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Economic Utilization of Crop Residues for Value Addition: A Futuristic Approach

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The agricultural products (cellulosic and ligno-cellulosic, including soft- and hard-wood) having being produced by the photosynthetic processes, are virtually inexhaustible, and could be converted into highly attractive substrate for bioconversion processes. The structure of ligno-cellulosic material is based on three main components: cellulose, hemicellulose and lignin. These three components offer tremendous biotechnological potential to be used as substrate in bioconversion processes and can be effectively exploited for the production of bulk chemicals and value-added products. It is pointed out there is an urgent need to develop processes for economic utilization of these residues through the advances in biotechnology. Some major products which could be produced from these sources include ethanol, enzymes (e.g. cellulases (endoglucanases, exo-cellobiohydrolase and β-glucosidase), ligninases (laccase, Mn-peroxidase and Li-peroxidase), xylanase, pectinase, amylases, α- and β-galactosidases, caffeinase, tannase), food and feed (probiotcis), etc. Some major residues which need special emphasis include sugar cane bagasse, cassava bagasse and coffee pulp. It is mentioned that additional research on the pre-treatment of feedstock is required to improve the components yield and the cellulose digestibility to the level, which would make usage of such residues economically viable. Similarly, although much efforts have been made in the past on the cellulose hydrolysis, its effective conversion into fermentable sugars is an area which needs further inputs in terms of R&D. It is argued that much need be done on the hydrolysis of hemicellulose (and soft-wood). An in-depth analysis is presented on the scenario of applications of crops residues in developing countries, particularly in India, with special reference to sugar cane bagasse, cassava bagasse and coffee pulp.

Introduction

The agricultural products (cellulosic and ligno-cellulosic, including soft- and hard-wood) having being produced by the photosynthetic processes, are virtually inexhaustible, and could be converted into highly attractive substrate for bioconversion processes. The structure of ligno-cellulosic material is based on three main components: cellulose, hemicellulose and lignin. Cellulose is a linear β-D-glucan, associated with an amorphous matrix of lignin and hemicellulose. Lignin is a highly abundant renewable aromatic material on the earth. It is a recalcitrant macromolecule, composed of highly branched polymeric molecules consisting of phenylpropane based monomeric units, linked together by different types of bonds. Hemicellulose is an heteropolysaccharide, composed of neutral sugars, uronic acid and acetyl groups. All the three components offer tremendous biotechnological potential for use as substrate in bioconversion processes and can be effectively exploited for the production of bulk chemicals and value-added products.

Untreated ligno-cellulose is degraded very slowly by the micro-organisms due to the compact and stringent structure of cellulose and its complex association with other components, thus leaving very few reactive sites for enzyme attachment[1-3]. Therefore, these resources are not directly accessible for enzyme hydrolysis and fermentation processes, necessitating a pre-treatment step[4].

Pre-treatment and Hydrolysis of Ligno-cellulose

The pre-treatment process results in the enlargement of inner surface area, accomplished by partial solubilization and/or degradation of hemicellulose and lignin. This leads the fractionation of three components and opening of the cellulose structure.

Physical and chemical methods are employed and steam pre-treatment (including steam explosion) could be an effective and economical method for fractionating

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ligno-cellulosic materials[5-7]. This results in the separation of cellulose, hemicellulose and lignin components, thus paving way for enhanced enzymatic hydrolysis. Evidently, additional research on the pre-treatment of feedstock is required to improve the component yield and the cellulose digestibility to the extent as would make usage of such residues economically viable.

Similarly, although much efforts have been made in the past on the cellulose hydrolysis, its effective conversion into fermentable sugars is an area which needs further inputs in terms of research and development. Much, however, needs to be done on the hydrolysis of hemicellulose (and soft-wood). One important component in this area is the production of enzymes required for the hydrolysis. Solid state fermentation holds promises on this aspect[8-12].

The hemicellulosic hydrolysate consists mainly of xylose, glucose, mannose, arabinose, galactose and traces of other sugars, depending on the kind of the ligno-cellulosic substrate or wood. Pentosan component of hemicellulose results in mainly D-xylose and a smaller quantity of arabinose.

Biotechnological Potential of Ligno-cellulosics

The earliest approach to convert the carbohydrate fractions (of ligno-cellulosics) started some 100 years ago when Klason lignin determination process was evolved in which the hemicellulose and cellulose fractions were gelatinized in 72% sulphuric acid, and after dilution with water, were hydrolyzed to get a mixture of five- and six-carbon (C_5 and C_6) sugars. With an abundant availability of ligno-cellulosic residues generated in the agricultural sectors and forests in India, there is an urgent necessity to develop processes for the economic utilization of these residues through the advances in biotechnology[13-15]. Some of the products, possessing economic potential in production from such substrates, are mentioned below.

Production of Ethanol Fuel

Cellulosic and hemicellulosic biomass can be utilized effectively for the production of biofuel ethanol. In several countries, for example in Canada, Denmark and Brazil, an extensive program is going on with a strategy that will reduce the cost of bioconversion of biomass to fuel ethanol in order to make it price competitive with gasoline by the turn of the century. In Canada, this program has a support of approximately US$ 600,000 per annum from the National Research Council of Canada under Bioenergy Development Program. USDA is also persuading an active program on similar lines since many years.

Some important aspects of the application of ligno-cellulosics for fuel ethanol program, which have a direct economical impact, are the delignification and the hydrolysis of these residues. Although a good amount of published work is available and continues appearing, not much has been attained in practical terms, which could pave way for economical feasibility. Hydrolysis using enzymes offers several advantages, but the cost of production of enzymes is very high and so is the case of hydrolysis.

Thus, the fuel ethanol production in biomass program needs strategic planning. Research and development in this area should focus on the pre-treatment of ligno-cellulosics, improved hydrolysis of the substrate, efficient fermentation utilizing xylose and other five-carbon sugars, and improved ethanol recovery.

Production of Enzymes

There exists an ample opportunity for the production of a number of enzymes from pre-treated ligno-cellulosic substrates[15-17]. These include: cellulases (endoglucanases, exo-cellobiohydrolase and β-glucosidase), ligninases (laccase, Mn-peroxidase and Li-peroxidase), xylanase, pectinase, amylases, α- and β-galactosidases, and some others as discussed in the subsequent pages of this article. As already mentioned, solid state fermentation may be an alternative methodology with economic feasibility.

Xylanases are typically important enzymes for the degradation of plant materials (hemicellulose, which comprises mainly of xylan). Xylans are formed mainly by heteropolysaccharide of a chain of β-1,4-xylanopyranose units highly substituted by acetyl, arabinosyl and glucopiranosyl residues[18]. Most of the commercially available xylanases are being produced from the 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[19].

One important aspect in the area of enzymes is the development of improved fermentation and control strategies, using improved strains with higher enzyme titres. Strain improvement should be focussed using traditional mutation/selection techniques as well as recombinant DNA technology[15]. Another important issue is the full characterization of cellulase (and ligninase) system(s) used for the hydrolysis and determination of optimum ratio of its components, with an aim to incorporate required genes in the producing organism.

Production of Food and Fodder

Bioconversion of crop residues for the production of food and fodder is an area, which needs much attention particularly in countries like India. Cultivation of food-grade cellulolytic micro-organisms, for example, *Neurospora sitophila*, would be an attractive possibility to convert cellulolytic materials to protein-rich products for food and fodder. Solid state fermentation is the most appropriate technology for the production of protein enriched feed. Mushroom cultivation is another example, which has already proved its commercial viability.

Cultivation of GRAS (generally regarded as safe) microbes such as yeasts and basidiomycetes, namely *Polyporus* sp., *Pleurotus* sp., *Trichoderma* sp., etc. on ligno-cellulosics should be considered for food and fodder production. It offers attractive socio-economic and employment opportunities. Such processes do not have serious legal, ethical or safety consequences. However, it would be necessary to look into the bio-safety aspects and analyse the above conditions adequately. Many of such processes provide useful enzymes, e.g. cellulases and ligninases, apart from improving the feed value of the fermented matter by increasing its protein contents[12,13,20-27].

Probiotics

Gastrointestinal diseases are one of the most common diseases responsible for a high number of infant deaths as well as health problems in children and adults. Intestinal micro-flora provides protection from such diseases. Considering the fact that India has a high rate of gastrointestinal diseases, it would be worthwhile to plan isolating of microbial strains from the intestines of human new-borns and select those strains, which show probiotic activity. These could be cultivated in culture media and after characterizing (especially anti-microbial activity) may be used as food supplements for the humans. Hemicellulosic hydrolysate, prepared from ligno-cellulosic residues could be a novel substrate for this purpose[7].

Other Products

The ethanol is a low value product, and it would be worthwhile to explore the possibilities of its end use for the production of value-added products. Also, it would be important to develop associated or complimentary technologies during fuel ethanol program, which could produce other value-added by-products so as to improve the overall economy of the ethanol production. Apart from this, there exists an ample opportunity in producing many novel value-added products from ligno-cellulosics. These include α-cellulose, acetone-butanol, xylitol, single-cell protein, aroma compounds, etc.[28]. Organic acids, such as lactic, fumaric, acetic, malic, etc. and other products such as gibberllic acid, glutamic acid, etc. could also be produced using fungi through the bioconversion of cellulosic residues or hemicellulosic hydrolysate[6,29,30].

Some Typical Agricultural By-products

Although, there are a number of crop residues available in huge quantities, some of these, like sugarcane bagasse, wheat straw, cassava bagasse, coffee pulp, etc. need special mention.

Sugarcane Bagasse

Sugarcane bagasse is a fibrous residue of cane-stalks left over after the crushing and extraction of the juice from the sugarcane. With a targeted (cane) sugar production of 171.19 lakh tones at the end of 9th Five-year plan in 1999-2000, it is expected that there would be huge amount of sugarcane bagasse available for bioconversion. It is a ligno-cellulosic residue and is almost completely used by the sugar factories themselves as fuel for the boilers. It consists of cellulose, hemicellulose and lignin in approx. 50, 25 and 25% ratio, respectively. It must be noted that hemicellulose and lignin are underutilized, as they do not offer adequate calorific value when burnt. 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%, ash contents respectively, 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 tones per hectare in comparison to about 1, 2 and 20 tons per hectare for wheat, other grasses and trees, respectively) and annual regeneration capacity.

In recent years, attempts are being made towards more efficient utilization of sugarcane bagasse. Several processes and products have been reported which utilize sugarcane bagasse as a raw material. These include electricity generation, pulp and paper production, and generation of products based on fermentation. In the present paper, we have limited our discussion to the application of bagasse for the bioconversion processes only. The products, obtained from the bagasse include chemicals and metabolites such as ethanol, organic acids, enzymes, aroma compounds, mushrooms and protein-enriched animal feed (single cell protein), etc.

One of the significant applications of the bagasse has been for the production of protein-enriched cattle feed and enzymes[31-39]. The awareness about the utilisation of renewable natural 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 affected severely, by the high cost of product isolation (and low value of the product), simultaneous isolation and marketing of cellulases enzymes compensate to some extent. Similarly, the enzymatic saccharification of cellulose though has been demonstrated to be uneconomical, yet 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 the fermented matter involving solid state fermentation of bagasse, making this system appropriate for protein enrichment and cellulases production from bagasse. However, it remains the fact that in spite of these advances, the commercial exploitation of bagasse-based processes remains limited.

Bagasse could also been used for the production of biofuel (ethanol)[40-42]. However, processes for ethanol production from bagasse do require the raw material in substantial quantities. This would adversely affect its use as fuel in the sugar mills and would necessitate search for same alternative fuel for that industry, which has largely been unsuccessful so far (mainly due to the economic reasons). In addition, ethanol production from bagasse needs its hydrolysis, which requires large quantity of cellulases enzymes for saccharification. Since processes for the production of cellulases are presently quite expensive and uneconomical, these bioconversions are not lucrative. Thus, much effort is needed to develop the appropriate technology for the economical production of saccharifying enzymes and also the improved conditions of hydrolysis. Another aspect in this regard is the development of technology for efficient utilisation of ethanol so produced. In this regard, it is appropriate to mention here the Brazilian Biofuel Program. This programm for cars, has not been fully successful due to some factors on a global scenario. The experiment itself has indicated that the system is not commercially viable at themoment. Such programmes, however need special considerations due to circumstances prevailing at specific geographical locations such as in Brazil or other countries with no or limited oil reserves.

As mentioned earlier, almost the entire quantity of the bagasse produced is used by the sugar industry as fuel for boilers, which is its necessity-based economical and efficient application. However, processes such as production of enzymes and other products (e.g. organic acids, etc.) utilizing bagasse as solid substrate/support would need relatively only its small fraction. This may not adversely affect its supply to the sugar mills and thus appears attractive for such bioprocesses. The negative effect, if any, could be compensated through the use of more efficient furnaces in the sugar mills.

Bagasse hemicellulose hydrolysate has been used as substrate for the production of some value-added products[43-50]. Although there are only a few groups working in this area, in Brazil, much efforts are required to be made to achieve efficient hydrolysis. Control of formation (or removal) of toxic compounds such as furan derivatives (2-furaldehyde and 5-hydroxymethyl-2-furaldehyde) and organic acids (e.g. formic acid, acetic acid, levulinic acid) which are formed during hydrolysis and which affect adversely the microbial growth are other important aspects to be worked out[50-52]. Efforts should be made to remove (or minimize) these inhibitory factors, using different pre-treatment process such as steam stripping, neutralization of the hydrolysate with the alkali or activated charcoal, ion exclusion chromatography, solvent-extraction, enzymatic detoxification and molecular sieving, etc. Enzymatic detoxification may hold promises in this direction. Lignin obtained by the hydrolysis could be a novel source for the production of many aromatic phenolic compounds. The development of improved strains is an important area for lignin degradation.

Since untreated bagasse is degraded very slowly by the micro-organisms, a pre-treatment step may be useful for the improved substrate utilization. Evidently, additional R&D on the pre-treatment of bagasse is required to improve the components yield and cellulose digestibility to the extent, which would make its use economically viable.

Cassava Bagasse

Cassava (*Manihot esculenta* Cranz) is an important source of food and dietary calories for a large population in tropical countries in the Asia, the Africa and Latin America. Cassava ranks sixth in the world's important food crops and is the basic food for more than 700 million people in several countries[53-55]. It has remarkable capacity to adapt various agro-ecological conditions. It is also considered as a low risk crop. In view of its drought-resistant nature and non-requirement of any specific growth conditions, much attention has been paid on its agricultural aspects for increasing its production all over the world during the past 15-20 years. Consequently, the global production of cassava has steadily increased from about 75 million tons in 1961-1965 to 162 million tons in 1998. India ranks third among the Asian countries in its production (about 5.0-5.5 million tons fresh roots), but the average yields on per hectare basis are highest in India, which are about 20 tons/hectare in comparison to 9-10 tons/hectare as the world average.

The industrial processing of cassava generates solid as well as liquid residues. Solid wastes include peels and bagasse (Figure 1). The processing of 250-300 tons of cassava tubers produces about 1.6 tons of solid peels and about 280 tons of bagasse with high moisture content (85%). Cassava bagasse is a fibrous residue, which contains about 20-50% starch on dry weight basis. Its composition may show variation probably due to most of its processing being done under poorly controlled technological conditions. In addition, the composition may also differ because of the use of different crop varieties. Starch is the main component determined as carbohydrates. Cassava bagasse does not show any cyanide content. However, its poor protein content makes it unattractive as animal feed.

Because of its low ash content, cassava bagasse also could offer numerous advantages for usage in bioconversion processes in comparison to other crop residues, such as rice straw and wheat straw, whose ash contents are 17.5 and 11.0%, respectively. In comparison to other agricultural residues, cassava bagasse can be considered as a rich solar energy reservoir due to its (cassava's) easy regeneration capacity. When compared with sugar cane bagasse, it offers advantages, as it does not require any pre-treatment and can be easily attacked by the microorganisms.

Cassava bagasse has been used for bioconversion mainly in SSF such as for aroma compounds, organic acids, and biotransformation into food and feed using edible fungal cultures. Cassava bagasse hydrolysate could be used for the cultivation of microbial strains to produce value added compounds such as organic acids, aroma compounds (in addition to the traditional applications for the production of food and fodder and other products).

Coffee Pulp

Coffee (*Coffea* sp.) is one of the most important agricultural commodities in the world. Its production is around one million tons per year in more than 50 countries. At different stages, from harvesting to the processing and consumption, several residues, viz. coffee husk, leaves and spent-ground are generated in more than two millions tons quantity yearly. Depending upon the processing method, wet or dry, used for coffee cherries, the solid residues obtained are termed as pulp or husk, respectively (Figure 2). Coffee pulp and husk are rich in organic compounds and nutrients but also contain compounds such as caffeine, tannins, and polyphenols, which make them toxic in nature, limiting their efficient utilization (Table 1). Their disposal rather poses serious environmental concerns.

Brazil is the largest producer of coffee, producing about 30 million sacs per year. India ranks fifth in its production along with Guatemala, Vietnam and Cote d'Ivoire, producing about five million sacs per year. In India, coffee cultivation is mostly distributed in the southern hill ranges. It is considered as an important export-oriented plantation crop, earning about 450 million dollars per year. During the past 50 years, many modifications have been made towards improving the quality of Indian coffee and controlling the diseases. Improved agricultural practices using hybrid varieties have resulted in high increases in the yields. However, not much attention has been paid on economic utilization of coffee pulp obtained from the processing of coffee. There is an urgent need to plan strategies for its efficient utilization through biotechnological means.

Some attempts have been made to use coffee pulp for composting or vermicomposting. Although coffee pulp has also been used as cattle feed, the presence of anti-physiological agents, such as caffeine etc. make it unsuitable for this. In recent years, focus has shifted on

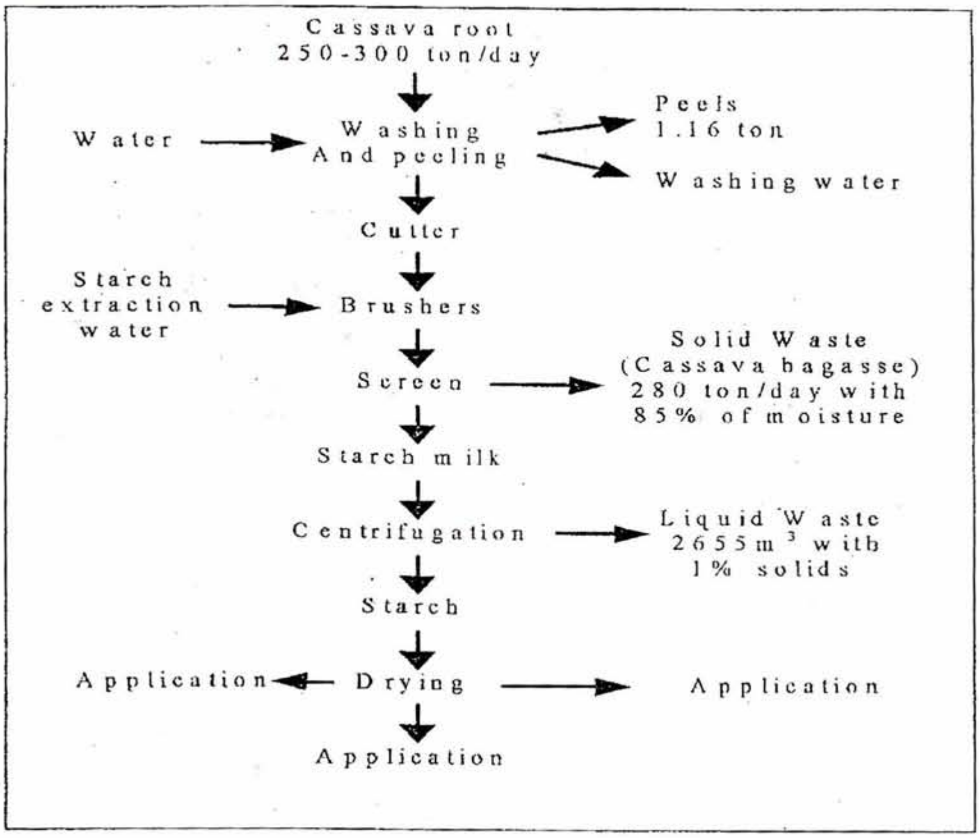

Figure 1 – Industrial processing of cassava

application of coffee pulp as substrate in bioprocesses for production of value-added products such as aroma compounds, organic acids, mushrooms, etc.

One of the most important applications of coffee pulp and husk has been in the production of enzymes such as pectinase, tannase, caffeinase, etc.[13,15,56,57]. Several mushrooms have been cultivated successfully on residues of coffee. These include *Lentinus edodes* (which is one of the most important edible mushrooms cultivated worldwide and has excellent organoleptical properties of flavour and aroma), *Pleurotus* sp. (which is also appreciated for its nutritional and therapeutical qualities), and *Volvariella* sp.[58-60]. The results showed that the coffee industry residues, viz. coffee husk, coffee spent ground and coffee leaves can be used for the cultivation of these mushrooms. The biotechnological advances could lead an effective and economical way to utilize these residues for mushroom cultivation and can improve the economy of the coffee industry.

Studies have also been made towards the detoxification of the coffee pulp and husk through biological means. If the toxic constituents could be removed, or at least degraded to a reasonably low level, it would open new avenues in their utilization as a cattle feed and as a substrate in bioprocesses. Several researchers have been working on the detoxification of coffee pulp and husk through different means including physical, chemical and microbial methods[61-66]. Some of the physical and chemical methods, though have demonstrated good successes, they worked out to be very expensive and could not provide economic feasibility. This has resulted in the entire focus now on the microbial methods.

In the microbial methods, the focuss has been generally on the application of filamentous fungi such as *Penicillium curtosum*, *P. rouquefortii*, *P. verrucosum*, *Phanerochaete chrysosporium* and *Rhizopus* sp.[64,67,68], mostly in the solid state fermentation (SSF), several strains of bacteria such as *Bacillus coagulans*, *Pseudomonas aeruginosa* and *P. putida* have also been found to have the capacity to degrade caffeine[69-72]. Brand *et al.*[65] reported that *Rhizopus* sp. appeared superior to *P. chrysosporium* as it resulted in better degradation of caffeine and tannin in relatively shorter period.

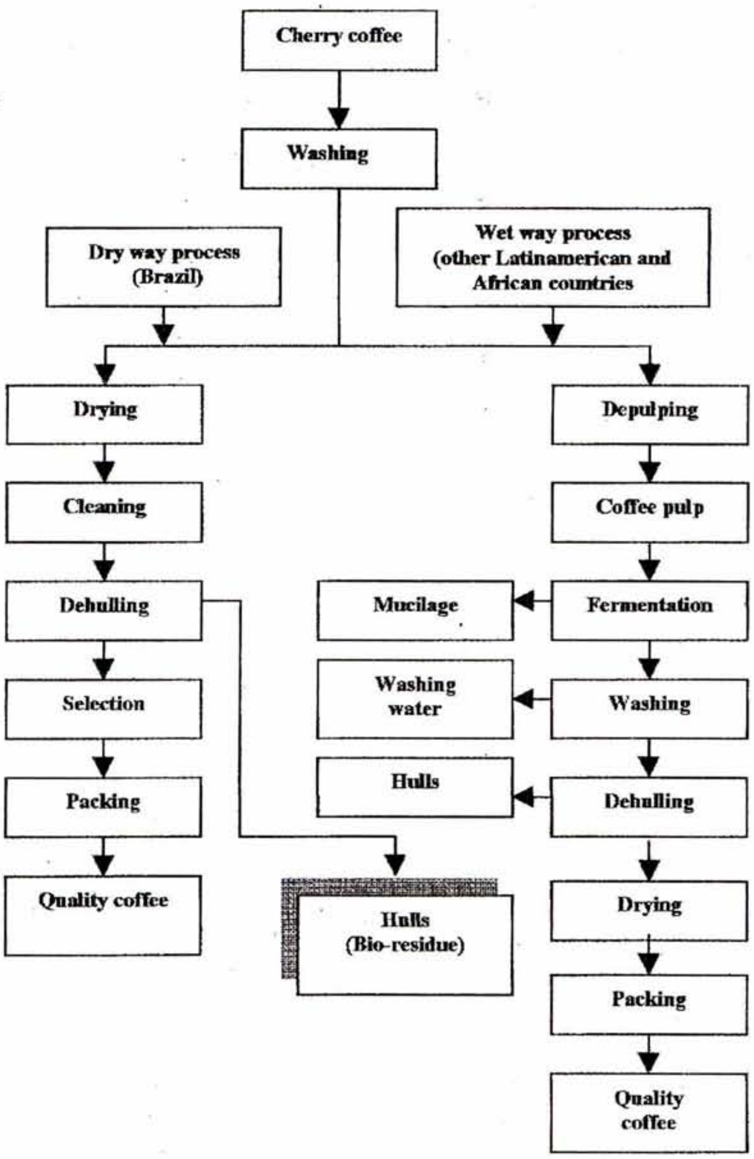

Figure 2 – Industrial processing of coffee

Table 1 – Composition of coffee pulp/husk

Components (dry wt basis)	Composition, %
Carbohydrates	45-50
Proteins	7-10
Fibres	18-21
Fat	2.5
Caffeine	0.9-1.3
Tannins	1.8-8.56
Polyphenols	0.8-1.2

Attempts have also been made to produce organic acids and some other products as aroma compounds, citric acid and gibberllic acid from the coffee husk[29,30,73,74].

Conclusions

It can be concluded that the bio-conversion of sugarscane bagasse could be advantageous economically in some cases, e.g. for the production of enzymes, amino acids and drugs. These processes require only small quantities of bagasse, which would not affect adversely the present usage of bagasse by the sugar factories. Slight downfall in its availability could be compensated through the improved fuel management such as by using more efficient furnaces in the mills or by controlling the losses, energy auditing, 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.

The 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 which could improve the overall economy of the ethanol production. However, since ethanol is a low-value product, it would be worth exploring the possibilities of its end-use for the production of value-added products.

Similarly, the bioconversion of cassava bagasse could be economically useful in some cases, e.g. for the production of enzymes, organic acids, feed, etc. Since cassava bagasse is degraded easily by microorganisms without any pre-treatment, it offers advantages in comparison with sugarcane bagasse. The production of microbial enzymes could be an area to be exploited using cassava bagasse. Biotransformed cassava bagasse could be used as cattle feed. However, these too require evaluation from economical considerations. In this regard, economic constraints may not favour liquid fermentation for such processes. However, SSF may hold promise and focus should be made on developing suitable SSF technologies. Development of efficient microbial strains, mainly fungal cultures, suitable for bioconversion of cassava bagasse is still a largely unexplored area. Efforts should also be made on improving the conditions of cassava bagasse hydrolysis. Its effective conversion into fermentable sugars is an area, which needs further inputs in terms of research and development. Cassava bagasse hydrolysate could serve as a good substrate for generating of value-added products.

Coffee pulp and husk contain small amounts of caffeine and tannins, which make it toxic in nature, resulting in their disposal problem. However, it is rich in organic nature, which makes it an ideal substrate for microbial processes for the production of value-added products. Attempts have been made to detoxify it for improved application as feed, and to produce several products such as enzymes, organic acids, flavours and aroma compounds, and mushrooms, etc. from coffee pulp/husk. Solid state fermentation has been mostly employed for bioconversion processes.

Future Programmes, Resources and Strategies

There is a strong need to continue the research and development projects exploring the biotechnological potential of agro-industrial residues. Financial supports for such projects should continue to be provided by the funding agencies under public sector/government, as it is less likelihood that Indian industries would support this activity in a strong way. These projects should be sponsored primarily to academic and research institutions, and encouragement should be given to link such projects with industries for the development and marketing.

Bioconversion of crop residues for value addition has significant potential. It is an environmentally sound biotechnological application for sustainable development. On one hand it helps in solving the disposal problem of many otherwise under/non-utilized residues, on the other hand it provides economic opportunities. With the advent of biotechnology, bioconversion of crop residues holds tremendous prospects and favourable socio-economic impacts.

References

1 Fan L T, Gharpuray M M & Lee H Y, *Cellulose Hydrolysis* (Springer-Verlag, Berlin) 1987.

2 Buchert J, *Biochemical Oxidation of D-xylose & Hemicellulose Hydrolyzate by Gluconobacter oydans,* PhD Thesis, Technical Research Centre of Finland, 1990.

3 Vikari L, Kantelinen Ratto M & Sundquist M, Enzymes in Biomass Conversion, *ACS Symp Ser* 460, (1991) pp. 12-21.

4 Ahring B K, Bjere A B, Nielsen, P & Schmidt A S, Conversion of wheat straw to ethanol, *Proc V Brazilian Symp Chemistry of Lignins and Other Wood Components,* edited by Ramos L P, Curitiba, Brazil, (1997), pp. 422-429.

5 Ramos L P & Emmel A, Fractionation of *Eucalyptus grandis* wood chips by steam explosion. I. Process Optimization, Conversion of wheat straw to ethanol, *Proc V Brazilian Symp Chemistry of Lignins and Other Wood Components,* edited by L P Ramos, Curitiba, Brazil, (1997), pp. 146-155.

6 Woiciechowski A L, Soccol C R, Ramos L P, Buczenko G M & Pandey Ashok, Experimental design to optimize the production of L-(+)-lactic acid from steam exploded wood hydrolysate using *Rhizopus oryzae*, *Process Biochem*. (in press).

7 Woiciechowski A L, Soccol C R, Pandey Ashok & Bustao E, Selective hydrolysis of hemicellulose fraction of coffee husk for production of sugars, *Third International Seminar on Biotechnology in the Coffee Agro-industry*, Londrina, Brazil, 24-28 May 1999, pp. 54.

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

9 Pandey Ashok, Solid state fermentation – An overview, in *Solid State Fermentation*, edited by A Pandey (Wiley Eastern Ltd, New Delhi, India) 1994, pp. 3-10.

10 Tengerdy R P, Solid substrate fermentation for enzyme production, in *Advances in Biotechnology*, edited by A Pandey (Educational Publishers & Distributors, New Delhi, India) 1998, pp. 13-16.

11 Pandey Ashok & Soccol C R, Bioconversion of biomass: A case study of lignocellulosics bioconversions in solid state fermentation, *Brazilian Arch Biol Technol*, **41** (1998) 379-390.

12 Pandey Ashok, Azmi W, Singh J & Banerjee U C, Fermentation types and factors affecting it, in *Biotechnology: Food Fermentation*, Vol. I, edited by Joshi V K & Pandey A (Educational Publishers & Distributors, New Delhi, India) 1999, pp. 383-426.

13 Pandey Ashok, Soccol C R, Nigam P & Soccol V T, Biotechnological potential of agro-industrial residues: I. Sugarcane bagasse, *Bioresource Technol*, accepted.

14 Pandey Ashok, Soccol C R, Nigam P, Mohan R & Vandenberghe L P S, Biotechnological potential of agro-industrial residues: II. Cassava bagasse, *Bioresource Technol*, accepted.

15 Pandey Ashok, Selvakumar P, Soccol C R & Nigam P, Solid state fermentation for the production of industrial enzymes, *Curr Sci*, **77** (1999) 149-162.

16 Pandey Ashok, Benjamin S, Soccol C R, Nigam P, Krieger N & Soccol V T, The Realm of microbial lipases in biotechnology, *Biotechnol Appl Biochem*, **29(2)** (1999) 119-131.

17 Pandey Ashok, Selvakumar P, Soccol C R, Soccol V T, Krieger N & Fontana J D, Recent developments in microbial inulinases – Its production, properties and industrial applications, *Appl Biochem Biotechnol*, **81**(1) (1999) 1-18.

18 Aspinall G O, Chemistry of cell wall polysaccharides, in *The Biochemistry of Plants*, Vol 3, *Carbohydrates: Structure and Function*, edited by J Preiss (Academic Press, NewYork) (1980) pp. 473-500.

19 Durate M C T, Portugal E P, Ponezi A & Franco T T, Alkalophilic xylanases production from bacteria, *Proc V Brazilian Symp Chemistry of Lignins and Other Wood Components*, edited by L P Ramos, Curitiba, Brazil, (1997) pp. 340-345.

20 Nigam P & Pandey Ashok, Utilization of by-products of cane sugar industry, *Coop Sugars*, **17** (1986) 539-42.

21 Nigam P, Pandey Ashok & Prabhu K, Mixed culture fermentation for bioconversion of whole bagasse into microbial protein, *J Basic Microbiol*, **26**(5) (1987) 323-28.

22 Nigam P, Pandey Ashok & Prabhu K, Ligninolytic activity of two basidiomycetes mould cultures in the decomposition of bagasse, *Biol Wastes*, **21**(1) (1987) 1-10.

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

24 Pandey Ashok, Nigam P & Vogel M, Process selection for single stage bioconversion of sugar beet pulp into microbial protein, *Biol Wastes*, **26**(1) (1988) 71-75.

25 Pandey Ashok, Nigam P & Vogel M, Simultaneous saccharification and protein enrichment fermentation of sugar beet pulp, *Biotechnol Lett*, **10**(1) (1988) 67-72.

26 Leifa F, Pandey Ashok & Soccol C R, Solid state fermentation and fructification of *Pleurotus ostreatus* on the residue of coffee, *Proc, Third International Seminar on Biotechnology in the Coffee Agro-industry*, Londrina, Brazil, 24-28 May, 1999, pp. 50.

27 Leifa F, Pandey Ashok & Soccol C R, Cultivation of *Pleurotus* sp. on coffee residues, *Proc, Third International Conference on Mushroom Biology and Mushroom Products*, Sydney, Australia, 12-16 October, 1999.

28 Medeiros A B P, Pandey Ashok, Soccol C R & Christen P, Optimization of production of aroma compounds by *Kluyveromyces marxianus* in solid state fermentation using factorial design and surface response, (1999) submitted.

29 Machado C M M, Oliveira B H, Soccol C R & Pandey Ashok, Coffee husk as a substrate for the production of gibberllic acid by fermentation, *Proc, Third International Seminar on Biotechnology in the Coffee Agro-industry*, Londrina, Brazil, 24-28 May, 1999, pp. 39.

30 Vandenberghe L P S, Soccol C R, Pandey Ashok & Lebeault J M, Citric acid production by *Aspergillus niger* in solid state fermentation, *Bioresource Technol*, (1999) in press.

31 Breccia J D, Bettucci L, Piaggio M & Sineriz F, Degradation of sugarcane bagasse by several white-rot fungi, *Acta Biotechnologica*, **17** (1997) 177-184.

32 Teunissen M J, Dekort G V M, Dencamp H J M O & Vogels G D, Production of cellulolytic and xylanolytic enzymes during growth of anaerobic fungi from ruminant and nonruminant herbivores on different substrates, *Appl Biochem Biotechnol*, **39** (1993) 177-189.

33 Milagres A M F, Lacis L S & Prade R A, Characterization of xylanase production by a local isolate of *Penicillium janthinellum*. *Enzyme Microbial Technol*, **15** (1993) 248-253.

34 Azzam A M, Pretreatment of cane bagasse with alkaline hydrogen peroxide for enzymatic hydrolysis of cellulose and ethanol fermentation, *Environ Sci Hlth, Part B*, **24** (1992), 421-433.

35 Rodriguez-Vazquez R & Diazcervantes D, Effect of chemical solutions sprayed on sugarcane bagasse pith to produce single cell protein - Physical and chemical analysis of pith, *Bioresource Technol,* **47** (1994) 159-164.

36 MooYoung M, Chisti Y & Vlach D, Fermentation of cellulosic materials to mycoprotein foods, *Biotechnol Adv,* **11** (1993) 469-479.

37 Puniya A K, Shah K G, Hire S A, Ahire R N, Rathod M P & Mali R S, Bioreactor for solid state fermentation of agro-industrial wastes, *Indian J Microbiol,* **36** (1996) 177-78.

38 Iritani S, Mitsuhashi M, Chaen H & Miyake T, New alkali treated bagasse used as livestock feed. PN : GB 2285806 (1995); 26.07.95.

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

40 Roberto I C, Lacis L S, Barbosa M F S & Demancilha I M, Utilization of sugarcane bagasse hemicellulose hydrolysate by *Pichia stipitis* for the production of ethanol, *Process Biochem,* **26** (1991) 15-21.

41 Gong G S, Chen C S & Chen L F, Pretreatment of sugarcane bagasse hemicellulose hydrolysate for ethanol production by yeast, *Appl Bioch Biotechnol,* **39** (1993) 83-88.

42 Vanwalsum G P, Allen S G, Spencer M J, Laser M S, Antal M J & Lynd L R, Conversion of ligno-cellulosics pre-treated with liquid hot water to ethanol, *Appl Bioch Biotechnol,* **57-58** (1996) 157-170.

43 Rodrigues D C G A, Silva S S, Prata A M R & Felipe M D A, Biotechnological production of xylitol from agroindustrial residues – Evaluation of bioprocesses, *Appl Biochem Biotechnol,* **70-72** (1998) 869-875.

44 Nigam P & Singh D, Processes for fermentative production of xylitol – A sugar substitute, *Process Biochem,* **30** (1995) 117-124.

45 Felipe M G A, Vitalo M, & Mancilha I M, Xylitol formation by *Candida guilliermondii* grown in a sugarcane bagasse hydrolysate – Effect of aeration and inoculum adaptation, *Acta Biotechnol,* **16** (1996) 73-79.

46 Silva S S, Ribeiro J D, Felipe, M G A & Vitolo M, Maximizing xylitol production from sugarcane bagasse hydrolysate by controlling the aeration rate, *App Biochem Biotechnol,* **63-65** (1997) 557-564.

47 Sene L, Felipe M G A, Vitalo M, Silva S S & Mancilha I M, Adaptation and reutilization of *Candida guilliermondii* cells for xylitol production in bagasse hydrolysate, *J Basic Microbiol,* **38** (1998) 61-69.

48 Felipe M G A, Vitalo M, Mancilha I M & Silva S S, Fermentation of sugarcane hemicellulose hydrolysate for xylitol production: Effect of pH, *Biomass Bioenergy,* **13** (1997) 11-14.

49 Pessoa A, deMancilha I M & Sato S, Evaluation of sugarcane hemicellulose hydrolysate for cultivation of yeasts and filamentous fungi, *J Ind Microbiol Biotechnol,* **18** (1997) 360-363.

50 Dominguez J M, Gong C S & Tsao G T, Pretreatment of sugarcane bagasse hemicellulose hydrolysate for xylitol production by yeast, *Appl Biochem Biotechnol,* **57-58** (1996) 49-56.

51 Roberto I C, Felipe M G A, Lacis L S, Silva S S & Demancilha I M, Utilization of sugarcane bagasse hemicellulose hydrolysate by *Candida guilliermondii* for xylitol production, *Bioresource Technol,* **36** (1991) 271-275.

52 Alves L A, Felipe M G A, Silva J B A E, Silva S S & Prata A M R, Pre-treatment of sugarcane bagasse hemicellulose hydrolysate for xylitol production by *Candida guilliermondii, Appl Biochem Biotechnol,* **70-72** (1998) 89-98.

53 Soccol C R, Aplicações da fermentação no estado sólido na valorização deresíduos agroindustriais, *França-Flash Agric,* **4** (1995) 3-4.

54 Kato M S A & Souza S M C, *Informe Agropecudrio,* **13** (1987) 9-16.

55 Soccol CR, Biotechnological products from cassava roots by solid state fermentation, *J Sci Ind Res,* **55** (1996) 358-64.

56 Antier P, Minijares A, Roussos S, Raimbault M & Viniegra-Gonzalez G, Pectinase hyper-producing mutants of *Aspergillus niger* C28B25 for solid state fermentation of coffee pulp, *Enzyme Microbiol Technol,* **15** (1993) 254-259.

57 Boccas F, Roussos S, Gutierrez M, Serrano L & Vineigra, G G, Production of pectinase from coffee pulp in solid state fermentation: selection of wild fungal isolate of high potency by a simple three step screening technique, *J Food Sci Technol,* **31** (1994) 22-26.

58 Leifa F, Pandey Ashok & Soccol C R, Growth of *Lentinus edodes* on the coffee industry residues and fruiting body production, *Third International Conference on Mushroom Biology and Mushroom Products,* Sydney, Australia, 12-16 October, 1999.

59 Leifa F, Pandey Ashok, Krieger M A & Soccol C R, Submerged fermentation of coffee pulp extract using *Volvariella volvacea, Third International Seminar on Biotechnology in the Coffee Agro-industry,* Londrina, Brazil, 24-28 May 1999, pp. 51.

60 Leifa F, Pandey Ashok & Soccol C R, Production of edible mushroom *Lentinus edodes* on the coffee spent ground, *Third International Seminar on Biotechnology in the Coffee Agro-industry,* Londrina, Brazil, 24-28 May 1999, pp. 49.

61 Molina M, Feunte de la G, Batten M & Bressani R, Decaffeination: A process to detoxify coffee pulp, *J Agric Food Chem,* **22** (1974) 1055-1059.

62 Bressani R, Anti-physiological factors in coffee pulp, in *Coffee Pulp: Composition, Technology, Utilization,* edited by J E Braham & R Bressani, (Publication 108e, International Development Research Centre, Ottawa) (1979), pp. 83-88.

63 Udayashankar K, Manohar B & Chokkalingam A, A note on super critical carbondioxide decaffeination of coffee, *J Food Sci Technol,* **23** (1986) 326-328.

64 Aquiahuatl M A, Raimbaukt M, Roussos M & Trejo-Hernandez M, Coffee pulp detoxification by solid state fermentation, in *Proc, Seminar Solid State Fermentation, Bioconversion of Agro-industrial Raw-materials*, edited by M Raimbault (ORSTOM, Montpellier) 1988, pp. 13-26.

65 Brand D, Pandey Ashok, Roussos S, Raimbault M & Soccol C R, Biological detoxification of the coffee husk using filamentous fungi in solid state fermentation, *Proc, Third International Seminar on Biotechnology in the Coffee Agro-industry*, Londrina, Brazil, 24-28 May 1999, pp. 38.

66 Leifa F, Pandey Ashok, Vandenberghe L P S & Soccol C R, Effect of caffeine on *Pleurotus* sp. and bioremediation of caffeinated residues, *IX European Congress on Biotechnology*, Brussels, Belgium, 11-15 July 1999, pp. 2664.

67 Roussos S, Hannibal L, Angeles-Aquiahuatl M de los, Trejo-Hernandez M del R & Marakis S, Caffeine degradation by *Penicillium verrucosum* in solid state fermentation of coffee pulp: Critical effect of additional inorganic and organic nitrogen sources, *J Food Sci Technol*, **31** (1994) 316-319.

68 Roussos S, Angeles-Aquiahuatl M de los, Trejo-Hernandez M del R, Gaime-Perraud, I, Favela E, Ramakrishna M, Raimbault M & Viniegra-Gonzalez G, Biotechnological management of coffee pulp- isolation, screening, characterization, selection of caffeine degrading fungi and natural micro-flora present in coffee pulp and husk, *Appl Microbiol Biotechnol*, **42** (1995) 756-762.

69 Schwimmer S & Kurtzman R H, Fungal decafeinization of roast coffee infusions, *J Food Sci*, **37** (1972) 921-923.

70 Blecher R & Lingens F, The metabolism of caffeine by *Pseudomonas putida* strains, *Z Physiol Chem*, **358** (1977) 807-817.

71 Kurtzman R H & Schwimmer S, Caffeine removal from growth media by micro-organims, *Experiencia*, **127** (1971) 481-482.

72 Woolfolk C A, Metabolism of *N*-methylpurines by a *Pseudomonas putida* strain isolated by enrichment on caffeine as the sole source of carbon and nitrogen, *J Bacteriol*, **123** (1975) 1088-1106.

73 Soares M, Soccol C R, Pandey Ashok, Fontana F & Christen P, Optimization of culture medium for production of volatile compounds by *Pachysolen tannophilus* grown on solid coffee wastes, *Proc. Third International Seminar on Biotechnology in the Coffee Agro-industry*, Londrina, Brazil, 24-28 May 1999, pp. 40.

74 Soares M, Christen P, Raimbault M & Soccol C R, Fruity aroma production by *Ceratocystis fimbriata* grown on solid coffee wastes, *Proc. Third International Seminar on Biotechnology in the Coffee Agro-industry*, Londrina, Brazil, 24-28 May 1999, pp. 52.