Solid-state Fermentation for the Production of Poly(hydroxyalkanoates)

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Poly(hydroxyalkanoates) (PHAs) can be synthesized by adopting different microbial fermentation strategies, of which submerged fermentation has been exploited largely. In the past few years, solid-state fermentation (SSF) has been reassessed as an alternative to submerged fermentation, and could be a possible strategy for the cost-effective production of PHAs. The capital investment for SSF is usually lower than that of submerged fermentation and the cost of raw materials for SSF would be cheap, since it uses waste agricultural residues. These positive factors make SSF a potential technique for PHAs production. However, this method is still too immature for commercialization. The major drawback to address is the proper maintenance of the culture conditions under SSF. The present review discusses the current developments in solid-state fermentation for the production of PHAs and addresses the various issues for its commercialization.

Key words:
solid-state fermentation, polyhydroxyalkanoates, biomass, bacteria

Introduction

Polyhydroxyalkanoates are biopolyesters stored as reserve source of energy by a great number of prokaryotes. It is stored as intracellular granules and during normal growth, its content in the cells usually does not reach high concentrations, usually in the range of 2–10 %, but the PHB contents can reach more than 80 % of the dry biomass if growth is limited by depletion of an essential nutritional compound in the production media. Nitrogen, phosphorus, sulfur or magnesium sources are usually limited for providing stress for the accumulation of PHA in excess available carbon source. In addition to these nutritional components, a low dissolved oxygen concentration also leads to enhanced PHA accumulation. Solid-state fermentation (SSF) is defined as the fermentation process in which microorganisms grow on solid materials in the absence of free water. This is probably the oldest method used by man to utilize microorganisms for specific purposes. In recent years, SSF has had wide acceptance and applications in the development of several commercially important bioprocesses and products[1].

Fermentation techniques are well exploited for the production of PHA. Both submerged and solid-state fermentation can be adopted for the synthesis of PHA. There are several reports available on the production of PHA under submerged fermentation, and very few reports are available on solid-state fermentation. The present review discusses the recent developments in the production of PHA under solid-state fermentation.

Solid-state fermentation

Solid-state fermentation is a low cost fermentation process, particularly suitable to the agro-industrial residues as the substrates for the bioprocesses. It deals with the controlled growth of the microorganisms, mainly on the surface of water-insoluble substrates, in the presence of varying amounts of available water. Moo-Young *et al.,* (1983)[2] proposed the term SSF for all those processes where growth of microorganisms on moist solid particles in substrate beds, in which the interparticle spaces are filled with a continuous gas phase with a minimum of liquid water present in these interparticle spaces. The solid material serves as nutrient source as well as support for microbial growth. For bacteria, the growth tends to be confined to a biofilm on the particle surface. The moisture content is to be maintained sufficiently high in order to achieve water activity levels that are not limiting to microbial growth and should not exceed the water absorption capacity of the solid material[3].

Water activity is a thermodynamic parameter defined in relation to chemical potential of water. It represents the availability of water for reaction in the solid substrate. Water present in SSF system exists in a complex form within the solid matrix or as a thin layer adsorbed on the surface of the solid[4]. Microbial growth will be critically affected by decrease of water activity (a_w). Reduction of a_w ex-

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tends the lag phase, which in turn decreases the specific growth rate and results in low amount of biomass[5]. Bacteria generally require a higher a_w when compared with fungi. The a_w of the medium is a crucial factor for mass transfer of the water and solutes across the cell membrane, and the control of this parameter could be used to modify the metabolic production or excretion of a microorganism[6,7].

The typical examples of SSF are the traditional fermentation, such as Japanese "koji", Indonesian "tempeh" and French "blue cheese". SSF processes have been proven to be particularly suitable for the production of the hydrolytic enzymes by the filamentous fungi, since they reproduce the natural living conditions of such fungi[8]. The selection of an adequate solid substrate (support) for performing the solid-state cultivation is essential, since the success of the process largely depends on it.

The wide range of solid materials used in SSF can be classified into two major categories: inert materials, which only act as a solid support offering an attachment place for the microorganism, and natural materials, which not only function as solid support but also act as carbon source for the microorganism. These materials are typically starch or lignocellulose-based agricultural products or agro-industrial sources, such as grains and grain by-products[9]. The utilisation of this kind of material helps in solving both the economic and the environmental problems caused by their disposal. SSF has found increased application using such materials (for example, straw, bran, oil cakes, etc.) for the production of antibiotics, surfactants, biocides and enzymes[9,10,11,12,13,14].

SSF process can also be classified based on whether the seed culture for fermentation is pure or mixed. In pure culture SSF, individual strains are used for substrate utilization and with mixed culture; different microorganisms are utilized for the bioconversion of agro-industrial residues simultaneously.

More than 40 % of the manufacturing costs of PHA are contributed by the raw material, and an additional 40 % of the cost is contributed by the downstream processes[15]. Adopting SSF as an alternative strategy for PHA production requires low capital investment and allows the usage of low-cost agro-residues as raw material, thus reducing the overall process economics[16]. Depending on the final application of polymer, the high-yield SSF processes can open up new avenues of completely eliminating downstream processing steps, and the fermented solids containing PHA can be directly processed[17].

Importance of solid-state fermentation

SSF offers several advantages in comparison to submerged fermentation (SmF), such as concentrated product formation, less water requirement, etc., but this system is susceptible to the water content, pH, oxygen gradients and accumulation of metabolic heat, making scaling up difficult.

Advantages of SSF compared to SmF

1) Quite simple media, generally consisting of an unrefined agricultural product, which may contain all the nutrients necessary for the microbial growth. The substrate may require less pretreatment, such as cooking with water to moisten or swell the substrate, or cracking of the substrate surface to increase the accessibility of the internal nutrients, or milling of large substrate pieces into small particles. In general, the pretreatment for SSF only has to increase the accessibility of the nutrients, while pretreatment for SmF must achieve extraction of the nutrients into the bulk liquid phase.

2) Restricted availability of water may help to select against the undesirable contaminants, especially the bacteria and yeast, although contamination by other fungi may be a problem. Low moisture availability may favor the production of specific compounds, which may not be produced or may be produced poorly in SmF. For example, *Monascus* produces up to 10-fold more red pigment in SSF than in SmF[18]. The products may have slightly different properties when produced in SSF and SmF. Deschamps and Huet, 1984[19] found that the glucosidase produced by *Aspergillus phoenicis* in SSF was more thermo-tolerant than that produced in SmF. From industrial applications point of view, this characteristic (thermo-tolerance) is desirable.

3) The substrates are more concentrated in SSF than SmF, which means that smaller reactors can be used in SSF compared to SmF to hold the same amount of substrate. Smaller reactor volumes result in lower capital and operating costs.

4) Forced aeration is often easier in SSF than in SmF because the interparticle spaces allow the transfer of the fresh air to thin films of water at the substrate surfaces. These thin films can have a high surface area allowing rapid oxygen transfer. Due to the nature of the substrate mass, aeration usually tends to require lower pressures than are needed for SmF, and vigorous agitation is not required[20].

5) Spore inoculum can often be used for processes involving fungi. This avoids the need for large seed tanks. Spores have the disadvantage of larger lag times due to the need for germination. However, the use of a spore inoculum facilitates uniform dispersion.

6) The downstream processing and waste disposal is often simplified or minimized. Often, the whole product is used, especially if it is intended as a feed supplement, in which case no wastes are generated. Kumar and Lonsane (1987)[21] calculated a 50–60 % saving in the downstream processing costs for the recovery of gibberellic acid from SSF compared to SmF.

7) Use of inert support conditions provides good conditions for fermentation along with the purity of the product[22,23].

Disadvantages of SSF compared to SmF

There are however some disadvantages of SSF over SmF:

1) SSF is restricted to the microorganisms, which can grow at reduced moisture level and therefore the range of possible processes and products are more limited than with SmF.

2) The removal of the metabolic heat generated during the growth may be a problem, especially at large-scale.

3) The solid nature of the substrate causes problems in the monitoring of the process parameters. The probes developed for SmF are often unsuitable for SSF. In addition, it is very difficult to ensure even distribution of any substances added during the process; hence, the effective control of parameters, such as the pH, moisture content, and substrate concentrations is virtually impossible. The biomass, which is a fundamental parameter in the characterization of the microbial growth, is almost impossible to measure directly in SSF involving fungi, because fungi penetrate into and bind themselves tightly to the substrate particles[2]. Indirect methods of the biomass estimation are of questionable reliability. In addition, studies are complicated by the poor reproducibility caused by the variation between the batches of the substrates, and the complex and heterogeneous composition of the substrate particles themselves.

4) The mass transfer in the solid phase is limited to the diffusion, while in SmF the mass transfer is facilitated by the agitation of the liquid. The intra particle diffusion may become the rate-limiting step in the growth in SSF[24].

5) The cultivation times are often longer in SSF compared to SmF due to the lower specific growth rate of the microorganisms. The yields are often lower in SSF than in SmF. This might be due to increased maintenance requirements in a physiologically demanding environment. Despite low growth rates and poor substrate conversion, the volumetric productivity may be similar or higher in SSF due to the highly concentrated nature of the solid substrate.

6) The extracts containing the products obtained by leaching of the fermented solids are often viscous in nature. This high viscosity restricts vacuum concentration of the crude extract or further concentration leads to paste formation. Enzyme precipitates from such crude extracts are also gummy and resist drying, powdering, blending and reconstitution. Due to these phenomena, the commercial enzymes produced by SSF are more dilute than those obtained in SmF, though they are much cheaper when considered on the basis of activities.

7) A major problem with SSF is that many important basic scientific and engineering aspects are yet to be characterized. Much of the work to-date is either qualitative or empirical due to the difficulties encountered in the quantification. Little is known about the mode of the growth of fungi within the substrate masses composed of irregularly shaped solid particles. Table 1 shows comparison of SSF and SmF.

8) One of the major inherent problems associated with adopting SSF strategy for PHA production is the difficulty in retrieving bacterial cells from the solid substrate after fermentation[25].

Table 1 *Comparison of SSF and SmF*

Factor	SmF	SSF
Substrates	Soluble substrates (sugars)	Insoluble substrates: starch, cellulose, pectin, lignin
Aseptic conditions	Heat sterilization and aseptic control	Vapour treatment, non- sterile conditions
Water	High volumes of water consumed and effluents discarded	Limited consumption of water; low a_w. No effluent
Metabolic heating	Easy control of temperature	Low heat transfer capacity
Aeration	Limitation by soluble oxygen, high level of air required	Easy aeration and high surface exchange air/ substrate
pH control	Easy pH control	Buffered solid substrates
Mechanical agitation	Good homogenization	Static conditions preferred
Scale up	Industrial equipment available	Need for engineering and new design equipment
Inoculation	Easy inoculation, continuous process	Spore inoculation, batch
Contamination	Risks of contamination for single-strain bacteria	Risk of contamination for low-rate growth fungi
Energy consideration	High energy consuming	Low energy consuming
Volume of equipment	High volumes and high cost technology	Low volumes & low costs of equipment
Effluent & pollution	High volumes of polluting effluents	No effluents, less pollution

Production of PHA under solid-state fermentation

Solid-state fermentation (SSF) serves as an alternative strategy to conventional submerged fermentation. The main advantage of this strategy is that agro-industrial residues can be used as substrate for the production of high value products. SSF can be used as an alternative strategy for the production of PHA. Few reports were available on PHA production by solid-state fermentation (SSF). One of the main limitations of adopting solid-state fermentation strategy for PHA production is the recovery of biomass from the substrate after fermentation.

Oliveira *et al.,* 2004[26] first reported the utilization of agro-industrial wastes for the production of PHA by *Cupriavidus necator*. Soy cake alone or supplemented with sugarcane molasses were used as substrate for PHB production. The PHB productivity and content were 4.9 mg g^{-1} medium in 60 h and 39 % w/w respectively. The results indicate that solid-state fermentation can be used as an interesting alternative for the production of PHB, allowing the production of biopolymers with adequate properties from low-cost, renewable resources. Oliveira *et al.,* 2007[27] characterized PHB produced by *Cupriavidus necator* under SSF. Various techniques like x-ray diffraction, differential scanning calorimetry, nuclear magnetic resonance and infra-red spectroscopy were carried out to investigate the chemical structure, thermal properties, as well as crystalline morphology of the samples produced by SSF using soy cake or soy cake supplemented with sugarcane molasses (2.5 %). Solid-state fermentation process provides a biopolymer that is identical to a commercial PHB produced by submerged fermentation, as well as to other PHB data reported in literature. The only differences noted for the polymers produced by SSF were a higher molar mass and a lower degree of crystallinity, which both represent advantages for the solid-state fermentation process, since these properties enable a broader range of applications for the PHB produced by this method.

Rocha, 2005[28] reported utilization of other residues like sunflower cake, soy bran, and a solid residue from biodiesel industry for the production of PHA by *C. necator*. The polymers produced with all these substrates were PHB, and the highest production was observed with biodiesel waste. The study revealed that supplementation with peptone does not improve PHB productivity. Biodiesel waste contains toxins derived from castor oil seeds and cannot be used as an animal feed. Utilization of this residue for value added biopolymer makes the process economically viable. Supplementation with sugarcane molasses, yeast hydrolyzate, corn steep liquor and glycerol revealed supplementation of sugarcane molasses improved a 1.85-fold increase in PHB production. Yeast extract and corn steep liquor supplementation has no influence on PHB production. Various process parameters like incubation temperature and inoculums concentration were optimized by adopting a fractional factorial design. The optimum PHB production (2.1 g kg^{-1}) was observed at an incubation temperature of 33 °C, 80 h of incubation time, and an inoculum concentration of 6 mg of cells g^{-1} of dry solid medium. In this study, the PHB production by *C. necator* was growth-associated, which is different from submerged fermentation, where PHB production by this bacterium is not growth-associated[29].

Ramadas *et al.,* 2013[25] developed a novel solid-state fermentation strategy for the production of poly-3-hydroxybutyrate using polyurethane foams by *Bacillus sphaericus* NII 0838. One of the major inherent problems associated with SSF for the production of PHB is the difficulty in retrieving bacterial cells from the solid substrate after fermentation. This can be overcome by using inert support like polyurethane foams (PUF) in SSF. PUF possess several advantageous characteristics like high porosity, low density, and high water absorption capacity. The use of inert support for SSF process has been reported earlier by Zhu *et al.,* 1994[30]. A statistical approach was adopted for optimization of various process parameters affecting growth as well s PHB production by *Bacillus sphaericus* NII 0838. The factors optimized by response surface methodology (RSM) were inoculum size, pH, and $(NH_4)_2SO_4$ concentration. Under optimized conditions – 6.5 % inoculum size, 1.7 % (w/v) $(NH_4)_2SO_4$, and pH 9.0, PHB production and biomass were 0.169 and 0.4 g g^{-1} PUF, respectively. Maximum PHB yield was observed with an inoculum size of $8 \cdot 10^8$ CFU mL^{-1}, 1.7 % (w/v) $(NH_4)_2SO_4$, and pH 9.0. Jack fruit seed hydrolyzate was used as carbon source. There was a fourfold increase in PHB production after statistical optimization. The inert nature of PUF helps in the easy recovery of bacterial cells, and the solid supports can be reused. This is the first report on the utilization of PUF as an inert support supplemented with mineral nutrients for the production of PHB.

PHB production from a marine sponge associated *Bacillus megaterium* MSBN 04 was reported by Sathiyanarayanan *et al.,* 2013[31]. Several substrates, like tapioca industry waste, palm jaggery, and horse gram flour supplemented with mineral salt solution were evaluated for PHB production. Maximum PHB and biomass yield (8.637 mg g^{-1} substrate and 15.203 mg g^{-1} substrate) was observed with tapioca industry waste. Among the various process variables selected for optimization, nitrogen source and trace element solution were found to be critical factors affecting PHB production by *Bacil-*

lus megaterium MSBN 04. This is the first report on PHB production by *Bacillus megaterium* MSBN 04 under solid-state culture.

Choice of substrates

Substrates used in SSF vary in composition, chemical nature, particle size, as well as mechanical properties[32]. These factors play an important role in the overall process design and product development. SSF gained importance in industrial fermentation due to several advantages of this process, like low energy requirement, high product yields, and generation of less wastewater. The solid matrix either acts as source of carbon or as inert material which supports growth of microorganisms on it.

Lignocellulosic biomass can be used as a substrate for SSF, but the major limitation for the utilization is the high stability of the material. Selection of an ideal strain for biosynthesis of PHA from lignocelluloses-derived substrates depends on the conversion rates of hexoses and pentoses by the organism[33]. If the sugars are not utilized as a substrate by the strain, this leads to accumulation in the fermentation broth and may cause a negative impact on growth, production kinetics, as well as yields.

Utilization of low-cost materials, like waste materials and by-products, will significantly reduce PHA production costs, since raw materials contribute significantly to production costs in conventional PHA production processes[3].

Several agro-residues, like rice bran, wheat bran, barley husk, banana waste, corn cob, sugarcane by-products, wheat straw, and molasses can be used as possible inexpensive substrates for PHA production. A large amount of work has been carried out for the production of PHA using molasses. Cane molasses have been found to be an excellent substrate for PHA production by *Bacillus megaterium*[34].

Up-stream operations

Fermentation involves two main operations – upstream and downstream operations. Operations before starting fermentation are called upstream operations. This includes selection of microbial strain, sterilization of the reactor, preparation and sterilization of culture media, preparation and growth of suitable inoculums of microbial strain, etc.

Microorganisms can be isolated using different strategies, like liquid culture method and solid culture method. Screening involves the use of highly selective procedures for the detection and isolation of microorganisms of interest from a large microbial population. Primary screening allows selection of microorganisms with specific applications, and secondary screening allows selection of microbes based on their production capabilities[35].

Selection of a suitable microbe-producing PHB is the first step. Several screening protocols were available for the selection of PHB-producing microbes. The commonly used dyes for PHB screening were lipophilic dyes, such as Sudan Black, Nile Blue, and Nile Red, which distinguishes between PHB-accumulating and non-accumulating strains. Screening using Nile Red produces an orange red fluorescence upon binding with PHB granules[36]. Lipophilic dyes are prepared in organic solvents, such as ethanol or acetone; hence, a master plate is to be stored before staining with the dye. Fig. 1 shows fluorescent microscopic image of PHB-producing strain. Spiekermann *et al.,* 1999[37] developed a viable colony staining method using incorporation of Nile Red or Nile Blue A in the medium.

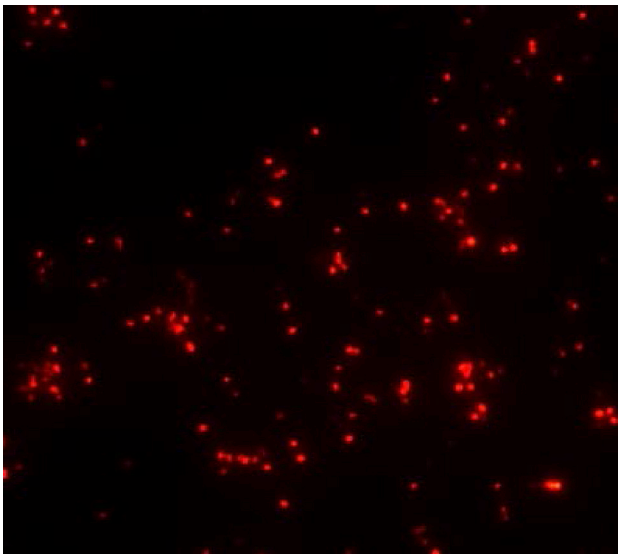

Fig. 1 – *Primary screening profile of bacteria based on Nile Blue staining*

Sterilization is one of the most important processes in industrial fermentation and is usually performed using pure culture in which only selected strains are allowed to grow. If a contaminant exists in the medium or in any parts of the equipment, the production organisms have to compete with the contaminants for limited nutrients. Hence, before starting fermentation, the medium, as well as the fermenter have to be sterilized.

Success of fermentation depends upon selection of a good media and the type of microorganism used for fermentation processes. Medium should contain an energy source, carbon, nitrogen, minerals, chelating factors, buffering agents, as well as antifoam agents. Ideal fermentation medium should contain minimum components to produce maximum product yield. Natural media ingredients have high batch variation, so it is desirable to use defined

or formulated media having very little batch variation. An ideal medium would have minimum components to produce maximum product yield. The essential minerals for all media include calcium, chlorine, magnesium, phosphorous, potassium, and sulphur, which supply the essential elements required for the cells during their cultivation. Other minerals like copper, cobalt, iron, manganese, molybdenum and zinc are required in trace amounts. The trace elements contribute to primary and secondary metabolite production. The specific concentration of the different minerals depends upon the type of microorganism being used. The functions of trace elements include coenzyme functions to catalyze many reactions, vitamin synthesis, and cell wall support[38]. For the development of a medium for large-scale fermentations, factors like nutrient requirement of the selected microorganism, composition of nutrients, as well as cost of ingredients must be considered. For the development of a medium for an industrial process, the stability of the nutrient components is very important. Product concentration, yield and productivity are the important process variables in determining conversion costs. Hence, a medium should be formulated in such a way that the process is economically feasible.

Inoculum media differ in composition from production media. The media are formulated in such a way to quickly yield a large number of microbial cells in their proper physiological and morphological states, maintaining genetic stability of the cells. The inoculum media usually contain a lower level of the main nutritive carbon source. The quality and reproducibility of the inoculum are critical factors that determine the reproducibility of product yields from one production run to another.

One of the main limitations for the commercial production of biopolymer is the high production cost. About 50 % of the production cost is contributed by the carbon source. Utilization of inexpensive carbon sources can reduce the cost. Utilization of agro-industrial residues can reduce the overall cost and make the process economically viable. PHB synthesis takes place when a carbon source is in excess and a limitation of nitrogen or phosphorous.

Downstream operations

All operations after fermentation are known as downstream operations. The methodology adopted for extraction and purification of the poly-3-hydroxybutyrate is one of the key steps in the bioprocess. An ideal purification method is that which leads to high purity and recovery level at a low production cost. Several strategies were adopted for extraction of PHB, which include chemical methods and biological methods.

Solvent extraction is the most commonly used method for recovering PHA from cell biomass. In solvent extraction method, different solvents like chloroform, acetone, propylene carbonate, methylene chloride and other halogenated solvents were used for extraction[39]. In solvent extraction process, the solvents alter cell membrane permeability and thereby solubilise and release PHA which is precipitated using non-solvent, such as methanol and ethanol. One of the main limitations of this technology is that these processes utilize large-scale application of solvents, which leads to a non-eco-friendly process. Extraction using chloroform is the common method, and a purity of 92 – 96 % was achieved by this method[40,41]. Extraction of PHA from *C. necator* using methylene chloride yielded 98 % purity[42].

Various surfactants and sodium hypochlorite were also used for recovery of PHA from cell biomass. In this method, the non-PHA biomass was solubilised. The commonly used surfactants for recovery are SDS, Triton X 100, etc. One of the main drawbacks of this method is that the PHA extracted by this method exhibited low purity. Studies conducted by Ramsay *et al.,* 1990[43] and Dong and Sun, 2000[44] revealed that sequential treatment with surfactant and hypochlorite leads to better and rapid recovery of PHA as well as 50 % reduction in overall cost when compared to solvent extraction.

Biological methods of extraction involve treatment using enzymes. Holmes and Lim, 1990[45] developed the enzymatic extraction process for the extraction of poly-3-hydroxybutyrate. Proteolytic enzymes were used for this process. The process involves a heat treatment followed by enzymatic hydrolysis, surfactant treatment and decolourisation with hydrogen peroxide. The advantage of this pretreatment is that the recovery rate was higher, and the major drawback of this technology is the high cost of the enzyme.

Ramadas *et al.,* 2013[25] extracted PHB from PUF by agitating each PUF cube with 50 mL of distilled water at 250 rpm for 20 minutes. The process was repeated four times to ensure maximum recovery of biomass from PUF cubes. This was centrifuged at 8000 g for 15 minutes. The pellet obtained was lyophilised and used for PHB quantification and biomass dry weight determination.

In addition to raw material cost, downstream operations is a critical cost-determining factor in biopolymer production. Depending on PHA-producing microorganisms, several strategies are available for PHA separation and purification, such as extraction or degradation of non-PHA biomass after harvesting the bacterial cells. None of the downstream processes possesses all the necessary requirements for an efficient and economical large-scale process. The major drawbacks for existing technologies are cost, safety and scalability[46].

Factors affecting solid-state fermentation

Solid-state fermentation is a low-cost fermentation, and one of its main advantages is that it can be carried out using agricultural by-products, like bran, straw, etc. Various physico-chemical parameters must be optimized to obtain the biopolymer at the highest yield. These parameters include incubation temperature, pH, water activity, moisture, aeration, particle size, nature of substrate, and bed properties[32]. Statistical design experiments were usually performed to select the critical factors as well as the interaction between these factors.

Selection of the microorganism for SSF is critical due to low water content and water activity of solid substrate. Water activity (a_w) is an important factor in the determination of microbial growth in SSF system. Due to absence of free water in the SSF system, only microbes that can grow and can carry out metabolic activities at low water activity are preferable[9]. Alteration of a_w can be used as a method to modify product formation and excretion from microbes, which can be done by adjusting relative humidity of the surrounding air.

Choice of substrate is also important in SSF. Generally, water-insoluble substrates were used for SSF. Maintaining temperature is also critical, and due to poor heat transfer leads temperature gradients. Heat generated during SSF is directly proportional to the metabolic activity, especially respiratory activities of microorganism. Heat removal in SSF system is difficult due to low thermal conductivity, and limited amount of water present in the system.

A pH change occurs by production of acids or utilization of nitrogenous compounds, which leads to a decrease or increase in pH. Maintenance of pH is also difficult in SSF, since free water is absent, and change of pH during fermentation cannot be monitored easily. One of the best methods for minimizing pH variation is by using buffers.

Commercialization possibilities

Optimization of bioprocess is one the major factor to reduce the production cost of all biotechnological commercial products. To achieve successful commercialization, economic production system must be sorted out. The cost of raw material is one of the major factors influencing the economy of production. SSF could be a cheap process as it uses cheap, readily available and renewable agro-residues.

The commercialization of PHA production dates back to 1980, when the British company Imperial Chemical Industries (ICI) developed a commercial process to produce poly-R-3-hydroxybutyrate–co-R-3-hydroxyvalerate. These polymers were produced under the tradename Biopol. However, ICI was unable to produce Biopol cheaply enough to compete with conventional plastics. Monsanto purchased Biopol from ICI in 1996. In 1998, Monsanto discontinued its bioplastics operations due to high costs and limited commercial opportunities. It sold its interests to the U.S. bioscience company Metabolix that began researching and developing a cost-effective process for manufacturing PHB-based plastics. In 2006, Metabolix formed a joint venture called Telles with the agricultural giant Archer Daniels Midland to commercialize a bioplastic under the name of Mirel. Tianan (China) market a PHB-coPHV co-polymer as Enmat. Similarly, Tianjin Green Bio-science produces PHB-based polymer called Sogreen. All these commercial processes are based on submerged fermentation.

For economic viability, the production process should be cheap enough to compete with petroleum-derived plastics. Solid-state fermentation would be a possible alternative for the commercial production of PHAs. However, much research and development needs to be performed for a mature process. A major hurdle in SSF for PHA production is the proper maintenance of the composition of the medium. Natural substrates like agricultural wastes are rich sources of carbon and nitrogen. During SSF culture for PHAs, the carbon concentration should be kept high, and it must induce some kinds of stress, like nitrogen or phosphorus limitations, once the biomass has attained its stationary phase. In submerged conditions, it is rather easy to control, but it would be difficult for SSF using heterogeneous substrates like agro-residues. Use of inert substrates like polyurethane foams can be a possibility. Another problem associated with SSF is the separation of biomass after fermentation. After fermentation, the fermented matter should be separated from the agro-residue in order to extract the polymer from microbial cells by solvent or enzymatic extraction. A schematic workflow for commercial production of PHAs under SSF is shown in Fig. 2.

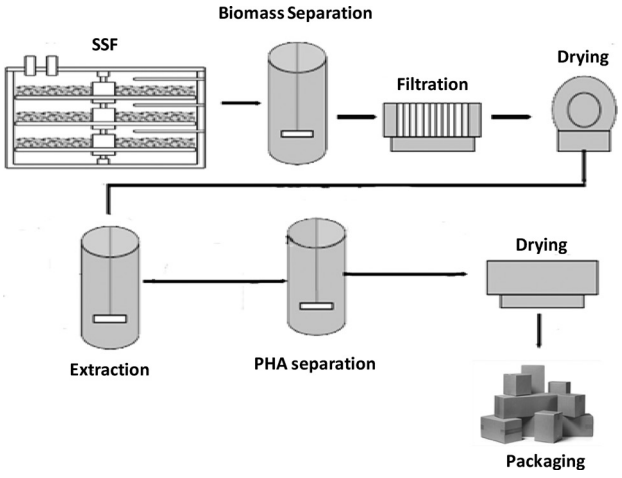

Fig. 2 – *Schematic representation of the workflow for PHAs production in SSF*

Conclusions

Solid-state fermentation offers a low-cost fermentation technique for the production of industrially important products. Even though there are several reports and commercial processes for PHA production under submerged fermentations, the use of SSF for PHAs production are very few. The major problems associated with SSF for the production of PHAs are the difficulties in maintaining the proper nutritional conditions for microbial cells for PHA production and its downstream processing. Another major challenge lies in the cost-effectiveness of the production process. There are many opportunities for research in this direction.

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