World Journal of Microbiology & Biotechnology 10, 165–168

Coffee husk: an inexpensive substrate for production of citric acid by *Aspergillus niger* in a solid-state fermentation system

V.S. Shankaranand and B.K. Lonsane*

***Aspergillus niger* CFTRI 30 produced 1.3 g citric acid/10 g dry coffee husk in 72 h solid-state fermentation when the substrate was moistened with 0.075 M NaOH solution. Production was increased by 17% by adding a mixture of iron, copper and zinc to the medium but enrichment of the moist solid medium with $(NH_4)_2SO_4$, sucrose or any of four enzymes did not improve production. The production of about 1.5 g citric acid/10 g dry coffee husk at a conversion of 82% (based on sugar consumed) under standardized conditions demonstrates the commercial potential of using the husk in this way.**

Key words: *Aspergillus niger*, citric acid, coffee husk, solid-state fermentation.

Coffee husk, a solid residue generated in the processing of coffee by the dry method, poses disposal problems and causes environmental pollution. Its present use as manure (Natarajan *et al.* 1952) leads to the loss of valuable organic matter while its use as fuel is highly inefficient due to its low calorific value. Exploratory studies on the potential of coffee husk for production of citric acid in a solid-state fermentation (SSF) system were therefore undertaken. No information is presently available on utilization of coffee husk for production of citric acid by any fermentation technique.

Materials and Methods

Microorganism and Substrate

Aspergillus niger CFTRI 30 was isolated locally, from a soil sample from an area where sugarcane bagasse is dumped by a sugarcane juice stall, using potato/dextrose/agar (PDA) containing 0.04% Bromocresol Green. The purified culture was identified by the procedure of Onions *et al.* (1981) and maintained on PDA slants by sub-culturing every month. The methodology for inoculum preparation was as described elsewhere (Shankaranand & Lonsane 1994). A single batch of dry coffee husk from a local coffee-curing works was pulverized to a particle size of 0.6 to 2.0 mm. All the chemicals and reagents used were of analytical grade. Pectinase, hemicellulase and cellulase were obtained from Novo Nordisk, Denmark, while amylase was from Sigma. All the experiments were carried out in duplicate and only means are reported as values varied by less than 5% around the mean. The key results were confirmed twice to establish the validity of the data.

Solid-State Fermentation

Coffee husk powder (10 g) in a 250-ml Erlenmeyer flask was mixed with 0.075 M NaOH solution to an initial moisture content of 50% (w/w). After autoclaving at 121°C for 15 min, the flasks were cooled to ambient temperature and inoculated with 1 ml of a spore suspension of *A. niger* CFTRI 30 (with about 1×10^7 spores/ml). The contents of the flasks were then mixed well and the flasks incubated in a slanting position at 30 ± 1°C. The pH of the autoclaved and uninoculated medium was 4.5 when measured by the method of Raimbault & Alazard (1980).

Optimization of Medium Parameters

The strategy adopted was to optimize one particular parameter at a time and then include it at its optimum value in the next optimization step, if found beneficial. The parameters optimized were, in chronological order: (1) supplementation with a mixture of iron, copper and zinc at 1.0, 0.2 and 0.1 p.p.m., respectively; (2) enrichment with 0.05% to 0.2% (w/w) $(NH_4)_2SO_4$; (3) addition of finely-powdered sucrose in varying amounts to increase the effective initial sugar concentration in the medium from 12.5% to 20% (w/w); and (4) enrichment of the medium at room temperature with each of four enzymes at 5% (v/w). The sucrose was sterilized by exposing it in an u.v. disinfector before adding it to the moist, autoclaved, uninoculated medium.

Extraction of Citric Acid

The fermented coffee husk was agitated on a rotary shaker for 60 min after mixing it with 10 vol. distilled water (based on

The authors are with the Department of Microbiology and Bioengineering, Central Food Technological Research Institute, Mysore - 570 013, India; fax: 0091-821-27697. *Corresponding author.

initial weight of the moist fermented coffee husk). The slurry was filtered through Whatman No. 1 filter paper and the resulting clear extract used in the analyses. Calculation of the conversion of sugar into citric acid was based on the difference in total sugar present in the system pre- and post-fermentation.

Analytical Techniques

Moisture content in the coffee husk was determined by drying the solids at 90°C to a constant weight. Reducing sugars, as glucose, were estimated using a glucose analyser after refluxing with distilled water. Total carbohydrate was determined by the phenol/H_2SO_4 method of Dubois *et al.* (1956) and citric acid by the colorimetric method of Marier & Boulet (1958) using pyridine and acetic anhydride reagents. Spot tests for citric acid were carried out according to Fiegl (1954).

Results and Discussion

Coffee Husk

Coffee husk contained 18% (w/w) reducing sugar as glucose when refluxed with distilled water for 2.5 h. The total sugar concentration was 20% (w/w) when extracted with distilled water at room temperature. The total sugars increased to 23% and 25% (w/w) after autoclaving with water and 0.075 M NaOH, respectively, probably due to the hydrolysis of some of the complex polysaccharides in the husk.

Citric Acid Production in Coffee Husk Medium

Aspergillus niger CFTRI 30 consumed 70% of the total sugars initially present in the medium and produced 1.3 g citric acid/10 g dry coffee husk at the end of 72 h fermentation (Figure 1). This represents 51% conversion based on the total initial sugar present in the medium and 73% based on the sugar consumed by the organism. Further fermentation decreased the content of citric acid with negligible utilization of sugar.

Citric acid production increased to 1.5 g/10 g dry coffee husk when the medium was supplemented with metal ions (Figure 1). Similarly, consumption of sugar increased to 74%, with simultaneous increases in conversions to 60% based on the total initial sugar and 82% based on the sugar consumed. In medium with metal ion supplementation 0.65 g sugar/10 g dry coffee husk remained unutilized after 72 h and the pH profile was different from that in the basal medium. Sugar (0.74 g/10 g dry coffee husk) also remained unutilized after 72 h fermentation in the medium without mineral salts (Figure 1) and little more was used after further fermentation up to 120 h. The amount of residual sugar is of importance from the economic point of view and the management of the spent solid residue that will arise from industrial plants.

Coffee husk, which contains 4.4% to 5.6% ash on a dry wt basis (Natarajan *et al.* 1952), slightly less than that in wheat bran, should be a more suitable substrate for the production of citric acid, at least in an SSF system (Shankaranand & Lonsane 1994). That adding metal ions to the husk increased citric acid production indicates that the metal ions in the husk are probably in bound form.

Enrichment of Coffee Husk with $(NH_4)_2SO_4$

Enrichment of the medium with 0.05% or 0.1% (w/w) $(NH_4)_2SO_4$ neither increased citric acid production nor decreased residual sugar concentration, and higher $(NH_4)_2SO_4$ concentrations (0.15% and 0.2%) decreased citric acid production (by 20% and 31%, respectively) without any effect on residual sugar (Figure 2). The basal medium was therefore not deficient in nitrogen and the residual sugars

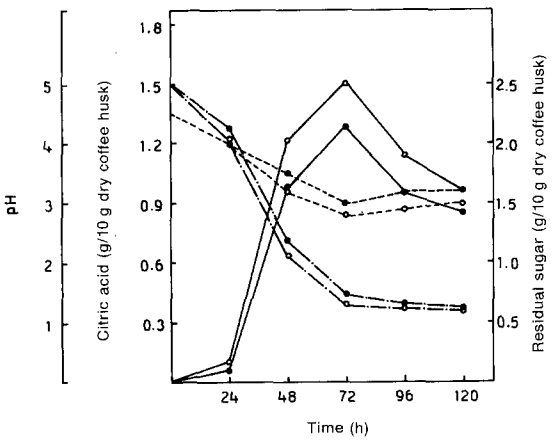

Figure 1. Effect of metal ions on the production of citric acid by *Aspergillus niger* CFTRI 30 in solid-state fermentation using basal coffee husk medium moistened with 0.075 M NaOH (●) or the same medium enriched with a mixture of iron (1.0 p.p.m.), copper (0.2 p.p.m.) and zinc (0.1 p.p.m.) (○). ——— Citric acid; —·—·— Residual sugar; – – – – – – pH.

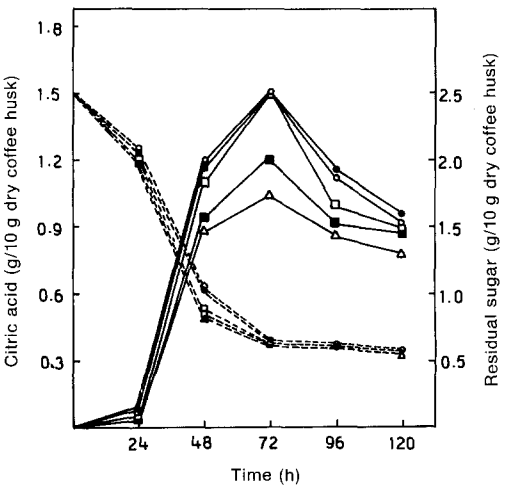

Figure 2. Effect of enrichment of coffee husk with 0 (○), 0.05 (●), 0.1 (□), 0.15 (■) and 0.2% (w/w) (△) $(NH_4)_2SO_4$ on citric acid production (——) and residual sugar (– – –).

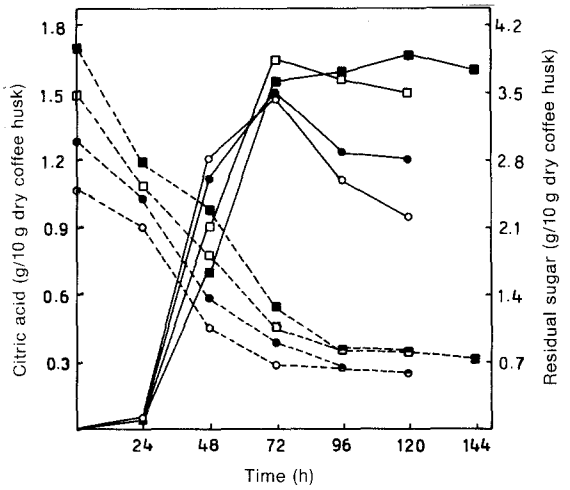

Figure 3. Effect of 12.5 (○), 15 (●), 17.5 (□) and 20% (w/w) (■) initial effective sugar concentration on citric acid production (——) and residual sugar (– – – –).

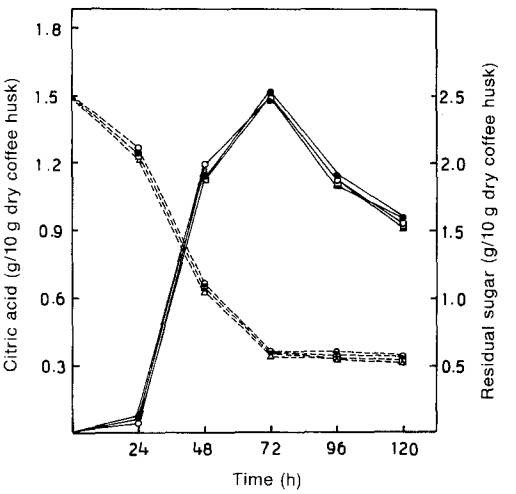

Figure 4. Effect of addition to the coffee husk medium of amylase (●), pectinase (□), hemicellulase (■), cellulase (△) or no enzymne (○) on citric acid production (——) and residual sugar (– – – –).

at the end of fermentation were probably non-utilizable in nature.

Effect of Enrichment with Sucrose

The effective sugar concentration in the moist coffee husk medium is 12.5% (w/w), which is slightly lower than the 14% to 22% commonly used in industrial submerged fermentation processes (Röhr *et al.* 1983). However, supplementation of the medium with sucrose to increase the effective sugar concentration to 15% (w/w) gave negligible improvement. Although citric acid production increased by 12% and 13% when the effective sugar concentration was increased to 17.5 and 20% (w/w), respectively (Figure 3), the peak in production was shifted by 48 h, due to a slower rate of production, and residual sugars increased to 0.82 and 0.81 g/10 g dry coffee husk, respectively (Figure 3). The results thus indicate that addition of sucrose to increase the effective initial sugar concentration is not economically viable.

Effect of Addition of Enzymes

Cellulase, hemicellulase, pectinase or amylase, when added at ambient temperature to the moist, autoclaved medium, neither improved the citric acid production nor reduced residual sugar (Figure 4).

Practical and Economic Considerations

The data indicate that about 1.5 g citric acid is produced per 10 g dry coffee husk in the optimized medium, conversion reaching about 80% based on sugar consumed. The husk is either similar to or far better than substrates such as sugar beet pulp impregnated with pineapple juice (Cahn 1935), sugarcane bagasse impregnated with molasses (Lakshminarayana *et al.* 1975), apple pomace (Hang & Woodams 1984), grape pomace (Hang & Woodams 1985), kiwi-fruit peel (Hang *et al.* 1987) and sugarcane-pressmud (Shankaranand & Lonsane 1993). There is therefore considerable potential for the commercial use of coffee husk for citric acid production and this would reduce the environmental pollution which husk disposal presently causes.

Citric acid is an industrially-important organic acid that finds extensive uses in numerous industries (Kapoor *et al.* 1982). Demand for the acid world-wide far exceeds current production (Röhr *et al.* 1983). Although countries such as India lack the indigenous technology and typical substrates, such as beet sugar molasses required for citric acid production, they may have other potential substrates in excess. India generates about 150,000 tonnes of coffee husk/year. The use of the husk as a component of animal feed (Adams & Dougan 1987) will pose severe problems due to the anti-nutritional factors in the husk, such as caffeine, chlorogenic acid and tannins (Natarajan *et al.* 1952; Reddy 1992). Extraction of caffeine and protein from coffee husk (Natarajan *et al.* 1952) may not prove economical due to their low concentrations. Use of the husk as an adjunct to coffee (Natarajan *et al.* 1952) may not be acceptable to regulatory agencies and the consumers. The use of coffee husk extract for production of yeasts (Adams & Dougan 1987) may not be practicable or economic in countries like India. Use of the husk to produce a value-added product such as citric acid, would appear to be the best possible solution. Moreover, the spent residue left after the leaching of the citric acid can be decontaminated to kill the microorganisms and then used as an animal feed component. The residual sugar concentration of about 6% (w/w) would

then gain in significance and the SSF may also reduce the concentrations of anti-nutritional factors (Reddy 1992).

It may be worthwhile to examine the coffee-processing industry in India and the availability of coffee husk. Karnataka state is the major producer of coffee, followed by Kerala, Tamil Nadu, Assam and Andhra Pradesh. Although the total production is 400,000 tonnes of cherries/year, only the robusta cherries (300,000 tonnes) are subjected to the dry processing that leads to the generation of coffee husk. Twenty-one of the 40 curing mills in India, are located in Karnataka and these process 77,000 tonnes of cherries/year. There are three mills in Mysore alone, (operating throughout the year and producing 20, 20 and 10 tonnes of husk/day) and the other curing mills in Karnataka lie within 150 km of Mysore.

One commercial SSF plant could process 5 tonnes of coffee husk and produce 0.75 tonnes of citric acid/day. The designs of larger SSF plants are not available within India and the removal of metabolic heat would cause insurmountable problems in very large bioreactors. Our calculations indicate that an SSF plant processing just 30,000 kg of husk/year would be more economical than one involving submerged fermentation (Ghildyal *et al.* 1985). One of the most exciting advantages SSF offers the developing countries is that it has economic characteristics even at relatively low capacity, unlike submerged fermentation. It should be possible and economic to establish 10 plants in India, each processing 5 tonnes coffee husk/day.

Acknowledgements

VSS is thankful to the Director, Central Food Technological Research Institute, Mysore, India, and to the Council of Scientific and Industrial Research (CSIR), New Delhi, India, for the award of a Senior Research Fellowship.

References

Adams, M.R. & Dougan, J. 1987 Waste Products. In *Coffee. Vol. 2: Technology*, eds. Clarke, R.J. & Macrae R. pp. 257–287. Essex: Elsevier Applied Science.

Cahn, F.J. 1935 Citric acid fermentation on solid materials. *Industrial and Engineering Chemistry* **27**, 201–204.

Dubois, M.K., Gilles, K.A., Hamilton, J.K., Rebers, P.A. & Smith, F. 1956 Colorimetric method for determination of sugars and related substances. *Analytical Chemistry* **28**, 350–356.

Fiegl, F. 1954 *Spot Tests*, Vol. 2. Amsterdam: Elsevier.

Ghildyal, N.P., Lonsane, B.K., Sreekantiah, K.R. & Murthy, V.S. 1985 Economics of submerged and solid state fermentations for the production of amyloglucosidase. *Journal of Food Science and Technology* **22**, 171–176.

Hang, Y.D., Luh, B.S. & Woodams, E.E. 1987 Microbial production of citric acid by solid state fermentation of kiwifruit peel. *Journal of Food Science* **52**, 226–227.

Hang, Y.D. & Woodams, E.E. 1984 Apple pomace: a potential substrate for citric acid production by *Aspergillus niger*. *Biotechnology Letters* **6**, 763–764.

Hang, Y.D. & Woodams, E.E. 1985 Grape pomace: a novel substrate for microbial production of citric acid. *Biotechnology Letters* **7**, 253–254.

Kapoor, K.K., Chaudhury, K. & Tauro, P. 1982 Citric acid. In *Prescott and Dunn's Industrial Microbiology*, ed. Reed, G. pp. 709–746. Westport, CT: AVI Publishing.

Lakshminarayana, K., Chaudhary, K., Ethiraj, S. & Tauro, P. 1975 A solid state fermentation method for citric acid production using sugar cane bagasse. *Biotechnology and Bioengineering* **17**, 291–293.

Marier, J.R. & Boulet, M. 1958 Direct determination of citric acid in milk with an improved pyridine-acetic anhydride method. *Journal of Dairy Science* **41**, 1683–1692.

Natarajan, C.P., Bhatia, P.S. & Subrahmanyan, V. 1952 Studies on the utilization of coffee husk. *Journal of Scientific and Industrial Research* **11a**, 410–411.

Onions, A.H.S., Allisop, D. & Eggins, H.O.W. 1981 *Smith's Introduction to Industrial Mycology*. London: Edward Arnold.

Raimbault, M. & Alazard, D. 1980 Culture method to study fungal growth in solid fermentation. *European Journal of Applied Microbiology and Biotechnology* **9**, 199–209.

Reddy, G.D. 1992 Biotechnological management of coffee hulls. M.Tech. Dissertation. University of Mysore, Mysore, India.

Röhr, M., Kubicek, C.P. & Kominek, J. 1983 Citric acid. In *Biotechnology. Vol. 3: Biomass, Microorganisms for Special Applications, Microbial Products I, Energy from Renewable Sources*, ed Dellweg, H. pp. 419–454, Weinheim: Verlagchemie.

Shankaranand, V.S. & Lonsane, B.K. 1994 Ability of *Aspergillus niger* to tolerate metal ions and minerals in a solid-state fermentation system for the production of citric acid. *Process Biochemistry* **29**, 29–37.

Shankaranand, V.S. & Lonsane, B.K. 1993 Sugarcane-pressmud as a novel substrate for production of citric acid by solid-state fermentation. *World Journal of Microbiology and Biotechnology* **9**, 377–380.

(Received in revised form 5 July 1993; accepted 7 August 1993)