutropha</i> (PHB) H.S 42.2% of CDW H.G 28.97% of CDW	[54]
Milk and icecream wastewater	Acidogenic fermentation	VFAs	Activated sludge	0.25 kg PHA/kg of COD	[11]
Palm oil mill effluents	Acidogenic fermentations	Acetate, propionate, and butyrate	<i>Ralstonia eutropha</i> JMP 134	At 20th and 40th hr 0.75 g PHA/g CDW At 40th and 60th hr 0.41 g PHA/g CDW	[55]
Rice straw	Acidic treatment	Pentose sugars and hemicelluloses	<i>Bacillus firmus</i> NII 0830	PHB; 89% of CDW	[56]
Seed oil of <i>Jatropha curcas</i>	Saponification	Oleic acid, linoleic acid, and palmitic acid	<i>Pseudomonas oleovorans</i> ATCC 29347	P(3HB-co-3HV); 26.06% CDW	[57]
Hemicellulosic feedstock (sugarcane bagasse)	Acid hydrolysis, boiling, precipitation, and activated charcoal	Xylose, arabinose, glucose, and acetic acids	<i>Burkholderia cepacia</i> IPT 048 and <i>Bacillus sacchari</i> IPT 101	IPT 048 PHB; 62% of CDW IPT 101 PHB; 53% of CDW	[58]
25% and 50% of vinasse (ethanol industry waste)	Activated charcoal	Sucrose, oxalate, lactate, malate, and pyruvate	<i>Haloferax mediterranei</i>	PHB; 70% of CDW	[59]
Paper mill wastewater	Acidogenic fermentation	VFAs	Activated sludge	PHB and PHV; 48% of SDW	[16]

HB: hydroxybutyrate, HV: hydroxyvalerate, HHx: hydroxyhexanoate, 3H2MB: 3-hydroxy-2-methylbutyrate, 3H2MV: 3-hydroxy-2-methylvalerate, PHB: poly-3-hydroxybutyrate.

under low nitrogen and phosphorus concentration was reported to be 45.1% and 54.2% of CDW, respectively. Optimizing the same cultural conditions enhanced copolymer P3(HB-co-HV) content by 14%, constituting 88% of HB and 8% of HV [116]. A study demonstrated that external metabolic limitation in addition to internal limitation could decrease the storage rate [41]. *Brachymonas* sp. P12, denitrifying phosphorus-removing bacteria, obtained from winery waste capable of producing PHB was studied to optimize the conditions for PHA production considering factors like oxygen or nitrate as electron acceptor, nitrogen concentration (NH_4Cl), phosphorus (KH_2PO_4), and carbon concentration (acetate). C-excess conditions had negative effect on PHB accumulation when compared to C-limited conditions in reference to its specific production rate. Thus, aerobic, C-limited, N-excess or N-limited, and P-limited or P-excess affected the growth-associated production of PHA [140].

Carbon/nitrogen (C/N) ratio is a very important variable to consider during PHA production supported by the fact that optimum C/N ratio of 25 showed maximum PHA productions [141]. PHB production increased by 1.8-folds with C/N ratio of 28.3, where sucrose as carbon source, ammonium sulfate as suitable nitrogen source, optimum pH of 6.5, and temperature of 33°C were provided in batch culture of *Alcaligenes latus* ATCC 29713 [142]. Oleic acid as carbon source (with C/N ratio of 20) enhanced PHA production in *Cupriavidus* sp. USMAA2-4 [143]. High C/N ratio of 144 for PHA production by activated sludge was found to be effective [144].

Designing of cultural conditions allows us to use suitable nutrients in order to enhance the PHA production. Box-Behnken design was undertaken to optimize the cultural conditions in respect to maximum PHB production. The authors considered cultivation parameters like ammonium sulfate as nitrogen source, glucose as carbon source, KH_2PO_4 , and Na_2HPO_4 as phosphorus source to successfully implement the idea which leads to maximum production of 1.45 g L^{-1} in *Bacillus megaterium* SW1-2 with 75% validity confirmed using verification experiment [145]. Similarly, PHB production by *Haloarcula* sp. IRU1 in batch culture with various constituents of production medium using Taguchi experimental design was done. Reports inferred that glucose at 2 g L^{-1} , nitrogen at 0.2 g L^{-1} , phosphorus as KH_2PO_4 , and temperature at 42°C resulted in the highest PHB accumulation up to 63.0% of CDW based on ANOVA. Glucose was actively utilized for PHB production; that is, carbon source plays a significant role in PHB production [146]. A five-level-three-factor central composite rotary design was employed to optimize magnetic field, $\text{NH}_3\text{-N}$, and initial pH on the PHA production by activated sludge under ADF strategy which led to accumulation up to 49.5% of CDW [147]. Central composite rotary design was also employed to optimize the production of PHB from dairy waste, rice bran, and sea water as nutrients source by *Bacillus megaterium* SRKP-3 [12]. Factorial design was adopted to study effect of ammonium, phosphate, and yeast extract on PHB production by *Halomonas boliviensis* [148]. The authors also reported high productivity

of PHB by *Halomonas boliviensis* in fed-batch culture [149]. Recently, to evaluate the influence and specific function of eight important factors (iron, glucose concentration, VFA concentration, VFA composition, nitrogen concentration, phosphorous concentration, pH, and microenvironment) on the PHA production, design of experimental methodology using Taguchi orthogonal array was implemented [150].

Different acids as possible carbon source showed very prominent result on PHA production. The supplementation by citrate (0.5%) and acetate (0.5%) individually under phosphate deficient conditions, followed by 5 days of dark incubation, results in 51% and 77% of PHB production, respectively, in case of *Aulosira fertilissima* [151]. PHB produced in acetate medium was 7 times higher than what was reported in succinate media by *Rhodobacter sphaeroides* KD131 [152]. Butyric acid showed the highest PHB productivity of 33% followed by acetic acid (32%), mixture of acids (butyric acid and acetic acid), and then propionic acid (11%). Mixture of butyric and acetic acids showed higher productivity than propionic acid since latter involved complex metabolism for its conversion to PHB [153]. VFA at concentration of 9 g L^{-1} and acetate : propionate : butyrate at ratio of 20 : 10 : 70 showed maximum PHB accumulation [150]. Different types of wastes used as substrate and microorganisms as PHA producers with their respective maximum PHA produced has been depicted in Table 2.

The composition of polymers was found to be varying with type of substrate used [136]. If acetate is used as carbon source, it results in production of copolymer P(3HB-co-3HV) with higher HB contents, whereas propionate (as carbon source) results in higher HV content. On the other hand, the butyrate results in the formation of HB units only. Mixture of all acids resulted in copolymer production with higher HV contents [26]. Butyrate and acetate were much effective towards polymer composition in comparison to propionate [150].

PHA polymer composition also varied with use of acidogenic effluents (AE). Contents of HB derived from synthetic acids (SA) and AE were found to be 91% and 84%, respectively, whereas HV derived from SA and AE was found to be 5% and 12%, respectively [25]. The authors also concluded that acidogenic effluents could be alternative and low cost substrate for P(3HB-co-3HV) production with higher concentration of HB and almost similar properties as obtained with synthetic acids. When PHA producers were subjected to SBR (with propionate) and SBR (with acetate), it resulted in production of copolymer P(3HB-co-3HV) and polymer (3HB), respectively [154]. 1-Pentanol as 3HV precursors together with γ -butyrolactone or 1,4-butanediol as 3HB precursors significantly produced terpolyester of different composition (monomers). Polymer obtained had five different monomers (3HB, 3HV, 3H2MV, 3H2MB, and 3HHx) with different proportions using fermented and synthetic VFAs as substrate and using GAOs as cultural organism and decomposition temperature of polymers obtained was independent of monomer compositions [28]. PHA yield and composition produced by *Pseudomonas aeruginosa* 47T2 Depends on carbon, nitrogen, phosphorus sources, and incubation temperature [155].

TABLE 2: Different types of wastes used as substrate and microorganisms as PHA producers with their respective maximum PHA produced.

Substrate type	Microorganisms	Type and maximum PHA produced	Reference(s)
Food processing wastewater and distillery spentwash	Activated sludge	D.J.G.B.D.S; PHB; 42.3% of CDW	[19]
		R.R.G.B.D.S + DAHP; PHB; 67% of CDW	
		F.F.P.I.W; PHB; 39.1% of CDW	
Cassava wastewater	<i>Pseudomonas aeruginosa</i>	PHA; 39% of CDW	[60]
Maple sap	<i>Alcaligenes latus</i>	PHB; 77.6% \pm 1.5 of CDW	[61]
Domestic wastewater	<i>Enterobacter aerogenes</i> 12Bi strain	PHB; Up to 90% of CDW	[62]
Molasses and dye textile dye	<i>Sphingobacterium</i> sp. ATM	PHD; 64% of CDW	[2]
Fatty wastes	Lipolytic bacterial strains (<i>Acinetobacter</i> sp., <i>Pseudomonas</i> sp., <i>Bacillus</i> sp.)	3HB, 3HV, 3Hx, 3HD, 3HDD, and 3HO; 0.1% to 32.2% of CDW	[63]
Glycerine pitch + 1,4-butanediol (5 g L ⁻¹)	<i>Cupriavidus</i> sp. USMAHM13	P(3HB-co-4HB); 49% of CDW	[64]
Starchy wastewater	<i>Alcaligenes eutrophus</i> ATCC17699	PHB and P(3HB-co-3HV); 50 g PHA/100 g TOC	[14]
Vinasse	<i>Haloarcula marismortui</i>	PHB; 23% of CDW	[29]
Waste potato starch	<i>Ralstonia eutropha</i> NCIMB 11599	PHB; 94 g L ⁻¹	[65]
Textile effluent and mixture of dyes	<i>Sphingobacterium</i> sp. ATM	PHD; 66% of CDW	[4]
Maple hemicellulosic hydrolysate	<i>Burkholderia cepacia</i> ATCC 17759	PHB; 51.4% of CDW	[66]
Whey lactose	<i>Haloferax mediterranei</i> ,	P-3(HB-co-8%-HV); 50% of CDW	[67]
	<i>Pseudomonas hydrogenovora</i>	PHB; 12% of CDW	
	<i>Hydrogenophaga pseudoflava</i>	P-3(HB-co-5%-HV); 40% of CDW	
Biodiesel waste water	<i>Novosphingobium</i> sp. THA_AIK7	PHB; 45% of CDW	[5]
Oil palm frond juice	<i>Cupriavidus necator</i> CCUG52238 ^T	PHB; 45 \pm 1.5 wt%	[68]

D.J.G.B.D.S: deproteinized jowar grain based distillery spentwash, R.R.G.B.D.S: raw rice grain based distillery spentwash, F.F.P.I.W: filtered food processing industrial wastewater, PHD: polyhydroxydecanoate, H.S: hydrolyzed seed, H.G: hydrolyzed grass, 3Hx: (R)-3-hydroxyhexanoate, 3HO: (R)-3-hydroxyoctanoate, 3HD: (R)-3-hydroxydecanoate, 3HDD: (R)-3-hydroxydodecanoate.

A linear correlation between monomer composition of PHA and mixture of saturated and unsaturated fatty acids has been elucidated in *Pseudomonas putida* GPO1 [156] and concept of PHA production as tailor made polymers was productively applicable, whereas polymer composition of PHA obtained from plant oils, glucose, and mixture of plant oils and glucose was dissimilar [157]. A study also reported that *Pseudomonas putida* and *Pseudomonas aeruginosa* as two different biological systems reflected differences between PHA synthase substrate specificity and route of PHA metabolic biosynthesis [158]. Plant oil and fats rich in different fatty acids when used as substrate have complex relation with respect to monomer composition of PHA formed and demand challenging research for PHA production as tailor made polymers [158].

3.2. Organic Loading Rate and Acids. Reports suggest that the increase in organic load is in direct relation to increase in PHB accumulation [159, 160]. High substrate availability (feast conditions) takes longer time to store maximum of PHB. Fewer organic loads result in faster PHB production. PHA production aptitude depends on substrate concentration because stored PHB was significantly high at higher substrate loading rate equal to 40.3% of CDW [161].

At different organic loading rate of 2.91 kg COD m⁻¹ day, 3.54 kg COD m⁻³ day, 4.58 kg COD m⁻³ day, and 7.53 kg COD m⁻³ day, PHA production was 25%, 15%, 8.5%, and 6%, respectively. Result obtained showed that maximum productivity was obtained with low substrate loading rate [153]. Effect of organic loading rate of synthetic acid and acidogenic effluent, namely, OLR1, OLR2, OLR3, and OLR4 (kg COD m⁻³ day), on PHA production and composition was evaluated in *Pseudomonas otitidis*. Synthetic acids (composed of acetate, propionic, and butyric acids) concentration varied for all different organic loading rates and acidogenic effluents had pH of 4.2, VFA at 9.85 g L⁻¹, COD of 24.52 g L⁻¹, and carbohydrate at 13.75 g L⁻¹. With increase in value of OLR from OLR1 to OLR4, time taken for maximum production also increased. Highest PHA accumulation was observed with OLR2 followed by OLR3 then OLR1 and last OLR4. With OLR4, cells experienced sudden shock load of concentrated acids which led to impeded growth and less PHA production. From AE, similar trend was followed in respect to time but OLR3 showed maximum accumulation followed by OLR4, then OLR2, and last OLR1 [25]. Effect of high organic load from 8.5 to 31.25 g COD L⁻¹ day on PHA production by mixed culture in SBR has been studied and concluded that OLR of 20 g COD L⁻¹ day showed the best performance

in regard to biomass productivity and storage capacity and further *Alcaligenes*, *Thauera*, and *Comamonas* genera spp. were reported as PHA producers based on DGGE [162].

3.3. pH. pH is also a very important factor in relation to PHA productivity and its monomer composition. Study on effect of pH on acidogenic fermentation shows that initial alkaline pH of 9 can generate good amount of VFAs [163]. Study on pH range also revealed that it affected composition of polymer formed in batch fermentation in respect to HV contents. PHA accumulated by mixed culture was higher in HV monomers when shifted from SBR maintained at pH 8.5 to batch reactors maintained at pH 9.5 without any decrease in PHA produced. They suggested that microorganisms maintain cytoplasmatic pH compatible with optimal cell functioning in response to change increase in pH of external environment. HV content of PHA produced increased from 10% to 30% mol with increase in pH from 5.5 to 9.5, respectively [41]. Operational condition at neutral redox condition (pH 7) resulted in PHB accumulation up to 25% of CDW when compared to basic pH 9 (8.5%) and acidic pH 6 (15%) [153]. Acetic acid and propionic acid mixture in different proportion was used as carbon substrate to enrich the culture with organic load of 8.5 g COD L⁻¹ day maintained at pH ranging from 7.7 to 9.5. Enriched culture showed high production rate and yields (389 mg PHA g⁻¹ of nonpolymer biomass) in SBR if maintained at pH of 7.5 with *Lamprospedia hyalina* as dominant bacterial species [164]. Different pretreatment methods applied to wastes, variables influencing selection of PHA producers in SBR, and PHA production reactor are shown in Figure 2.

3.4. Feeding Strategy. Production and composition of PHA varied with feeding strategy employed. Feeding regime affects the monomers compositions of PHA produced. Study by ¹³C NMR spectroscopy showed that the chemical composition and microstructure of produced copolymer P(3HB-co-HV) were significantly affected by feeding regime [165]. Feeding with acetate as carbon source under aerobic conditions in SBR showed no effect on microbial composition whereas substrate utilization rate was higher with pulse feeding mode than continuous feeding mode [166], whereas continuous feeding led to accumulation of PHA up to 64.5% in SBR by activated sludge collected from wastewater treatment plant and food wastes [163]. HV contents of PHA increased by 8% when fermented molasses as source of VFAs was fed continuously compared to pulsewise feeding strategy [22]. Increase by 4.8-fold was reported when whey, a dairy waste product rich in sugars, as substrate was fed intermittently with ammonium sulfate in fed-batch culture of *Methylobacterium* sp. ZP24 under oxygen limiting conditions [167].

Periodic feeding (after every 2 hr day⁻¹) of carbon substrate constituting acetic, propionic, and lactic acids at 8.5 g COD L⁻¹ D⁻¹ resulted in production of co-polymer P(3HB-3HV) and polymer (PHB) from activated sludge enriched by selective pressure in SBR [168]. The increase in OLR (up to 12.75 g COD L⁻¹ D⁻¹) resulted in populations

with high storage capacity and yields. Copolymer P(3HB-co-3HV) with 18% of HV content was produced by periodically feeding the mixture of acetic, lactic, and propionic acids at frequency of 2 hr with dilution rate of 1 day⁻¹. A *Methylobacteriaceae* bacterium, *Flavobacterium* sp., 28 Candidatus *Meganema perideroedes*, and *Thauera* sp. were dominant genera obtained under provided conditions [41]. Culture of *Cupriavidus necator* DSM 545 when pulse fed with soybean oil, rich in fatty acids, produced PHB (81%) on onset of growth limited conditions with exhaustion of P, Cu, Ca, and Fe [169]. PHB production was enhanced in 2-stage fed-batch cultivation of *Pseudomonas putida* when pre-grown cells in glucose were transferred to medium containing octanoate under nitrogen- and oxygen-limiting conditions [170].

4. PHA and Simultaneously Produced Metabolites

4.1. PHA and Biosurfactants. Biosurfactants are amphiphilic compounds with polar and nonpolar heads, known for their interface surface tension reducing capabilities and solubilization of aromatic compounds [171]. Biosurfactants commercialization is still a cost effective process. Biosurfactant production from different renewable substrates and fermentative aspects has been clearly discussed [88]. There is a metabolic resemblance between biosynthetic pathways of PHA and rhamnolipids. Rhl is the enzyme which catalyses the reaction for formation of monorhamnolipids in *Pseudomonas aeruginosa*. It consists of two subunits RhlA and RhlB. RhlA subunit is responsible for β -hydroxy fatty acid dimer moiety formation. Rhl can use both ACP-fatty acids and CoA-fatty acids for rhamnolipids formation. PHA is formed by polymerisation of β -hydroxy acids catalysed by enzyme PhaC, PHA synthase. Thus, PHA and rhamnolipids metabolic pathways compete for β -hydroxy-acids available in the vicinity. PhaG plays a significant role hereby in the formation of CoA-fatty acids from ACP-fatty acids followed by its conversion into polyhydroxyalkanoic acids [172].

Simultaneous production of PHA and rhamnolipids using decanoate as the most appropriate carbon source by *Pseudomonas aeruginosa* IF03924 has been studied. They were produced at stationary phase and there existed a time lag between the attainment of maximum production of PHA and rhamnolipids [33]. Later, it was reported that β -oxidation of fatty acids is rate-determining step for simultaneous production of PHA and rhamnolipids in *Pseudomonas aeruginosa* IF03924. The optimum temperatures for PHA and rhamnolipid syntheses were 30°C and 28°C, respectively, suggesting that the product ratio between these two products can be controlled by changing temperature. Similarly, *Pseudomonas aeruginosa* IF03924 as inoculum and hydrolyzed palm oil as possible source of fatty acids and glycerol showed simultaneous production of PHA and rhamnolipids at the end of growth phase when nitrogen source gets exhausted [34]. In a similar investigation, *Thermus thermophilus* HB8 as inoculum and sunflower seed oil or oleic acid as substrate showed simultaneous production of PHA and rhamnolipids and also depicted that cells can be considered as "microbial

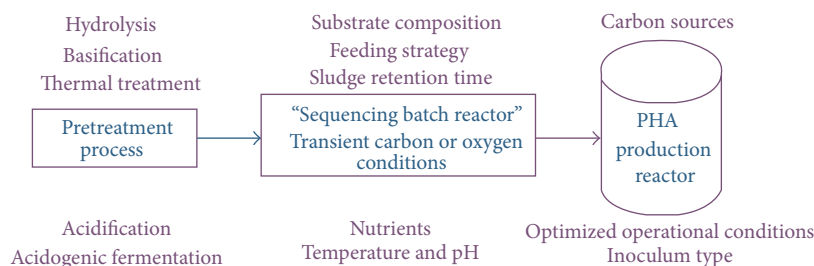


FIGURE 2: Different pretreatment methods applied to wastes, variables influencing selection of PHA producers in SBR, and PHA production reactor.

cell factories” to separate exoproducts (rhamnolipids) before disruption to recover stored intracellular inclusions (PHA) [173].

Both PHA and rhamnolipids, produced by *Pseudomonas* sp. have profound application in industries in reference to its eco-friendly properties but economy of individual production at large scale limits its applicability. Rhamnolipids and PHA could be separated using conventional techniques as discussed [33]. The use of nonpathogenic strains of *Pseudomonas* sp. to simultaneously produce rhamnolipids and PHAs using renewable resources can be helpful towards reducing the cost of overall production [174].

4.2. PHA and Extracellular Polymeric Substances. Extracellular polymeric substances protect and supply energy to cells when subjected to unfavourable growth conditions. Basically it is mixture of high molecular polymers which serve as supplier of carbon units when substrate is limited. As reported, EPS an extracellular product and PHB an intracellular product both are produced when organisms are under starvation conditions [37]. Simultaneous production of EPS and PHB was reported in *Ralstonia eutropha* ATCC 17699 [37], *Azotobacter beijerinckii* WDN-01 [175], and *Anabaena cylindrica* 10 C [176]. Positive and negative effects of nitrogen concentration on EPS production were also reported [37, 176]. EPS production was initiated in nitrogen-limiting conditions with fructose and glucose as efficient carbon sources in *Azotobacter chroococcum* [36], whereas increase in supply of nitrogen and glucose enhanced EPS production by *Ralstonia eutropha* ATCC 17699 in batch cultures [37]. Decomposition of EPS served as nitrogen source for PHB synthesis. On the other hand, PHB decomposition served as supplier of carbon units when external carbon source gets depleted [94].

Pseudomonas mendocina NK-01 produced simultaneously alginate oligosaccharides extracellularly and *mcl*-PHAs intracellularly using glucose as main carbon source. Glycolysis converts glucose to acetyl-CoA which either enters into TCA cycle or gets converted into PHA. Acetyl-CoA via TCA cycle is converted to oxaloacetate and then to fructose-6-phosphate via gluconeogenesis. Fructose-6-phosphate is converted to mannose 6-phosphate and then subsequently to alginate oligosaccharide [35]. A diagrammatic representation of simultaneous production of extracellular and intracellular products is shown in Figure 3.

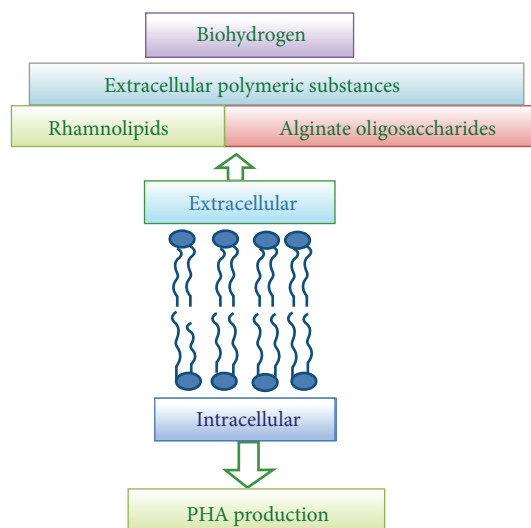


FIGURE 3: A diagrammatic representation of simultaneous production of extracellular and intracellular products.

4.3. PHA and Biohydrogen. Biological production of H_2 gas is a promising technology attributed to its purity and less energy requirements [177]. Many purple nonsulfur bacteria like *Rhodobacter sphaeroides*, *Rhodospirillum rubrum*, *Rhodopseudomonas palustris* WP3-5, *Rhodopseudomonas palustris* 420 L, and *Bacillus* sp. are reported to produce H_2 and PHA under nutrient-limited conditions [38, 39, 178]. The literature shows that although PHB synthesis does compete with H_2 production in *R. palustris* WP3-5, it is still conducive to H_2 production when strain WP3-5 is in a stressful condition [39]. H_2 gas production occurred at first stage (nitrogen-limited) and subsequent transfer of culture to second stage (phosphorus-limited) where PHB production takes place and it could be efficient light-dependent process to reduce the cost of bioproducts [178]. Some nonphotosynthetic bacterium like *Bacillus thuringiensis* UGU45 and *Bacillus cereus* EGU44 produced H_2 gas in range of 1.67 to 1.92 mol H_2 mol $^{-1}$ glucose $^{-1}$ and 11.5% of CDW of PHB when grown on glucose in two-stage systems [38]. A study reported that stored PHB facilitated *Rhodopseudomonas palustris* WP3-5 bacterium to retain its H_2 producing ability at optimum pH when subjected to extra environmental stress conditions like suboptimal pH value [39]. Integration of effluent generated

from biohydrogen producing reactor serving as primary substrate for PHB production under anoxic conditions by aerobic consortia can be effective and efficient process [153].

5. Discussion

Hereby we have discussed how different types of physiological stress or ecological conditions could stimulate the PHA production in microbes as to survive and reproduce. Carbon and nutrients content of wastes vary depending on the point sources. Coupling of waste effluents or solid organic matter to acidogenic fermentation results in generation of VFAs. Anaerobic fermentation of olive oil mill effluents increased the percentage of VFAs from 18% to 32%. Also, the concentration of produced PHA increased linearly with increase in concentration of substrate fed [114]. With control on pH, different composition of VFAs could be obtained after acidogenic fermentation of palm oil mill effluents [179]. This can be more pronounced if hydraulic retention time (HRT) is kept for longer duration. Research on effect of HRT on acidogenic fermentation of dephenolized olive mill wastewater showed that 5 days of HRT could result in maximum conversion of COD into VFAs, that is, 78% of effluent COD [180].

PHA accumulating population varies with type of substrate being fed and substrate uptake capabilities of the microbial population. Butyrate and acetate were preferably taken up by *Azoarcus* and *Thauera* and broad range of substrates were taken up by *Paracoccus* [42]. PHB long-standing famine phase results in selection of those accumulating good amount of PHB [181]. It had been elucidated that, irrespective of sludges origin, PHA production was significantly dependent on operational sludge retention time [182]. Sludge retention time of 5 to 10 days showed maximum PHA production [141]. Sufficient supply of oxygen shifts cellular activities towards protein, glycogen, and other metabolic activities and thus inhibits PHA production because of availability of ATP and NAD^+ reducing equivalents, thereby necessitating proper oxygen management [23, 183]. PHB produced under anoxic condition was higher than under aerobic conditions [116]. Two hours of aeration was very effective towards PHA production [141]. Thus, all together various biotic and abiotic variables interact with each other and result in effective PHA production with varying composition in SBR.

Concluding, it had been observed that number of bacterial genera are actively involved in PHA accumulation with silent features of degrading hydrocarbon, dyes, exhibiting PGPR traits, accumulating glycogen and polyphosphate, synthesising antibiotics, inhabiting high or low salts, and synthesis of food using sunlight as energy source. PHA production and simultaneous degradation of toxic effluents from industries can be effective towards sustainable development. Evaluating the capabilities of archaea tendency to grow under salty conditions with no stringent and nonsterilized conditions suggests a cost effective process. Salts in the sludges can be recycled to solve or minimize entrenched ecological problem related to postfermentation residues disposal. Thus,

use of extremophiles biomass seems to develop attractive option to produce metabolites at commercial scale [184].

Organisms reported for simultaneous production of endopolymers and exopolymers are seemingly promising. Rhamnolipids and PHA are produced simultaneously by some *Pseudomonas* sp. and have common steps in respect to their metabolic biosynthesis [174]. Biosurfactants and PHA can be produced using broad range of substrate and organic wastes [24, 88]. Similarly, extracellular polymeric substances and biohydrogen gas [37] were reported to be produced simultaneously with PHA as discussed earlier in this paper. Thus, with proper understanding of ecological niches of strain of interest, future research should be towards use of wastes as renewable substrates, organisms with possibilities of simultaneously producing other metabolites as inocula, and subsequent production under well-optimized conditions in either batch or continuous production system.

6. Conclusion

PHA, a biodegradable stored substance of microbes, is alternative to petroleum-derived plastics. A technology needs serious remarks to develop an integrated system for separation of high-valued microbial synthesised products in concern with reduced cost production. Under environmental stress with excess of carbon available some microbes reportedly produce PHA intracellularly with simultaneous production of other metabolites. PHA production growth kinetics showed it to be produced at stationary phase in most of reported cases. These significantly important biologically produced by-products demand a vigorous establishment of industrial process contributing as key element towards high-cost production. The advent research on simultaneous production of polymeric substances (intracellularly as well as extracellularly) opens the door to new aspects of understanding the metabolic links and ecological prospects (i.e., defining role, diversity, and evolution). To have this high-valued endopolymers and exopolymers simultaneously produced using the same organisms under optimized conditions using domestic, industrial, agricultural, or industrial effluents waste may help us to combat the issues related to environmental pollution, cost production, and its commercialization into the market as well. Thus, we can have a real question to our mind: if waste is really “waste” in relation to production of high value products from its residing microbes.

Objectives

The main objectives of the review are

- (1) comprehensive discussion on main producers of PHA and their ecological niches,
- (2) to have idea about the main factors which affect the PHA production and composition,
- (3) understanding of PHA and simultaneous production of metabolites.

Highlights

The highlights of paper are

- (i) comprehensive summary of PHA producers and their ecological niches,
- (ii) discussion on main factors which affect the PHA production and composition,
- (iii) biological processes of PHA and simultaneous production of metabolites using wastewaters as alternative resources.

Conflict of Interests

The authors declare that there is no conflict of interests.

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