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Production of New Bio Fertilizer from Waste of Halieutic Activities, Brewing Industry and Brandy Distilleries in Morocco

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***Abstract*- The aim of this study is to improve natural biotransformation properties of fish wastes by combining them with brandy distillery wastes and industrial brewery wastes. The tested product (compost) was followed for 15 days by different physicochemical and microbiological parameters of control. Fertilization tests were also performed on a crop of barley. The product was optimized while better formulas were proposed. After eight days, the product was mature, odorless, had a stable pH, with favorable hygienic qualities. In regards to the tested products' nutritive qualities, an interesting rate of phosphorus and nitrogen was obtained; these unique nutritive qualities were responsible for improving the yield of a barley crop. The resulting compost product was better than commercially tested fertilizer products.**

Keywords— biotransformatio,n brandy distilleries, fertilizer, waste of halieutic, , brewing waste.

I. INTRODUCTION AND OBJECTIVES

In Morocco, the halieutic sector has an important role in the national economy, but it can generate a lot of waste. Due to lack of funds oran absence of binding legislation, these wastes are usually rejected into the environment without any treatment [1, 2].

In order to reach sustainable production and consumption with respect to the environment, the goal is to develop industrial processes that are sustainable and focus on three strategies: prevention, recovery and recycling.

Based on this premise, this study adopted a strategy in biofertilizer production by coupling fish waste with brewing waste and waste from brandy distilleries.

The product of the biotransformation of this ternary mixture is carbon/nitrogen/phosphorus and will be monitored for a period of 15 days under the parameters of being used as fertilizer for agricultural soil amendment.

II. MATERIALS AND METHODS

A. *Preparation of mixtures of biotransformation*

The mixture of products used is a ternary combination of three components:

- Fish waste, undergoing grinding in an ice-crusher.
- Waste of figs and dates brandy distilleries in Morocco. These wastes are considered essential sources of carbon.
- Third generation of Saccharomyces cerevisiae yeast (GFS2) recovered from the tanks of fermentation of Company Breweries Morocco, rejected and not reused. It is exploited in this study as agent of biotransformation because of its fermentative power and probiotic activity [3].

Each mixture was placed in plastic bins that measured l x w x h (cm): 30x18x11. To ensure that ventilation of the agitation of the contents was performed, a benchtop stirrer (Heidolph, Rotamax 120) was used at the rate of 100 rotations per minute.

Several fractions containing these three components were tested. The obtained mixtures were incubated at room temperature for 15 days. Table I shows the six prepared compositions.

TABLE I. COMPOSITION OF INITIAL TESTS

Composition	Fish waste (%)	Waste of figs and dates brandy (%)	Waste of beer yeast (%)
C1	100	0	0
C2	33	33	33
C3	50	0	50
C4	50	10	40
C5	50	25	25
C6	50	40	10

B. *Physicochemical analysis*

The acidity of each composition was measured daily by a pH meter (Fisher Scientific, Basic AB15).

The dry matter (DM) was determined daily, three times per day, by oven drying of 3g at 60 °C for 24h. Conductivity and temperature were measured daily by HANNA Instruments, EC215. Total nitrogen was determined according to the Kjeldahl method [4].

Dosage of trimethylamine (TMA) was performed by distillation, according Regulation EC No. 2074/2005 for TVB-N determination (total volatile basic amines), with an additional step between the sample deproteinization with perchloric acid and distillation; formaldehyde at a basic pH level was added to block the primary and secondary amines, which thus remained to be determined if the amine form was TMA [5]. Finally, the rate of phosphorus was determined by spectrophotometric assay according to the French standard NF V18-106 [6].

C. *Microbiological analysis*

Microbiological testing was performed on the first and last day. A Columbia blood medium was prepared as it can determine the specific presence of streptococcus to proteolytic effects [7]. Presence of staphylococci (lipolytic marker) was identified on Chapman agar [8]. A MacConkey medium ~~is~~was used to visualize the presence of Escherichia coli (health indicator). [9]

D. *Toxicity test and fertilization*

The study of the toxicity of this product on plant germination was carried out on the Hordeum vulgare species of barley of the Amira variety that is marketed in Morocco and ~~(~~provided by the National Institute for Agricultural Research. The barley was grown in cases with a depth of 6 cm; the seeds were deposited at a depth of 3 cm and covered with soil. The application rate was 0.009g per 200g (6 cm2 of soil), 150Kg/hectare equivalent (the minimum- recommended by the FAO fertilizer use for cereal crops in Morocco dose) [10].

Plastic square plug trays that measured 6x4 were used for barley crop testing. Column 1 and 2 contained the control soil without amendment, column 3 and 4 contained commercial fertilizer (Algoflash), and column 5 and 6 contained natural compost developed during this study, representing optimal physicochemical results.

III. RESULTS AND INTERPRETATIONS

A. *Changes in physical appearance*

Initially, all mixtures were a very heterogeneous thick paste. During the biotransformation, liquefaction was observed; the mixtures became pasty in the ascending order of C1, C3, C4, C5, C2, and C6. C1 was the only mixture to have developed the smell of rot, and after 5 days there was the appearance of worms.

B. *pH and conductivity*

With the exception of C1, based on the results of monitoring the pH (Figure 1) through various pH tests, the pH was maintained at an acidity of 4.8 during the 15 days of observation. The pH of C1 seemed to evolve differently, with its pH that rose to become alkaline (pH = 10.2).

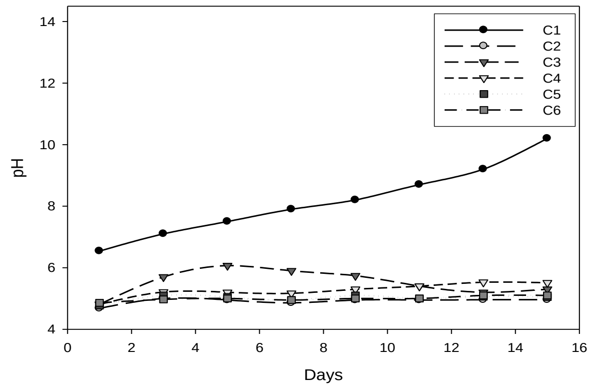

Fig. 1. Evolution of pH.

The conductivity (Figure 2) maintained an almost stagnant rate of 5 mS for all tests except C1during the 15 days. For C1, the conductivity reached 28.2 mS at the 15th day.

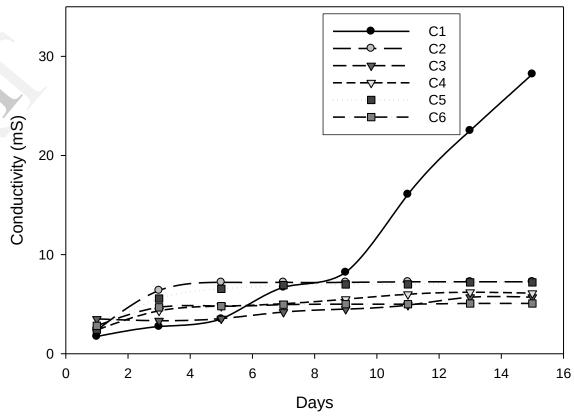

Fig. 2. Evolution of the conductivity.

C. *Temperature and dry matter*

From Figure 3, we can see that the temperature presented insignificant variability less than 6°C, probably due to the low thickness of the manipulated testing and regular agitation applied.

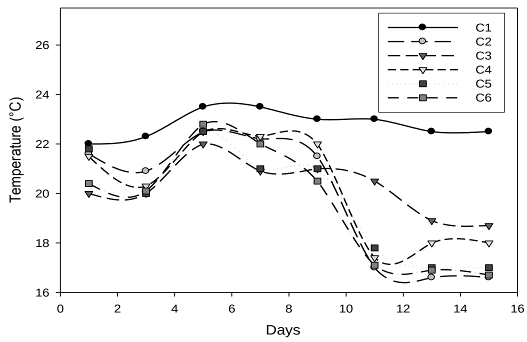

Fig. 3. Evolution of temperature.

In conjunction with previous results, the dry matter of the various tests was stagnant, except for compost C1, which underwent a significant decrease (Figure 4).

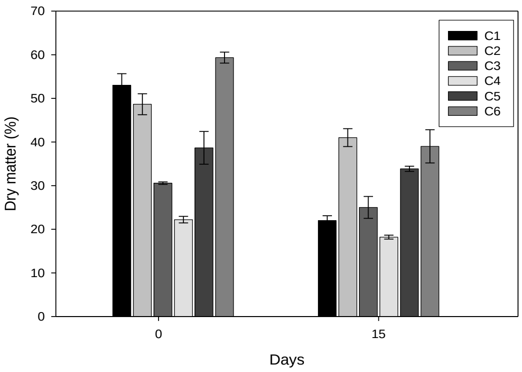

Fig. 4. Evolution of dry matter content.

D. Phosphorus, total nitrogen and trimethylamine

Table 2 shows the evolution of the rate of phosphorus total nitrogen and trimethylamine in different compositions.

TABLE I. TABLE II. EVOLUTION OF PHOSPHORUS, TOTAL NITROGEN AND TRIMETHYLAMINE.

	Days	***Phosphorus (g/100g) (% evolution)***	***Nitrogen (g/100g) (% evolution)***	***Trimethylamin (g/100g) (% evolution)***
C1	***0***	28	0.55	0.22
	15	8.2(-70.71%)	0.22 (-60%)	1.8 (+718.18%)
C2	***0***	25.43	1.47	0.048
	15	110 (+332.55%)	3.2 (+117.68%)	0.05 (+4.16%)
C3	***0***	55.22	1.51	0.16
	15	127 (+130%)	2.01 (+33.11%)	0.098 (-38.75%)
C4	***0***	30.32	1.67	0.115
	15	92.2 (+204.08%)	2.84 (+70.05%)	0.084 (-26.95%)
C5	***0***	21.35	1.34	0.17
	15	96.66 (+352.7%)	2.78 (+107.46%)	0.15 (-11.76%)
C6	***0***	17.81	0.97	0.14
	15	72.3 (+305.95%)	1.72 (+77.31%)	0.13 (-7.14%)

From table 2 and figure 5, we note that rate of phosphorus exhibits a significant decrease in C1. Unlike other tests, these rates increased during the fermentation process.

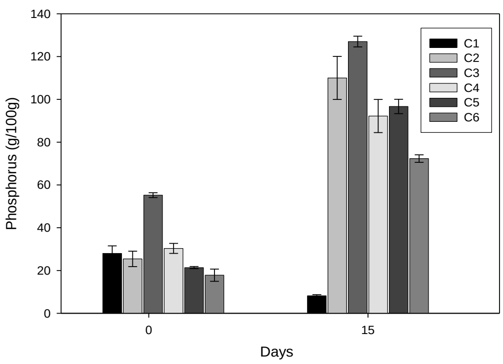

Fig. 5. The rate of mineral phosphorus evolution.

By presenting results of nitrogen's evolution (Figure 6), we can see a similar change.

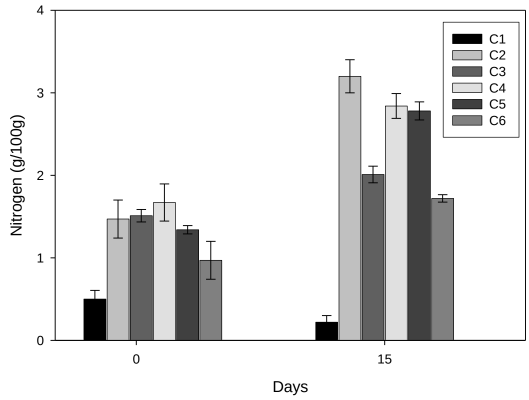

Fig. 6. Evolution of the rate of total mineral nitrogen.

Simultaneously, the rate of evolution of trimethylamine (Figure 7) had a slight decrease in C2, C3, C4, C5 and C6 during the process, unlike C1, which experienced a considerable increase.

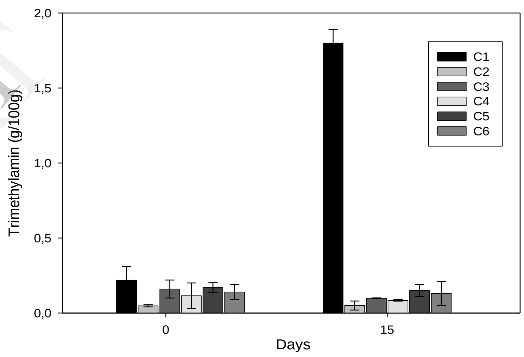

Fig. 7. The rate of mineral TMA Evolution.

According to the various parameters studied (pH, conductivity, MS, TMA, total nitrogen and phosphorus), C2, C3, C4, C5 and C6 evolved better than C1. It is noted that C2, comprised of 1/3 of fish waste, 1/3 of figs and dates brandy waste, and 1/3 of beer yeast waste, expressed relatively better results than the other compositions.

These results demonstrate the favorable impact of Brewer's yeast on the performance and optimization of the biotransformation.

E. Microbiological tests

Table 3 presents the results of the bacteriological tests conducted on the first and last day, to identify the presence of strain indicator of hygiene and alteration.

TABLE II. MICROBIOLOGICAL ANALYSIS (++:> 100UFC; +:> 10 CFU -: ABSENCE).

	C1		C2		C3		C4		C5		C6	
Days	0	15	0	15	0	15	0	15	0	15	0	15
Escherichia coli	++	++	++	-	++	-	++	-	++	-	++	-
Staphylococcus	+	+	+	-	+	-	+	-	+	-	+	-
Streptococcus	+	+	+	-	+	-	+	-	+	-	+	-

The product must have a number of staphylococcus and streptococcus that does not exceed ten Colony Forming Unit per gram of product (<10 CFU / g), and have less than one hundred CFU per gram (<100 CFU / g Unit) for Escherichia coli [11].

As displayed in Table 3, C2, C3, C4, C5 and C6 showed no presence of bacteria at the end of the fermentation process. Alternatively, in C1 the persistence of hygiene indicator strains, proteolysis and lipolysis bacteria, existed.

F. Toxicity test and fertilization

Table 4 shows the results of the germination tests on barley over the course of 21 days (carried out four times). Fertilization tests were performed with C2 since it had the best physico-chemical results.

TABLE III. FERTILIZATION TEST ON BARLEY CROP

	Germination (%)	Average stem length (cm)	Average root length (cm)	Dry weight (g)
Sol + Commercial Fertilizer	**87**	14.5	16.5	0.9
Sol + C2	**91**	15	20	1.07

The results in Table 4 show that C2 had no inhibitory effect on the germination and had optimal growth for the barley crop tested, which was better than the commercial fertilizer. This compost allowed an improvement in the lengths of the barley's stems and roots.

V. DISCUSSION

The stabilization of pH in the testing of C2, C3, C4, C5, C6 was due to the fermentative activity of the yeast, Saccharomyces cerevisiae [12]. The reported values were optimal for yeast and bacteria activity in the biotransformation (with a pH between 4.5 and 8.5) [12].

Monitoring the pH indicated that after 8 days of testing C2, C3, C4, C5 and C6 were mature. PH stabilization was due to the reduction of activity of micro-organisms [13]. In C1, which only contained fish waste, the pH became alkaline, and according to microbiological results, underwent alteration.

Change in conductivity was correlated to pH; thus, C1 was the only compost that presented a rise in conductivity. This result was due to the presence of ionic forms, and a large liquefaction and degradation of the material in the compost. Generally, the presence of ions could be beneficial to plants, but it has been shown that too high of conductivity threatens the survival of microorganisms and reduces the quality of the compost [14].

C1 experienced an extraordinary evolution of dry matter compared to the other composts. In fact this parameter experienced a reduction of about 80%. Based on the work of Lourhzal, this may be due to the total degradation of carbohydrates by yeast and the release of volatile substances [14].

The initial rate of phosphorus was between 18 and 55mg / 100g for all tests, shown in ascending order of C6> C5> C2> C1> C4> C3. Fish and brandy distilleries wastes are a source of this element, known for their richness in minerals and phosphorus [15, 16].

For all the tested compositions, the increased rate of phosphorus was explained by the mineralization performed by micro-organisms, which convert phosphorus in protein to inorganic phosphorus [13]. The increased rate of phosphorous was larger for C2 by approximately 333% (Figure 5). Uploading of Brewer's yeast was beneficial and promoted the conversion of phosphorus.

During processing, the total nitrogen content increased by 33%, 77%, 70%, 107% and 118% respectively, for compost C3, C6, C4, C5 and C2 (Figure 6). Unlike other tests, C1 expressed a decrease of 56% of nitrogen. This decrease can be explained by the activity of micro-organisms and by lossing of volatile liquid fraction in C1

The constancy of the rate of trimethylamine proved the stability of our product. The C1 value of 1.8% was considered significant and reflected the alteration of the product [17,18].

Due to microbial activity, composting is a generally a transformation marked by a rise in temperature [14,20]. It is not so in this study; the transformation was due to the small thickness of tests (area/volume) and the regular homogenization applied [19].

The microbiological test was also favorable. In contrast to C1, the composts of C2, C3, C4, C5 and C6 experienced the disappearance of E. coli, staphylococci, and streptococci. Additionally, there was a hygienisation and inhibition of proteolysis and lipolysis in the composts containing Brewer's yeast, due to its probiotic activity [20].

All these tests showed the importance of Brewer's yeast in the making of a favorable biotransformation of fish waste, which inhibits pathogenic bacteria without any degradation of nutritional qualities of the product nor produces a toxic effect.

The fertilization tests performed on the barley crop showed that our product did not have a phytotoxic or inhibitory effect.

However, compared to the witness and commercial fertilizer, plant growth was optimal with application of the compost. The compost promoted improvement in the lengths of the plant stems and roots, with a germination index of 91%. According to agricultural standards, (G> 80%), these growth rates are there by judged quite interesting [21].

IV. CONCLUSION

This study proposes a way of valuing abundant waste and pollutants from part of the food industry in Morocco. It shows the possibility of producing a good quality agricultural fertilizer from a ternary mixture of industrial waste, using an easy and inexpensive process that is both environmentally friendly and sustainable.

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