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REVIEW ARTICLE

Solid state fermentation for cellulase production

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ABSTRACT
Successful utilization of cellulosic material as a renewable carbon source depends on the development of economically feasible process technologies for the production of cellulose. This enzyme has various industrial applications and is now considered as major group of industrial enzyme. The review discusses the advantages of cellulase production by solid state fermentation as compared to submerged fermentation, the types of cellulolytic bacteria and different agro-industrial wastes utilized for cellulose production. The biotechnological aspect of cellulase research and their future prospects are also discussed.

KEY WORDS: *Cellulase, Solid-state fermentation, agro-industrial wastes, bioreactors, submerged fermentation.*

Introduction

Presently, cellulases constitute the third largest class of industrial enzymes worldwide, in terms of volume of dollar. The immense potential of cellulase enzymes to hydrolyze cellulosic compounds makes them extremely applicable to industrial processes like paper recycling, cotton processing, starch processing, extraction of juice form fruits and vegetables and extraction of green-tea components, modification of food tissues, grain alcohol fermentation, malting and brewing, removal of soybean seed coat, improving cattle feed quality, detergent enzymes and animal feed additives and most significantly, for production of ethanol from enzymatic hydrolysis of lignocellulosic biomass (Rani *et al*., 2010; Roussos, 1989).

However, besides recombinants, the level of cellulase production from majority of the microorganisms has been usually less and contrary to this, an efficient cellulose hydrolysis necessitates a high concentration of enzyme. Reportedly, cellulases solely contribute to 22.5–43.4% of the overall cost of the cellulosic ethanol production, when enzymes are procured from commercial sources. The cost of pure enzyme is considered to be a major obstacle in the widespread commercialization of enzymatic hydrolysis of lignocellulosic biomass and therefore consequently, large-scale strategies yielding low cost cellulases are incredibly important. Utilization of abundant renewable lignocellulosic biomass, predominantly agricultural or agro-industrial wastes or their by-products as substrates and also the use of cheaper fermentation technologies can further improve the production economics along with reduction in cellulase prices (Deswal *et al*., 2011; Dhillon *et al*., 2011; Roussos, 1989).

SSF Vs SmF

Cellulases were traditionally produced using the submerged fermentation (SmF), in which the microorganisms are cultivated in an aqueous environment containing nutrients. Contrastingly, Solid-state fermentation (SSF) is a process whereby an insoluble substrate is fermented with sufficient moisture but in the absence of free-flowing water. The low moisture content means that fermentation can only be carried out by a limited number of microorganisms, mainly yeasts, fungi and some bacteria. The previous decade has

noticed a renewed interest in SSC partly, due to the realization that many microorganisms, along with GMOs, may produce their products more effectively by SSF. Cellulases produced in solid-state culture shows remarkable stability towards temperature, pH, metal ions, etc. Optimization of SSF conditions may further improve the overall production economics and moreover can make it an attractive technique for cellulase production. A 10-fold reduction in the production cost when SSF is employed for production. Table 1 shows list some parametric comparisons between SmF and SSF for cellulose production (Acharya *et al*., 2010; Lever *et al*., 2010; Rani *et al*., 2010; Roussos, 1989; Sadhu and Maiti, 2013).

Microorganisms for SSF

Cellulolytic microbes usually utilize carbohydrates for growth and are generally unable to use proteins or lipids as energy sources. Cellulolytic bacteria and most fungi can utilize a range of carbohydrate sources, in addition to cellulose, while on the contrary anaerobic cellulolytic species can utilize limited carbohydrate sources, often cellulose and or its hydrolytic products only. Among different microorganisms, fungi seems to be the most adaptive to SSF because their hyphae can grow on particle surfaces and penetrate into the inter particle spaces and thereby colonizing solid substrates. Fungal species- *Trichoderma, Humicola, Penicillium, Aspergillus*, *Thermomonospora fusca, H. insolens*; Bacterial species-Bacilli, Pseudomonads, *Cellulomonas*; and among Actinomycetes-*Streptomyces, Actinomucor* are commonly used for cellulose production (Mussatto and Teixeira, 2010; Rani *et al*., 2010; Sukumaran *et al*., 2005). Alternatively, artificial cellulosomes can be generated by engineering cellulosome bearing bacteria to express bacterial or fungal cellulases in cellulosomes by genetic engineering (Rani *et al*., 2010).

Substrates for SSF

Apple pomace

Apple pomace is the main solid waste generated in cider and apple juice making factories and is a poor animal food because of its extremely low protein content. A variety of researchers have evaluated the potential of apple pomace as a substrate for the production of cellulase. Sun et al., (2010) were the first to report production of cellulase using apple pomace as substrate. They explored that the optimum initial moisture level, incubation temperature and inoculum size were 70%, 32°C and 2 × 108 spores/flask, respectively for production of cellulose by *Trichoderma* sp. GIM 3.0010 along with supplementation of apple pomace with 1% corn-steep solid as nitrogen source resulted in an enzyme activity of 7.6 U/gds.. Although apple pomace can support the growth of *Trichoderma* sp. GIM 3.0010 and cellulase production, it may not provide enough nutrients needed by the organism for maximum enzyme production. Hence, the exogenous addition of various nutrients to the medium may improve cell growth and enzyme production. The supplementation of apple pomace with lactose, lactoserum and corn-steep solid further favors the enzyme formation markedly. The higher FPase and BGL activities of 133.68 ± 5.44 IU/gram dry substrate (gds) and 60.09 ± 3.43 IU/gds, respectively were observed while using $CuSO_4$ and veratryl alcohol after 48 h of incubation time. The higher CMCase activity of 172.31 ± 14.21 IU/gds was obtained with lactose after 48 h of incubation period. Solid-state tray fermentation resulted in cellulase activities (IU g^{-1} dry weight basis) of 383.7 ? 17.9, 425.3 ? 21.3, 336.1 ? 16.2 and 4868 ? 39.8, respectively for FPase (filter paper cellulase), CMCase (carboxymethyl cellulase), BGL (b-glucosidase) and xylanase using A. niger NRRL 567 (Singh *et al*., 2012a, 2012b; Sun *et al*., 2010).

Distillery spent wash.

Distillery spent wash is the residual liquid waste generated during alcohol production and it also contains considerable nutrients in terms of potassium, sulphur, nitrogen and phosphorus as well as large amount of micronutrients like Ca, Cu, Mn, and Zn . Acharya *et al*., (2010) for the first time reported the direct use of high strength anaerobically treated distillery spent wash in combination with wheat straw for the production of cellulases by *A. ellipticus* under solid-state fermentation. Under optimized conditions, filter paper activity, β- glucosidase and endo-b-1, 4-glucanase activities were found to be 13.38, 26.68 and 130.92 U/g of substrate respectively.

Table 1: Comparisons between SmF and SSF for cellulose production (Rani *et al*., 2010; Sadhu and Maiti, 2013).

Parameter	SmF	SSF
Cellulosic substrate	Pure cellulose	Utilization of the natural cellulosic wastes as substrates,
Aspetic conditions	Required to avoid contamination under high moisture conditions	Aseptic conditions can be curtailed under water limited conditions of fermentation.
Substrate utilization	A maximum utilization of only 5% in SmF process	The amenability of SSF technique to utilize 20-30% of the substrate, makes it more promising.
Moisture requirements	Large volumes of water needed	Carried out in the absence of free-flowing water.
Process parameters	Generally involves mixing, aeration, control and monitoring of temperature, pH, dissolved oxygen and gas flow rates and therefore profitable in terms of its higher degree of process control and monitoring	Generally operated under static conditions.
Effluent generation	Large volumes of effluents discarded	Virtually, no effluent generation
Scale up	Easy scale up and industrial equipments are available	Scale up is problematic, new design equipments are needed.
Energy consumption	High	Low
Productivity	30-80 g/L	100-300 g/L, 2-3 times higher enzyme production as well as protein rate also enhanced titres of the product in the medium
Downstream processing	Requires downstream processing	Air-dried fermented solids can be directly used as source of enzyme eliminating the need of expenses on downstream processing,

Water Hyacinth

However, no data about cellulase production from water hyacinth has been reported at present. Water hyacinth have high content of hemicellulose (35 to 55% of dry mass), cellulose (18% of the dry mass) and protein (13% of the dry mass), which can provide enough nutrients for cellulase production by the *T. reesei* SEMCC- 3.217. In this study, water hyacinth was firstly used as the main substrate for cellulase production in solid-state fermentation by the strain *T. reesei* SEMCC-3.217. The statistical analysis of the results showed that, the optimum composition were: 5 g of substrate containing 3.9 g water hyacinth, 1% corn steep liquor, 1% soybean meal, 0.2% NH_4NO_3, 0.2% KH_2PO_4, 0.08% $MgSO_4 \cdot 7H_2O$, 2.8% $(NH_4)2SO_4$, 1.5% urea, 13.9% wheat bran, 0.08% $ZnSO_4 \cdot 7H_2O$, 0.08% $FeCl_2$ 0.05% $CaCl_2$, 0.08% $NaNO_3$, 0.08% KCl and 0.27% (v/v) Tween-80. Under these conditions, the cellulase production was 4-fold increased (13.4 FPIU/g dry solid) compared with the initial level (3.4 FPIU/g dry solid) after 7 days of fermentation in a 250 ml Erlenmeyer flask (Zhao *et al*., 2011).

Jatropha carcass

The residual protein-rich *Jatropha curcas* (Jatropha) (Physic Nut) seed cake, remaining after extraction of the oil, could form a protein-rich substrate for use as a solid state fermentation substrate for production of cellulolytic enzymes

by *Aspergillus niger*. Maximum cellulase was maximally produced at 40◦C, pH of 5. Under optimised conditions, 3974 U of cellulase were obtained per gram of substrate. Zymograms of crude enzyme extracts showed six active bands ranging from 20 kDa to 43 kDa for cellulase (Ncube *et al*., 2012).

Sugarcane bagasse

Sugarcane bagasse is abundantly and cheaply available as a byproduct from sugar industry. Bagasse consists of cellulose 43.8%, hemicellulose 28.6%, lignin 23.5%, ash 1.3%, and other components 2.8%. Direct use of sugarcane bagasse is not susceptible to exploit as growth substrate for cellulase production, therefore pretreatment is usually done (Haq *et al*., 2006). Various physical and chemical pretreatment methods either individually or in combination, have been developed and include bail uùlling, compression uùlling, grinding, cryomiling, gamma ray dosage, microwave irradiation, steam explosion. rapid depressurization and auto-hydrolysis by various chemicals such as acids, alkalies. solvents, gaseous ozone etc. (Dwiarti *et al*., 2012). Haq *et al*., (2006) utilized a thermophilic strain of *Humicola insolens* TAS-13 for cellulases production under solid-state fermentation conditions using sugar cane bagasse, pretreated with 2.0% H_2O_2 and 1.5% NaOH, as substrate. Under optimized conditions; thickness of the fermentation medium of 0.8 cm, pH 5.5 and temperature 50°C; the yield of the enzyme reached maximum with CMC-ase (18.98 U/g/min), FP-ase (13.63 U/g/min), β-glucosidase (19.54 U/g/min) 72 h after inoculation. Cunha *et al*., (2012) used sequential solid-state and submerged cultivation with sugarcane bagasse as substrate for cellulase production by *Aspergillus niger* A12 in a 5-L bubble column bioreactor and an endoglucanase productivity of 57 ± 13 IU/L/h was achieved.

Paper sludge

The first step in conversion of PS to ethanol is saccharification of PS cellulose to reducing sugars. Enzymatic saccharification of cellulose materials has been improved for generation of fermentable sugars during the ethanol production process. The possibility of combining sterilization of the substrate and its pretreatment was, therefore, conceived in the present studies. For saccharification of PS cellulose, cellulase activity was improved from 1.28 x 107 FPU m^{-3} (wild type) up to 1.52 x 10^7 FPU m^{-3} by mutation of *Acremonium cellulolyticus* C-1. Besides this different agro-industrial wastes like rice straw and bran, wheat bran and straw, corn cob etc. have been utilized for production of cellulases (Alonso *et al*., 2005)

Future Perspectives

The low thermal conductivity of the solid medium used in SSF restricts the removal of excess heat generated by microbial metabolism and therefore SSF requires aeration. The increase in temperature in bioreactors may lead to denaturation of thermolabile proteins. Secondly, SSF processes lead to development of different gradients (moisture, temperature, substrate concentration and others) along the bioreactor, which may negatively influence the process. The main drawbacks of SSF are therefore the scale up of the process, mainly because of heat transfer and culture homogeneity problems. Several researches have aimed towards the development of bioreactors for SSF systems; however, the available information has not indicated any ideal bioreactor yet. There are many research and development problems related to cellulase process technology, and a great deal more work needs to be done before its practical applications are realized.

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