Biotechnological potential of agro-industrial residues. I: Sugarcane bagasse

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Review paper

Biotechnological potential of agro-industrial residues. I: sugarcane bagasse

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Abstract

Advances in industrial biotechnology offer potential opportunities for economic utilization of agro-industrial residues such as sugarcane bagasse. Sugarcane bagasse, which is a complex material, is the major by-product of the sugar cane industry. It contains about 50% cellulose, 25% hemicellulose and 25% lignin. Due to its abundant availability, it can serve as an ideal substrate for microbial processes for the production of value-added products. Attempts have been made to produce from bagasse substrate protein-enriched animal feed, enzymes, amino acids, organic acids and compounds of pharmaceutical importance, etc. Often, a pretreatment process has resulted in improved substrate utilization by the microbes. Application of solid-state fermentation technology could be an attractive possibility for such bioconversions. This article reviews the recent developments on processes and products developed for the value addition of sugarcane bagasse through the biotechnological means. Emphasis has been given on more recent developments of the past 8–10 years.

Keywords: Sugarcane bagasse; Submerged fermentation; Solid state fermentation; Biotechnological applications

1. Introduction

Cellulose, the major constituent of all plant materials, forms about half to one-third of plant tissues and is constantly replenished by photosynthesis. One of the largest cellulosic agro-industrial by-products is sugarcane bagasse (or, 'bagasse' as it is generally called), a fibrous residue of cane stalks left over after the crushing and extraction of the juice from the sugar cane. It is a ligno-cellulosic residue (by-product) of the sugar industry and is almost completely used by the sugar factories themselves as fuel for the boilers.

In recent years, there has been an increasing trend towards more efficient utilization of agro-industrial residues, including sugarcane bagasse. Several processes and products have been reported that utilize sugarcane bagasse as a raw material. These include electricity generation, pulp and paper production, and products based on fermentation. In the present article, we intend to limit our scope on the application of bagasse for the bioconversion processes only. The various products, which have been obtained from the processes involving bagasse include chemicals and metabolites such as alcohol and alkaloids, mushrooms, protein-enriched animal feed ('single cell protein'), and enzymes.

One of the significant applications of bagasse has been for the production of protein-enriched cattle feed and enzymes. The new awareness of the importance of utilizing renewable resources such as bagasse for value addition has led to the development of several processes for the production of protein-enriched cattle feed. Although the economy of such processes in submerged fermentation is severely affected by the high cost of product isolation (and low value of the product), simultaneous isolation and marketing of cellulases enzymes have made economics to recover somewhat. Similarly, although enzymatic saccharification of cellulose has been demonstrated to be uneconomical, cellulases are increasingly being used for the extraction of fruit juices, starch, and oil from woody materials. These enzymes can be recovered rather easily from fermented matter involving solid-state fermentation of bagasse, making this system appropriate for protein enrichment

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and cellulases production from bagasse. However, it remains a fact that in spite of these advances, the commercial exploitation of bagasse-based processes remains limited.

Bagasse could also have been used for the production of biofuel (ethanol). However, processes involving bagasse for ethanol production do require it in substantial quantity. This would affect the supply of fuel for the sugar mills and would necessitate the search for an alternative fuel for them, which has so far largely been unsuccessful (mainly due to economical reasons). In addition, ethanol production from bagasse needs its hydrolysis, which requires large quantities of cellulase enzymes for saccharification. As processes for the production of cellulases are presently quite expensive and economically unfeasible, such bioconversion appears unattractive. Thus, much effort would be needed to develop the technology for economical production of saccharifying enzymes and also improve the condition of hydrolysis. Another aspect in this regard would be the final application of ethanol so produced. In this regard, it would be worth mentioning here the Brazilian Biofuel Programme for cars, which largely has not been successful, due to various reasons, on a global scenario. The experiment has shown that the system is not, overall, commercially viable. Such a programme, however, may eventually be considered useful under special circumstances at special geographical locations such as Brazil or other countries with no or limited oil reserves.

As mentioned previously, almost the entire quantity of the bagasse produced is used by the sugar mills themselves as fuel for boilers, which is necessity-based economical and an efficient application. However, processes such as production of enzymes and other products (e.g., drugs) utilizing bagasse as solid substrate/support would need relatively a small fraction of total bagasse. This may not affect its supply to the sugar mills and thus appears attractive for bioprocesses. If such demands still disrupt or tend to disrupt the bagasse supply to the mills (although surely at a much lesser extent), this could be covered by more efficient furnaces in the mills.

2. Composition of bagasse

Bagasse consists of approximately 50% cellulose and 25% each of hemicellulose and lignin. Chemically, bagasse contains about 50% α-cellulose, 30% pentosans, and 2.4% ash. Because of its low ash content, bagasse offers numerous advantages in comparison to other crop residues such as rice straw and wheat straw, which have 17.5% and 11.0%, respectively, ash contents, 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 t/ha in comparison to about 1, 2, and 20 t/ha for wheat, other grasses and trees, respectively) and annual regeneration capacity.

3. Microbial strains cultivated on bagasse

Over the years, a large number of micro-organisms including bacteria, yeasts and fungi have been used for cultivation on bagasse. However, filamentous fungi, especially basidiomycetes are the preferred choice for enzyme production and protein enrichment and have most widely been employed. A list of different micro-organisms cultivated on the bagasse for varying purpose by different workers is given in Table 1.

4. Pre-treatment of bagasse

Pre-treatment of bagasse has often been found useful to improve its digestibility and easy access for microbial attack (by removing core and noncore lignin fractions) (Alani and Smith, 1988; Doran et al., 1994). The pre-treatment results in enlargement of the inner surface area of substrate particles, accomplished by partial solubilization and/or degradation of hemicellulose and lignin. This leads the fractionation of the three components and opening of cellulose structure. Several physical and chemical methods are employed for the pre-treatment, which include steam explosion, gamma radiation, treatment with alkali, hydrogen peroxide, solvents, etc. Among these, chemical pre-treatments (e.g., treatment with alkali such as NaOH solution) have been found effective and economical.

Rodriguez-Vazguez et al. (1992) treated bagasse (pith) with a solution of sodium hydroxide in such a low volume that no free liquid was present. They referred it as a dry pre-treatment and compared it 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 the dry process. Rodriguez-Vazgues and Diazcervantes (1994) compared various chemical solutions, such as hydroxides of sodium, ammonium, and calcium and hydrogen peroxide, for their efficiency of use in a dry process, which revealed fermentation data in decreasing order as NaOH, $Ca(OH)_2$, NH_4OH, and H_2O_2. Bravo et al. (1994) treated bagasse with water or alkali at three liquid/solid ratios before using it as substrate for microbial protein production. The treatment significantly enhanced fungal growth compared to nontreated bagasse. Aiello et al. (1996) also used sodium hydroxide at various temperatures to pretreat the bagasse for fungal cultivation. DuToit et al. (1984) compared pre-treatments of bagasse with dilute alkali and acid for the determination of the monosaccharides present in bagasse hemicellulose. The

Table 1
Micro-organisms cultivated on bagasse

Micro-organism	References
Acinetobacter calcoaceticus	Valino et al. (1997a,b)
Agrocybe aegarita A1	Zadrazil and Puniya (1995)
Aspergillus ellipticus	Gupte and Madamwar (1997a)
A. fumigatus	Gupte and Madamwar (1997a,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 and Tengerdy (1998), Duenas et al. (1995)
Athelia sp.	Breccia et al. (1997)
Brevibacterium sp.	Nampoothiri and Pandey (1996)
Candida blankii	Meyer et al. (1992)
C. tropicalis	Pessoa et al. (1996)
C. utilis	Christen et al. (1993), Zayed and Mostafa (1992)
Cellulomonas flavigena	Rodriguez-Vazquez et al. (1992), Perezavalos et al. (1996)
Cephalosporium sp.	Valino et al. (1997)
Ceratocystis fimbriata	Christen et al. (1994)
Chaetomium cellulolyticum	Bravo et al. (1994)
Claviceps purpurea	Harnandez et al. (1993)
Clostridium saccharoperbutylacetonicum	Chin et al. (1991)
E. faecium	Iritani et al. (1995)
F. velutipes	Pal et al. (1995)
Fusarium oxysporum	Sharma et al. (1991)
Ganoderma applantum	Breccia et al. (1997)
Gibberella fujikuroi	Tosmani et al. (1997)
Hyphodontia sp.	Breccia et al. (1997)
Klebsiella oxytoca	Doran et al. (1994)
Kuehneromyces mutabilis	Zadrazil and Punia (1995)
Melanocarpus albomyces IIS-68	Jain (1995)
M. purpureus	Chiu and Chan (1992)
Neocallimastix	Teunissen et al. (1993)
Neurospora sitophila	MooYoung et al. (1993)
Panus tigrinus	Breccia et al. (1997)
P. chrysogenum	Barrios-Gonzalez et al. (1993), Sharma et al. (1991)
Phellinus punctatus	Breccia et al. (1997)
Phlebia sp.	Breccia et al. (1997)
Pichia stipitis	Roberto et al. (1991a)
Piromyces sp.	Teunissen et al. (1992, 1993)
Pleurotus sp. P7	Zadrazil and Puniya (1995)
P. cornucopiae	Chaudhary et al. (1994)
P. eryngii	Zadrazil and Puniya (1995)
P. florida	Chaudhary et al. (1994)
P. ostreatus	Elsayed et al. (1994)
P. sajor-caju	Puniya et al. (1996)
Polyporus sp.	Nigam (1990), Nigam et al. (1987a,b)
R. oryzae	Soccol et al. (1994)
Schwanniomyces castellii	Saucedo-Castaneda et al. (1992)
Spongipellis pachyodon	Breccia et al. (1997)
Stereum sp.	Breccia et al. (1997)
Streptomyces sp.	Iyo and Antai, 1991, Modi et al. (1994)
T. versicolor	Pal et al. (1995)
Trichoderma harzianum	Roussos et al. (1992a), Kalra et al. (1984)
T. longibranchiatum	Sidhu et al. (1983)
T. reesei	Gutierrez-Correa and Tengerdy (1997), Gutierrez-Correa and Tengerdy (1998), Duenas et al. (1995), Aiello et al. (1996)
T. viride	Sharma et al. (1991)
Xanthomonas sp.	Roudriguez-Vazquez et al. (1992)

pentosan fraction of the bagasse was successfully hydrolysed and extracted with 5% (m/v) HCl. Treatment with dilute alkali resulted in 39.8% solubilization of bagasse, but only about 72% of the available hemicellulose could be extracted in this way.

A thermochemical pre-treatment of bagasse involved autoclaving with a binary solvent, composed of water and organic solvent having an upper critical temperature (UCT) on the mutual solubility curve. The pre-treatment was termed as 'UCT-solvent pre-treatment' and

proved to be of significant potential (Kurakake et al., 1991). Alkaline hydrogen peroxide treatment of bagasse was also found effective in improving its digestibility (Amjed et al., 1992; Azzam, 1989). Azzam studied the pre-treatment of bagasse with gamma irradiation, coupled with an acid or alkali, which resulted in improved production of biomass protein and in vitro rumen digestibility.

Kling et al. (1987) studied the possibilities of a steam explosion pre-treatment of bagasse in terms of hemicellulose solubilization and enhancement of enzymatic hydrolysis. The pre-treatment led to a significant improvement of sugar yield through enzymatic saccharification.

5. Bioprocess techniques

The processes involving cultivation of microbes on bagasse can be broadly classified into two groups: processes based on liquid fermentation, and processes based on solid-state fermentation (SSF). Liquid fermentation processes (submerged fermentation (SmF)) can be subdivided into two categories: one in which the whole bagasse is used as the substrate, and others in which bagasse is hydrolysed and the hydrolysate is used as the substrate. SSF can also be divided into two sub-groups: one in which bagasse is used as the source of carbon (energy), and others in which it is used as an inert solid support.

6. Application of bagasse in SmF processes

6.1. Processes involving whole-bagasse

Several processes have been reported for the production of enzymes, ethanol, single-cell protein (SCP), etc., on whole-bagasse or treated-bagasse in SmF (Nigam and Prabhu, 1991; Nigam et al., 1987a,b; Nigam et al., 1988; Zayed and Mostafa, 1992; Azzam, 1992; Rodriguez-Vazquez et al., 1992; Rodriguez-Vazquez and Diazcervantes, 1994; Perezavalos et al., 1996; Aiello et al., 1996; Breccia et al., 1997). Table 2 shows some examples of the application of bagasse in SmF.

One of the most widely studied aspects of bagasse application has been on cellulolytic enzymes production. Generally basidiomycetes have been employed for this purpose, the isolates produced extra-cellular cellulases (exo-glucanase, endo-glucanase and β-glucosidase) and ligninases (Kalra et al., 1984; Sidhu et al., 1983; Nigam and Prabhu, 1991; Nigam et al., 1987a, 1988; Sarkar and Prabhu, 1983). Aiello et al. (1996) used a strain of *Trichoderma reesei* QM-9414 for cellulase and biomass production from bagasse. Enzyme yields were higher when alkali-treated bagasse was used, although the difference was very small. Several white-rot fungi were successfully used by Breccia et al. (1997) for the degradation of long-fibre bagasse. All the cultures showed ligninolytic enzymes activity but no correlation was found between the amount of enzymes secreted and the residual composition of the bagasse. Most of the strains caused an increase in the relative concentration of residual cellulose, indicating that hemicellulose was the preferred carbon source.

Teunissen et al. (1992, 1993) used three anaerobic fungi to produce cellulolytic and xylanolytic enzymes from a range of substrates including bagasse. Bagasse was a good inducer for xylanolytic enzymes but not for the cellulolytic enzymes. Enzyme activities were generally lower after growth on glucose and other soluble sugars. SDS-PAGE pattern showed that the differences in enzyme activities were not the result of secretion of different sets of isoenzymes, although it could be possible that the relative amount of each isoenzyme produced was influenced by the growth substrate. These enzymes were produced constitutively. Milagres et al. (1993) reported the production of xylanase by a local fungal isolate in which the enzyme activity was inducible by bagasse.

Table 2
Products of SmF of bagasse

Products	References
Ethanol	Roberto et al. (1991a), Vanzyl et al. (1991), Katzen and Fowler (1994), Gong et al. (1993), Vanwalsum et al. (1996)
Xylitol	Roberto et al. (1991b, 1995), Gurgel et al. (1995), Dominguez et al. (1996), Felipe et al. (1996, 1997a,b), Rodrigues et al. (1998), Alves et al. (1998), Sene et al. (1998), Silva et al. (1997)
SCP/protein enriched feed	Nigam et al. (1987a,b), Zayed and Mostafa (1992), Azzam (1992), Rodriguez-Vazquez et al. (1992), Katzen and Fowler (1994), Rodriguez-Vazquez and Diazcervantes (1994), Elsayed et al. (1994), Pessosa et al. (1996), Aiello et al. (1996)
Mycoprotein	MooYoung et al. (1993)
Aroma	Christen et al. (1994)
Cellulases and ligninases	Nigam and Prabhu (1991), Nigam et al. (1991a), Teunissen et al. (1992, 1993), Ray et al. (1993), Aiello et al. (1996), Breccia et al. (1997)
Xylanases	Milagres et al. (1993), Teunissen et al. (1992, 1993), Jain (1995), Perezavalos et al. (1996)

Another important application of bagasse has been for the production of SCP or protein-enriched cattle feed. Attempts have been made to develop mixed cultures for simultaneous saccharification and fermentation, a process that offers unique advantages (Pandey et al., 1988). Azzam (1992) used a defined mixed culture for biomass production on bagasse. The growth of the two micro-organisms was followed by the production of biomass protein and the in vitro rumen digestibility. The biomass contained 35.5% crude protein and had 69.8% digestibility. Mixed cultures were also used by other workers for SCP production from bagasse or bagasse pith (Rodriguez-Vazquez and Diazcervantes, 1994; Ponce and de-la-Torre, 1993; Molina et al., 1983, 1984). Elsayed et al. (1994) cultivated a fungal strain on bagasse (whole and treated), and after 14 days, an increment of 22.6% of crude protein content in the fermented substrate was observed. MooYoung et al. (1993) cultivated a food-grade fungus on bagasse for food- and fodder-grade mycoprotein production.

Felber et al. (1988) carried out an extensive study on the pre-treatment, enzyme production, hydrolysis, by-product utilization, and energy supply in the degradation of bagasse. Ethanol, SCP, furfural or furfurylic alcohols were the main products produced.

6.2. Processes involving bagasse hydrolysate

The hemicellulose fraction of bagasse has no utility for steam and power generation. Thus, if it can be hydrolysed (partially or completely), it can provide a good substrate for microbial cultivation. The hemicellulosic hydrolysate consists of, mainly, xylose, glucose, mannose, arabinose, galactose and traces of other sugars. The pentosan component of hemicellulose contains mainly D-xylose and a smaller quantity of arabinose. It, however, may also contain substances (depending upon the type of hydrolysis), which could exert toxic effects on micro-organisms. These inhibitory effects, however, could be overcome by the treatment of hydrolysate by various methods, such as treatment with bases or acid, etc. (Roberto et al., 1991b; Dominguez et al., 1996; Alves et al., 1998).

Bagasse hemicellulose hydrolysate has been used for the production of enzymes, SCP, ethanol, xylitol, etc. (Chin et al., 1991; Meyer et al., 1992; Roberto et al., 1991a,b; Katzen and Fowler, 1994; Purchase, 1995; Felipe et al., 1997a,b; Pessoa et al., 1996, 1997; Dominguez et al., 1996; Sene et al., 1998). Ethanol production has been widely studied, from bagasse hydrolysate. An advanced technology was developed by BioEnergy International to convert 5- and 6-carbon sugars into ethanol. The technology utilized novel recombinant strains of bacteria to ferment. A patent was granted to the Purdue Research Foundation (1982) on production of ethanol from hemicellulose waste, such as sugarcane bagasse. Roberto et al. (1991a) investigated ethanol formation by four yeast strains in the bagasse hydrolysate. They compared yeast performance in alkali-treated- and untreated-hydrolysate. Fermentation with treated hydrolysate showed an extended lag phase. Gong et al. (1993) reported inhibition of cell growth and ethanol production by yeasts in alkali-treated bagasse hydrolysate. Treating hydrolysates with either ion-exchange resins or with acidified, activated charcoal, however, could alleviate this inhibition. An acid hydrolysate of bagasse containing xylose, glucose, arabinose, and acetic acid was fermented to ethanol with a yield of 0.27 g. g^{-1}. Fermentation with hydrolysate after removing (84%) acetic acid resulted in higher ethanol yields (0.37 g. g^{-1}) (Vanzyl et al., 1991). Vanwalsum et al. (1996) developed a process to convert bagasse into ethanol using hydrolysate prepared from hot water under pressure. This involved the treatment of bagasse with water at 220°C (5 Mpa, 120 s) and batch simultaneous saccharification and fermentation with *S. cerevisiae* and *T. reesei* cellulase. The hydrolysate produced showed a slight inhibition for the yeast strain.

Xylitol, which is an important substitute for sucrose and finds many applications in the food industry, is another important product produced from bagasse hydrolysate. In a recent review, Nigam and Singh (1995) discussed processes for fermentative production of xylitol. A comparative study on xylitol production with an approach for the utilization of agro-industrial residues revealed bagasse hydrolysate as the one giving the highest xylitol production rate (Roberto et al., 1995). Rodrigues et al. (1998) evaluated batch, fed-batch, and semi-continuous fermentation for xylitol production from bagasse hydrolysate. Best results were achieved by a semi-continuous process. Felipe et al. (1996, 1997a,b) studied environmental parameters affecting xylitol production from bagasse hydrolysate. The bioconversion was affected by cell inoculum level, age of inoculum, hydrolysate concentration, and pH. Xylitol production also markedly depended on aeration rate and on the adaptation of the yeast culture to the hydrolysate. A suitable control of the oxygen input was necessary for efficient xylitol production (Silva et al., 1997). Sene et al. (1998) found that adaptation and reutilization of yeast cells increased xylitol productivity by 15%. Xylitol recovery from the fermented hydrolysate has been an important aspect. Gurgel et al. (1995) used activated carbon to clarify the fermented broth, which after treatment with ion-exchange resin was used for crystallization of xylitol.

7. Application of bagasse in SSF processes

Bioprocessing of agro-industrial residues in SSF has often been found very efficient. There has been a

wide-spread resurgence of SSF all over the world due to several advantages it offers, mainly on engineering aspects (Hesseltine, 1977; Aidoo et al., 1982; Pandey, 1991a,b, 1992, 1994; Nigam and Singh, 1994; Soccol and Krieger, 1998). Numerous SSF processes have been developed in which bagasse has been used as the solid substrate. While in most of the processes, it has been used as the carbon (energy) source, in some processes it has been used as the solid inert support.

7.1. Processes involving bagasse as C-source

Bagasse has most commonly been used for the production of protein-enriched animal feed by SSF, employing yeasts and fungi. A number of reports have appeared on production of animal feed in recent years (Table 3). Nigam et al. (1987a) and Nigam (1990) investigated solid state fermentation of bagasse for animal feed production using basidiomycetes. The C/N ratio and initial moisture were critical factors. Zadrazil and Puniya (1995) differentiated bagasse into four fractions of particle size (<1, 1–3 mm, 3–5 mm and 5–10 mm) with a view to enhancing its nutritive value as animal feed. They found varying degrees 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 sizes 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 optimising 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. Iyo and Antai (1991) achieved 21% crude protein in bagasse after 12 weeks cultivation of a fungal strain of *Streptomyces,* which resulted in 45% depletion of lignocelluloses. A patent was obtained on the application of bagasse, softened with alkali treatment, for feedstuff, fertilizer, and sweetener by cultivating *Enterococcus faecium* in SSF (Iritani et al., 1995). 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 enzymes largely depends upon the cost of the production of cellulases. Application of bagasse in SSF for this purpose appears attractive. Recently, Pandey et al. (1998a,b, 1999) discussed bioconversion processes involving agro-industrial residues, such as bagasse, for their effective utilization to produce value-added products. Sharma et al. (1991, 1995) reported the production of cellulases from different fungal strains. A significant FPD activity was noted from *Pencillium. chrysogenum*, which, apart from the enzyme, also showed high levels of reducing sugars (glucose and xylose). They suggested an integral process for the

Table 3
Products of SSF of bagasse

Products	References
a. Used as carbon/energy source	
Protein enriched feed	Nigam (1990), Nigam et al. (1987a,b), Zadrazil and Puniya (1995), Bravo et al. (1994), Iyo and Antai (1991), Rodriguez-Vazquez 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 and Madamwar (1994, 1997a,b), Gutierrez-Correa and Tengerdy (1997, 1998), Duenas et al. (1995), Roussos et al. (1992a,b)
Laccase	Pal et al. (1995), Machado et al. (1996)
Ligninase	Nigam et al. (1987a,b), 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 and 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 and Chan (1992)
Composting/Ensiling	Baca et al. (1993), Roussos et al. (1992b)
b. Used as inert carrier	
Glutamic acid	Nampoothiri and Pandey (1996)
Ergot alkaloids	Hernandez et al. (1993)
Lactic acid	Soccol et al. (1994)
Citric acid	Lakshminarayana et al. (1975), Manonmani and Sreekantiah (1987),
Pectinases	Solis-Pereyra et al. (1996), Huerta et al. (1994), Acuna-Arguelles et al. (1994)
Penicillin	Barrios-Gonzalez et al. (1993)
Ethanol	Navarro et al. (1982)

production of ethanol, furfural, fermentable sugars and biogas from bagasse. Roussos et al. (1992a) 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 higher yields of cellulase from a strain of *Streptomyces* sp. HM29 when grown on bagasse instead of rice straw, rye straw or corncobs. The 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 has shown a desirable impact on fermentation. Gupte and Madamwar (1997a, 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 improved the enzyme production (Gupte and Madamwar, 1994). Similarly, Gutierrez-Correa and Tengerdy (1997) also reported higher cellulase productivity in coculturing of a basidiomycete strain with another filamentous fungus. A mutual synergism was observed between the parent strain of *T. reesei* LM-UC4 and *A. phoenicis* QM 329, resulting in enhanced combined biomass production and corresponding increase in cellulase, endo-glucanase and β-glucosidase activities. When coculturing was carried out using a mutant strain of *T. reesei* LM-UC4E1, such synergism was absent, suggesting that in the hypermutation the ability for cooperative interaction with other microbes was lost. Treatment of bagasse with ammonia (80%, w/w moisture content) resulted in 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. *Trametes versicolor* produced laccase and manganese-peroxidase activities, showing a simultaneous degradation of lignin and holocellulose. However, only phenol-oxidase activity was found with *Flammulina 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. Beaux et al. (1996) used a mixture of sugarcane bagasse with cassava bagasse for mushroom cultivation.

Xylanase has been another enzyme produced in SSF of bagasse. Xylanases are typically important enzymes for the degradation of plant materials (hemicellulose, which is comprised mainly of xylan). Xylans are formed mainly by a chain of β-1,4 xylanopyranose units highly substituted by acetyl, arabinosyl, and glucopiranosyl residues. Most of the commercially available xylanases are being produced from fungi which are active at neutral or acidic pH and their optimum temperature for activity is below 45°C. Thermophilic xylanases, which are active at alkaline conditions, have great potential for industrial applications. Jain (1995) used a thermophilic fungus for the production of extra-cellular xylanase on various agro-residues, including bagasse. The 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 carried out xylanase production in SSF using bagasse. They co-cultured *T. reesei* and *A. niger* or *A. phoenicis* and achieved high xylanase titres (2600–2800 IU/g dry wt.).

The range of SSF of bagasse increased further with the report appearing on production of other products, such as gibberellic acid. Tosmani et al. (1997) compared gibberellic acid production in SmF with SSF when the latter showed excellent fungal growth.

7.2. Processes involving bagasse as solid inert support

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 developments of SSF systems (Aidoo et al., 1982; Pandey, 1991b, 1992). The use of a 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.

7.2.1. Production of value-added 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. Impregnated bagasse was also used by Hernandez et al. (1993) to grow a fungus culture for the production of ergot alkaloids. They used a total of 16 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 an inert support. The use of a large particle size (14 mm) bagasse increased penicillin production by 37%. Christen et al. (1994, 1997) reported production of a fruity aroma on bagasse when it was fermented with a nutritive medium containing glucose (200 g/l). Twentyfour compounds were separated and 20 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 the potential of bagasse, impregnated with a liquid medium containing glucose and calcium carbonate, to be used as an inert support, for lactic acid production from a strain of *Rhizopus oryzae* NRRL 395. Keeping glucose at 120 and 180 g/l for liquid and solid-state fermentation, yields of 93.8 and 137.0 g/l of L(+)-lactic acid were obtained, respectively. The productivity was 1.38 and 1.43 g/l/h in liquid- and solid-fermentations, respectively. Citric acid was another organic acid, which was produced in SSF using bagasse as an inert carrier (Lakshminarayana et al., 1975). Manonmani and Sreekantiah (1987) conducted citric acid production, using an enzymatic hydrolysate of alkali-treated bagasse, by SSF.

Pectinases were produced in SSF using bagasse, impregnated with a high glucose concentration solution (Solis-Pereyra et al., 1996). The fermentation was carried out in a packed-bed column fermenter for SSF. In a similar study, Huerta et al. (1994) concluded that SSF carried out on inert substrates (they referred to it as the 'adsorbed substrate fermentation' technique) not only allowed the design of culture medium to produce important metabolites, but also the study of fungal metabolism in the artificially controlled SSF processes. Acuna-Arguelles 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 was 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*. The fungus was cultivated in wet bagasse containing PGY medium with corn oil in SSF when it produced red and yellow pigments.

7.2.2. Growth and model studies

Solid substrates of an inert nature offer several advantages in measurements of growth in SSF and have 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 *C. 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) used bagasse impregnated with a liquid growth medium for studying growth kinetics of *A. niger*. Sugarcane bagasse pith has also been used to immobilize yeast cells for the ethanol production (Navarro et al., 1982).

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 seven min. allowed maximum protein extraction and there was no colour interference by the medium components. They claimed that the method was useful for direct biomass estimation in SSF. Valino et al. (1997a) determined the effect of molasses B on the sugar cane bagasse microbiote and *T. viride* fungi. They used a completely randomized design with a 4×3 factorial arrangement. The fermentation process was controlled by evaluating ammonia, total and individual VFA and pH. It was observed that the various proportions of molasses B used did not affect the ammonia concentration produced by the native bagasse microbiote and had a lethal effect on fungus sporulation. Valino et al. (1997b) have also studied the interactions between the microbiotes of bagasse and the strains of fungus *Cephalosporium* sp. and the bacterium *Acinetobacter calcoaceticus* in solid-state fermentation. The results showed a better adaptation of bagasse microbiote, which was more efficient in overcoming any undesirable effect when comparing the cultivation of the mixture of bacteria and fungi with each one separately.

8. Conclusions

It can be concluded that bioconversion of bagasse could be economically advantageous in some cases, e.g., for the production of enzymes, amino acids, and drugs. Such processes require only small quantities of bagasse, which would not be difficult to obtain from the sugar factories. If such demands still disrupted or tended to disrupt the bagasse supply to the mills, this could be covered by improved fuel management, such as by using more efficient furnaces in the mills or by controlling the losses, etc. Diversion of bagasse in large quantity for any other purpose may disrupt the present set-up of sugar factories (its present use as fuel). This could be possible only if some alternative economical fuel for the sugar factories could be found (which so far has largely been unsuccessful). Hence, bioprocesses which need large quantities of bagasse could eventually be considered only if surplus bagasse availability were ensured to meet such demands.

Ethanol production from bagasse needs renewed considerations. One important aspect in this regard would be to develop associated or complimentary technologies during the fuel ethanol programme, which

could produce other value-added by-products whose sale would improve the overall economy of ethanol production. However, as ethanol is a low-value product, it would be worth exploring the possibilities of its end-use for the production of value-added products.

As the untreated bagasse is degraded very slowly by micro-organisms, a pre-treatment step may be useful for improved substrate utilization. Evidently, additional research on the pre-treatment of bagasse is required to improve components yield and cellulose digestibility to the extent which would make its use economically viable. Similarly, although many efforts have been made on hemicellulose hydrolysis, its effective conversion into fermentable sugars is an area which needs further inputs in terms of research and development. Bagasse hemicellulose hydrolysate is a good substrate for production of value-added products. Efforts are also needed to control the formation (or removal) of toxic compounds, such as furan derivatives (2-furaldehyde and 5-hydroxymethyl-2-furaldehyde) and organic acids (formic acid, acetic acid, laevulinic acid), if formed during hydrolysis, as these could affect microbial growth. Enzymatic detoxification may hold promises for this. Lignin obtained by hydrolysis could be a novel source for the production of many aromatic phenolic compounds. Development of improved microbial strains remains an important area for lignin degradation.

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