Solide-State Fermentation Technology for Bioconversion of Lignocellulose

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Abstract—Solid-state fermentation (SSF) is envisioned as a prominent bio conversion technique to transform natural raw materials into a wide variety of chemical as well as biochemical products. SSF is being successfully exploited for food production, fuels, enzymes, antibiotics, animal feeds and also for dye degradation. The reuse of agro-industrial wastes in SSF processes is of particular interest due to their availability and low cost, besides being an environment friendly alternative for their disposal. In this paper the art of lignocellulose bioconversion by solid substrate fermentation (SSF) is presented.

Keywords—Bioprocess, fungus, SSF, substrates, valorisation.

I. INTRODUCTION

Lignocellulosic biomass is a renewable and abundant resource with great potential for bioconversion to value-added bioproducts. Lignocellulosic materials consist mainly of cellulose (40–60%) with lesser, but significant, amounts of hemicellulose (20–30%) and lignin (15–30%) [1]. There are multiple sources of lignocellulosic waste from industrial and agricultural processes, e.g., citrus peel waste, sawdust, paper pulp, industrial waste, municipal solid waste, and paper mill sludge. Solid substrate fermentation (SSF) plays an important role, and has a great perspective for the bioconversion of this biomass. Finding alternatives for the reuse of these wastes is an objective that has been strongly taken into account by countries around the world, considering environmental and economical aspects. The agroindustrial wastes reuse in SSF processes is of particular interest due to their availability, low cost, and characteristics that allow obtaining different value-added compounds, besides being an environment friendly alternative for their disposal. Additionally, the agroindustrial wastes may be used in these processes as solid support, carbon, nitrogen and/or mineral sources, which would allow obtaining more economical fermentation processes avoiding the use of expensive chemical components in the media formulation.

Cellulase has been demonstrated very effective to the biodegradation and bioconversion of cellulose to monomeric sugar, and many efforts have been made to develop new enzyme with high activity and high stability for the lignocellulose conversion [2,3]. It has proved that lignocellulose can be biodegraded by three types of cellulases: endoglucanases (EC 3.2.1.4, endo-β-D-1, 4-glucanases) (EG), cellobiohydrolases (EC 3.2.1.91) (CBH), and β-glucosidases (EC 3.2.1.21) (BGL) [4-5]. These cellulases can be produced by a wide range of cellulolytic microorganisms [6-7-8].

II. SOLID-STATE FERMENTATION

Solid-state fermentation (SSF) is defined as the fermentation process in which microorganisms grow on solid materials without the presence of free liquid [9]. The concept of using solid substrates is probably the oldest method used by man to make microorganisms work for him. In recent years, SSF has shown much promise in development of several bioprocesses and products. This bioprocess has been subject of several studies and it has been proved that SSF has the important advantage of leading to higher yields and productivities or better product characteristics than submerged fermentation (SmF), which is characterized by the cultivation of the microorganisms in a liquid medium. Another great advantage of SSF compared to SmF is the lower capital and operating costs due to the utilization of low cost agricultural and agro-industrial wastes as substrates. The low water volume used in SSF has also a large impact on the economy of the process mainly because of the smaller fermenter size, the reduced downstream processing, the reduced stirring and lower sterilization costs [10]. Several advantages and disadvantages of SSF over SmF are summarized in table 1.
There are remarkable changes, which occur in the pH of the substrates. These are mainly for the production of acids due to incomplete oxidation of the substrate or uptake of ammonium ions, which will cause the pH to fall, while the release of ammonia by desamination of urea, or other amines will increase the pH. As we cannot monitor pH in the SSF it is very difficult to control the pH. So, it is desirable to use microorganisms which can grow over a wide range of pH and which have broad pH optima.

The most important parameters characterizing an SSF process are the: water activity and moisture content of the substrate, temperature and heat transfer, pH, aeration, mixing, substrates used in SSF and microbial types [12-13].

A. Water Activity And Moisture Content Of The Substrate

Water activity, Aw is defined as the ratio of vapor pressure of an aqueous solution to that of pure water at the same temperature [14]. Water has a solvent function providing nutrients and scavenges wastes. It also holds a structural role involved in the stability and the function of the biological structures. Water activity of substrates has a strong influence on microbial activity. Aw determines the type of organisms that can grow in SSF. Aw of the medium has been attributed as a fundamental parameter for mass transfer of water across the microbial cells. The control of this parameter could be used to modify microbial metabolic production and its excretion [15]. In solid-state, the low moisture within the substrate limits the growth and metabolism of microorganisms when compared to submerged fermentation. Production of secondary metabolites also depends on water activity. It was found that there is a direct relation between the amounts of enzyme produced and activity of water [16-17].

B. Temperature and heat transfer

Fungal growth and secondary metabolite production in SSF are greatly influenced by temperature and heat transfer processes in the substrate bed. During SSF a large amount of heat is generated, which is proportional to the metabolic activities of the microorganism. However, fungus can grow over a wide range of temperatures from range of 20 °C to 55 °C. Nevertheless, optimum temperature for fungal growth could be different from that required for product formation[18]. High temperature effects fungal germination, metabolites formation and sporulation [19]. The fungal activity declined exponentially when optimum temperature for growth reached above maximum [20].

C. Effect of pH and its control

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III. PARAMETERS AFFECTING THE BIOPROCESS OF SSF

A considerable amount of work has been done to understand the engineering aspects of solid-state fermentation.
By using different ratios of ammonium salts and urea in the substrate, pH control in both natural and model SSF system can be obtained [21]. The effect of increasing buffer concentration, effect of pH on the maximum specific growth rate and the optimization of different buffers for the growth and enzyme parameter were investigated [22]. They have also calculated the required buffer concentration by utilizing expected biomass production, initial pH and pH change. Exact control of pH is very difficult in SSF process, but one can maintain pH during the process by using pH-correcting solutions [23]. Many substrates are effective buffers. This is particularly true for protein rich substrate, especially if desamination of protein is minimal. Model has been proposed which relate growth rate to pH based on empirical equations describing experimental data for the effect of pH on growth [23].

D. Aeration

Aeration establishes the O2 and CO2 concentration inside the bioreactor, regulating the removal of CO2 and volatile compounds, the relative humidity, and it also can improve the heat transfer. Limitation of O2 affects the aerobic SSF performance. In SSF fungal mycelia develops on a solid surface and in a void area within the substrate. Fungal mycelia are filled with water and oxygen in the space between substrate [24]. During the FMS, the aeration culture is carried by injection of compressed sterile air through the forced air fermentors [21]. Its flow rate depends on the required O2 of the microorganism involved [25].

E. Mixing

Mixing is a crucial parameter in SSF because it aids to obtain uniformity of the substrate and enhance heat removal and gas exchange. It also influences the process conditions and in particular the water content.

F. Substrates Used In SSF

Substrates used in SSF are generally insoluble in water. In practice, water is absorbed onto the substrate particles, which can then be used by microorganisms for growth and metabolic activities. Bacteria and yeast grow on the surface of the substrate while fungal mycelium penetrates into the particles of the substrates [26].

Lignocellulosic solid-substrate includes wheat straw, corn, rice stover, wheat bran, sugar-beet pulp and wood Substrates containing soluble sugars include grape pomace, sweet sorghum, sugar-beet, pineapple waste, carob pods and coffee pulp. Another strategy is to impregnate inert solid material such as bagasse or hemp with soluble sugars in order to provide a SSF environment for the growth [27].

Natural substrates are easily available and are cheaper than synthetic substrates. But, they generally require pretreatment to make their chemical constituents more accessible and their physical structure more susceptible to mycelial penetration. Physical treatment includes chopping or grinding to reduce size and cracking to make the interior of the particle more accessible. Chemical treatment includes high temperature cooking and acid or alkali treatment. Supplementation of additional nutrients may be required in order to stimulate growth, induce enzyme synthesis or to prolong secondary metabolite production such as the supplementation of 0.5% of glucose or cellobiose, 0.5% peptone, asparagines or yeast extract are in use. The size of substrate particles affects the extent and rate of microbial colonization, air penetration, CO2 removal and downstream extraction. The optimum particle size often represents a compromise between the accessibility of nutrients and the availability of oxygen. Particle size from 1 mm to 1 cm is often used in SSF.

G. Microbial Types

The low amount of free liquid in the substrate affects the whole process of SSF and therefore it becomes the most important feature of SSF. Due to the less availability of free water in SSF process than the majority of liquid fermentations, most of the SSF processes involve fungi, although there are number of reports involving bacteria and yeast. The filamentous fungi are the major group of microorganisms, which predominate in the SSF process. Different species of fungi used in SSF process include many species of Aspergillus, Rhizopus, Alternaria, Fusarium, Mucor, Trichoderma and some species of Penicillium [28]. Most of the species belong to filamentous fungi, as these are best suited because of their ability to spread over and to penetrate inside the solid-substrate. The other advantage of using filamentous fungi is that the fungal mycelia synthesize and release large quantity of extra-cellular hydrolytic enzymes.

IV. POTENTIAL APPLICATION OF AGRO-INDUSTRIAL WASTES IN SSF PROCESSES

The SSF applications for the valorization of agro-industrial products are many and varied. The applications of SSF in biorefineries (biogas, bioethanol) revealed his interest in the recovery of waste agricultural [29-30]. Furthermore, cellulases production by SSF on agro-industrial residues for the production of biofuels is experiencing increased demand.
Thus, by using the SSF, the production of hydrogen, organic acids, ethanol and biodiesel has been realized successfully using solid substrates [31-32]. FMS are also applicable in many other areas. The soil remediation, biodegradation of hazardous compounds, detoxification of agro-industrial residues, biotransformation of crops and their residues improve their nutritional qualities, are among the areas of potential research.

V. FUTURE PERSPECTIVE

Lignocellulose bioconversion by SSF will have an important role in future biotechnologies, mainly because of its favorable economy, and ease of on site operation in agricultural facilities. The focus in SSF application will be on searching for host-specific, SSF targeted fungi, and on their genetic improvement for desired tasks. Those applications will have the greatest perspective, where the transformed lignocellulose is a value added product, biopulp, compost, biofertilizer, biopesticide, biopromoter, or where a fermented SSF product (enzyme, chemicals, etc.) may be used directly in animal feeds or in biofuel reactors. The engineering aspects of SSF must be further developed, with special attention to mixed culturing and the behavior of lignocellulose during SSF.

The advantages of SSF processes overweight the obstacles due to engineering problems involved in fermentation processes. Presently, in most SSF systems fungi are more suitable than bacterial strains and yeasts, but genetically improved or genetically modified bacterial and yeast strains may be made to suite SSF processes. Bacterial cultures decrease the time required for fermentation and hence reduce the capital involved. Many difficulties are involved in SSF, that require extensive attention, such as: difficulty in scale-up, requirement for controlling process variables like heat generation, unavailability of direct analytical procedures to determine the biomass directly in the substrate bed, and heterogeneous fermentation conditions. It has been noted that the use of inert support conditions provides good conditions for fermentation along with the purity of the product [33]. Improvement in bioreactors, process control for continuous SSF is required in the biotechnology industry for producing most value added products. Analysis of existing literature has proved that most value added products could be produced in higher amounts by SSF than by submerged fermentation. Optimization of the proper substrate and additives are an important part of the process. Recent developments made by various researchers, show that control of heat transfer, scale-up in SSF should be solved through prior laboratory-scale mathematical modeling.

REFERENCES


