Solid-State Bioconversion and Animal Feed Production: Present Status and Future Prospects

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Abstract

An overview of solid-state bioprocesses and its relevance to India is presented. The latest developments, existing problems, and future areas of research arising in methods of analysis, process optimization and scale-up, new reactor designs, instrumentation and control of solid-state bioprocesses, and systems analysis for screening and strain improvement have been discussed in detail. This chapter brings forth the need to integrate the biological and engineering sciences to catalyze the progress in this field to the next level where quantitative analysis, accuracy, and standardization will be achieved.

Keywords

Solid-state bioconversion • Animal feed • Lignocellulosic residues • Solid-state reactors • Optimization • Scale-up

Introduction

Lignocellulosic residue is a vast renewable energy resource abundantly available. About 2×10^{11} metric tons of carbon and 3×10^{13} J of energy (ten times the total energy presently consumed in the world) are fixed annually by photosynthesis in green plants. Agricultural residues comprise a substantial component of this in the form of cellulose, hemicellulose, and lignin. Lignocellulosic residues contain about 70% carbohydrates that can be used effectively and economically in animal feed preparations, provided some measures are taken to improve its utilization. Lignocellulose in wood may be transformed into paper products with the help of solid-state bioconversion (SSB), biopulping, and biobleaching processes. Agricultural residues may be converted into animal feed enriched with microbial biomass, enzymes, and biopromoters and made more digestible by SSB (Villas-Bôas et al. 2002). Lignocellulosic waste may be composted for the manufacture of biofertilizer, biopesticide, and biopromoter products. Postharvest residue may be decomposed on site by filamentous fungi and recycled into the soil

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with improved biofertilizer and bioprotective properties. In this chapter, the state of the art of lignocellulose bioconversion has been presented—the microbes used in the process, the fermentation technology with its engineering aspects, the main products of the bioconversion, and future trends in practical applications.

Most of the carbohydrates fractions in lignocellulosic residues are not available for utilization because of the presence of the recalcitrant lignin molecule. Physical or mechanical treatments, such as milling, grinding, chopping, and steaming, have long been used to improve the feed value (digestibility) of lignocellulosic residues (da Costa Souza et al. 2009; Bhatnagar et al. 2008). However, the small effect in improving digestibility by physical treatment is mainly due to the increase in surface area. Chemical methods have also been used, but with limited success, to improve the digestibility of lignocellulosic residues (Sahoo et al. 2002; Kumar and Gomes 2008). Alkaline agents can chemically break the ester bonds between lignin and hemicellulose and cellulose. Alkali makes the structural fibers swell thus enabling rumen microbes to attack the structural carbohydrates more easily, thereby improving digestibility. However, chemical methods require large volumes of water for removal of chemicals after treatment and add to the already existing problems of chemical pollutants and treatment cost. In addition, it increases the risk of exposing cattle to the danger of latent chemicals in the feed. Among the methods employed, the most promising results have been obtained from biological methods of degrading lignin. The use of intact microorganisms or enzymes produced by them, for the conversion of lignocellulosic residues into animal feed, has been an active area of research (Howard et al. 2003; Shrivastava et al. 2011; Goff et al. 2012).

Solid substrate bioconversion (SSB) is an important process applicable to most kinds of plant biomass. Lignocellulose, the primary constituent of plant biomass, is a feedstock that has been exploited for the production of biofuels, enzymes, and other biochemical products. Crop residues (straw, corn by-products, bagasse, etc.) are particularly suitable for this purpose, since they are available in large quantities in processing facilities. SSB may be defined as a process where microorganisms or enzymes act upon insoluble solid materials derived from natural resources, in the absence of free flowing liquid. Due to low-moisture content, bioconversion can only be effectively carried out by a limited number of microorganisms, mainly yeasts and fungi, although some bacteria have also been used (Singhania et al. 2009). As a major research topic, however, SSB process has not received much attention in the past, and the major efforts were focused on submerged fermentation (SmF). A closer evaluation of these two processes in recent years in several research centers throughout the world has revealed that for certain processes, there are enormous economical and practical advantages of SSB over SmF (Gamarra et al. 2010). These include non-aseptic conditions, use of economical raw materials as substrates, use of a wide variety of matrices (varied composition, size, mechanical resistance, porosity, and water holding capacity), low capital cost, low energy expenditure, lesser solvent for downstream processing, lesser water usage and lower wastewater output, and higher product concentration.

Solid-State Bioconversion for Animal Feed Production

The utilization of solid-state bioprocesses for converting lignocellulosic materials to animal feed has been practiced for more than five decades. However, the literature reveals that development of processes in this field has not integrated the microbiological and engineering sciences to yield what may be called *animal feed production technology*. A recent review restates this problem (Ajila et al. 2012). Other reviews also acknowledge the existence of problem in the development of a viable technology for animal feed production and examine relevant issues (Chiesa and Gnansounou 2011). There is a general agreement that the first period of development of solid-state bioprocesses took place between 1970 and 1975 and the second period of development took place between 1990 and 1999.

There are many important issues related to nutrition, palatability, large-scale production, and process economics that have never been adequately addressed in the literature. In particular, these issues are relevant, important, and of far-reaching consequence in the context of Indian scenario.

Relevance to India

There has been a gradual depletion of grazing land for cattle in India because of the tremendous population pressure on agricultural land. Although the impact of this trend has not yet been felt by farmers holding cattle for irrigation and milk production, a simple extrapolation shows that there will be insufficient green fodder in the near future. This in turn will affect various industries dependent on cattle and cattle products. In particular, it is a matter of concern that the dairy industry may be adversely affected. Although the dairy industry has been generating substantial revenue, to maintain their present turnover, it would be necessary to ensure alternative sources and alternative methods of fodder production. Insufficient fodder is already a problem in arid regions in India, especially in peak summer months. Therefore, developments in the technology for large-scale production of animal feed are needed.

Current Status and Future Prospects

Methods of Analysis

The variables identified and measured to characterize solid-state bioprocesses are lignin, cellulose, and hemicellulose degradation; oxygen uptake rate; and the temperature and humidity of the inlet and exit gases from the reactor. The quantitative methods of analysis for lignin, cellulose, and hemicellulose degradation are the methods that were developed in the 1970s (Updegraff 1969; Morrison 1972). These methods are cumbersome to carry out and are prone to large experimental errors. If these problems are addressed, and faster and accurate methods are developed, it will have a significant impact on scale-up and engineering improvements. Reports on the development of new methods of lignin and cellulose analysis show that researchers have recognized the magnitude of this problem (Agblevor et al. 1994; Dupont and Mortha 2004; Ohra-aho et al. 2005; Scarlata and Hyman 2010; Goff et al. 2012).

The measurement of oxygen uptake rate is well established and may be carried as routinely as it is done in submerged cultivation. Analysis of inlet and exit gases using paramagnetic oxygen sensors and infrared carbon dioxide sensors can give accurate measurements of the oxygen and carbon dioxide concentrations and their rates of utilization and evolution, respectively. An important precaution especially applicable in SSB is to ensure that all the moisture is removed from the sample gas before it reaches the probes. Humidity measurements of the inlet and exit gas streams may also be made using humidity probes. Since these probes are prone to giving saturated readings if condensation occurs on the active element, correct direction of the airflow stream is necessary to ensure that trouble-free online monitoring of humidity.

Process Optimization and Scale-Up

Solid-state bioprocesses are three-phase processes and are difficult to optimize experimentally. Theoretical methods of optimization require first a detailed and accurate model of the process and extensive mathematical analysis to determine the optimum in terms of maximizing or minimizing a given objective function. Many practical problems can only be described qualitatively; hence, only partial solutions from the perspective of the individual researcher appear in the literature (Basu et al. 2002; Raghavrao et al. 2003; Kumar and Gomes 2008). A complete optimization would incorporate both experimental and mathematical treatments in reasonable detail and the application of statistical methods for the evaluation of performance of the optimization exercise. The optimization exercise itself will depend

on the type of reactor and the scale of operation. Therefore, the future of this area of research in terms of underutilized information and its potential application in both existing and new processes is far reaching. What is required at this point is a proposition of an acceptable exhaustive optimization methodology and proof of its success in a process environment.

One such proposition outlined below considers the experimental plan in which the seed culture stage is also included and a theoretical plan that includes modeling and mathematical determination of the optimum. The reactor performance is analyzed statistically:

1. Design experiments based on statistical design methods for carrying out SSB in which the effect of a critical variable, for example, the biomass of inoculum, is accounted for.
2. Write detailed models and identify parameters of the model (Mitchell et al. 2000). Often simple models may give the required information being sought, and in such cases, the simpler model should be accepted. In addition, one may consider the development of the model based on variables, such as lignin degradations (or content) that can be directly measured and offer better process understanding.
3. Describe an objective function for the process considering market forces. Compute either numerically or analytically the optimum conditions.
4. Perform experiments and verify the predictions from experimental and mathematical optimization.
5. Analyze reactor or process performance based on productivity and profit considerations.

Scale-up follows optimization and pertains to the exercise that converts production of small volumes to large volumes. Normally, it is based on one of the variables to which the performance of the reactor exhibits high sensitivity. A detailed analysis has been presented by Mitchell et al. (2000). In their analysis, temperature plays an important role. Clearly, other variables, such as lignin degradation, that also have an impact on performance cannot be included until issues related to accurate measurement of lignin are resolved. Bed height and particle size are physical variables that strongly influence performance because of the effect these variables exert on mass and heat transfer (Mitchell et al. 2000; Valera et al. 2005). Above all, scale-up and design must address practical issues of enabling conductive and convective heat transfer, adequate mass transfer, and material handling (loading and unloading) convenience (Suryanarayan and Mazumdar 2001; Valera et al. 2005). Often, these cannot be achieved without sacrificing an advantage. Further, what is desirable is the development of a procedure using dimensionless variables and is a parallel of that which exists for submerged cultivation bioprocesses (Hardin et al. 2000).

Engineering Problems Associated with Solid-State Bioconversion

The reasons why SSB has not yet found a broad use in India are related to the engineering issues, mainly the low amenability of the processes to standardization and the limited reproducibility of the results. Scale-up represents a particular bottleneck because several different parameters (e.g., temperature, humidity, substrate concentration), which can arise during the course of the process, can have an adverse effect not only in static solid bed processes but also in processes involving reactor content mixing, such as those performed in rotating drums. Interacting relationships among environmental factors such as oxygen content, moisture level, and temperature contribute to the difficult regulation of these parameters. The microbial growth under aerobic conditions in the bioreactor results in a considerable production of heat that causes rapid increase in temperature. This effect, which results in hotspots, is undesirable in bioconversion because the normal temperature of operation is below 40°C, and there exists a danger of products being heat denatured. In the absence of a free aqueous phase, the produced heat is difficult to remove, for example, via the bioreactor double walls. Instead, the cooling of the process takes place through evaporation. This requires very high aeration rates that increase with increasing metabolic activity. Since the reactor contents in a SSB

processes are poor heat conductors, high aeration rates needed for heat removal usually overcompensate for the heat produced within the reactor. This results in loss of humidity, and the water lost must be replenished by spray or mist devices. In a large-scale reactor, water replenishment can cause local condensation that adversely affects reactor performance. In semi-sterile processes, increased water activity can in turn facilitate the growth of bacterial contaminants, whereas in sterile fermentations, it may create local anoxic zones detrimental to the proliferation of microorganisms. Substrate mixing may help, but it is not recommended because many microorganisms respond very sensitively to the shear stress caused by it. Another factor that is difficult to account for is the production of metabolic water by aerobic microorganisms, which can cause problems especially in the formation of conidiospores (Rahardjo et al. 2006).

Reactor Designs

The design of an efficient industrial-scale reactor for SSB is of significance because it produces less effluent than SmF. However, it shows considerable drawbacks such as heat and mass transfer resistance, steep gaseous concentration, and thermal gradients that develop within the medium bed, which may adversely affect solid-state fermenter performances. Agitation and rotation in SSF were often carried out to improve mass and heat transfers, but the shearing force caused by agitation and rotation has adverse effects on medium porosity and disrupts fungal mycelia.

There are four types of reactors used in SSB processes, and each of these designs possesses features that favor certain types of SSB process conditions. The bioreactors commonly used, which can be distinguished by the type of aeration or the mixing system employed, include the following:

Tray: It consists of an ensemble of flat trays. The substrate is spread onto each tray forming a thin layer, only a few centimeters deep. The reactor is kept in a chamber at constant temperature through which humidified air is circulated. The main disadvantage of this configuration is that numerous trays and large floor area are required, making it an unattractive design for large-scale production.

Packed bed: It is usually composed of a column made of plastic, glass, or steel with the solid substrate retained on a perforated base. Through the bed of substrate, humidified air is continuously forced (Durand et al. 1993; Raimbault 1998; Rodríguez Couto et al. 2000). It may be fitted with a jacket for circulation of water to control the temperature during fermentation. This is the configuration usually employed in commercial koji production. The main drawbacks associated with this configuration are the following: difficulties in obtaining the product, nonuniform growth, poor heat removal, and scale-up problems.

Horizontal drum: This design allows adequate aeration and mixing of the substrate, whilst limiting the damage to the inoculum or product. Mixing is performed by rotating the entire vessel or by various agitation devices such as paddles and baffles (Domínguez et al. 2001; Nagel et al. 2001a, b; Prado et al. 2004; Stuart et al. 1999). Its main disadvantage is that the drum is filled to only 30% capacity; otherwise, mixing is inefficient.

Fluidized bed: In order to avoid the adhesion and aggregation of substrate particles, this design provides continuous agitation with forced air. Although the mass heat transfer, aeration, and mixing of the substrate are increased, damage to inoculum through a sheer forces and lower fluidity of material under high humidity conditions may affect the final product yield.

The production of animal feed from lignocellulosic residues in various types of bioreactors has been studied at the laboratory scale and pilot scale (Dasthban et al. 2009; Kumar and Gomes 2008; Bhatnagar et al. 2008). Designing of large-scale bioreactors for solid-state bioconversion (SSB) is different from the design of submerged reactors due to difference in physical characteristics of the medium. It is important to carry out the bioconversion under conditions such that

lignin is degraded as much as possible with minimum utilization of cellulose. In this way, the energy content of the residue is preserved, and the cellulose is exposed for easy digestion in the rumen of the animal. The heat transfer with the solid substrate is one of the major problems of the SSB due to poor conductivity of the lignocellulosic residues (Ashley et al. 1999; Raghavrao et al. 2003; Singhania et al. 2009). The metabolically generated heat during growth creates spatial temperature gradients. Mass transfer is another problem of the SSB process. During scale-up of bioreactors for animal feed production by SSB process, heat and mass transfer problems are major considerations.

The history of design of reactors for bioconversion of lignocellulosic residues to various value products is more than 50 years old. The current status of industrial implementation is constrained to a maximum processing of only a few tons of raw materials. Traditionally, rotary drum reactors have been used for solid-state bioconversion. However, problems of material handling, nonuniform mixing, and low overall yield in these reactors are well known. This had initiated the research for new designs more than two decades ago. Several new designs have also been implemented by the industry, among which the Plafractor designed by Biocon Ltd., India, has been particularly successful (Suryanaryan and Mazumdar 2001). Bhatnagar et al. (2008) developed operating conditions for a 200 L staged vertical reactor for bioconversion of wheat straw by *Phanerochaete chrysosporium*. Kumar and Gomes (2008) and Gomes et al. (2006) developed a vertical reactor, and its performance evaluation was reported for animal feed production. No single reactor design can solve all the problems faced in solid-state bioconversion processes and can provide solutions only to particular problems.

Among the many engineering considerations, the ones that strongly influence the design and performance analysis of reactors are (1) provisions for material handling, (2) accuracy of temperature control, (3) accuracy of humidity control, (4) efficiency of in situ sterilization, (5) efficiency of air circulation and nutrient supply, and (6) ease of reactor cleaning and maintenance. An example of chronology of reactor design from laboratory scale to pilot scale for the bioconversion of lignocellulosic residues to animal feed can take years of development (see Fig. 2.1).

Fig. 2.1 1,200 L packed bed bioreactor. The reactor is a stand-alone unit and operates in all weather conditions

Instrumentation and Control of Solid-State Bioprocesses

The primary difficulty that faces an engineer implementing instrumentation and control for SSB is the heterogeneity of the solid matrix and the poor conductivity of lignocellulosic materials. Along with these difficulties, the three-phase SSBs cannot be provided with instrumentation to measure directly variables such as lignin and cellulose content. The only variables that seem to be amenable to instrumentation are humidity and temperature. Consequently, control of SSBs will continue to be a challenge to the control engineer.

Temperature and humidity, along with mixing (agitation speed) and airflow rate, can be

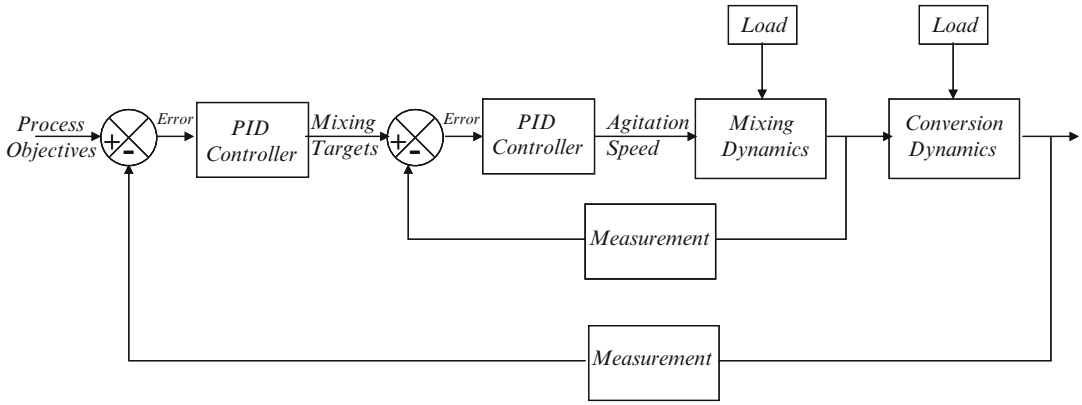

Fig. 2.2 A general cascade control loop applied to solid-state bioprocesses

independently controlled by feedback PID (proportional-integral-derivative) controllers, and at best, cascade controllers may be implemented (Fig. 2.2). Although the classical PID controllers can be used for controlling simple loops, it is not possible to implement goal-oriented time-varying trajectories in solid-state bioprocesses. Advanced control strategies are required. Considering the complexity of modeling three-phase systems in which partial derivatives appear as a regular feature, the application of nonlinear control strategies may not be straightforward, and considerable theoretical developments will be required. However, it will definitely be easier to develop Artificial Neural Networks for prediction and control of solid-state bioprocesses (Nayak and Gomes 2006). In particular, the development of continuous solid-state bioprocesses will mean that this area of research can no longer be ignored.

Systems Analysis for Screening and Strain Improvement

The environment contains a myriad of microorganisms, few of which may be satisfactory with respect to a desired purpose. The diversity of microorganisms may be exploited by searching the strains from the natural environment, which are capable of producing the product of interest. Useful microorganisms performing desired reactions or producing desired products are the unique subsets of all the microorganisms that are available. Microorganism isolated from nature exhibits cell growth as their main physiological property. However, in order to survive in special environments to which they are adapted, then evolve and acquire special characteristics that may be exploited for commercial applications. Some of the commonly used conventional methods for screening of useful microorganisms include the following: (1) isolation of microorganisms in the neighborhood of habitats with enhanced concentration of the substrates, (2) selection of strains based on taxonomic closeness to prior successful strains, and/or (3) enrichment culture. Such screening strategies are empirical, labor intensive, and have low success rate. Screening becomes more difficult in the absence of suitable selection criterion such as antibiotic resistance or production of any specific distinguishable characteristic (such as pigment production). Screening becomes more tedious if one has to search for an organism carrying out a particular type of reaction to give a particular product without any observable property. There are innumerable possibilities with no guaranteed assurance of obtaining the desired organism. In addition, every organism has different culture conditions that may not be provided during initial isolation and screening experiments (due to lack of knowledge of specific growth conditions), and this may lead to non-cultivability of some of the organisms.

Since extensive databases of suitable microorganism are available, it is possible to conceive

that a preliminary search may be possible to target a set of candidate organisms (Malviya and Gomes 2009). This preliminary theoretical analysis based on data-mining techniques may reduce time, effort, and cost of isolating new microorganisms with unknown potential for solid-state bioprocesses. Later successful candidate microorganisms can be improved via modern molecular protocols. Here again, it is possible to carry out a systems based (systems biology) analysis whereby key genes and enzymes can be determined and targeted.

Concluding Comments

Research in solid-state bioprocesses has gained renewed vigor. The trend now is to bring in quantitative analysis and accuracy in characterizing solid-state bioprocesses. Various aspects of this field have opened up a series of research issues that must be addressed to advance this field into the next stage. The progress in the 1990s has been tremendous and has brought solid-state bioprocesses to a threshold of new milestones. It may be expected the coming decade of research will address and solve many bottleneck problems presented in this chapter.

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