BIOTECHNOLOGICAL MANAGEMENT OF COFFEE PULP

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General inroduction

Agro-industrial residues/wastes are generated in large quantities throughout the world. Their non-utilization results in loss of valuable nutrients and environmental pollution (Zuluaga, 1989). Their better utilization by biotechnological means assumes social, economic and industrial importance. Considering these facts, ORSTOM participated into a scientific collaboration with Universidad Autonoma Metropolitana (UAM), Mexico, for the development of biotechnological processes for better utilization of agro-industrial byproducts/wastes, especially the coffee pulp (Viniegra *et al*, 1991). Coffee pulp, generated to the extent of 40% in the fermentation of coffee berries (Zuluaga, 1989), poses many problems in the coffee producing tropical countries. Its disposal in nature, without any treatment, causes severe environmental pollution, due to putrefaction of organic matter (Zuluaga, 1989). Hence, the possibility of utilizing coffee pulp in the biotechnological processes for production of different metabolites was investigated thorought by Roussos *et al* (1993).

Coffee pulp is the main byproduct on coffee explotation industry. Two tons of green coffee produces one ton of coffee pulp (dry matter). Its production on world scale rised 2.400.000 tons (two million four hundred thausand tons) in the harvest cycle from 1986 to 1987. Coffee pulp is essentially composed of carbohydrates, proteins, aminoacids, mineral salts, tannins, poly phenols and caffeine. The last two compounds are reported to be antiphysiological factors on animal feed. Hence, coffee pulp has to follow a preliminary treatment before is used. Moreover, this byproduct can occurs in the nature and spoiling hardly the environment.

Research on this field is been carried out to study its further utilization in biotechnological processes. Several alternatives were founded and developed these decades to avoid and minimized the environmental impact due to coffee pulp. Previous assays of yeast culture on coffee pulp were realized by The Colombian Research Center CENICAFE in 1951, conducted by Dr. Calle. Later, INCAP and ICAITI in Guatemala also reported research on this matter. However, the most important contribution was the happening of the First International Conference on Coffee Byproducts Utilization for Animal Feed and its furtherIndustrial Applications, wellcomed by the Costarican Center CATIE in 1974. More recently, in 1989 INMECAFE in Veracruz, Mexico and in 1991 CENICAFE, Manizales, Colombia, there are organized an International Symposium on Biotechnology of Agroindusty of Coffee, called SIBAC; where sessions and contributions presented original research on the potential of coffee pulp byproducts (aquaculture and feed).

Aquaculture : Fish farming could be a way to produce animal protein using locally available feedstuffs but taking into account present market limitations (Ramos-Henao, 1988). Some

work has been done on feeding coffee pulp to Tilapia (Garcia and Baynes, 1974), carp and catfish (*Clarius mossenbicus*) as indicated by Christensen (1981). The level of coffee pulp used in the experimental diets was closed to 33% without negative effects on the growth rate and yields of the fish. Lagooning may be also used as a secondary water treatment process, after anaerobic primary treatment of spent waters in the coffee mill.

Ruminant nutrition: Proximate composition of coffee pulp shows a relative low nutritive value due to high level of wall materials, lignin and also due to the presence of cafeine, tanins and chorogenic acid. Therefore, the use of raw coffee pulp is been suggested to be lower than 20% in ruminant diets (Ruiz and Ruiz, 1977; Vargas et al. 1982; Abate and Pfeffer, 1986). High raw coffee pulp intake has been associated to negative nitrogen balance because of the cafeine diuretic effect (Cabezas, et al. 1974). Coffee pulp silage seems to correct this problem probably because of cafein leaching in the silage liquor (Cabezas et al. 1976). On the other hand, solid-state culture of fungal organisms such as *Penicillium roquefortii* or *Aspergillus niger* may reduce to less than 10% the level of cafeine in coffee pulp, leaving a probiotic activity in the fungal biomass as indicated above (Tapia et al. 1989; Campos-Montiel, 1995). Therefore, despite the nutritional limitations of raw coffee pulp, solid-state fungal culture and ensiling (the two step fermentation process discussed above) may increase the ruminant nutritional and market value of this material. This is an interesting feature which remains to tested *in vivo*.

Recent work done int the Biotechnology Laboratory of Centre ORSTOM, Montpellier (France) and UAM (Mexico) has shown that it is possible to keep and improve the biochemical quality of coffee pulp by using a mixture of selected strains of lactic bacteria and filamentous fungi. Solid-state fermentation of this material yields a decafeinated product which can be dried or rensiled (Roussos et al., 1989; Perraud-Gaime, 1995) Some HPLC measurements suggest that a major fraction of phenolic compounds is broken down (Perraud-Gaime, 1995). On the other hand, work by Antier et al. (1993 a,b), has shown that coffee pulp is en excellent substrate for pectinase production by selected strains of *Aspergillus niger* (Boccas et al. 1994). The solid residue after such fermentation is done has been found to have *probiotic* effect when assayed *in vitro* by Tapia et al. (1988). This probiotic effect is apparently linked to a water soluble enhancement growth factor present in fungal biomass and acting on rumen cellulolytic bacteria (Campos-Montiel and Viniegra-Gonzalez, 1995, Islas et al. 1995).

In the first transperency the New alternative for the Biotechnological Upgradation of Coffee Pulp is presented. Fresh Coffee Pulp is subjected to Lactic Acid Fermentation and Coffee Pulp Silage thus obtained can be used as substrate for solid State Fermentation system for the production of Probiotics, enzymes, animal feeds and phytohormona.

Content: During these presentation 1 will be covering mainly about production of coffee pulp, its biochemical composition, natural microflora, oriented silage, caffeine degradation and pectinases production by filamentous in SSF system by filamentous fungi like *Aspergillus* and *Penicillium* species.

Word production: The data on the Worl Green coffee and Coffee Pulp Production for the period 1989-90 are presented in Table 1. The World Coffee Production is five and a half

million tons. For every ton of coffee produced half a ton of coffee pulp is generated in humid process. This is applicable only when the coffee is processed by humid process. In India the total production of coffee during this period was one hundred thirty thousand (130,000) tons. the exact quantity of coffee pulp produced is not definitely known because both humid and dry process are used.

Coffee pulp chemical composition: The chemical composition of coffee pulp is presented in Table 2. Coffee pulp is essentially composed of carbohydrates, protein, amino acids, mineral salts, tannins, and caffeine (Zuluaga 1989). The last two compounds are reported to be antiphysiological factors for animal consumption (Bressani et al. 1972)..

The composition of soluble sugars present in coffee pulp are presented in Table 3. These represents about 23% of total solids by dry weight (Zuluaga, 1981). The presence of protein, sugars, minerals and water in coffee pulp obtained by humid process, offers itself as an excellent substrate for the growth of microorganisms. If it is not utilized immediately it causes environmental pollution particularly in the rivers surrounding the factory processing area. In order to conserve the nutritional factors present in the coffee pulp and to maintain its quality throughout the year we have used sillage process (Perraud-Gaime and Roussos, 1997).

Natural Microflora of coffee pulp: In the first instance the natural microflora of coffee pulp was evaluated and the data is shown in Table 4. Bacteria represents nearly 95% of the microflora whereas filamentous fungi and yeast population was only about 5% (Gaime-Perraud et al. 1993). Figure 1 gives an idea of groups of microorganisms (bacteria, yeast molds) present in Mexican and Columbian coffee pulp with its nutritional capacities such as amylolytic, cellulolytic, pectinolytic and lactic acid bacterial population.

LONG TIME CONSERVATION OF COFFEE PULP

1- Preservation of coffee pulp by ensilage: Influence of biological additives

Coffee pulp, as it is generated, contains 80-85% moisture (Bressani *et al*, 1972), in addition to appreciable quantities of sucrose, proteins, amino acids and other nutrients. All these factors and nutrients allow various microflora to develop quickly on the coffee pulp and the development of the microorganisms cause the putrefaction of coffee pulp (Gaime-Perraud *et al*, 1993 ; Roussos et al, 1995). It is also not practicable to utilize the coffee pulp immediately, after its generation during coffee berry treatment mainly because the season of coffee berry processing lasts for 3-5 months. During this season, the industry cannot divert attention to this waste, as its priority is focused on the quality of coffee seeds during the entire season. Moreover, quick dehydration of the coffee pulp is impracticable, considering the huge quantity of the waste, high energy requirement, larger capacity of machinery needed and heavy investment on space and building, not only for dehydration, but also for stocking of the dehydrated pulp, till its utilization.

Ensilage of coffee pulp, for its preservation and improvement of feed value, is one of the avenues for value-added utilization of coffee pulp. Ensilage, a quick anaerobic process involving lactic acid bacteria, has been extensively used for preservation of forage in the

temperate regions. It allows the prevention of putrefaction of the forage with minimum degradation of organic matter. The process is quicker and it also improves the nutritive quality of the forage (Mc Donald *et al*, 1991).

Ensilage factors: A number of factors are of vital importance in obtaining a good silage. The substrate to be ensiled should have 30-40% dry matter, should be compactable to the desired level, amenable for anaerobiosis and contain utilizable sugars in sufficient quantities (Bertin, 1986). It must also have the colour, which is most nearer to the raw material, the fruity aroma and slightly acidic taste. In terms of chemical characteristics and achievement of the organic matter stability, the ensilage should involve a minimum loss of dry matter and the resulting silage should have a pH value lower than 4.5, higher than 3% lactic acid, but less than 0.5 and 0.3% acetic and butyric acid, respectively (Mc Donald *et al*, 1991).

A number of chemical and biological additives are mixed with the substrate for improving silage or reducing fermentation time. In the case of biological additives, a lactic acid bacterial inoculum is added, as a minimum of 10^5 lactic acid bacteria per g dry matter is required (Gouet, 1994) to convert the carbohydrates into lactic acid, but not into butyric acid. Enzymes are also added, when the rate of assimilation of sucrose by the endogenous lactic acid bacteria is slower (Bertin, 1986).

Ensilage is also practiced in tropical countries, despite the problems in terms of temperature, humidity and rains. Consequently, the rate of ensilage is slower, putrefaction is common and there is need to use a number of additives.

A number of reports have been produced on ensilage of coffee pulp (Bohkenfor and Fonseca, 1974; Murillo, 1978; Carrizalez and Gonzalez, 1984). But, most of these are associated with the development of the ensilage technique or the effect of chemical additives on the process. For example, Murillo (1974) compared the silage of coffee pulp, obtained by natural microflora based fermentation, with that involving the use of molasses or organic acids as additives. After 90 days of ensilage, the loss of dry matter was as high as 26.8%, in the case of the use of organic acids as additive, though it allowed to attain a pH of less than 4.0. Caffeine content of the drained water was reported to increase significantly, in the case of the use of organic acids, probably because it became more soluble in acidic pH.

Table I. Comparative physico-chemical characteristics of the coffee pulp ensiled using natural microflora and biological additives (Perraud-Gaime, 1995).

Parameters	Initial pulp	Ensilage of coffee pulp (28 days)			
		Without additives	Natural microflora	*L. plantarum* A6	Commercial inoculum
Moisture	62,55	61,63	66,78	66,78	66,19
pH	4,44	3,90	3,91	3,92	4,11
DM losses (%)	-	0,80	1,73	1,41	0,38
Lactic acid (%DM)	0,00	2,39	3,35	2,14	0,08
Acetic acid (%DM)	0,00	0,29	0,68	0,48	0,05
Reducing sugars (%DM)	4,72	4,85	4,56	3,67	8,32
Caffeine (%DM)	1,04	0,95	1,02	0,93	0,90

The ensilage of coffee pulp was investigated by Perraud-Gaime (1995) with respect to the microbiology and biochemistry of the process, along with the evaluation of biological additives, for improving the process and also the quality of the silage. Accordingly, the studies involved a) allowing the endogenous lactic microflora to grow on coffee pulp for using the fermented mass as inoculum for the next batch, b) use of monoculture of *Lactobacillus plantarum* A6 as a biological additive (Giraud et al. 1991) and c) the use of commercial inoculum as yet another biological additive. The latter contained two lactic bacteria and an enzyme complex.

Data on the influence of three biological additives on the ensilage of coffee pulp for its preservation show that the endogenous microflora of the coffee pulp is efficient enough to produce good quality silage, with acceptable levels of organic acid, dry matter loss and final pH. The use of inoculants, as biological additives, showed the efficiency of natural microflora grown on coffee pulp and the monoculture of *Lactobacillus plantarum* A6 in improving the physico-chemical characteristics of the silage, though commercial inoculum was not efficient, due to several reasons (Perraud-Gaime, 1995). Degradation of caffeine was absent in all the cases. Cellulases as a biological additive showed increased sugar production during ensilage. The results on the kinetics of different microflora development and physico-chemical characteristics during ensilage provide the insight into the microbiology and physiology of the process and point out a number of possibilities for improving the ensilage process as well as the quality of the silage (Perraud-Gaime & Roussos, 1997).

Data allow us to conclude that ensilage is a good technique for preservation of wet coffee pulp. The endogenous lactic acid flora of dry coffee pulp is sufficient enough to produce a good quality of silage (Table 5). However, addition of biological additives, such as lactic acid bacterial inoculants and enzymes, allows the improvement of the quality of the silage, in terms of increesing of lactic acid production, without concomitant production of volatile organic acids and ethanol. Caffeine is not degraded during the silage and hence it is necessary to decaffeinate the coffee pulp with appropriate fungi by solid state fermentation (Perraud-Gaime and Roussos, 1997), if the ensiled coffee pulp is to be used for animal feeding, as caffeine has antiphysiological effects (Bressani *et al*, 1972).

2.- Selection of filamentous fungi for coffee pulp decaffeination in SSF

It is of economic and industrial importance to note that only 5.8% of the solids of the coffee berry result in the ultimate coffee drink and the remaining 94.2% forms water and various byproducts (Zuluaga, 1989). Among the latter, the coffee pulp is the maximum and represents 40% of the coffee berry in wet form (Tauk, 1986), corresponding to 29% of dry matter (Bressani *et al*, 1972). This large quantity of the coffee pulp poses problems of disposal to coffee berry producers, due to putrefaction and causes environmental pollution if not disposed after appropriate treatment (Zuluaga, 1989). Due to its high organic matter content, coffee pulp can be utilized for beneficial purposes and intensive research on this topic has been carried out at ORSTOM (Roussos *et al*, 1995) and also in collaboration with Universidad Autonoma Metropolitana (UAM-I), Mexico (Viniegra-Gonzalez *et al*, 1991).

Direct use of coffee pulp in animal feeding poses problems, due to its chemical composition (Viniegra-Gonzalez *et al*, 1991). For example, the coffee pulp of *Coffea arabica* contains approximatelly 1% caffeine and has antiphysiological effects on the animals (Braham *et al*, 1973; Cabezas *et al*, 1974, 1976; Vargas *et al*, 1982). It is, therefore, necessary to decaffeinate the coffee pulp, before its use as animal feed. Moreover, the coffee pulp gets putrified, because of its high content of water and, hence, needs preservation by appropriate economic technique. At ORSTOM, Montpellier, the techniques of ensilage and fungal degradation of caffein by solid state fermentation (SSF) have been selected for preservation and decaffeination of the coffee pulp, respectively, because of their economic character. If these two techniques are applied in succession, it is of vital importance that the decaffeination by fungi is achieved before the formation of conidiospores. In the case of conidiospore formation, it will be essential to sterilize the decaffeinated coffee pulp, before ensiling. However, mycelial cells of fungi can be eliminated during ensiling and hence sterilization step can be avoided to achieve economy (Perraud-Gaime, 1995).

Isolation of new fungi strains: Isolation, purification and conservation of filamentous fungi capable of degrading caffeine was carried out as shown in figure 2.
A total of 350 fungi have been isolated from coffee domains (coffee plants, soils of coffee plantation, coffee byproducts, fermenting coffee berries, etc.) during the research at ORSTOM and UAM (Aquiahuatl *et al*, 1988; Viniegra-Gonzalez *et al*, 1991; Roussos *et al*, 1995). From this collection, a total of 8 filamentous fungi, representing two strains of *Penicillium* and 6 strains of *Aspergillus*, were selected for use in the present studies, based on their higher capacity to degrade caffeine to the extent of 90 to 100% in liquid culture (Roussos *et al*, 1989). One of the *Penicillium* strains selected (V33A25) showed negative effect on caffeine degradation, upon the addition of inorganic nitrogen to the medium in SSF process (Roussos *et al*, 1994).

Selection of filamentous fungi to Caffeine degradation: The objective of this study was to select one or more of the filamentous fungi to grow in SSF and to degrade caffeine to the extent of 80%, before the initiation of conidia formation. Work was also carried out to develop a simple criterion, to correlate growth of the fungi, degradation of caffeine and sporulation time, so that it can be used to stop fermentation at the most appropriate stage. The ability of seven fungal isolates which can degrade caffein totally is shown in table 6. They belong to the genus of *Aspegillus* and *Penicillium*. Of these, only two strains are belonging to the genus of *Aspergillus* species (V12A25) and *Penicillium* species (V33A25) was selected to study the kinetic and biochemical pathway of caffeine degradation.

Decaffeination of coffee pulp in Solid State Fermentation, to eliminate its antiphysiological effects on animals, was studied by aerobic fungal solid state fermentation, prior to the stage of initiation of conidiospore formation (Perraud-Gaime, 1995). Comparative data on performance of two strains of *Penicillium* and six strains of *Aspergillus* spp., selected for their high ability to degrade, indicated the potential of *Penicillium* sp V33A25 for caffeine degradation in aerobic solid state fermentation, before the initiation of sporulation by the culture. Kinetic studies pointed out that the evolution of CO_2 is the reliable criterion for the determination of the phase of fermentation, caffeine degradation, increase in medium pH and initiation of sporulation, without taking sample and subjecting it to analyses or disturbing the

fermentation. These advantages are not available, if rise in pH the medium is selected as a criterion. Amongst 7 different factors, the fermentation temperature, level of $CaCl_2$ in the medium and autoclaving or non-autoclaving of the medium exhibited strong effects on the initial time of sporulation, extent of CO_2 evolution, pH of the medium and caffeine degradation (Figure 3). The data allow to envisage the use of mixed culture of lactic acid bacteria and filamentous fungi for decaffeination and ensilage of the coffee pulp, or in two stage fermentation, involving any of the simpler order.

Table II. Comparative data on growth and metabolism of the filamentous fungal cultures in column fermenters under solid state fermentation (Perraud-Gaime, 1995).

Strains	Lag phase h	CO_2 production ml/g MSI	Respirometry coefficient h^{-1}	Caffeine degradation at 30 h, %	Time of initiation of sporulation
Penicillium sp. V26A25	12,5	115	0,34	91	32 h
Penicillium sp. V33A25	11,5	95	0,34	94	30 h
Aspergillus sp. C16A25	13,0	100	0,30	80	32 h
Aspergillus sp. V12A25	10,5	130	0,29	82	28 h
Aspergillus sp. C17B25	17,0	65	0,34	87	32 h
Aspergillus sp. C11B25	20,0	65	0,26	12	42 h
Aspergillus sp. C28B25	11,0	100	0,30	94	32 h
Aspergillus sp. C23B25	11,0	85	0,34	79	30 h

It can be concluded that it is possible to decaffeinate the coffee pulp in 30 h under aerobic conditions by using selected fungal culture, i.e., *Penicillium* sp. V33A25 in solid state fermentation, before initiation of the sporulation by the strain. It is also not necessary to sterilize the substrate. It is, therefore, possible to envisage the inoculation of the coffee pulp with mixed culture of lactic acid bacteria, for the ensilage preservation of coffee pulp, along with the selected filamentous fungi, for degradation of the caffeine. It can lead to decaffeinated and stabilized coffee pulp, which is suitable for animal feeding (Perraud-Gaime, 1995).

It is also possible that the stages of the fermentation can be observed visually on the computer, through respirometric parameters, without removing the sample and subjecting it to analyses and also without disturbing the culture medium. This factor of CO_2 evolution permits to reliably estimate different phases of the development of *Penicillium* sp. V33A25, in terms of degradation of caffeine and time of the sporulation of the filamentous fungi.

Biochemical pathway for caffeine degradation by filamentous fungi: In order to understand the biochemical pathway for caffeine degradation by filamentous fungi, synthetic caffeine was used under submerged fermentation system with defined synthetic medium. Metabolic pathway of caffeine degradation by *Pseudomonas putida* is shown in figure 4. The caffeine is degraded to urea as indicated in this figure. The same pathway may not hold good for filamentous fungi.

The kinetic of caffeine degradation by *Penicillium* and *Aspergillus* species is presented in figure 5. In both cases the degradation of caffeine is total in 50 hours, but the intermediate metabolites produced are different (Denis, 1996). In the case of *Penicillium* only theophyline appeared, whereas with *Aspergillus* species, Theobromine, paraxanthine and 3-methyl xanthine appeared as intermediates. The proposed pathway for the degradation of caffeine by filamentous fungi is shown in Figure 6.

Pectinases production from coffee pulp in SSF: Another utilization of coffee pulp is for the production of enzymes (Antier et al. 1993). Coffee pulp is an excellent substrate for pectinase production (Boccas *et al.* 1994, Augur *et al.* 1997). The ability of four wild fungal isolates capable of producing pectinase in solid state fermentation system using coffee pulp is shown in table 9. Cafeine degradation by *Aspergillus oryzae* and *Penicillium roquefortii* was also studied (Denis, 1996).

Conclusion

In conclusion it may be said that by selecting proper filamentous fungi it is possible to detoxify coffee pulp (caffein degradation) and upgrade the coffee pulp for animal feed. The sillage of coffee pulp permit to conserve the good potentiallities of the pulp for various uses as indicated in the trophic chain (figure 9). The silage of coffee pulp under anaerobic conditions inhibits polyphenol oxidation. Uner aerobic conditions the caffeic acid, chlorogenic acid and tannic acid forms polyphenols which can be further oxidized in presence of air to form quinones (fig. 10). These quinones in presence of proteins and free amino acids form a black water insoluble product (fig. 11). In order to overcome this anaerobic fermentation of coffee pulp with selected lactic acid bacteria is most efficient for the total detoxification of coffee pulp, polyphenols.

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