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Solid state fermentation for microbial products : A review

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ABSTRACT

Solid state fermentation (SSF) technology has been widely used in the industry over the last three decades. The advantages of SSF processes attract the researchers and industrialists to pay attention onto this system than the other fermentation systems even though there are some concerns in this system such as: problem in scale-up, higher requirement for controlling process, unavailability of direct analytical procedures to determine the biomass directly in the substrate and heterogeneous fermentation conditions. Wide ranges of microbes are used in the SSF system to produce diverse economically important products such as enzymes, organic acids, secondary metabolites, antibiotics, biofuels, biocontrol agents and vitamins at low cost and less labor. If the culture growing conditions are optimized and the issues related to scaling up for larger level and controlling the system under different environmental conditions are solved, then SSF could be efficiently used to produce extensive human needs through microbial products without causing any environmental pollution.

Key words: Solid state fermentation; enzyme; scale up; optimize.

INTRODUCTION

Solid state fermentation (SSF) is defined as the microbial cultivation process in the absence or near absence of free water in the substrate [19]. However, there must be enough moisture present to support cell growth [20]. Many organisms produced xylanase enzymes extracellularly with a wide range of activities from 4-400 IU/mL using various substrates both in submerged and solid state fermentation (SSF) processes. Bacteria and filamentous fungi grow typically in nature on solid substrates, such as wood, seeds, stems, roots and leaves of plants in symbiotic associations. Extracellular enzymes are considered important from the industrial viewpoint as they ease the extraction procedure [9]. Diverse group of enzymes have been produced by submerged fermentation techniques at the large scale industrial level. However, the generation and application of these groups of enzymes produced by solid state fermentations are comparatively scarce in with submerged system.

In addition to the conventional applications in food and fermentation industries, microbial enzymes have attained significant role in biotransformations involving organic solvent media, mainly for bioactive compounds. This system offers numerous advantages over submerged fermentation (SMF) system, including high volumetric productivity, relatively higher concentration of the products, less effluent generation, requirement for simple fermentation equipments, etc [7]. However, SSF is currently used only to a small extent for enzyme and secondary metabolite production because of several process engineering problems [16].

Gas phase in SSF is affected by the molecular size and shape, and nature of the gas-filled pores. Availability of spaces between the particles used in SSF ensures the supply of more oxygen. This will improve the enzyme

production in aerobic conditions. Usage of naturally occurring waste materials such as rice bran, wheat bran, saw dust etc, resulted in the improvement in the morphology of the substrate and increase the yield during solid-state fermentation [9]. The growth of microbs in natural substrates is generally very slow. This limitation is overcome by the addition of substances such as carbon and nitrogen sources, regulators ions and vitamins. Application of appropriate mechanical and chemical pretreatment of the raw substrate improves the productivity in shorter time. However, pretreatments should not induce any structural changes in the substrates, that could lead to the alteration of the natural physicochemical properties of the substrate [19, 20, 21]. A scale-up of solid-state processes seems to be difficult due to the generally known problems of heat transfer, the fact that the media is not homogeneous, and difficulties with aeration. During cultivations, these problems could be made worse by the shear sensitivity of the microbe [23].

MICROORGANISMS USED FOR THE ENZYME PRODUCTION IN SSF

A large number of microorganisms, including bacteria, yeast and fungi produce different groups of enzymes. The selection of a suitable strain for the required purpose depends upon a number of factors, in particular upon the nature of the substrate and environmental conditions. Generally, hydrolytic enzymes, e.g. cellulases, xylanases, pectinases, etc. are produced by fungal and bacterial cultures, since such enzymes are used in nature by fungi and bacteria for their growth. In order to achieve high productivity with less production cost, apparently, genetically modified strains would hold the key to enzyme production.

SUBSTRATES USED FOR THE PRODUCTION OF ENZYMES IN SSF

A number of agro-industrial residues have been employed for the cultivation of microorganisms to produce host of enzymes. Some of the substrates that have been used included sugar cane bagasse, wheat bran, rice bran, maize bran, gram bran, wheat straw, rice straw, rice husk, soyhull, sago hampas, grapevine trimmings dust, saw dust, corncobs, coconut coir pith, banana waste, tea waste, cassava waste, palm oil mill waste, aspen pulp, sugar beet pulp, sweet sorghum pulp, apple pomace, peanut meal, rapeseed cake, coconut oil cake, mustard oil cake, cassava flour, wheat flour, corn flour, steamed rice, steam pre-treated willow, starch, etc [4, 7, 10, 22]. Wheat bran however holds the key, and has most commonly been used, in various processes.

The selection of a substrate for enzyme production in a SSF process depends upon several factors, mainly related with cost and availability of the substrate, and thus may involve screening of several agro-industrial residues [10]. In a SSF process, the solid substrate not only supplies the nutrients to the microbial culture growing in it but also serves as an anchorage for the cells. SSF processes are distinct from submerged fermentation (SMF) culturing, since microbial growth and product formation occurs at or near the surface of the solid substrate particle having low moisture contents. Moreover, water has profound impact on the physico-chemical properties of the solids and this, in turn, affects the overall process productivity.

MERITS OF SOLID-STATE FERMENTATION METHOD

Although a number of xylanase productions were performed using submerged systems, SSF was more economical mainly due to the cheap and abundant availability of agricultural wastes which can be used as substrates [19, 21]. Besides that SSF offers distinct advantages over submerged fermentation including economy of space needed for fermentation; simplicity of the fermentation media; higher fermentation productivity, higher end-concentration of products, higher product stability, lower catabolic repression, cultivation of microorganisms specialized for water-insoluble substrates, lower demand of sterility due to the low water activity used in SSF no requirement for complex machinery, greater compactness of the fermentation vessel owing to a lower water volume; greater product yield; reduced energy demand; lower capital and low recurring expenditures in industrial operation; easier scale up processes; lesser volume of solvent needed for product recovery; superior yields; absence of foam build up; and easier control of contaminants due to the low moisture level in the system [9, 19, 20]. SSF has been used for the production of fine chemicals of commercial value from microbial sources such as enzymes, antibiotics, flavouring compounds, secondary metabolites and also microbial biomass which was used as animal feeds (Kang *et al*., 2004). At the end of the cultivation, enzymes can be extracted from the substrate by simply percolating the bioreactor with appropriated buffers which is an easy, economical operation (Person *et al*., 1990). The submerged fermentation process needs close control of the conditions and is expensive [1]. The greatest advantage of SSF over submerged fermentation is the considerable reduction in the cost of plant, machinery, equipment, raw materials and labor [20]. Therefore SSF system, using paddy husk as the support was selected for further studies.

DEMERITS OF SOLID-STATE FERMENTATION METHOD

Delay in the enzyme production in solid state fermentation could be due to the accumulation of nutrients within the cells while they were growing in the activation medium and utilizing it after being transferred to the fermentation medium. As the flasks were not agitated, the distribution of nutrients and oxygen in the medium was not uniform. To maintain uniform bacterial growth, the cells must produce xylanase to utilize the xylan in the medium [13]. Hence there is a delay in the enzyme production in SSF and the organism continuously produced the enzyme for 6 days. Non-production of xylanase beyond 10 days may be due to the accumulation of the toxic substances which might have prohibited the growth of the bacterial cells and caused death. If the flasks have been agitated, higher litres of xylanase production would have been obtained, with a reduction in the production time. The particle size of substrate like paddy husk would not be satisfactory in giving a higher surface area which would ease oxygen diffusion and nutrient absorption and assimilation by the bacteria [2]. The suitability of a substrate depends on the availability of the nutritional content. In SSF, free movement of air and better oxygen transfer can be achieved by incorporating fibrous substrate or porous coarsely granulated substrate to the solid medium [17]. Paddy husk contains higher moisture content and satisfactory porous structure [1]. However submerged fermentation broth can be concentrated to that level obtained by SSF but it involves additional cost [19].

APPLICATION OF SOLID STATE FERMENTATION

1.Enzyme production

Agro-industrial substrates are considered best for enzyme production in SSF. The cost of enzyme production by submerged fermentation is higher compared to SSF. Ideally, almost all the known microbial enzymes can be produced under SSF systems. Literature survey reveals that much work has been carried out on the production of enzymes of industrial importance, like proteases, cellulases, ligninases, xylanases, pectinases, amylases, glucoamylases, proteases, lipases, peroxidases, and xylanases, etc. SSF process for the production of thermostable xylanase by thermophilic *Bacillus licheniformis* was established and the enzyme production was 22-fold higher in SSF system than in SmF system. Enzyme produced in SSF system was more thermostable than in SmF system [5]. The bacterial strain extracted from open xylan agar plate and characterized as *Bacillus pumilus* was able to produce xylanase by both submerged and SSF fermentation systems. In submerged fermentation, *Bacillus pumilus* produced highest xylanase activity of 29.35UmL^{-1} at 42 hours. This system needs continuous electricity supply and the highest activity was obtained within a shorter period (42h) of fermentation than the SSF. Time for higher xylanase production in SSF system is higher (6 days) than that of submerged fermentation system (42 hours). The yield of xylanase production per one gram of xylan was 1465UmL^{-1} in SMF and 8598UmL^{-1} in SSF. Xylanase production by SSF process is more than 5.87 times as compared to that of submerged culture process [12, 13]. Rice bran was used to produce acid proteases with *Rhizopus oligosporus*, and there was no toxin production occurred during SSF.

2. Organic acids

Some of the acids produced by SSF system are citric acid, lactic acid, gallic acid, fumaric acid, and kojic acid. Various agro-industrial wastes such as sugarcane, coffee husk, wheat bran, de-oiled rice bran, carob pods, apple pomace, grape pomace, kiwi fruit peels, pineapple wastes, etc. are most efficient substrates for citric acid production in SSF. Pine apple waste was used as substrate to produce citric acid from *Aspergillus* [18]. *Rhizopus oryzae* produced lactic acid on sugarcane bagasse impregnated with glucose and $CaCO_3$ in SSF [24].

3. Secondary metabolites

Gibberellic acid is a fungal that produces secondary metabolite in its stationary phase. The SSF system increases the yield of gibberellic acids [15]. Accumulation of gibberellic acid was 1.626 times higher in SSF than SMF using wheat bran as substrate with *Gibberella fujikuroi*.

4. Antibiotic

Many antibiotics such as penicillin, cephamycin C, neomycin, cyclosporin A, cephalosporins and iturin are produced by SSF. *Penicillum chrysogenum* produced penicillin with wheat bran and sugarcane bagasse as substrate under high moisture content (70 %). Cephamycin C is produced by *Streptomyces cattleya*, *Streptomyces clavuligerus* and *Nocardia lactamdurans* [6]. Wheat raw supplemented with cottonseed-de-oiled cake and sunflower cake was used as substrates for the production of cephamycin C in SSF system. Antibiotic penicillin was produced by actinomycetes and fungi on solid state fermentations in mixed cultures [8].

5. Biofuel

Ethanol is the most widely used biofuel today. Although it is easier to produce ethanol using submerged fermentation, SSF is preferred due to its lower water requirement, smaller volumes of fermentation mash, prevention of end product inhibition, and disposal of less liquid water, which decreases pollution problems. Cellulosic materials are receiving major attention for ethanol production because of their abundant availability [11]. Solid-state fermentation of apple pomace supplemented with ammonium sulfate and controlled fermentation with *Saccharomyces cerevisiae* has been reported to produce ethanol. Various substrates could be used for alcohol production using *Saccharomyces cerevisiae*. They are sweet sorghum, sweet potato, wheat flour, rice starch, soluble starch and potato starch. However, maximum ethanol production is obtained by when mixed substrates are used [3].

6. Biocontrol agents

Among the various microbial agents, fungal agents are found to have greater potential to act as biocontrol agents because of their different modes of action. *Liagenidium giganteum*, a fungal agent used for control of mosquitoes, act by encysting on their larvae. It uses the larvae as a substrate for growth.

7. Vitamin

Water soluble vitamins B6, vitamin B12, thiamine, nicotinic acid, nicotinamide and riboflavin were produced on SSF with the usage of diverse species of *Rhizophus*. *Klebsiella* is one of the very active vitamin B 12 producer on SSF system [14].

CONCLUSION

SSF technology has been showing a developing trend over the last three decades. The advantages of SSF processes attract the researchers and industrialists to pay attention onto this system than the other fermentation systems. There are concerns involved in SSF that require extensive attention, such as: problem in scale-up, higher requirement for controlling process, unavailability of direct analytical procedures to determine the biomass directly in the substrate and heterogeneous fermentation conditions. If the usage of substrates and additives are optimized and the problem of usage of SSF system is minimized, then SSF would be efficiently used in meeting the demands of the future.

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