REVIEW
Bioconversion of Biomass: A Case Study of Ligno-cellulosics Bioconversions in Solid State Fermentation

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ABSTRACT

Lignocellulosic residues obtained from crops cultivation form useful sources to be used as substrate for bioconversion processes. Sugarcane bagasse, which is a complex substrate obtained from the processing of sugar cane, is an important biomass among such sources. Due to its abundant availability, it can serve as an ideal substrate for microbial processes for the production of value added products. This paper reviews recent developments on biological processes developed on production of various products in solid state fermentation using sugarcane bagasse as the substrate and describes production of protein enriched feed, enzymes, amino acid, organic acids and compounds of pharmaceutical importance, etc. through microbial means.

Keywords: Ligno-cellulosics, sugarcane bagasse, bioconversion, solid state fermentation

1. INTRODUCTION

1.1. Sugar Cane

Sugar cane is a crop being cultivated in countries with a moderate tropical climate. It requires an average rainfall, fertile soil and good sunshine for high productivity. A number of countries in the world produce sugar cane and most of the crop is used for the production of sugar. India and Brazil are among the countries having largest land area under this crop. As such, sugar factories are not the only outlet for sugarcane (especially in India) as conversion of sugar cane into other type of sweetening products, like *jaggery* and *khand* is relatively common in villages.

In India, approximately 3500,000-hectare land cover is estimated to be under sugar cane. Although the average yields of about 65 tonnes per hectare have been generally obtained, yields as high as 90-105 tonnes per hectare are achieved in certain areas of the country, which are as good as the best yields in the world (these values are similar to those of Australian production which is considered highest in the world). The quality of sugar cane cultivated in India is considered fairly good which contains about 12.5% sugar, although there are varieties cultivated in Western part of the country which contain 13.5% sugar. There has been a gradual and constant increase in sugar production in the country. With 403 functional sugar factories (against the 503 as licensed) in 1991-92, the sugar production touched a peak of 132.77 tonnes against 85.02 tonnes in 1986-87 and it is expected that at the end of 9th five-year plan in 1999-2000, it would reach 171.19 tonnes as against 134.13 tonnes in 1994-95.

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During the production of sugar from sugarcane, a number of by-products are produced. Sugarcane bagasse and molasses are the major ones among these.

1.2. Sugar Cane Bagasse

Cellulose, the major constituent of all plant materials, forms about half to one-third of the plant tissues and is constantly replenished by photosynthesis. One of the largest cellulosic agro-industrial by-products is sugar cane bagasse, a fibrous residue of cane-stalks left over after the crushing and extraction of cane juice from sugar cane. It is a ligno-cellulosic waste (by-product) of sugar industry, which consists of approximately 50% cellulose and 25% each of hemicellulose and lignin. Sugar cane bagasse (or, bagasse as it is generally called) is almost completely used by the sugar factories themselves as fuel for the boilers. Chemically, bagasse contains about 50% α-cellulose, 30% pentosans and 2.4% ash. Thus, bagasse offers numerous advantages in comparison to other crop residues (e.g. rice straw and wheat straw which have 17.5 and 11.0%, respectively ash) for usage in bioconversion processes using microbial cultures. Also, in comparison to other agricultural residues, bagasse can be considered as a rich solar energy reservoir due to its high yields (about 80 tonnes per hectare in comparison to about 1, 2 and 20 tonnes per hectare for wheat, grass and tree, respectively) and annual regeneration capacity.

2. SOLID STATE FERMENTATION

Solid state fermentation (SSF) can be defined as the fermentation process in the absence or near-absence of free water in the substrate (Aidoo *et al.* 1982, Hesseltine 1977, Pandey 1992, 1994, Nigam & Singh 1994). However, there must be enough moisture present in the substrate to support the growth of micro-organisms. There has been wide-spread resurgent of SSF all over the world due to several advantages it offers, mainly on engineering aspects. SSF has successfully been carried out using a number of ligno-cellulosic residues and bagasse is no exception to that.

3. MICROBES FOR SSF

As is evident from Table 1, although there are reports on cultivation of bacteria, yeast as well as fungi on bagasse, filamentous fungi have most widely been studied. Valino *et al.* (1997) used strains of *Acinetobacter calcoaceticus* and *Cephalosporium* sp.for studying interactions between microbiote of sugar cane bagasse. The results showed that a better adaptation of bagasse microbiote in solid fermentation, being able to more efficiently overcome any harmful effect compared to the mixture of bacteria and fungi each one separately. A strain of *Aspergillus niger* was used for biomass estimation on real and model supports (amberlite IRA-900 and bagasse) in SSF (Cordova-Lopez *et al.*1996).

Zadrazil and Puniya (1995) employed white-rot fungus strains to study the effect of particle size of bagasse in SSF for the production of animal feed with a view to enhancing the nutritive value of the bagasse. They used *Pleurotus* sp. P7 and P1, *P. eryngii*, *Agrocybe aegarita* A1, and *Kuehneromyces mutabilis.* The *P. eryngii* improved the digestibility of all the four experimental fractions of the bagasse. Importance of particle size in fungal SSF has also been emphasised by Pandey (1991 a). Gupte and Madamwar (1997 a, b) carried out co-culturing of *A. ellipticus* and *A. fumigatus* for the production of cellulolytic enzymes. Cellulases and β-glucosidase were also produced by a strain of *A. niger* (Ray *et al.* 1993). Machado *et al.* (1996) screened 44 basidiomycete strains for their ability to produce ligninolytic enzymes. Among the tested strains, 12 and 7 failed to produce detectable peroxidases and phenol-oxidases, respectively. Eleven showed good production, and belonged to the genera *Lentinus, Melanoporia, Peniophora, Tramets, Trichaptum* and *Trogia.*

There are other studies also on co-culturing of fungal strains in SSF using bagasse. For

example, Gutierrez-Correa and Tengerdy (1997, 1998) and Duenas *et al.* (1995) used strains of *Trichoderma reesei* and *A. niger* or *A. phoenicis* for SSF of bagasse for the production of enzymes. Rodriguez-Vazquez *et al.* (1992) reported co-culturing of *Cellulomonas flavigena* and *Xanthomonas* sp. for the production of single-cell protein (SCP) using bagasse as substrate.

Sharma *et al.* (1991) used strains of *T. viride*, *Penicillium chrysogenum* and *Fusarium oxysporum* for enzyme production from bagasse. *P. chrysogenum* appeared best among these for cellulase production. Pal *et al.* (1995) cultivated the mushroom *Flammulina velutipes* and white-rot fungus *Trametes versicolor* separately on bagasse for the production of enzymes. Soccol *et al.* (1994) used *Rhizopus oryzae* NRRL 395 for solid culturing on bagasse for lactic acid production. Two strains of *Streptomyces* sp. were used for the protein enrichment of bagasse (Iyo & Antai 1991). Several other microbial cultures like *Chaetomium cellulolyticum* (Bravo *et al.* 1994) and basidiomycetes strains (Nigam 1990, Nigam *et al.* 1987) were also used for protein enrichment of bagasse.

As generally bacteria require high water activity, they have not been regarded suitable micro-organisms for SSF of ligno-cellulosics. In a significant study, however, a bacterial strain of *Brevibacterium* sp. was shown to grow and produce amino acid in SSF of bagasse (Nampoothiri & Pandey 1996) in which bagasse was used as inert substrate. Christen *et al.* (1993) used bagasse as inert support for monitoring the growth of *Candida utilis* in SSF. Tosmani *et al.* (1997) cultivated a strain of *Gibberella fujikuroi* for production of gibberllic acid in SSF of bagasse and *Claviceps purpurea* was grown on bagasse in SSF for the production of ergot alkaloids (Harnandez *et al.* 1993).

4. BIOCONVERSION PROCESSES

In recent years, there have been a number of reports on the application of sugar cane bagasse as substrate for the production of various products by microbial bioconversions. For the sake of clarity and convenience, we will classify the processes in two categories, one, in which bagasse has been used as the substrate (carbon source) itself while the other, in which it has been used as inert carrier (inert substrate).

4.1 Processes Involving Bagasse as Solid Substrate (Carbon Source)

Bagasse has most commonly been used for the production as protein enriched animal feed by solid state fermentation, employing yeast and fungi. A number of reports have appeared on production of animal feed in recent years (cf. table 2). Nigam (1990) and Nigam *et al.* (1987) investigated solid state fermentation of bagasse for animal feed production using basidio-mycetes. C/N ratio and initial moisture were critical factors. Zadrazil and Puniya (1995) differentiated bagasse into four fractions of particle size (>1mm, 1-3 mm, 3-5 mm and 5-10 mm) with a view to enhancing its nutritive value as animal feed. They found a varying degree of degradation by white-rot fungi and also variation in *in vitro* rumen digestions. It was concluded that the mechanical separation of a substrate into different particle size could be useful if it was utilized as a substrate to be fermented by filamentous fungi to produce animal feed. Puniya *et al.* (1996) subjected bagasse to SSF using a strain of *P. sajor-caju* in a closed system, with the aim of optimizing the gaseous atmosphere and developing a cost-effective and simple technology for animal feed production. They found that the application of gases during SSF without disrupting mycelial growth and substrate content was the key to the suitability of this technology. Bravo *et al.* (1994) treated bagasse with water- or alkali- at three-liquid/solid ratio before using it as substrate for microbial protein production. The treatment significantly enhanced fungul growth compared to non-treated bagasse. Rodriguez-Vazguez *et al.* (1992) also treated bagasse (pith) with a solution of sodium hydroxide in such a low volume that no free liquid was present. They referred it as dry pre-treatment and compared with a wet pre-

treatment. Maximum digestibility with dry and wet pre-treated bagasse was 75 and 71%, respectively. Biomass production was also higher in dry process. Iyo and Antai (1991) achieved 21% crude protein in bagasse after 12 weeks cultivation of a fungal strain of *Streptomyces*, which resulted 45% depletion of lignocelluloses.

A patent was obtained by Hayashibara Biochem. Res. (Iritani *et al.* 1995) on the application of bagasse, softened with alkali treatment, for feedstuff, fertilizer, and sweetener by cultivating *Enterococcus faecium* in SSF. Chaudhary *et al.* (1994) also reported feedstuff production from bagasse using two strains of *Pleurotus* sp.

Amongst the various enzymes produced in SSF of bagasse, cellulases have most extensively been studied. It is well established that the hydrolysis of the lignocellulosic residues using enzyme largely depends upon the cost of the production of cellulases. Application of bagasse in SSF for this purpose appears attractive. Sharma *et al.* (1991, 1995) reported production of cellulases from different fungal strains. Significant FPD activity was noted from *P. chrysogenum*, which, apart from the enzyme, also showed high levels of reducing sugars (glucose and xylose). They suggested an integral process for the production of ethanol, furfural, fermentable sugars and biogas from bagasse. Roussos *et al.* (1992) used a mixture of bagasse and wheat bran (4:1) for the production of cellulases. They suggested hydraulic pressing as a good technique to leach out the enzymes from the fermented matter. Modi *et al.* (1994) reported better yields of cellulase from a strain of *Streptomyces* sp. HM29 when grown on bagasse in comparison to rice straw, rye straw and corncobs. Yields were comparable with those obtained from rice bran but lower than those from wheat straw, wheat bran and newspaper.

Often, cultivation of two different strains as mixed culture and pre-treatment of bagasse showed desirable impact on fermentation. Gupte and Madamwar (1997 a, b) reported that production of cellulolytic enzymes under SSF by co-culturing of two fungal strains showed improved hydrolytic and β-glucosidase activities as compared to the occasions when they were used separately. Alkali pre-treatment further improved the enzyme production (Gupte & Madamwar 1994). Similarly, Gutierrez-Correa and Tengerdy (1997) also reported higher cellulase productivity in co-culturing of a basidiomycete strain with another filamentous fungi. A mutual synergism was observed between the parent strain (of *T. reesei* LM-UC4) and the *A. phoenicis* QM 329, resulting in enhanced combined biomass production and corresponding increase in cellulase, endo-glucanase and β-glucosidase activities. When co-culturing was carried out using a mutant strain of *T. reesei* LM-UC4E1, such synergism was absent, suggesting that in the hypermutation the ability for co-operative interaction with other microbes was lost. Treatment of bagasse with ammonia (80%, w/w moisture content) resulted higher enzyme productivity (Duenas *et al.* 1995).

An extensive study was carried out by Pal *et al.* (1995) on SSF of bagasse using a strain of mushroom fungus and another of white-rot fungus separately for 40 days. *T. versicolor* produced laccase and manganese-peroxidase activities, showing a simultaneous degradation of lignin and holocellulose. However, only phenol-oxidase activity was found with *F. velutipes*. A preferential degradation of lignin was detected in this case, which exhibited a greater reduction in the ratio of weight loss to lignin loss than the other culture.

Xylanase was another enzyme produced under SSF of bagasse. Jain (1995) used a thermophilic fungus for the production of extra-cellular xylanase on various agro-residues, including bagasse. Fungus grew well on untreated bagasse and enzyme titres were lower when fungus was grown on treated (alkali or acid chlorite treatment) bagasse. Acetyl esterase was produced concurrently, maximal activity being with bagasse in comparison to other substrates. Gutierrez-Correa and Tengerdy (1998) also performed xylanase production in SSF using bagasse. They co-cultured *T. reesei* and *A. niger*

Table 1. Micro-organisms employed in SSF of bagasse

Micro-organism	*References*
Acinetbacter calcoaceticus	Valino *et al.* (1997)
Agrocybe aegarita A1	Zadrazil & Punia (1993)
Aspergillus ellipticus	Gupte & Madamwar (1997 a)
A. fumigatus	Gupte & Madamwar (1997 a, b)
A. niger	Acuna-Arguelles *et al.* (1994) Cordova-Lopez *et al.* (1996) Huerta *et al.* (1994), Ray *et al.*(1993), Solis-Pereyra *et al.* (1996)
A. ochraceus	Biswas *et al.* (1988)
A. phoenicis	Gutierrez-Correa & Tengerdy (1998) Duenas *et al.* (1995)
Brevibacterium sp.	Nampoothiri & Pandey (1996)
Candida utilis	Christen *et al.* (1993)
Cellulomonas flavigena	Rodriguez-Vazquez *et al.* (1992)
Cephalosporium sp.	Valino *et al.* (1997)
Ceratocystis fimbriata	Christen *et al.* (1994, 1997)
Chaetomium cellulolyticum	Bravo *et al.* (1994)
Claviceps purpurea	Harnandez *et al.* (1993)
Enterococcus faecium	Iritani *et al.* (1995)
Flammulina velutipes	Pal *et al.* (1995)
Fusarium oxysporum	Sharma *et al.* (1991)
Gibberella fujikuroi	Tosmani *et al.* (1997)
Kuehneromyces mutabilis	Zadrazil & Punia (1995)
Melanocarpus albomyces IIS-68	Jain (1995)
Monascus purpureus	Chiu & Chan (1992)
Penicillium chrysogenum	Barrios-Gonzalez *et al.* (1993) Sharma *et al.* (1991)
Pleurotus sp. P7	Zadrazil & Puniya (1995)
P. cornucopiae	Chaudhary *et al.* (1994)
P. eryngii	Zadrazil & Puniya (1995)
P. florida	Chaudhary *et al.* (1994)
P. sajor-caju	Puniya *et al.* (1996)
Polyporus sp.	Nigam (1990), Nigam *et al.* (1987)
Rhizopus oryzae	Soccol *et al.* (1994)
Schwanniomyces castellii	Saucedo-Castaneda *et al.* (1992)
Streptomyces sp.	Iyo & Antai 1991, Modi *et al.* (1994)
Trametes versicolor	Pal *et al.* (1995)
Trichoderma harzianum	Roussos *et al.* (1992)
T. reesei	Gutierrez-Correa & Tengerdy (1997), Gutierrez-Correa & Tengerdy (1998), Duenas *et al.* (1995)
T. viride	Sharma *et al.* (1991)
Xanthomonas sp.	Roudriguez-Vazquez *et al.* (1992)

Table 2. Products of SSF of bagasse (used as carbon/energy source)

Products	*References*
Protein enriched feed	Nigam (1990), Nigam *et al.* (1987), Zadrazil & Puniya (1995), Bravo *et al.* (1994), Iyo & Antai (1991), Rodriguez-Vazguez *et al.* (1992), Puniya *et al.* (1996), Iritani *et al.* (1995), Chaudhary *et al.* (1994),
Cellulases	Sharma *et al.* (1991, 1995), Ray *et al.* (1993) Gupte & Madamwar (1994, 1997 a, b) Gutierrez-Correa & Tengerdy (1997, 1998), Duenas *et al.* (1995), Roussos *et al.* (1992)
Laccase	Pal *et al.* (1995), Machado *et al.* (1996)
Ligninase	Nigam *et al.* (1987), Machado *et al.* (1996)
Mn-peroxidase	Pal *et al.* (1995), Machado *et al.* (1996)
Phenol oxidase	Pal *et al.* (1995), Machado *et al.* (1996)
Xylanase	Jain (1995), Gutierrez-Correa & Tengerdy (1998), Biswas *et al.* (1988)
Aroma production	Christen *et al.* (1994)
Acetyl esterase	Jain (1995)
Gibberllic acid	Tosmani *et al.* (1997)
Fruity aroma	Christen *et al.* (1997)
Pigments	Chiu & Chan (1992)
Composting/Ensiling	Baca *et al.* (1993), Roussos *et al.* (1992)

Table 3. Products of SSF of bagasse (used as inert carrier)

Products	*References*
Glutamic acid	Nampoothiri & Pandey (1996)
Ergot alkaloids	Hernandez *et al.* (1993)
Lactic acid	Soccol *et al.* (1994)
Citric acid	Lakshminarayana *et al.* (1975), Manonmani & Sreekantiah (19987)
Pectinases	Solis-Pereyra *et al.* (1996), Huerta *et al.* (1994), Acuna-Arguelles *et al.* (1994)
Penicillin	Barrios-Gonzalez *et al.* (1993)

or *A. phoenicis* and achieved high xylanase titres (2,600-2,800 IU/g dry wt).

The spectra of SSF of bagasse increased further with the report appearing on production of other products, like gibberllic acid. Tosmani *et al.* (1997) compared gibberllic acid production in liquid fermentation with SSF. Different SSF systems were used. SSF of bagasse showed excellent fungal growth but presented extraction problem.

4.2 Processes Involving Bagasse as Solid Inert Substrate (Inert Carrier)

SSF carried out on inert support materials, which differs from the process of microbial growth on or in solid particles floating in a liquid medium has been regarded as one of the future development of SSF systems (Aidoo *et al.* 1982, Pandey 1991b, 1992). The use of solid inert material impregnated with suitable liquid media would provide homogenous aerobic conditions throughout the bioreactor and the purity of the product would also be relatively high.

4.3.1 Processing Involving Bagasse as Solid Inert Substrate for Products

In a unique study, the first of its type, Nampoothiri and Pandey (1996) reported production of L-glutamic acid in which bagasse was impregnated with a medium containing glucose, urea, mineral salts and vitamins. Maximum yields (80-mg glutamic acid/g dry bagasse) were obtained when bagasse of mixed particle size was fermented with 85-90% moisture and 10% glucose. Yet, in a significant finding, Hernandez *et al.* (1993) reported production of ergot alkaloids from a fungus culture grown on bagasse, impregnated with a liquid medium. They used a total of sixteen different combinations of liquid media and concluded that there existed the possibilities of achieving tailor-made spectra of ergot alkaloids by changing the liquid nutrient media composition used for impregnation. Barrios-Gonzalez *et al.* (1993) studied the effect of particle size, packing density and agitation on penicillin production in SSF using bagasse as inert substrate. The use of a large particle size (14-mm) bagasse increased penicillin production by 37%. Christen *et al.* (1997) reported production of fruity aroma on bagasse when it was fermented with a nutritive medium containing glucose (200 g/L). Twenty-four compounds were separated and twenty of them were identified from the headspace analysis of the fermenter by GC. Aroma production was dependent on the growth and the maximum aroma intensity was detected at about time of the maximum respirometric activity.

Soccol *et al.* (1994) evaluated potential of bagasse to be used as inert substrate, impregnated with a liquid medium containing glucose and calcium carbonate, for lactic acid production from a strain of *Rhizopus oryzae* NRRL 395. Keeping glucose level at 120 and 180g/L for liquid and solid state fermentation, an yield of 93.8 and 137.0g/L of L(+)-lactic acid was obtained, respectively. The productivity was 1.38 and 1.43g/l/h in liquid and solid fermentation, respectively. Citric acid was another organic acid, which was produced in SSF using bagasse as inert carrier (Laxminarayana *et al.*, 1975). Manonmani and Sreekantiah (1987) conducted citric acid production using enzymatic hydrolyzate of alkali treated bagasse by SSF.

Pectinases were produced in SSF using bagasse impregnated with high glucose concentration (Solis-Pereyra *et al.* 1996). They used packed bed column fermenter. In a similar study, Huerta *et al.* (1994) concluded that SSF carried out on inert substrates (they referred it as adsorbed substrate fermentation technique) not only allowed the design of culture medium to produce important metabolites, but also the study of fungal metabolism in artificially controlled SSF processes. Acuna-Argulles *et al.* (1994) studied the effect of water activity on pectinases production using bagasse impregnated with a medium containing pectin and sucrose. Ethylene glycol, sorbitol and glycerol were used as water activity depressors. Results indicated that

although polygalacturonase production decreased at low a_w values, this activity was present at a_w values as low as 0.90. The specific activity increased up to 4.5-fold by reducing a_w from 0.98 to 0.9.

Chiu and Chan (1992) described production of pigments using bagasse in roller bottle cultures of *Monascus purpurea*. Fungus was cultivated in wet bagasse containing PGY medium with corn oil in SSF when it produced red and yellow pigments.

4.3.2 Processing Involving Bagasse as Solid Inert Substrate for Growth/Model Studies

Solid substrates of inert nature offer several advantages on measurements of growth in SSF and made it possible to study growth kinetics in SSF. Bagasse has been commonly employed for this purpose. Christen *et al.* (1993) successfully monitored the growth of *Candida utilis* in a bagasse medium in SSF. Auria *et al.* (1993) conducted a study on the influence of mould growth on the pressure-drop in aerated SSF using bagasse and wheat bran. They proposed the measurement of pressure-drop (DELTAP) across an aerated fermentation bed as an alternative on-line sensor for the qualitative and, in some cases, quantitative, macroscopic changes in static SSF. Oriol *et al.* (1987, 1988) also used bagasse impregnated with a liquid growth medium for studying growth kinetics of *A. niger*.

In an attempt to estimate fungal biomass in SSF, Cordova-Lopez *et al.* (1996) carried out direct hydrolysis of fungal mycelium grown on bagasse in SSF, followed by the analysis of soluble protein by the dye binding method. Hydrolysis with phosphoric acid for 7 minutes allowed maximum protein extraction and there was no colour interference by the medium components.

Valino *et al.* (1997) carried out an experiment with the objective of studying the interactions between the microbiotes of bagasse and fungal and bacterial strains being cultivated on it in SSF. A factorial arrangement with replications was used. The results showed a better adaptation of bagasse microbiote in SSF, being able to more efficiently overcome any undesirable effect compared to the mixture of bacteria and fungi, each one separately.

RESUMO

Resíduos celulósicos obtidos a partir do cultivo de plantas podem ser utilizados como substratos nos processos de bioconversão. Bagaço de cana é um importante substrato e fonte de biomassa obtido a partir do processamento da cana de açucar. Em razão da sua produção em grandes volumes o mesmo pode ser utilizado como substrato ideal em processos microbianos para obtenção de produtos de elevado valor comercial. Esse trabalho de revisão apresenta os recentes desenvolvimentos em processos biológicos utilizando a técnica da fermentação no estado sólido na obtenção de enzimas, aminoácidos, ácidos orgânicos e componentes farmacêuticos de interesse industrial.

REFERENCES

Acuna-Arguelles, M, Gutierrez-Rojas, M, Viniegra-Gonzalez, G, & Favela-Torres, E. (1994) Effect of water activity on exo-pectinase production by *Aspergillus niger* CH4 on solid state fermentation. *Biotechnol Letts*, **16,** 23-28

Aidoo, KE, Hendry R & Wood, JB. (1982) *Adv Appl Microbiol*, **28**, 201-237

Auria, R, Morales, M, Villegas, E, & Revah, S. (1993) Influence of mold growth on the pressure-drop in aerated solid state fermenters. *Biotechnol Bioeng*,**41**, 1007-1013

Baca, MT, Steban, E, Almendros, G & Sanchezraya, AJ. (1993) Changes in gas phase of compost during solid state fermentation of sugar cane bagasse. *Biores Technol*, **44**, 5-8

Barrios-Gonzalez, J, Gonzalez, H, & Mejia, A. (1993) Effect of particle size, packing density and agitation on penicillin production in solid

state fermentation. *Biotechnol Adv,* **11**, 539-547

Biswas, SR, Mishra, AK & Nanda, G. (1988) Xylanase and beta glucosidase production by *Aspergillus ochraceus* during growth on lignocelluloses. *Biotechnol Bioeng,* **31**, 613-616

Bravo, O, Ferrer, A, Aiello, C, Ledesma, A & Davila, M. (1994) Growth of *Chaetomium cellulolyticum* in solid state fermentation of sugar cane bagasse treated with water and alkali at several liquid/solid ratios. *Biotechnol Letts,* **16**, 865-870

Chaudhary, LC, Singh, R, & Kamra, DN. (1994) Biodelignification of sugar cane bagasse by *Pleurotus florida* and *Pleurotus cornucopiae. Indian J Microbiol,* **34**, 55-57

Chiu, SW & Chan, SM. (1992) Production of pigments by *Monascus purpurea* using sugar cane bagasse in roller bottle cultures.*World J Microbiol Biotechnol,* **8**, 68-70

Christen, P, Auria, R, Vega, C, Villegas, E, & Revah, S. (1993) Growth of *Candida utilis* in solid state fermentation. *Biotechnol Adv,* **11**, 549-557

Christen, P, Meza, JC, & Revah, S. (1997) Fruity aroma production in solid state fermentation by *Ceratocystis fimbriata:* influence of the substrate type and the presence of precursors. *Mycol Res,* **101**, 911-919

Christen, P, Villegas, E, & Revah, S. (1994) Growth and aroma production by *Ceratocystis fimbriata* in various fermentation media. *Biotechnol Letts,* **16**, 1183-1188

Cordova-Lopez, J, Gutierrez-Rojas, M, Huerta, A, & Saucedo-Castaneda, G. (1996) Biomass estimation of *Aspergillus niger* growing on real and model supports in solid state fermenation. *Biotechnol Tech,* **10**, 1-6

Duenas, R, Tengerdy RP, Guierrez-Correa, M. (1995) Cellulase production by mixed fungi in solid sibstrate fermentation of bagasse. *World J Microbiol biotechnol,* **11**, 333-337

Gupte, A & Madamwar, D. (1994) High strength cellulase and beta-glucosidase formation from *Aspergillus* sp. under solid state fermentation. *IN- Solid State Fermentation.* Ed. A. Pandey, Wiley Eastern Publishers, New Delhi, pp. 130-133

Gupte, A & Madamwar, D. (1997a)Production of cellulolytic enzymes by co-culturing of *Aspergillus ellipticus* and *Aspergillus fumigatus* grown on bagasse under solid state fermentation. *Appl Biochem Biotechnol,* **62**, 267-274

Gupte, A & Madamwar, D. (1997b) Solid state fermentation of ligno-cellulosic wastes for cellulase and beta-glucosidase production by co-culturing of *Aspergillus ellipticus* and *Aspergillus fumigatus*. *Biotechnol Progress,* **13**, 166-169

Gutierrez-Correa, M & Tengerdy RP. (1997) Production of cellulase on sugar cane bagasse by fungal mixed culture solid substrate fermentation. *Biotechnol Letts,* **19**, 665-667

Gutierrez-Correa, M & Tengerdy RP. (1998) Xylanase production by fungal mixed culture solid state fermentation on sugar cane bagasse. *Biotechnol Letts,* **20**, 45-47

Hernandez, MRT, Lonsane, BK, Raimbault, M & Roussos, S. (1993) Spectra of ergot alkaloids produced by *Claviceps purpurea* in solid state system - influence of the composition of liquid medium used for impregnating sugar cane bagasse. *Process Biochem,* **28**, 23-27

Hesseltine, CW. (1977) Solid-state fermentation. *Process Biochem,* **12**, 22-24

Huerta, S, Favela, E, Lopez-Ulibarri, R, Fonseca, A, Viniegra-Gonzalez, G, & Gutierrez-Rojas, M. (1994) Absorbed substrate fermentation for pectinase production with *Aspergillus niger*. *Biotechnol Tech,* **8**, 837-842

Iritani, S, Mitsuhashi, M, Chaen, H & Miyake, T. New alkali treated bagasse used as livestock feed. PN : GB 2285806; 26.07.95

Iyo, AH & Antai, SP. (1991) Protein enrichment of lignocellulose resulting from the growth of two *Streptomyces* strains. *World J Microbiol Biotechnol,* **7**, 624-625

Jain, A. (1995) Production of xylanase by thermophilic *Melanocarpus albomyces* IIS-68. *Process Biochem,* **30**, 705-709

Lakshminarayana, K., Chaudhary, K., Ethiraj, S. and Tauro, P. (1975) *Biotechnol. Bioeng.,* **7**, 291-293

Machado, KMG, Silva, WRF & Bononi, VLR. (1996) Screening of ligninolytic fungi for soil remediation. II-Production of peroxidases and phenol oxidases. *Int. Biodeterio. Biodegrd.*, **37**, 130

Manonmani, HK & Sreekantiah, KR. (1987) Studies on the conversion of cellulase hydrolysate into citric acid by *Aspergillus niger*. *Process Biochem*, **22**, 92-94

Modi, HA, Patel KC & Ray, RM. (1994) Solid state fermentation for cellulase production by *Streptomyces* sp HM-29. *IN- Solid State Fermentation*. Ed. A. Pandey, Wiley Eastern Publishers, New Delhi, pp. 137-141

Nampoothiri, KM & Pandey, Ashok. (1996) Solid state fermentation for L-glutamic acid production using *Brevibacterium* sp. *Biotechnol Letts*, **18,** 199-204

Nigam, P. (1990) Investigation of some factors important for solid state fermentation of sugar cane bagasse for animal feed production. Enz Microb Technol, **12**, 808-811

Nigam, P & Singh, D. (1994) Solid state (substrate) fermentation systems and their applications in biotechnology. *J. Basic Microbiol*, **34,** 405-14

Nigam, P, Pandey, Ashok & Prabhu, KA. (1987) Cellulase and ligninase production by basidiomycetes in solid state fermentation. *Biol wastes*, **20,** 1-9

Oriol, E, Contreras, R & Raimbault, M. (1987) Use of microcalorimetry for monitoring the solid state culture of *Aspergillus niger*. *Biotechnol Tech*, **1**, 79-84

Oriol, E, Schettino, B, Viniegra-Gonzalez, G & Raimbault, M. (1988) Solid state culture of *Aspergillus niger* on support. *J Ferment Technol*, **66**, 57-62

Pal, M, Calvo, AM, Terron, MC & Gonzalez, AE. (1995) Solid state fermentation of sugar cane bagasse with *Flammulina velutipes* and *Trametes versicolor*. *World J Microbiol Biotechnol*, **11**, 541-545

Pandey, Ashok. (1991a) Effect of particle size of the substrate on enzyme production in solid state fermentation. *Biores. Technol.*, **37,** 169-172

Pandey, Ashok. (1991b) Aspects of design of fermenter in solid state fermentation. *Process Biochem*, **26**, 355-361

Pandey, Ashok. (1992) Recent developments in solid state fermentation. *Process Biochem*, **27**, 109-117

Pandey, Ashok. (1994) Solid state fermentation: An ioverview. *IN – Solid State Fermentation*. Ed. A. Pandey, Wiley Eastern Publishers, New Delhi, pp. 3-10

Puniya, AK, Shah, KG, Hire, SA, Ahire, RN, Rathod, MP & Mali, RS. (1996) Bioreactor for solid state fermentation of agro-industrial wastes. *Indian J. Microbiol*, **36**, 177-78

Ray, L, Pal, A, Ghosh, AK & Chattopadhyay, P. (1993) Cellulases and beta-glucosidase from *Aspergillus niger* and saccharification of some cellulosic wastes. *J. Microbial Biotechnol*, **8**, 85-94

Rodriguez-Vazquez, R, Villanuevaventura, G & Riosleal, E. (1992) Sugarcane bagasse pith dry pretreatment for single cell protein production. *Biores Technol*, **39**, 17-22

Roussos, S, Raimbault, M, Geoffroy, F, Saucedo-Castaneda, G & Lonsane, BK. (1992) Efficient leaching of cellulases produced by *Trichoderma harzianum* in solid state fermentation. *Biotechnol Tech*, **6**, 429-432

Roussos, S, Raimbault, M, Saucedo-Castaneda, G & Lonsane, BK. (1992) Potential of ensiling for efficient management of spent residue from solid state fermentation system. *Biotechnol Tech*, **6**, 87-90

Saucedo-Castaneda, G, Lonsane, BK, Navarro, JM, Roussos, S, & Raimbault, M. (1992) Control of carbondioxide in exhaust air as a method for equal biomass yields at different bed heights in a column fermenter. *Appl Microbiol Biotechnol*, **37**, 580-582

Sharma, DK, Niwas, S & Behera, BK. (1991) Solid state fermentation of bagasse for the production of cellulase enzyme from cellulolytic fungi and extent of simultaneous production of reducing sugars in the fermenter. *J. Microbial Biotechnol*, **6**, 7-14

Sharma, DK, Tiwari, M & Behera, BK. (1995) Solid state fermentation of new substrates for production of cellulase and other biopolymer hydrolysing enzymes - scientific note. *Appl Biochem Biotechnol*, **51-52**, 495-500

Soccol, CR, Marin, B, Raimbault, M & Lebeault, JM. (1994) Potential osf solid state fermentation for production of L(+)-lactic acid by *Rhizopus oryzae*. *Appl Biochem Biotechnol*, **41**, 286-290 (

Solis-Pereyra, S, Favela-Torres, E, Gutierrez-Rojas, M, Roussos, S, Saucedo-Castaneda, G, Gunasekaran, P, & Viniegra-Gonzalez, G. (1996) *World J Microbiol Biotechnol*, **12**, 257-260

Tosmani, A, Fajardo, C & Barrios-Gonzalez, J. (1997) Gibberllic acid production using different solid state fermentation systems. *World J Microbiol Biotechnol*, **13**, 203-206

Valino, E, Elias, A, & Albelo, N. (1997) Interactions between the microbiote of sugar cane bagasse and *Cephalosporium* sp. and *acinetobacter calcoaceticus* strains in solid state fermentation. *Cuban J Agri Sci*, **31**, 279-285

Zadrazil, F & Puniya, AK. (1995) Studies on effect of particle size on solid state fermentation of sugar cane bagasse into animal feed using white-rot fungi. *Biores Technol*, **54,** 85-87

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