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Review paper

Biotechnological potential of agro-industrial residues. II: cassava bagasse

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Abstract

Advances in industrial biotechnology offer potential opportunities for economic utilization of agro-industrial residues such as cassava bagasse. Cassava bagasse, which is a fibrous material, is the by-product of the cassava-processing industry. It contains about 30–50% starch on dry weight basis. Due to its rich organic nature and low ash content, it can serve as an ideal substrate for microbial processes for the production of value added products. Attempts have been made to produce several products such as organic acids, flavour and aroma compounds, and mushrooms from cassava bagasse. Solid-state fermentation has been mostly employed for bioconversion processes. This paper reviews the developments in processes and products developed for the value addition of cassava bagasse through biotechnological means.

Keywords: Cassava bagasse; Submerged fermentation; Solid-state fermentation; Biotechnological applications

1. Introduction

In recent years, there has been an increasing trend towards more efficient utilization of agro-industrial residues such as cassava bagasse, sugar cane bagasse, sugar beet pulp, coffee pulp/husk, apple pomace, etc. Several processes have been developed that utilize these as raw materials for the production of bulk chemicals and value-added fine products such as ethanol, single-cell protein (SCP), mushrooms, enzymes, organic acids, amino acids, biologically active secondary metabolites, etc. (Pandey, 1992, 1994; Pandey et al., 1988; Nampoothiri and Pandey, 1996; Pandey and Soccol, 1998). Application of agro-industrial residues in bioprocesses on the one hand provides alternative substrates, and on the other hand helps in solving pollution problems, which their disposal may otherwise cause. With the advent of biotechnological innovations, mainly in the area of enzyme and fermentation technology, many new avenues have opened for their utilization. In this article, we intend to discuss the biotechnological potential of cassava bagasse for value addition.

2. Cassava

Cassava (*Manihot esculenta* Cranz) is considered an important source of food and dietary calories for a large population in tropical countries in Asia, Africa and Latin America. It is known as tapioca in Asian countries, as aipin, castelinha, and macaxeira in Brazil, as yuca in Spanish-speaking countries of Latin America, and as manioc in French-speaking countries in Africa (Soccol, 1996; Rosemberg 1957; Laukevics et al., 1985). Cassava is considered to have originated in Venezuela during 2700 B.C. (Soccol, 1996). In a significant research published during May 1999, biologists from the Washington University in St. Louis discovered that cassava originated from the southern border of the Amazon River basin in Brazil. They used a sophisticated DNA sequencing technique that traced variation in a single gene (*G3p*dh) found in cultivated and wild cassava (Sachaal and Olsen, 1999). It was introduced in Africa during the 16th century and from there into Asia during the 18th century. It is a bushy plant producing tubers

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and is made up of an aerial part and an underground part. The aerial part can be as high as 2–4 m with a trunk and branches on it. The underground part consists of two types of roots: the ones responsible for the plant nutrition, and the others with axial disposition surrounding the trunk. These are called tubers and are the edible parts of the plant. Each plant may have 5–20 tubers, and each tuber may attain a length of 20–80 cm and a diameter of 5–10 cm. The fresh weight of each tuber may vary between a few hundred grams and 5 kg. Table 1 shows the chemical composition of cassava tubers. As is evident, the cassava tubers are rich in starch but poor in protein.

Cassava ranks as the world's sixth most important food crop and is the basic food for more than 700 million people in several countries (Soccol, 1996; Kato and Souza, 1987; Cereda et al., 1996). It has the remarkable capacity to adapt to 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 in the past 15–20 years to its agricultural aspects, for increasing its production all over the world, which has been well achieved. World production of cassava has steadily increased from about 75 million ton in 1961–1965 to 153 million ton in 1991. During 1994, the world production touched approximately 167 million ton (FAO, 1995), but fell slightly in 1998 to 162 million ton (FAO/GIEWS, 1999). Africa is the largest producer with about 53% of the world's production, followed by Asia with about 29% and Latin America with about 18%. Although cassava is cultivated in about 88 countries, only five countries account for about 67% of the production. These are Nigeria (approximately 31 million ton), Brazil (23 million ton), Thailand, 19 million ton), Zaire (18 million ton), and Indonesia (16 million ton) (Soccol, 1996). Brazil ranks first in the production in Latin America. Although India ranks third among the Asian countries in the production (about 5.0–5.5 million ton fresh roots), the average yields on a per hectare basis are highest in India, and are about 20 ton/ha in comparison to 9–10 ton/ha, the world average (Pandey and Damodaran, 1991).

Table 1
Physico-chemical composition of cassava tubers (100 g basis)[a]

Composition	Fresh weight	Dry weight
Calories	135	335
Moisture (%)	65.5	15.7
Proteins (g)	1.00	1.4
Lipids (g)	0.2	0.5
Starch (g)	32.4	80.6
Fibres (g)	1.1	1.2
Ash (g)	0.9	1.8
Calcium (mg)	26	96
Phosphorus (mg)	32	81
Iron (mg)	0.9	7.9
Sodium (mg)	2	–
Potassium (mg)	394	–
Vitamin B2 (mg)	0.04	0.06
Vitamin C (mg)	34	0
Niacine (mg)	0.6	0.8
Cyanide (%)	–	1.6

[a] Source: Hohnholz, (1980), Soccol (1996) and Cereda and Takahashi (1996).

About 60% of the cassava produced all over the world is used for human consumption. It is consumed in natural form as flour or in fermented forms such as *gari*, *fufu*, etc. The most important consumer is Africa with an average of about 102 kg/person/year or 220 kcal/person/day (Giraud, 1993). Another large consumer of cassava is the animal food industry, using about 33% of the world production. The remaining 7% is used by industries such as textile, paper, food and fermentation. With the advent of biotechnological approaches, focus has shifted to widening the application of cassava and its starch for newer applications with the aim of value addition.

2.1. Industrial processing of cassava

Industrial processing of cassava is done mainly to isolate flour (which generates more solid residues) and starch (which generates more liquid residues) from the tubers. Most of the industries are of small or medium size. Fig. 1 shows the processing of cassava tubers for isolating starch with the mass balance. Two types of

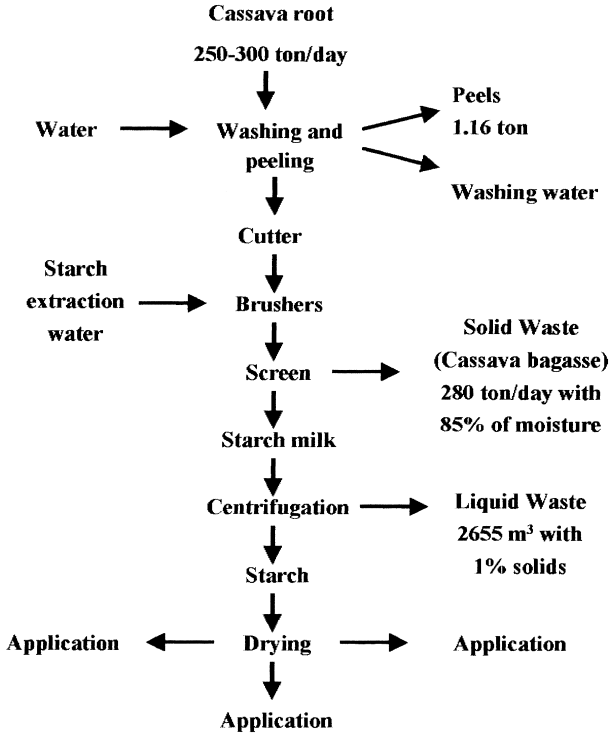

Fig. 1. Industrial processing of cassava.

wastes are generated: solid and liquid. Solid wastes include peels and bagasse. As is evident, processing of 250–300 ton of cassava tubers results in about 1.6 ton of solid peels and about 280 ton of bagasse with a high moisture content (85%). Liquid wastes include wastewater (about 2655 m^3) with about 1% solids. Solid wastes are generally discarded in the environment as landfill without any treatment. Their disposal is a serious concern to the environment.

2.2. Cassava bagasse

Cassava bagasse is a fibrous residue, which contains about 50% starch on a dry weight basis (Carta et al., 1999). Table 2 shows the composition of cassava bagasse as determined by various authors. These analyses (Table 2) were conducted on the bagasse samples obtained from different processing units at different times in the State of Parana, Brazil. The composition shows variation probably due to the fact that most of the processing is done under poorly controlled technological conditions. In addition, the composition may also differ due to 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 an animal feed.

Because of its low ash content, cassava bagasse could offer 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. 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 pretreatment and can be easily attacked by micro-organisms.

3. Microbial strains cultivated on cassava bagasse

Micro-organisms which utilize starch as the substrate for growth and activity have generally been preferred for bioconversion processes utilizing cassava bagasse because of its high starch content. Some yeasts and fungi have been used for cultivation on cassava bagasse. However, filamentous fungi have been most widely employed. Table 3 cites different micro-organisms cultivated on cassava bagasse for various purposes.

Table 2
Physico-chemical composition of cassava bagasse (g/100 g dry weight)

Composition	Soccol (1994)	Cereda (1994)	Sterz (1997)	Vandenberghe (1998b)
Moisture	5.02	9.52	10.70	11.20
Protein	1.57	0.32	1.60	1.61
Lipids	1.06	0.83	0.53	0.54
Fibers	50.55	14.88	22.20	21.10
Ash	1.10	0.66	1.50	1.44
Carbohydrates	40.50	63.85	63.40	63.00

Table 3
Bioprocesses involving cassava bagasse[a]

Micro-organism	Process	Application	Reference
A. niger LPB 21	SSF	Citric acid	Kolicheski et al. (1995)
A. niger NRRL 2001	SSF	Citric acid	Vandenberghe et al. (1999)
A. niger CFTRI 30	SSF	Citric acid	Shankaranand and Lonsane (1994)
Candida lipolytica	SmF	Citric acid	Vandenberghe et al. (1998b)
C. fimbriata	SSF	Aroma compounds	Christen et al. (1997)
C. fimbriata	SSF	Aroma compounds	Bramorski et al. (1998a)
K. marxianus	SSF	Aroma compounds	Medeiros (1998)
L. edodes	SSF	Mushroom	Beux et al. (1995)
P. sajor-caju	SSF	Mushroom	Barbosa et al. (1995)
Rhizopus sp.	SSF	Biotransformation	Soccol et al. (1995a,b,c)
R. arrahizus	SmF	Fumaric acid	Carta et al. (1999)
R. ciricians	SmF	Fumaric acid	Carta et al. (1999)
R. delemer	SmF	Fumaric acid	Carta et al. (1999)
R. formosa	SmF	Fumaric acid	Carta et al. (1998, 1999)
R. oligosporus	SmF	Fumaric acid	Carta et al. (1999)
R. oryzae	SmF	Fumaric acid	Carta et al. (1999)
R. oryzae	SSF	Aroma compounds	Bramorski et al. (1998b)

[a] SSF: solid-state fermentation, SmF: submerged fermentation.

4. Cultivation systems

The processes involving cultivation of microbes on cassava bagasse can broadly be classified into two groups: processes based on liquid fermentation, and processes based on solid-state fermentation (SSF). Most of the work has been carried out in SSF systems (Soccol, 1996). High water retention capacity (85–90%) also makes it an ideal substrate for SSF processes. Submerged fermentation (SmF) processes have rarely been utilized due to obvious reasons of cost effectiveness.

5. Bioprocesses involving cassava bagasse

5.1. Production of aroma compounds

One of the important areas of cassava bagasse utilization in bioprocesses has been on the production of flavour and aroma compounds. Bramorski et al. (1998a) compared fruity aroma production by *Ceratocystis fimbriata* in solid cultures from several agro-industrial wastes: cassava bagasse, apple pomace, amaranth and soya bean. Cassava bagasse was used in combination with soya bean or apple pomace. All the media supported fungal growth. Media containing cassava bagasse with apple pomace or soya bean produced a strong fruity aroma. The aroma production was growth dependent and the maximum aroma intensity was detected a few hours before or after the maximum respirometric activity. Sixteen compounds were separated by gas chromatography of the components present in the headspace and 15 of them were identified as acid (1), alcohols (6), aldehyde (1), ketones (2) and esters (5). Christen et al. (1997) studied fruity aroma production from various agro-industrial residues; wheat bran, cassava bagasse and sugar cane bagasse. All the substrates were shown to be adequate substrates for the growth and aroma production by the mould *C. fimbriata*. Addition of glucose to the solid medium (200 g l^{-1}) resulted in the production of a fruity aroma, whereas addition of leucine or valine resulted in a strong banana aroma. In these studies also aroma production was dependent on growth and the maximum aroma intensity was detected at about the time of the maximum respirometric activity. Twentyfour compounds were separated by headspace analysis using GC and 20 were identified as aldehyde (1), alcohols (7), ketones (4) and esters (8). It was clearly demonstrated that the chromatographic profile of the headspace of the culture was dependent on the substrate used and on the eventual precursor added. When leucine or valine was added to the substrate, the production of total volatiles in the headspace was ten times higher than that for ripe bananas. The Gompertz model, a logistic-like equation, was used to fit the integrated CO_2 and volatiles production data.

Bramorski et al. (1998b) also studied the production of volatile compounds by the edible fungus *Rhizopus oryzae* during solid-state cultivation on tropical agro-industrial substrates. When *R. oryzae* was grown on a medium containing cassava bagasse plus soybean meal (5:5 w/w), CO_2 production rate was at its highest (200 ml/l), whereas the highest volatile metabolite production was with amaranth grain as the sole substrate (282.8 ml/l). In the headspace, ethanol was the most abundant compound (more than 80%). Acetaldehyde, 1-propanol, ethyl propionate and 3-methyl butanol were also present. CO_2 and volatile metabolite productions reached their maxima around 20 and 36 h, respectively.

A strain of the yeast *Kluyveromyces marxianus* was used for the production of a fruity aroma in SSF using cassava bagasse as substrate (Medeiros, 1998). Experiments were performed with a 2^5 statistical experimental design. The parameters studied were cultivation temperature, pH, initial water content and C/N ratio of substrate, and inoculum size. The volatile compounds were measured by the headspace analysis on a HP 5890 GC. A sensorial evaluation was employed to characterize the aroma of the cultures. The initial pH and the C/N ratio of the medium were statistically significant at 5% level for the production of volatile compounds. Aroma production increased in acidic pH (3.5) medium with a C/N ratio of 100. Sensorial evaluation of the produced volatiles revealed a fruity aroma, probably due to the production of isoamyl acetate and ethyl acetate. These compounds were identified by comparing with standard compounds. The results showed the feasibility of using cassava bagasse as a substrate to produce a fruity aroma with *K. marxianus* in SSF.

Flavour and aroma (including fragrance) compound synthesis by biotechnological processes nowadays plays an increasing role in the food, feed, cosmetic, chemical and pharmaceutical industries. This is mainly due to an increasing preference by the consumer for natural food additives and other compounds of biological origin. Attempts are being made to produce such compounds by fermentation of simple nutrients such as sugars and amino acids. In this regard, the works mentioned above have good implications. However, it will be necessary to develop efficient extraction processes for isolating these compounds from the fermentation media.

5.2. Biotransformation of cassava bagasse

In view of the high starch contents of cassava bagasse, an approach was applied to biotransform this into food and feed using edible fungal cultures. Soccol et al. (1995a, 1995b, 1995c) explored the possibilities of cultivating *Rhizopus* strains capable of attacking raw cassava starch present in cassava bagasse. They used 19 *Rhizopus* strains in SSF, but only three of them were capable of attacking significantly raw starch present in

cassava bagasse, six attacked moderately, seven weakly and three did not grow at all (Soccol et al., 1995a). Based on the results, a strain of *R. oryzae* 28627 was selected for further studies. Ideal conditions for biotransformation of cassava bagasse in SSF were determined. These were: temperature, 28–32°C, inoculation rate, 10^5 spores/g dry bagasse, substrate initial moisture and pH, 70% and 5.7–6.4, respectively, and C/N, 4.7–14. After 24 h of fermentation, the fermented matter showed 12 g protein/100 g cassava bagasse on a dry weight basis, which was almost sevenfold more than the initial protein content of cassava bagasse (1.67 g/100 g dry bagasse). The yield coefficient between consumed starch and synthesised protein was about 0.50 (Soccol et al., 1995b). The results on scale-up showed that tray-type bioreactors were most suitable. Six to eight cm thick substrate bed in the trays was good to ferment without any considerable loss in the growth of the fungus, as compared to initial experiments in the flasks. The biotransformed cassava bagasse showed a satisfactory quality on microbiological evaluation required by the existing law in Brazil. The final protein content in this case was about 13.5 g/100 g of dried cassava bagasse (Soccol et al., 1995c). These results are important, but it needs further studies to improve the protein content.

5.3. Production of mushrooms

Cassava bagasse has also been used for mushroom cultivation in SSF. Beux et al. (1995) compared the cultivation of *Lentinus edodes* on cassava bagasse and sugarcane bagasse, individually or in their mixture. Both the substrates were found suitable for mushroom production, but the best results were obtained when a mixture of cassava bagasse (80%) and sugarcane bagasse (20%) was used. Data on kinetics of starch consumption (present in cassava bagasse) showed that about 77% of the starch was used during the biotransformation process. The protein content of the substrate was improved three times. The results were claimed to be useful in providing a novel alternative technology for *shiitake* production. Barbosa et al. (1995) also compared cassava bagasse and sugarcane bagasse for mushroom production. They used a different fungal culture, *Pleurotus sajor-caju*. Cassava bagasse showed good potential for mushroom cultivation, but the best results were obtained when cassava bagasse was used in a mixture with sugarcane bagasse (8:2, dry weight basis). The results were claimed to be useful for upgrading the cassava bagasse for animal feed.

5.4. Production of organic acids

Among the various products produced through microbial cultivation on cassava bagasse, organic acids are important ones. Among these, citric acid production has been well studied and reported. Citric acid is used in several industrial processes, such as food and pharmaceutical industries. It is also used in cosmetics and plastic industries. Most of the production of citric acid is by fermentation (submerged or liquid-surface methods), employing agro-industrial residues such as sugar beet molasses (Milson and Meers, 1985; Wang, 1998). Attempts have also been made to use alternative substrates such as carob pod extract (Roukas, 1998). In recent times, much attention has been paid to the production of citric acid from various other agro-industrial residues through the SSF route (Hang and Woodams, 1998; Pintado et al., 1998; Pintado and Lonsane, 1998). Kolicheski et al. (1995) studied citric acid production on three cellulosic supports in SSF. Out of the six strains of *Aspergillus niger* one, LPB 21, was selected for cultivation on cassava bagasse, sugarcane bagasse and vegetable sponge. Cassava bagasse was found to be a good substrate, giving 13.64 g citric acid per 100 g dry substrate. This corresponded to 41.78% yield. Under improved fermentation conditions, the citric acid production increased to 27 g/100 g dry substrate, which corresponded to 70% yield (based on sugars consumed). Shankaranand and Lonsane (1994) presented a comparative profile of citric acid production from various agro-industrial residues, such as cassava bagasse, wheat bran, rice bran, sugarcane pressmud, coffee husk, etc., using an indigenous strain of *A. niger* CFTRI 30. Cassava bagasse gave the highest yield of citric acid based on the total starch or sugars present initially in the medium (Shankaranand and Lonsane, 1994). Vandenberghe et al. (1999) used three substrates, sugarcane bagasse, coffee husk and cassava bagasse for citric acid production with *A. niger* NRRL 2001. Cassava bagasse best supported the mould's growth, giving the highest yield of citric acid among the tested substrates. The citric acid production reached a maximum (88 g/kg dry matter). The results were of significant importance for commercial production.

5.5. Hydrolysis of cassava bagasse and application of hydrolysate in bioprocesses

One alternative approach for utilizing cassava bagasse has been to subject it to enzymatic hydrolysis using amylolytic enzymes and then to use the hydrolysate for cultivation of micro-organisms for bioprocesses. Vandenberghe et al. (1998a) and Carta et al. (1997) conducted a study to optimize the hydrolysis parameters of cassava bagasse using commercially available α-amylase (Termamyl 120 L) and glucoamylase (AMG, Novo Nordisk). A statistical experimental design was used to obtain a hydrolyzate with high concentration of reducing sugars, mainly glucose. Parameters studied were the concentration of enzyme, period of enzyme action and bagasse particle size. Best results were obtained when

hydrolysis was carried out using 100 μl of Termamyl 120 L/100 g of starch at 90°C for one h, and 471 μl of AMG/100 g of starch at 60°C for 24 h with a particle size <0.84 mm. Hydrolysates reached 110 g/l of reducing sugars, demonstrating a good conversion efficiency.

Cassava bagasse hydrolysate was used for the production of citric acid by fermenting with a yeast culture of *Candida lipolytica* NRRL Y-1095 (Vandenberghe et al., 1998b). Batch fermentations were performed in two stages, a carbon-limited growth phase and a nitrogen-limited phase. In the first stage, growth was carried out at 28°C, pH 5.5 and 150 rpm for 40–48 h, and then the cells were separated from the medium by centrifugation. In the second stage, fermentation was carried out using these cells (28°C, pH 5.5, 200 rpm, 96 h). Citric acid yields were 10 g/l. However, 2.5 g/l of isocitric acid was also produced simultaneously.

Another important organic acid produced using cassava bagasse hydrolysate is fumaric acid ($C_4H_4O_4$). Fumaric acid has a wide range of applications. It is an interesting intermediate in chemical synthesis involving esterification reactions. It is non-toxic and non-hygroscopic in nature and due to these properties is also used as an acidulant in the food and pharmaceutical industries. Carta et al. (1998, 1999) studied the prospects of production of fumaric acid from cassava bagasse. First, cassava bagasse was subjected to enzymatic hydrolysis and the hydrolysate so obtained was used for fumaric acid production. Submerged fermentation was carried out using several *Rhizopus* strains. Six different media were constituted using different nitrogen sources and hydrolysate. An experimental design was used to optimize the media and cultivation conditions. The strain *Rhizopus formosa* MUCL 28422 was found to be the best fumaric acid producer, yielding 21.28 g/l in a medium containing cassava bagasse hydrolysate as the sole carbon source, KNO_3 as nitrogen source (C/N ratio of 168), 20 g/l of $CaCO_3$, 10 μg/l of biotin, 0.04 g/l of $ZnSO_4 \cdot 7H_2O$, 0.25 g/l of $MgSO_4 \cdot 7H_2O$, 0.15 g/l of KH_2PO_4, and 15 ml/l of methanol. The process was found feasible for commercial fumaric acid production.

6. Conclusions

It can be concluded that bioconversion of cassava bagasse could be economically useful in some cases, e.g. for the production of enzymes, organic acids, feed, etc. As cassava bagasse is degraded easily by micro-organisms without any pretreatment, it offers advantages in comparison with sugarcane bagasse. 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 would require economical considerations too. 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 SSF technologies. Development of efficient microbial strains, mainly fungal cultures, suitable for bioconversion of cassava bagasse is still a largely unexplored area. Efforts should be also made for improving cassava bagasse hydrolysis conditions; 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 production of value-added products.

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