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Biotransformation of Liquid Wastes from Gut-Dressing Works by a Fermentation Process

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ABSTRACT

Gut-dressing work effluents were first characterized for their microbiological and physico-chemical properties. Assays of biotransformation of these effluents into a stable ingredient through a fermentation process by lactic acid bacteria were carried out in duplicates. The liquid effluent was mixed with 20% molasses and inoculated with a starter made up of *Lactobacillus delbrueckii*. The pH and the microbiological characteristics including standard plat count (SPC), coliforms, S*almonella*, *Clostridium* and staphylococci were followed up during a fermentation period of 10 days. Results relative to the characterization showed that the microbial load was very high. So, the SPC reached 10^6 cfu mL^{-1} in the effluent before transformation. Coliforms reached 10^4 cfu mL^{-1}, *Salmonella* was not detected. Results of the second part relative to the stabilization of theses effluents showed a rapid decrease of the pH during the first two days of fermentation and reached 3.6 after 48 h. In parallel, a potent decrease was obtained for the coliforms and staphylococci to reach less than 1 cfu mL^{-1} after two days. LAB population increased significantly to reach 10^9 cfu mL^{-1}. The results indicated that the process by fermentation was successful for the stabilization of the effluent.

Keys Words: Liquid wastes; Gut- dressing works; Biotransformation; Lactic acid bacteria; Fermentation

INTRODUCTION

Wastes from the gut-dressing works are produced in huge amounts in Morocco. These wastes consist of solid and liquid materials generated by the intestinal cleaning. All the wastes produced by the preparing units are discarded in the waste water collectors. Up to now, no study has been carried out on these wastes to determine their polluting power or their possible recycling except that done by Elmjadli *et al.* (1999) on the solid part. In a previous study, we have characterized the liquid part of the wastes. Results from this work indicated that the liquid wastes of the gut-dressing works showed a pH around 6.35, a dry matter of 2.03 g 100 m^{-1}, a COD of 8.65 g L^{-1} and a BOD of 8.14 g L^{-1}. The microbiological profiles were high with a standard plate count of 10^7cfu mL^{-1}, the total coliforms reached 5.8 10^6 cfu mL^{-1} while fecal coliforms were around 1.4 10^4 cfu mL^{-1}. Staphylococci reached 5.3 10^5 cfu/ml. Among the coliforms *Escherichia coli* was the most frequent species with 85% of the isolates (El Akhdari *et al*., 2003).

These materials are not known to all firms concerned in wastes treatment and the possible known way for treating these materials is an anaerobic digestion which may uses a biological process in which organic matter is transformed to methane under anaerobic conditions (Salminen & Rintala, 2002). Chemical acidification of wastes by acetic acid and/or propionic acid was also used to eliminate pathogens (Harmon *et al.*, 1975). However, none of these methods used a microbial controlled fermentation. New techniques using biotechnological process are more interesting for the control of food and feed systems. Transformation and preservation through a microbial fermentation by acid-producing microorganisms (lactic acid bacteria) would seems to be the most suitable procedure for recycling wastes from the food industry (Kherrati *et al*., 1998). Wastes are not only preserved but they are transformed into new ingredients required for animal nutrition because of its potential protein source.

The aim of the present study is to investigate the effluent of gut dressing works to stress the problem of high contamination and also to solve this problem by a biotechnological process using strains of lactic acid bacteria (LAB).

MATERIALS AND METHODS

Samples collection and preparation. Samples of liquid wastes of gut-dressing works were collected from a unit in Rabat (Morocco). Amounts of about 20 L of wastes were transported to the laboratory, dispensed in 10 L containers to make two trials (trial n°1 & trial n°2). Cane molasses were added in the proportion of 20%.

Microorganisms used- pH test. LAB strains in single starter cultures and in combinations were screened for their activity in biopreservation in previous works during the last decade. Strains of LAB, isolated from different materials (fish silage & vegetables) through fermentation in Morocco, were studied and characterized for their activities (pH decrease & gas production). The pH was checked by the use of a pH meter Crisson Micro-pH 2000. Strains were stored on MRS (De Man, Rogosa & Sharpe, Merck, Germany) slants at 4°C.

Inhibition test. The antimicrobial activity of LAB used in this work was studied using the spot test (Harris *et al*., 1989) and by the agar diffusion test (Piddock, 1990). The antimicrobial activity was determined by measuring the clear zone around the colonies. A diameter of 1.5 mm or greater around the culture was considered as significant inhibition. Strains used included *Escherichia coli* (4 strains), *Pseudomonas aerogenosa* (4 strains), *Klebsiella pneumonia* (4 strains), *Bacillus subtilus* (1 strain), *Bacillus megaterium* (1strain) and *Bacillus sp* (3 strains). All of theses strains were obtained from the collection of micro-organisms of the Department of Food Microbiology and Biotechnology, IAV Hassan II (Rabat).

Inoculation. The prepared mixture was dispensed in sterilized flasks. The flasks were inoculated with a starter culture. Strains of *Lactobacillus* were grown on MRS (De Man, Rogosa & Sharpe) broth incubated at 30°C for 48 h. Cultures were centrifuged at 3000 g to separate the biomass from the supernatant. The biomass obtained from 1 L of culture was used to inoculate 10 L of liquid waste. The fermentation process was monitored by the pH determination as well as the microbial counts. LAB were also evaluated to follow up their growth pattern during the incubation period by pour plating appropriate dilutions (10^{-4}-10^{-7}) on MRS agar incubated at 30°C for 24 h.

Microbiological determinations. 10 mL of each sample were blended with 90 mL of saline water (8.5 g L^{-1}) to make the initial dilution (10^{-1}). Serial dilutions up to 10^{-6} were then prepared. The Standard plate count (SPC) was determined by plating appropriate dilutions on plate count agar (PCA, Difco, USA). The plates were incubated at 30°C for 48 h. LAB were plated on MRS (De Man Rogosa Sharp medium) (Difco, USA), incubated at 30°C for 48 h. Staphylococci were determined on Mannitol Salt Agar (Merck, Germany), the plates were incubated at 37°C for 24 h. Coliforms were cultured on Desoxycholate citrate agar lactose (DCL), the plates were incubated for 24 h at 37°C for total coliforms and at 44°C for fecal coliforms. *Salmonella* was determined on 25 mL of the sample according to the method described by Poelma and Silliker (1975). For the spore forming bacteria (*Clostridium*), the initial dilution was heat-activated at 80°C for 10 min and immediately cooled in iced water. These anaerobic sulphite-reducing bacteria were grown on Reinforced *Clostridium* medium (Merk, Germany) in tubes. Just after inoculation and while the medium was still liquid, 0.1 mL of a 5% sodium sulfite solution and 0.1 mL of a 5% ferric ammonium citrate solution were added. Tubes were incubated at 30°C for 24 to 48 h. Yeasts were plated on Potato Dextrose Agar (Difco, USA), acidified by a lactic acid solution to pH 3.5. The plates were incubated at 30°C for 48 to 72 h.

RESULTS AND DISCUSSION

The selection of suitable strains of *Lactobacillus* for the fermentation was based on the pH decrease evaluation and growth of microorganisms. A good fermentation process was shown by the waste odor disappearance during the batch assay. Best results were obtained with the strain L1 (Table I), that was identified as *Lactobacillus delbrueckii* using API 50 CHL identification kit (Biomérieux, France)

Table I. Selection of lactic acid bacteria strains by their acidification and fermentation activities

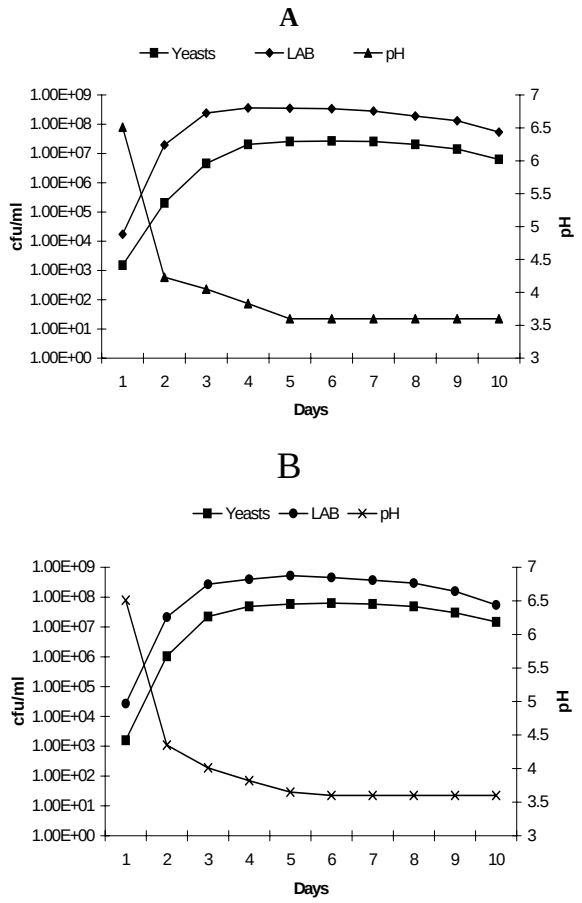
LAB strains	20% pH	Molasses Fermentation	30% pH	Molasses Fermentation
L1	3.60	+++	3.66	+
L 2	3.90	+	3.68	+
L 3	4.10	+	3.79	+
L 4	3.93	+	3.88	+
L 5	4.50	+	4.11	-
L 6	3.63	+	3.63	+
L 7	3.78	+	3.86	-
L 8	3.65	+	3.65	+
L 9	3.76	+	3.88	-
L 10	3.67	+	3.87	+

+++: strong activity; +: weak activity; -: no activity

Table II. Inhibitory effect of *Lactobacillus delbrueckii delbrueckii* Strain L1 on Gram-postive and Gram-negative bacteria

Strains		Zone diameter (mm)
Escherichia coli	1	2.8
	2	2.0
	3	2.1
	4	1.9
Pseudomonas aerogenosa	1	1.8
	2	1.7
	3	1.7
	4	2.0
Klebsiella pneumonia	1	1.6
	2	1.4
	3	1.5
	4	1.4
Citrobacter sp	1	1.4
	2	1.6
Staphylococcus aureus	1	1.5
	2	1.6
	3	1.3
	4	1.7
Bacillus subtilus	1	2.5
Bacillus megaterium	2	1.7
Bacillus spp1	3	2.7
Bacillus spp2	4	1.7
Bacillus spp3	5	1.5

Fig. 1. pH pattern and growth profile of yeasts and lactic acid bacteria during fermentation. (A: Trial n°1, B: Trial n°2)



according to Benno (1990).

The inhibitory test showed that *Lactobacillus delbrueckii* strain L1 was inhibitory to all Gram+ and Gram- bacteria (Table II). The diameters of all the inhibition zones ranged from 1.3 to 2.8.

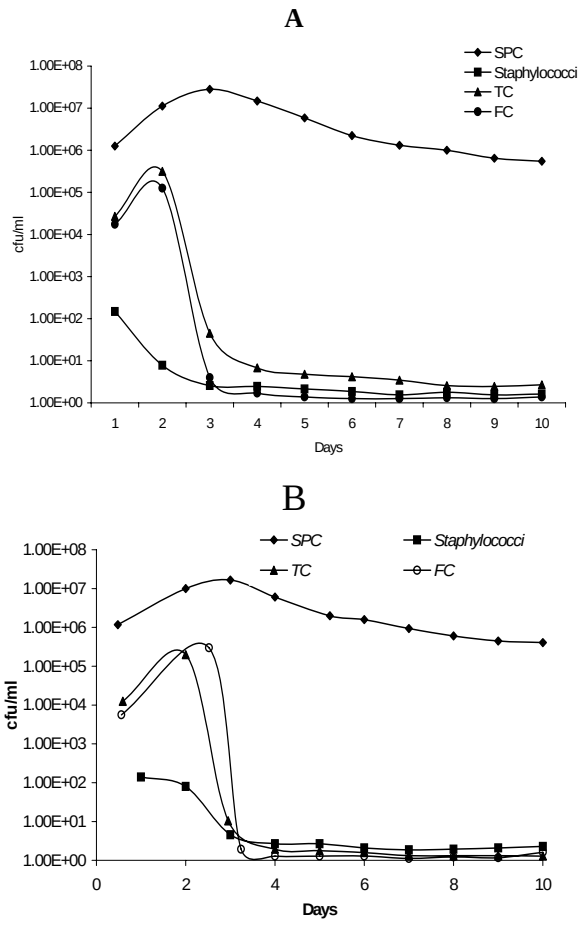
The microbiological characteristics and the pH values of the two trials of the mixture effluents/molasses during the fermentation process are reported in (Fig. 1). As Shown, the pH decreased from an average pH of 6.5 to 3.6 after 5th day of fermentation for trial 1 and 2. These values remained constant for the last five days of fermentation. The pH decrease in the product gives evidence of a good acidification through lactic acid fermentation by the starter culture. The most important factor to control in the biotransformation is the pH decrease (Haaland *et al*., 1990; Faid *et al*., 1997) which must be achieved as quickly as possible in order to inhibit the growth of spoilage microorganisms in the product.

Fig. 1 shows the growth pattern of the starter cultures during the fermentation, this showed that the growth of the two strains of yeast and LAB was simultaneous and without antagonism. Both strains showed good logarithmic phases which may indicate suitable conditions for growth during fermentation. The stationary phase was reached after 4 days indicating a maximum growth level about $3.5.10^8$ and $5.2.10^8$ cfu mL^{-1}, respectively for trial n°1 and trial n°2. The strain used for the inoculation of the mixture would constitute a suitable starter culture for the biotransformation of the effluent from the gut-dressing works. The high population of LAB found at the end of the fermentation pointed out the suitable conditions for growth of the culture and also the efficiency of the strain used.

The total plate count was also followed during the period of fermentation. Profiles reported in Fig. 2 showed an initial phase where microorganisms grew and reached high numbers. This phase lasted two days. A stationary phase then observed during which growth was stopped. This phase may correspond to the logarithmic growth of the LAB and yeasts. A third phase was observed after the 3rd day of fermentation during which low counts of microorganisms (staphylococci, fecal & total coliforms) were reached.

Coliforms showed a net decrease during the fermentation to reach a minimum of <1 cfu mL^{-1} after three days for both trials. The reduction of coliforms number may ensure a good biopreservation against undesirable and/or hazardous microorganisms. Indicator microorganisms (coliforms) were eliminated in the product obtained after two days of fermentation; this could be due to the acidification by lactic acid formed by the LAB. Owens and Mendoza (1985) reported that pathogens (*Salmonella*) and toxigenic microorganisms (Clostridium & Staphylococci) are sensitive to low pH values. *Clostridium* counts had also been decreased during the fermentation and reached count after two days of fermentation were less than 1 cfu mL^{-1}. The reduction of *Clostridium* counts could be due to the acidification and/or some inhibitory compounds formed by the starter culture. The suppression of growth of *Clostridium* is very important during fermentation, because these bacteria produce organic acids with offensive odours such as butyric acid (Wang *et al*., 2001). *Salmonella* was not isolated in the product and this may give evidence of the process success in inhibiting hazardous microorganisms. All suspected isolates were identified as species of *Proteus*. This would not ensure the absence of *Salmonella* in the effluent, but it could be due to the method used or to the low number of *Salmonella* in the liquid wastes (El Akhdari *et al*., 2003). The presence of *Proteus* in the gut dressing works seems tangible since this microorganism can grow and reached a high level after washing the intestines and can also survive in high salt concentrations. The initial population of Staphylococci was found in high counts before the fermentation. Counts showed a net decrease to reach a minimum of 1 cfu mL^{-1}. Their presence is not unexpected because of the high contamination of the effluent by these microorganisms and their resistance to salting.

Fig. 2. Microbial profiles during fermentation by lactic acid bacteria (A: Trial n°1, B: Trial n°2)



Although the suppression of growth of putrefactive and food poisoning bacteria was mainly due to the fall in pH during lactic acid fermentation. Recent studies indicates that various inhibitory substances (organic acids, diacetyl, bacteriocins, hydrogen peroxide) generated from LAB would also suppress the growth of these putrefactive bacteria (Callewaert & De Vuyst, 2000). In particular, bacteriocins (substances of low protein) show a wide range of antibacterial effects on Gram-positive cells in putrefactive bacteria such as *Clostridium* and *Staphylococcus* (Wang *et al*., 2001).

Yeast counts showed a high level during the fermentation period. This may show a net increase from low numbers to high count concentrations (10^7 cfu mL^{-1}) in the product. The high counts of yeasts would result in alcohol and some metabolites production, which could hide the effluent odor in the final product. Fermentation by yeast may require high amounts of sugar relative to growth. Faid *et al*. (1995) demonstrated that fish waste silage in a controlled fermentation by LAB can be improved by yeasts addition for a combined alcohol/ lactic fermentation.

CONCLUSION

It could be pointed out that the biotechnological process used hereby may constitute a very convenient process for the stabilization and recycling of the huge amounts of liquid wastes from gut-dressing works produced in different food treating units in Morocco. Occurrence of mixed fermentation by pure culture of LAB could be involved in both preservation and improvement of the organoleptic quality of the final product. This process would provide a value added product because of the inhibition of pathogenic microorganisms, the transformation of some compounds and the elimination of undesirable odour from the gut-dressing works effluents.

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