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Research Paper

Sustainable Utilization of Agro Wastes for the Production of Bioactive metabolites of Industrial Importance through SSF Technique

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Abstract

Kodagu District of Karnataka state is blessed with abundant natural resources and bears a favorable climate for commercial cultivation of crops such as coffee, areca nut, pepper and cardamom. The waste from these crops at various stages of processing include mainly husks, bran, effluent generated during pulping, washing and cleaning up processes, have limited applications such as fertilizer, livestock feed, compost and such others. These wastes are organic in origin, rich in plant nutrients and contain cellulosic material, hemicelluloses, lignin compounds, poly phenols, proteins etc. Biotechnological applications such as Solid Substrate Fermentation (SSF) technique promote sustainable utilization of agro wastes and development of country's economy. SSF technique using soil microbes holds tremendous potential for the production of bioactive metabolites such as enzymes, natural antioxidants, vitamins, pigments, antibiotics etc. With the background of high crop production in the upcoming years, there is an imperative need to counterpart this production with some utilization and industrial application of agro wastes. The present review highlights the parameters governing SSF of agro wastes for enzyme production by soil microorganisms and their industrial application.

Keywords: enzyme production; solid substrate fermentation; agriculture waste; soil micro flora

INTRODUCTION

Kodagu with its varied agro climatic zone is amenable to grow a wide variety of crops and horticultural products comprising of cereals, spices, condiments, plantation crops, medicinal and aromatic plants, which forms a significant part of the total agricultural produce of our country. Agricultural production leaves considerable amount of agricultural waste. Some of it is recycled into the agricultural production as fertilizer, while large amounts remain unused. In many instances they pose a disposal problem. Uncontrolled burning in the fields is not only a hazardous disposal solution but also wasting useful energy. With efficient collection systems, the cheapest and abundantly available natural waste from agricultural production such as coffee husk, areca husk, sugarcane bagasse, wheat bran, rice bran, corn cob and wheat straw can be utilised for the production of compounds of industrial importance. The use of agro wastes of plant origin for enzyme production using microorganisms appears to be the best option to solve the dilemma of using plant and animal enzymes in various industries, since it adds value to these wastes in eco-efficient processes [1]. Agro waste management can contribute in a significant way to maintain healthy environment.

Solid Substrate Fermentation (SSF) Technique

Solid Substrate Fermentation technique is a biotechnological approach for producing various fermented foods, enzymes, organic acid, ethanol etc. and for the conversion of wastes. The term SSF refers to the growth of the microorganisms on solid media where the liquid phase is bound with the solid phase through physico-chemical ways. In practice growth of microorganisms occur on and inside the solids in near–absence of free liquid [2]. SSF is a biomolecule manufacturing process by microorganisms grown on a solid support and are widely used in the food, pharmaceutical, cosmetics, fuel and textile industries. SSF holds tremendous potential in producing protein rich biomass or enzymes because of the inherently favourable energetic of SSF [3].

Agro wastes as substrates for SSF systems

Substrate expenses can easily accounts for up to 80% of the production cost and fermentation process. It is therefore important that the producer microorganisms be able to grow rapidly on a cheap medium. Agro wastes are generally considered the best substrate for the SSF process. The selection of a solid substrate for a fermentation process depends upon availability of the substrate, and thus may involve screening a large number of agricultural materials for microbial growth and product formation. Some of the commonly used substrates are rice kernels, rice bran, pearled barley, pearled wheat, cracked corn, sugar cane bagasse, wheat bran, maize bran, gram bran, saw dust, tea waste, cassava waste, steamed rice etc. In SSF process, the solid substrate not only supplies all the nutrients to the microbial culture growing in it but also serves as an anchorage for the cells. However, some of the nutrients may be available in limited concentrations, or even absent in the substrates. Then, it would become necessary to supplement them externally with these. It has also been a practice to pre-treat (chemically or mechanically) some of the substrates before using in SSF processes, thereby making them more easily accessible for microbial growth [4]. Among the several factors that are important for microbial growth and enzyme production using a particular substrate, particle size and moisture level and water activity are the most critical. Generally, smaller substrate particles provide larger surface area for microbial attack and, thus, are a desirable factor. However, too small a substrate particle may result in substrate accumulation, which may interfere with microbial respiration and therefore result in poor growth. In contrast, larger particles provide better respiration efficiency, but provide limited surface for microbial attack. This necessitates a compromised particle size for a particular process [5].The optimum moisture level of 65-90% and pH6-7 with incubation period of 21 days and incubation temperature between 27-30^{0}c was found to favor good growth of fungi on paddy straw [6].

Applications of SSF Technique

Current trends on SSF have focused on application of SSF for the development of bioprocess such as bioremediation and biodegradation of hazardous compounds, biological detoxification of agro-industrial residues, biotransformation of crops and crop-residues for nutritional enrichment, bio pulping. SSF processes using agro-industrial residues is extensively used for production of value-added products such as biologically active secondary metabolites, such as enzymes, organic acids, food aroma compounds, antibiotics, alkaloids, single cell protein, Poly unsaturated fatty acids, bio pesticides, bio surfactants, bio fuel, pigments, xanthan, gum, and hormones like gibberellic acid [7].

Production of enzymes by SSF

Enzyme production is one of the most important applications of SSF. SSF using agro wastes has advantages over submerged fermentation, such as high volumetric productivity, low cost of equipment involved, better yield of product, lesser waste generation and lesser time consuming processes etc. The type of strain, culture conditions, nature of the substrate and availability of nutrients are the other important factors affecting yield of enzyme production [8]. It is crucial to provide optimized water content and control the water activity for good 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. Tangerdy et al.,(1998) have also proved this by comparing cellulase production costs in SSF and SMF [9]. Proteases, Lipase, Cellulase, Ligninase, Xylanase, Amylase, Glucoamylase, glutaminase, Pectinase, Phytase are successfully being produced using SSF process.

Bioreactors for SSF using agro wastes

In fermentation processes, bioreactor systems provide the environment for growth, cultivation of microbes. However, some of the factors affecting the growth of the product in SSF bioreactors are susceptibility of the substrate to increase in temperature, height and humidity of substrate bed, type of substrate used, size of the bioreactor, aeration, cooling rate, height of bed and fungal morphology. SSF is carried out in simple bioreactor systems. SSF bioreactors are fitted with a humidifier and with or without an agitator unit. Poor thermal conductivity of the substrate bed presents a great challenge to bioreactor design, but composition, particle size, porosity and water-holding capacity of the substrate used also affects the bioreactor [10].

SSF at laboratory scale is carried out in Petri dishes, jars, wide mouthed Erlenmeyer flasks, roux bottles, and roller bottles. These systems are simple and experiments are carried out easily. INRA (Institute National de la Recherché Agronomique, Dijon, France) team has developed a reactor with one liter working volume fitted with a relative humidity probe, a cooling coil in heating circuit, and a cover for the reactor vessel. These reactors will be filled with pre-inoculated solid material, and all growth parameters of the reactor are computer controlled. Sampling for analysis is also easier in INRA bioreactors. The automatic control of relative humidity and temperature make these reactors useful in scale-up studies [11].

In large scale bioreactors, the successful operation depends on design features obtained after mathematical modeling. Koji types of bioreactors, also known as tray bioreactors are the simplest and without forced aeration. The trays are made of wood, plastic, metal. It is not necessary that trays should be perforated. Trays are arranged one above the other with suitable gaps between them and placed in climatic controlled chamber under circulating air, which maintain uniform temperature. An advantage of this technology is that by increasing the number of trays, the scale-up is easier. However, the requirement of sterility, large space and labor makes the process difficult. BIOCON India has used this technology for large-scale production of immuno-suppressants. They have simplified problems of sterility by keeping the trays in HEPA (High Efficiency Particulate Air) filtered air, using automated machines for layering the substrate in trays [12]. The feasibility of using low cost production system favors SSF technique at a large scale.

Microorganisms used for enzyme production

Microorganisms Bacteria, yeasts and fungi can grow on solid substrates and find application in SSF processes. Bacteria are mainly involved in composting, ensiling and some food processes. Yeasts can be used for ethanol and food or feed production. But filamentous fungi are the most important group of microorganisms used in SSF process owing to their physiological, enzymological and biochemical properties. Filamentous fungi are the best adapted for SSF and dominate in research works. The hyphal mode of fungal growth and their good tolerance to low water activity and high osmotic pressure conditions make fungi efficient and competitive in natural micro flora for bioconversion of solid substrates.

Main groups of microorganisms involved in SSF process include Bacteria like species of *Clostridium,Lactobacillus,Streptococcus,Pseudomonas, Serratia and Bacillus.* Fungi like *Altemaria sp. Penicilium notatum, Lentinus edodes, Pleurotus oestreatus, Aspergillus niger, Rhizopus oligosporus, Aspergillus oryzae, Amylomyces rouxii, Beauveria sp.,Metharizium.,Trichoderma sp.,Phanerochaete chrysosporium, Rhizopus sp., Mucor sp., Monilia sp,. Fusarium sp., Endomicopsis burtonii, Schwanniomyce castelli, Saccharomyces cerevisiae* etc [13].A large number of microorganisms including bacteria, yeast, and fungal culture are employed for enzyme production in SSF systems. The fungi are amongst the largest producers of enzymes in the natural state growing on organic wastes [14]. It has been reported that while a strain of *Aspergillus niger* produces 19 types of enzymes, a α-amylase was being produced by as many as 28 microbial cultures. Thus, 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 cultures, since such enzymes are used in nature by fungi for their growth [15]. Fungi are used successfully for the production of vast range of enzymes at various industries and used in bioremediation processes.

Factors affecting enzyme production in SSF systems

The major factors that affect microbial synthesis of enzymes in a SSF system include: selection of a suitable substrate and microorganism; pre-treatment of the substrate; particle size of the substrate; water content and water activity of the substrate; relative humidity; type and size of the inoculums; control of temperature, removal of metabolic heat; period of cultivation; maintenance of uniformity in the environment of SSF system, and the gaseous atmosphere.

Advantages of microbial enzyme production by SSF

Microbial enzymes have many advantages over the animal and plant enzymes. They are economical, grown on large scale within limited space and time. They can grow in a wide range of environmental conditions. They show genetic flexibility and can resist the minor changes in the culture environment. The culture and maintenance of microbial cells are easier than those of plants and animals [16].

Fungal enzymes are gaining importance in agriculture and industry as they are often stable at high temperature and extreme pH than the enzymes derived from plants and animals [17]. Commercial fungal enzymes are increasingly replacing conventional chemical catalysts in many industrial processes. Proteases, Ligninases, Amylases, xylanases, Cellulases and Pectinases represent one of the largest groups of fungal enzymes and find wide application in industries and bioremediation process [18]. Fungal enzymes have a wide application in beverages and brewing industry, animal feeds, textile industry, leather industries, dairy processes and starch processing [19].

Significance of SSF using agro wastes

SSF has been considered superior in several aspects to submerged fermentation [SmF] due to various advantages it renders. It is cost effective due to the use of simple growth and production media comprising agro-industrial residues, uses little amount of water, which consequently releases negligible or considerably less quantity of effluent, thus reducing pollution concerns. SSF processes are simple, use low volume equipment (lower cost), and are yet effective by providing high product titers (concentrated products). Further, aeration process is easier since oxygen limitation does not occur as there is increased diffusion rate of oxygen into moistened solid substrate, supporting the growth of aerial mycelium [20]. These could be effectively used at smaller levels also, which makes them suitable for rural areas also.

Summary

One of the major challenges in Agricultural production is to reduce the waste generated and ensure proper management of agro wastes. Agricultural practices produce significant amounts of waste which contain high quantities of organic matter. The agricultural waste produced in particular period of the year pose potential pollution problems. Therefore an efficient utilization of such waste is of great importance, not only minimizing environmental impact, but also for obtaining higher profit.

SSF systems, which during the previous decades were termed as a ‘low-technology’ system appear to be a promising ones for the production of value-added ‘low volume–high cost’ products such as biopharmaceuticals and other bio molecules such as enzymes using agro residues. SSF processes offer potential advantages in bioremediation and biological detoxification of hazardous and toxic compounds.

CONCLUSION

Critical analysis of the literature shows that production of industrial enzymes by SSF offers several advantages. It has been well established that enzyme titers produced in SSF systems are many-fold more than in SmF systems. Although the reasons for this are not clear, this fact should be kept in mind while developing novel bioreactors for enzyme production in SSF systems using agro wastes. Genetically improved strains, suitable for SSF processes, would play an important role in this. It is hoped that enzyme production processes based on SSF systems employing agriculture related wastes will be the technologies of the future.

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REFERENCES

[1] F.Gonsalves, E. Sanjinez-Argandoña and G. Fonseca, "Utilization of agro-industrial residues and municipal waste of plant origin for cellulosic ethanol production", Journal of Environmental Protection, 10th ed., vol. 2, pp. 1303-1309, 2011.

[2] L. Demain, and N.A. Solomon, ’Solid state fermentation’, A manual of industrial microbiology and biotechnology. Washington dc, pp 66-68, 1996.

[3] H. Meade, J. White, P. Shoemaker, H. Gelf, Chang and A. Innis, Modification and bioconversion of raw materials, Food Biotechnology , 1st ed.,. Library of Congress, pp. 399-400, 2006.

[4] A.N.Glazer, and Nikaido, Microbial enzymes, Microbial biotechnology, WH freeman and co, New York, pp.247, 1995.

[5]P. Nigam and D. Singh, J. Basic Microbiol., vol.34, pp.405–422, 1994.

[6] P.A. Geethanjali, A study on lignin degrading fungi isolated from the litter of evergreen forests of Kodagu (D), Karnataka, International Journal of Environmental Sciences, vol.2, No.4, pp.2053-2058, May 2012.

[7]S.R. Carlos, S.P.Luciana and P.S.Vandenberghe, Overview of applied solid-state fermentation in Brazil, Biochemical Engineering Journal, vol.13, Issue 2-3, pp.205–218, March, 2003.

[8] A.Pandey, C.Soccol, J.Rodriguez-Leon and P. Nigam, Solid-state fermentation, Biotechnology-Fundamentals and Applications, Asiatech Publ. Inc., New Delhi, pp. 50-225, 2001.

[9] R.P.Tengerdy and A. Pandey, Solid substrate fermentation for enzyme production, Advances in Biotechnology, Educational Publ. and Distributors, IP Ext., New Delhi, pp. 13, 1998.

[10] S. Bhargav, B. P. Panda, M. Ali and S. Javed, Solid-state fermentation: An Overview, Chem. Biochem. Eng. Q. 22 (1), pp.49–70, 2008.

[11] A.Durand, Biochem. Eng. J. vol.13, pp.113, 2003.

[12] D. Mitchell, N. Krieger, D. M. Stuart, and A.Pandey, Process Biochem, vol. 35, pp.1211, 2000.

[13] M.S. Bashir, I. Ahmad and U.H. Abdul, Microbiological features of solid state fermentation and its applications - an overview, Research in biotechnology, vol.2, 6th ed., pp.21-26, 2011.
[14] C. Sandhya, A. Sumanth and A.Pandey, Proteases, Enzyme biotechnogy, 1st ed., Asiatech Publishing Ltd, New Delhi,pp.319-328, 2004.
[15] A. Pandey, Process Biochem., vol.27, 109–117, 1992.
[16] Kumaresan, Enxzymes, Biotechnology, 6th ed., Saras Publication, Kanyakumari, pp.650-656, 2010.
[17] M.J. Morgan, N.L. Rockey, G. Higton, Microbial enzymes, Industrial microbiology,1st ed.,. Blackwell publishing, New Delhi, pp.113, 2007
[18] G.Walsh, Industrial enzymes, Proteins Biochem and Biotech, 1st ed., John Wiley and sons Ltd, England, pp.419-435, 2002.
[19] J.Waiter, L. Morgan, S.Rockey and Higton, Industrial process and products, Industrial Microbiology, 1st ed., Blackwell Science, UK. pp.193, 2001.
[20] A.Pandey, Solid-state fermentation, Sci Topics. June13, 2008.