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Full Length Research Paper

Utilization of Algal Waste: A Study on Biological Fermentation Techniques

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The garbage of red algae, *Gelidium sesquipedale*, from a factory after extraction of agar-agar, is indiscriminately discharged into the public environment. Microbiological analyses confirmed that this garbage contains different groups of bacteria whose number is variable: the total aerobic mesophilic flora, 4 x 10^6 cfu/g; total coliforms, 6 x 10^5 cfu/g; fecal streptococci, 2 x 10^2 cfu/g; staphylococci, 180 cfu/g; lactic bacteria, 7 x 10^3 cfu/g and yeasts 2 x 10^5 cfu/g. Analyses showed that clostridiums, salmonellas and faecal coliforms are absent. To treat this garbage of algae, we employed a biological fermentation process using lactic acid bacteria (BL11) and yeast (THE 16). These were isolated and selected for their acidifying and fermentation qualities, respectively. The fermentation resulted in a decrease of pH from 7.4 to 3.75 and a reduction of the different pathogenic groups of bacteria; total coliforms, streptococci and staphylococci. On the other hand, the number of the lactic bacteria increased. Physical and chemical analyses showed that this garbage of algae is rich in mineral elements, proteins, sugars and a small amount fat. This fermentation product can be used as fertilizer and/or integrated in animal feed.

Key words: *Gelidium sesquipedale*, Agar, fermentation, ferment, ingredient, food.

INTRODUCTION

Increase in population, development, diversification of individual consumption, urban concentration, increase of the number of industrial units and the lack of a strategy of treatment of garbage by satisfactory techniques can cause big problems for the environment and human health. Morocco possesses several coasts with a marine biodiversity of fish, molluscs and algaes. These algae constitute a source important of sulphated galactanes like the agar-agar and the carrageenans. The abundance of the rhodophycées encourages the development of the industrial units specialized in the production of the phyco-colloides particularly agar-agar. The *Gelidium* represents 90% of the harvest of the marine algae treated locally and that generate an important quantity of garbage that is generally rejected in the public dump. This causes a serious environmental problem for the city of Kenitra in Morocco.

The present work is essentially focused on the physical, chemical and microbiological characterization of the residues of algae after extraction of agar-agar. Also, this work provides a biological process for the treatment of this garbage. The treatment of unwanted products of agro-food waste has been the subject of the previous work on sugar cane Ouhssine et al., 2000b), the break-down of the cellulose (Fann and Lee, 1992) and fermentation of olives (Asehraou A, 1993). Other processes have been used for the transformation of garbage of animal origin (Hammoumi A., 1998). These treatment processes used some chemicals such as acetic acid, sulphuric acid and hydrochloric acid (Tatterson and Windsor, 1974). Fermentation has been used for the transformation of different products or unwanted products of plant or animal origin. This technique is based on the production of lactic acids due to the naturally present lactic bacteria in the garbage or the bacteria are added.

This work consists of characterizing and determining the chemical and microbiological composition of algal garbage and developing a biological treatment process with acidifying and fermenting features.

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Table 1. Physical and chemical analysis of the algae garbage after extraction of agar-agar.

Parameters	Unprocessed algae
Humidity (%)	5.50 – 9.90
Dry matter (%)	94.50
Ashes (%)	7.10 – 11.70
Organic matter (%)	87.40
Organic carbon (%)	22.30 – 25.3
Total sugars (%)	54.95 – 49.10
Lipids (%)	0.85 – 0.60
Crude proteins (%)	31.60 – 30.00
Total nitrogen (%)	3.61- 4.01
Total phosphor (%)	0.686 – 0.770
Total potassium (%)	4.230 – 4.801
Calcium (mg/l)	180
Magnesium (mg/l)	18.8
Iron (mg/l)	1.38
Zinc (mg/l)	0.37
Energetic value	3167.5 Kcal/Kg

MATERIALS AND METHODS

Physical and chemical analyses

The humidity was determined on the different ground and dried samples using automatic desiccators at 140°C for 15 min. The total nitrogen was determined by the Kjeldahl method (APHA, 1992). Fat was extracted using soxhlet apparatus with hexane as solvent. Total sugar was determined by phenol method as recommended by Sattler L. (1948). Dry matter was determined by drying 10 g of sample put at 105°C for 24 h. Total mineral matter was obtained after heating to a temperature of 550°C for 24 h. The amount of Mg, K, Zn and Fe was measured with the help of the atomic absorption device and phosphorus by spectrophotometer. The analysis of the trace elements was done on 500 mg of ashes of every sample diluted in one litre of HCL to 1 N. pH of the samples was also determined. Acidity wass measured by titration of 10 ml of solution in presence of phenolphtalein as colour indicator. The acidity is expressed in % of lactic acid.

Microbiological analyses

The total aerobic mesophilic flora was enumerated on PCA media after incubation at 30°C for 2 to 3 days. The coliforms were enumerated and isolated on MacConkey agar. 1 ml of every dilution (10^{-1} to 10^{-6}) was plated. This was kept at 37°C to count the total coliforms and at 45°C to enumerate the fecal coliforms after 24 h of incubation. The isolation of the staphylococci is done on Chapman media after incubation at 37°C for 24 h. The salmonellas were enumerated on SS (Salmonella - Schigella) media after incubation at 37°C for 24 h. The identification of the characteristic isolates was achieved by API 20 E (Bio Mérieux). Clostridium spores were counted on RCA media (reinforced clostridium agar). The sample was heated to 80°C for 10 min to activate the spores, and then incubated at 44°C for 24 h. Lactic acid bacteria were counted on Man Rogosa and Sharpe (MRS) media, using 10^{-3} to 10^{-9} dilutions and incubation at 30°C for 48 h. Only the colonies having the suitable shape were counted. Yeasts were counted on PDA (potato dextrose agar) media, after incubation at 30°C for 72 days. The amount of streptococci was counted on sodium azide media after incubation at 30°C for 24 h.

Isolation and purification of lactic bacteria

Lactic bacteria are isolated and are selected according to two criteria: the acidifying quality and production of bactericidal sub-stances. The isolation and the purification of isolates of lactic bacteria of different biotopes were achieved on MRS media. The cultures of the lactic bacteria were incubated at 30°C for 24 h. The purification is done after 4 successive cultures in solid MRS media. This was kept at 4°C for identification and further use.

Antibacterial activity of selected lactic bacteria

Several methods have been described for the detection of lactic acid bacteria producing bactericides. They are all based on the principle that the bactericides are the proteins substances that are distributed in a solid or semi solid milieu inoculated previously (Kashket ER, 1987) . The production of bactericides is detected by the inhibitory power of the microorganism tested by the filtrate of its culture on the growth of target bacteria.

Isolation and characterization of yeasts

The yeasts were isolated from different biotopes: milk and sugar cane juice and kept on PDA media. The yeasts, selected with respect to their fermentation and acidifying featues measured by the final pH, are cultivated on a semi-synthetic media of yeast extract 3 g, sucrose 3 g, magnesium sulphate 3 g, manganese sulphate 0.5 g, dipotassium phosphate 0.1 g, mineral solution 1 ml and made up to 1000 ml distilled water and kept at or below 4°C.

Biotransformation of the garbage of algae

The algal garbage was collected from an industrial unit extracting agar-agar and brought in plastic bags to the laboratory for physico-chemical and microbiological analyses. About 200 kg of the algae was collected. After drainage, 150 kg of garbage are treated in a mixture garbage/molasses and used like source of carbon. Every barrel was inoculated by a mixture of yeasts and lactic bacteria. The barrels are filled to 2/3 to facilitate the agitation of the content of the barrels and to prevent the possible overflow due to the rise of the product following production of gas during fermentation. The pH and acidity were measured to optimize the progress of fermenta-tion. The product obtained after fermentation was submitted to physicochemical and biological analyses previously described.

RESULTS AND DISCUSSION

Physicochemical analyses

The use of algae for various applications notably as a fertilizer or as an ingredient for animal feed requires previous survey of its mineral and organic composition. The results obtained (Table 1) show that the garbage of algae is very rich in organic matter (87.4%) of which 31.60% is made up of proteins, 54.95% of total sugars and 0.85% of fat matter. The dry matter represents 94.5%. It is important to note that sugars represent more than half of the organic matter in the algae garbage. These sugars include the fermentable sugars and the lignocellulosic fibres that are difficult to digest.

Furthermore, the algae is rich in calcium (180 mg/l),

Table 2. Microbiological analyses of algae garbage before treatment.

Microorganism	Count (cfu/g)
FMAT	4×10^6
Total Coli forms	6×10^5
Fecal Coli forms	Absent
Fecal streptococci	2×10^2
Staphylococci	180
Clostridium	Absent
Salmonellas	Absent
Lactic bacteria	7×10^3
Yeast	2×10^{15}

Table 3. Selection of acidifying bacteria lactic. Cultures achieved on a MRS milieu to 30° during 24h incubation

Isolate	Initial pH	Final pH	Final acidity (%)
BL1	5.70	4.15	0.94
BL2	5.52	4.05	0.90
BL3	5.71	3.82	0.96
BL4	5.70	4.02	0.92
BL5	5.58	4.35	0.89
BL6	5.73	3.90	0.93
BL7	5.63	4.2	0.88
BL8	5.49	3.88	0.95
BL9	5.73	4.12	0.93
BL10	5.75	4.3	0.89
BL11	**5.54**	**3.72**	**1.04**
BL12	5.60	4.02	0.95

magnesium 18.8 (mg/l). Potassium and the total phosphorus are also present in smaller quantities. Iron and zinc are also present.

Microbiological analyses

The microbiological analyses of the algae garbage show that the microbial load is very important (Table 2) . The total aerobic mesophilic flora was estimated to be 4×10^6 cfu/g followed by the total coliforms, 6×10^5 cfu/g. Salmonellas and clostridiums were not detected. The number of the lactic bacteria is 7×10^3 cfu/g, whereas the number of the yeasts is about 2×10^{15} cfu/g. This microbial load is high, indicating that this garbage represent a serious problem for public health. This is why treatment is obligatory before using the garbage.

Selections of lactic bacteria

The lactic bacteria are isolated from different biotopes

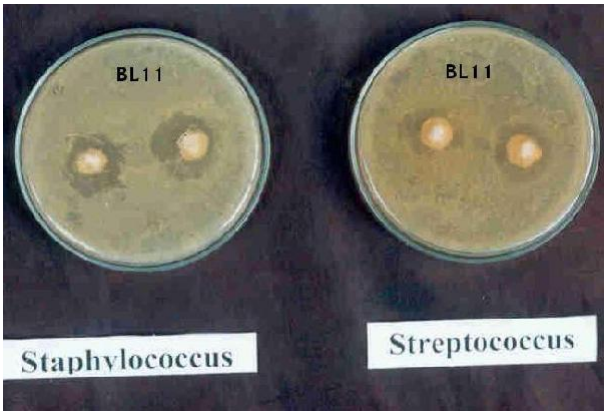

Figure 1. Antibacterial activity of lactic acid bacteria BL11 on staphylococci and streptococci. Cultures were made on TSA media at 30°C for 24 h.

Table 4. Antibacterial activity of the lactic bacteria selected on staphylococci and streptococci.

Lactic bacteria	Inhibition (mm)	
	Staphylococcus	Streptococcus
BL 4	26	26.5
BL3	29	31
BL6	28	29
BL8	27	30
BL11	**32**	**31**

such as milk and sugar cane juice. The results obtained show that all isolates of the lactic bacteria present a strong acidity with a reduction of pH (Table 3). A profile of remarkable acidity was obtained for BL11. In the same manner, the antibacterial activity of BL11 was effective against staphylococci and streptococci (Figure 1, Table 4). Therefore, the BL11 was employed for the fermen-tation of the algae garbage.

Isolation and characterization of the yeasts

Among 16 acidifying yeasts isolated from different biotopes, only two were selected and purified (Table 5). LE8 and LE16 showed high acidifying feature (1.15 and 1.20%, respectively). LE16 has been kept for the mixed fermentation (lactic bacterium and yeast) of the algae garbage.

Fermentation of algae garbage using mixed culture

150 kg of algae garbage is treated in barrels by adding 20% of molasses. Every barrel was inoculated by a mixture of lactic bacteria and yeast. The obtained results

Table 5. Selection of saccharolytic and fermentative yeasts.

Yeast	Initial pH	Final pH	Initial OD	Final OD	Final acidity (%)
LE1	5.59	4.87	0016	0.45	0.4
LE2	5.58	4.66	0.16	0.36	0.6
LE3	5.32	5.02	0.19	0.14	0.3
LE4	5.59	4.86	0.14	0.89	0.4
LE5	5.62	5.75	0.12	0.33	0.2
LE6	5.08	4.29	0.09	0.32	0.5
LE7	5.05	4.89	0.16	0.82	0.4
LE8	5.56	4.19	0.23	1.178	1.15
LE9	5.50	4.29	0.23	0.94	0.7
LE10	5.09	3.90	0.08	0.85	0.8
LE11	5.06	3.88	0.18	1.121	0.9
LE12	5.52	4.96	0.20	0.56	0.4
LE13	5.50	4.28	0.17	0.97	0.7
LE14	5.41	4.23	0.23	1.05	0.6
LE15	5.33	4.25	0.33	0.75	0.8
LE16	5.41	4.11	0.37	1.3 72	1.2

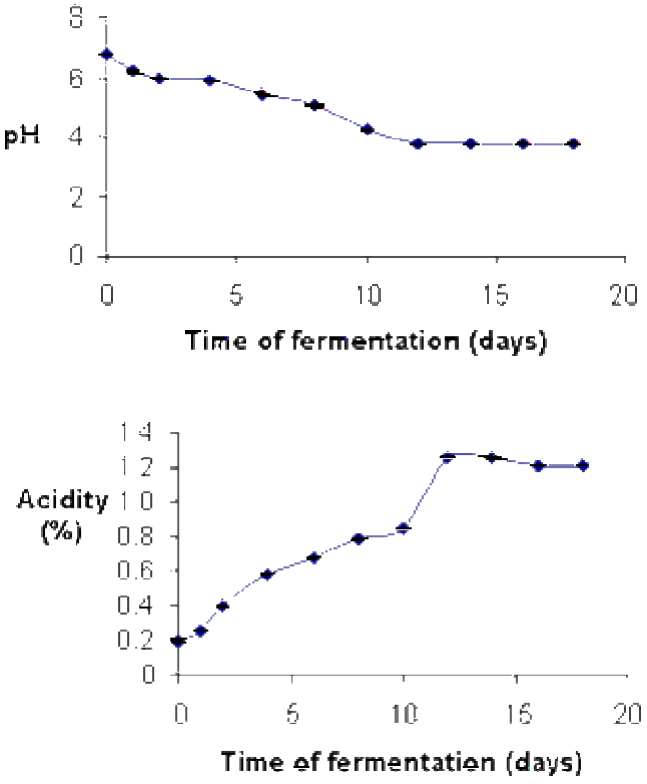

Figure 2. Variation of the pH and the acidity of the algae garbage during fermentation.

show a progressive reduction of the pH from the third day of fermentation to 3.8 on the tenth day. In the same way, the acidity increased to 1.25% on the tenth day (Figure 2). Fermentation in presence of the lactic bacteria is

Table 6. Physical and chemical analysis of the algae garbage after biological fermentation.

Parameters	Value
Humidity (%)	8.46
Dry matter (%)	91.48
Ashes (%)	8.11-13.25
Mat organic (%)	80.60
Organic carbon (%)	25.80 - 28.20
Sugars (%)	37.82
Lipids (%)	0.33 – 0.48
Raw proteins (%)	25-27
Total nitrogen %	3.19 - 3.48
Total Phosphorus (°/°°)	8.02 - 8.76
Total potassium (°/°°)	54.05-59.02
Calcium (mg/l)	180.26
Magnésium (mg/l)	18.9
Potassium (mg/l)	11.9
Iron (mg/l)	1.40
Zinc (mg/l)	0.40
Energizing value	-

usually accompanied by the production of the antibac-terial substances that can be useful for the conservation of the fermented products.

Some trace elements increased slightly due to the addition of molasses at the beginning of fermentation (Table 6). The reduction of sugars during fermentation is due to the presence of lactic bacteria and yeasts which consume the fermentable sugars and produce organic acids responsible for the pH fall. The fat did not show any

meaningful variation; this is due to the absence of lipolytic bacteria. The amount of proteins of the treated garbage shows a slight decrease due to the use of these proteins by the microorganisms (lactic bacteria and yeasts). The loss of nitrogen as ammonia results from the hydrolysis of the proteins by proteolytic enzymes of microbial origin. The consumption of nitrogen by the microorganisms during fermentation is also one of the reasons for the reduction of total nitrogen in the finished product.

The reduction of the number of bacteria at the end of fermentation can be explained by the acidification of the media and/or by the formation of some inhibitory compounds liberated by the lactic bacteria whose number increased considerably. Owens J>D and Mendoza (1985) reported that the pathogenic (salmonella) and the toxic microorganisms (clostridiums and staphylococcus) are sensitive to the reduction of pH. Dahia and Speck (1978) showed the effect of the fermentation products on *Staphylococcus aureus*. At the beginning of fermentation, the number of yeasts was very high, due to their consumption of sucrose which is in the molasses, but this number was reduced at the end of fermentation. The lactic bacteria are present in the finished product in highamounts (34×10^8 cfu/g). This can be explained by the presence of resident bacteria in the algae garbage and by the contribution of the inoculums.

The total aerobic mesophilic flora decreased to 16×10^5 cfu/g; probably due to the presence of yeast that is able to reduce the pH. The total coliforms, staphylococci and streptococci also decreased. Faecal coliforms, clostridiums and salmonellas were undetectable. This reduction of the pathogenic germs is essentially due to the increase of the acidity of the media, the reduction of pH and the antibacterial substances synthesized by BL11. The lactic bacteria reached high values of 34×10^8 while the yeasts reduced to 15×10^3 cfu/g.

Conclusion

The microbiological and chemical analyses of the algae garbage show that these garbage can constitute precious raw material but their bacterial load makes them potentially dangerous for direct uses in animal feed or in the fertilization of soils. Biological fermentation achieved by using a mixed inoculum of lactic bacteria and yeasts can be used to avoid these potential dangers. Fermentation is an efficient means, which permits the stabilization and the transformation of this garbage to a more hygienic quality product. Because of its rich organic and mineral matter, this finished product that was obtained after fermentation can be exploited as an ingredient in animal nutrition or used as a fertilizer. More interesting applications of this process are under investigation.

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