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Optimizing biogas and biofertilizer production from abundant Moroccan industrial organic wastes by the formulation and the use of a fungal inoculum

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ABSTRACT

In this study, the production of biogas using two fungal strains, *Aspergillus niger* and *Saccharomyces cerevisiae,* was studied. In fact, three different waste components consisting of sardine waste (SW), potato peels (PP), and poultry waste (PW) were successfully combined in mesophilic bio-digestion with fungal strains to enhance the production capacities of gas. This work also exhibited the effect of the formulation using a 10-point simplex-centroid mixture design strategy on biogas optimization. The results showed that 12 days was sufficient to achieve stability in mesophilic bio-digestion. This paper proved that the use of fungal inoculum with the mixture of organic and agro-industrial wastes, balanced in chemical elements necessary for cell growth (M7: 66% SW;17% PP;17% PW), led to higher production capacities of biogas. Therefore, the germination and fertilization tests carried out by the digestates resulting from these mixtures showed that they did not inhibit growth and proved to be suitable to improve the crop yields of bell peppers.

1. Introduction

The food industry sector in Morocco represents an important part of the national economy; its development has faced a multitude of environmental and economic challenges. Moreover, food waste constitutes a silent disaster; many economic losses are generated through the food value chain, making food products more expensive and less accessible to the poorest and most vulnerable population [1-5]. According to the UNEP Food Waste Index Report (2021), 91 kilograms of food per person are wasted annually in Morocco. Nationwide, about 30% of global CO_2 emissions come from food production, and food waste accounts for over 3.3 million tons per year [6]. Food wastage is also a waste of energy because organic matter can be used to produce biogas via the anaerobic bio-digestion process. And this is one of the best solutions for recovering and creating added value from organic industrial waste. The

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application of anaerobic digestion would indeed lead to the production not only of biogas but also an organic digestate that represents an excellent quality of biofertilizer rich in various macronutrients, including nitrogen (N), phosphorus (P), potassium (K), and sodium, as well as an organic matter [7-9]. Morocco ranks first in the world as a sardine producer/exporter country; this sector is considered a polluting industry and causes many environmental problems [10,11]. Meanwhile, the poultry sector also continues to industrialize in Morocco, according to the figures communicated by the Interprofessional Federation of Poultry Sector (FISA). The increase in meat production generates a tremendous amount of waste resulting from inadequate management [12-14]. The potato is among the most practiced vegetable crop in the world and in Morocco. It holds an important place in the diet of Moroccans; moreover, this sector also produces a significant amount of waste that must be treated [15,16]. The aim of this work was to study the effect of formulation by using a 10-point simplex-centroid mixture design strategy and the anaerobic fermentation of three abundant wastes of strategical agroalimentary products in Morocco: sardine waste, potato peel, and poultry wastes. The mixtures were inoculated with two different fungal strains of *Aspergillus niger* and *Saccharomyces cerevisiae*. The digestate quality was assessed using physicochemical and microbiological testing in order to determine the possibility for digestate use as a biofertilizer according to the NF U 44-051 standard.

2. Materials and methods

2.1. Preparation of the fermentation mixture

The sardine waste containing mainly bones, viscera, and heads was crushed in an ice crusher; then, it was mixed with potato peels crushed by an electric chopper and poultry wastes, which were recovered after slaughtering. The yeast *Saccharomyces cerevisiae* was used as a methanation agent and added at a set ratio of 1 percent [11]; reference strains of *Aspergillus niger* (11G323A) were also used in this study. The two species of fungal *Saccharomyces cerevisiae* and *A. niger* were chosen as bioconversion agents because they are ubiquitous and usually inoffensive; therefore, their usage is not hazardous. These species also demonstrated their ability to resist a variety of stressful conditions [10,17]. They are also of biotechnological and economic value, as confirmed by several authors [18]. *Aspergillus niger* is a thermotolerant and osmotolerant fungus that is used in industrial fermentation to produce citric and gluconic acids as well as enzymes. *Saccharomyces cerevisiae* is known to have a high fermentative capacity capable of bioconversion of a wide range of substrates. These two species are used in the agricultural industry to ensure the fermentation of food products such as cereal, wine, and tea. Other industrial activities are interested in their applications in the biological treatment of industrial effluents using biofilm reactors [17]. Contrary to these species, common bacteria associated with anaerobic fermentation (e.g., *Clostridium*) pose a significant pathogen risk, allowing us to sustain and justify the benefits of using the studied fungal strains. A quantity of 200g of waste was introduced into an Erlenmeyer flask with a capacity of 500 ml. A digester was provided with a hole where a capillary was fixed, which transported the biogas from the tank to the test tube, and a thermostatic bath that allowed the temperature to be fixed at 37°C in the bioreactor (mesophilic fermentation). The continuous control of the temperature was carried out by a Smartsensor AR300+ Infrared Thermometer, effectively proving the stability of this parameter. The volume of biogas formed was measured using the displaced liquid method (Figure 1).

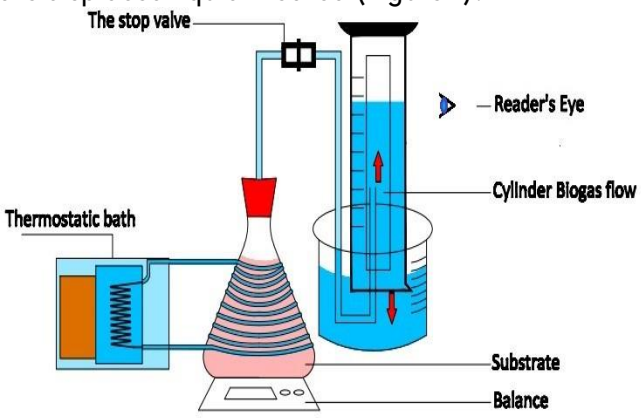

Fig. 1. Schematic presentation of the reactor of the anaerobic digester.

The biogas production potential represents the maximum amount of biogas produced by a given

organic substrate. This measurement is specific to each mixture (Table 1) and representative of its level of biodegradability under anaerobic conditions. The experiments continue until the biogas volume is stabilized.

Table 1. Initial compositions of the mixtures.

	Sardine wastes (SW)	Potato peels (PP)	Poultry wastes (PW)
M1	100.00%	0.00%	0.00%
M2	0.00%	100.00%	0.00%
M3	0.00%	0.00%	100.00%
M4	50.00%	50.00%	0.00%
M5	50.00%	0.00%	50.00%
M6	0.00%	50.00%	50.00%
M7	66.67%	16.67%	16.67%
M8	16.67%	66.67%	16.67%
M9	16.67%	16.67%	66.67%
M10	33.33%	33.33%	33.33%

2.2. Physicochemical and nutritional analysis

The pH of each test was measured at the beginning and end of anaerobic digestion by a pH meter (Fisher Scientific, Basic AB15). The conductivity was measured by a conductivity meter (HANNA instruments, EC215). The dry matter (DM) was determined by steaming at 60°C for 24 hours (Lovergrove, 1966). The organic matter (OM) is obtained by weighing the difference between the mass of the dry waste and the mass of the waste calcined at 600°C for six hours [10]. The total organic carbon and nitrogen were determined by catalytic thermal oxidation with a high temperature TOC analyzer (Model: TOC-L Shimadzu, Japan) [19]. The phosphorus was measured by a spectrophotometric; the potassium was measured by flame emission spectrophotometry using a flame photometer (Digital Flame Photometer PFP7/C, Jenway®) [10]. The contents of the metallic elements (cadmium, nickel, lead, and copper) were analyzed by atomic absorption spectrophotometry (AAS).

2.3. Microbiological analysis

Microbiological analysis was performed before and after the anaerobic biotransformation of the waste according to the NF U 44-051 standards. The presence of *Escherichia coli* (hygiene indicator) was determined on a Mac Conkey agar. The SS medium was used to determine the presence of salmonella [1,19].

2.4. Statistical analysis

The study of the influence of each type of waste was studied by the methodology of mixing plans for the elaboration of Principal Component Analysis (PCA) [20]. The graphs were drawn by the STATISTICA ® software.

2.5. Tests of germination and fertilization

In the study of the toxicity of biofertilizer on the germination of the bell pepper culture, 5 mL of the filtrate was introduced on a double lined Whatman filter paper into a sterile petri dish. Ten bell pepper seeds were deposited in a similar manner in each dish (three replicates for each sample) [21].

3. Results and discussion

3.1. Production of biogas and the mass of digestate evolutions

The results presented in Figures 2 show that the biotransformation process is described according to three phases:

- The first phase, before the 5th day: slow and relatively small production of biogas.
- The second phase, between the 5th and the 12th day: important and exponential production of biogas in variable proportions towards the maximum values.
- The third phase, after the 12th day: stabilization and saturation phase and end of the significant biogas production.

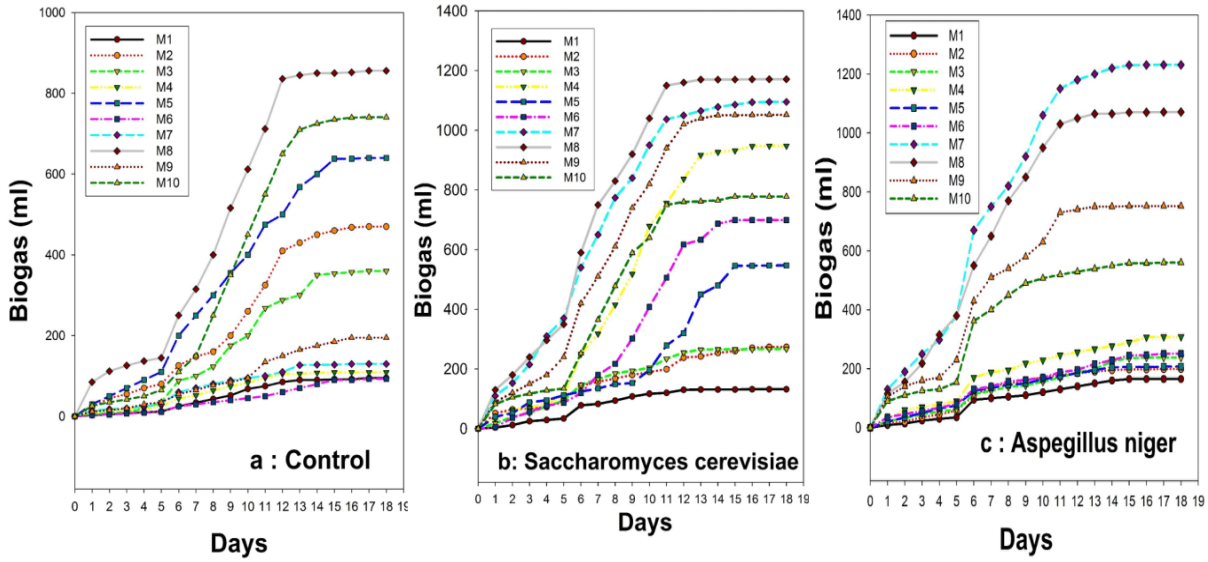

Fig. 2. Kinetics of cumulative biogas production of (a) Control (b) *A. niger* (c) *S. cerevisiae*.

The results showed that the optimal biogas production for the control was 856 ml; however, the addition of 1.0 percent yeast resulted in an increase of 1171 ml, which was mainly due to the yeast increasing the activity of methane-producing bacteria in anaerobic digestion. These results are in agreement with [22]. The inoculation with *Aspergillus niger* increased biogas production up to 1231 ml, facilitating anaerobic digestion, improving biodegradability, and reducing its inhibitory effect. The biogas production (Figure 2) was faster compared to the control substrate. This could be explained by the fact that the fungi used *Aspergillus niger* and *Saccharomyces cerevisiae* that contained carbon and nitrogen-rich nutrients needed for the growth of methane-producing bacteria [23]. Kinetics of mass evolution for the ten mixtures follows the same variations as the biogas production as showing in Figure 3. So the two results corroborate each other and confirm that effectively the progress of the biotransformation process according to the three phases.

3.2. Physicochemical and nutritional characteristics

The specific characteristics of each simple, binary, and ternary mixture are presented in Table 2.

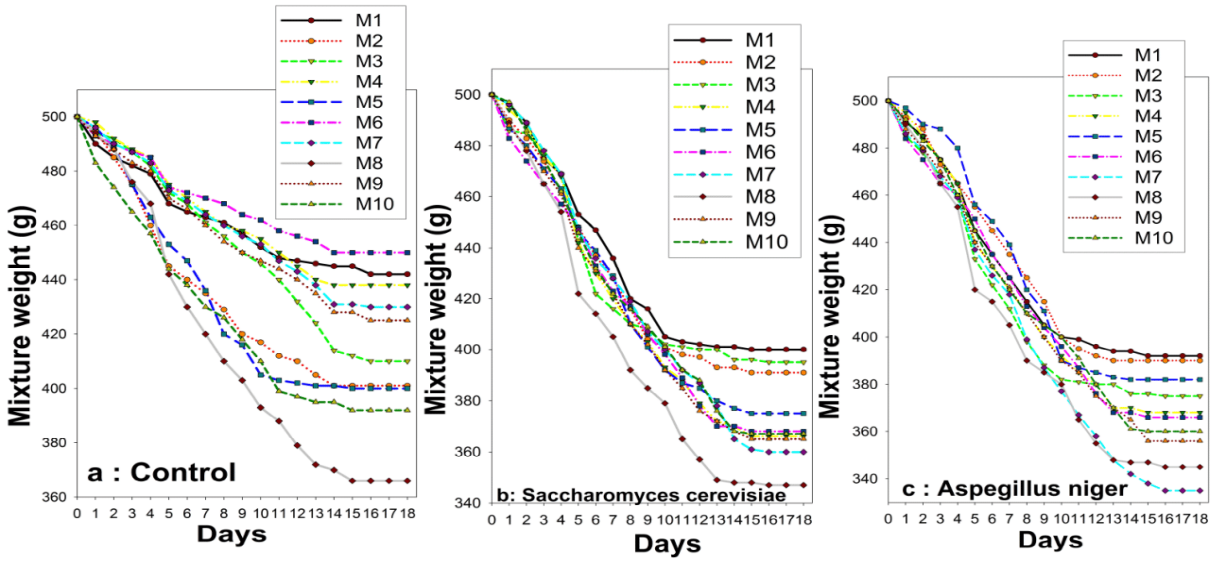

Fig. 3. Kinetics of mass evolution for the ten mixtures of (a) Control (b) *A. niger* (c) *S. cerevisiae*.

Table 2. Physicochemical and nutritive parameters of studied initials mixtures.

	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
pH	6.39 (± 0.02)	6.08 (± 0.03)	7.05 (± 0.1)	6.93 (± 0.13)	6.43 (± 0.06)	7.18 (± 0.08)	7.53 (± 0.12)	7.3 (± 0.17)	7.06 (± 0.15)	7.08 (± 0.03)
C	2.63 (± 0.02)	1.19 (±0.00)	2.76 (± 0.00)	2.8 (± 0.05)	2.46 (± 0.00)	2.71 (± 0.01)	2.66 (± 0.13)	2.52 (± 0.01)	2.81 (± 0.01)	2.91 (±0.04)
DM	47.47 (± 1.37)	37.21 (± 0.18)	52.73 (±0.60)	41.28 (± 1.08)	49.38 (± 1.37)	47.48 (± 0.13)	46.63 (± 0.86)	46.98 (± 0.52)	51.29 (± 1.16)	48.36 (± 1.2)
OM	43.9 (± 1.02)	33.1 (± 0.15)	50.58 (± 1.53)	38.4 (± 0.34)	46.87 (± 0.04)	43.75 (± 0.12)	44.77 (± 0.02)	43.68 (± 1.18)	46.57 (± 0.05)	44.81 (± 1.14)
C/N	13.55	17.2	18.21	19.17	17.7	18.87	25.09	23.11	20.72	19.57

Table 2 shows that the waste used contains more than 33.10 percent organic matter, making our substrates more appropriate for anaerobic digestion. The use of simple mixtures allowed for a pH value between 6.08 and 7.05, a C/N ratio between 13.55 and 17.00, and a less important biogas production; thus, leading to the elaboration of the mixing plan to increase the C/N ratio towards the optimum and to have a balance between the three mixtures. The use of the binary and ternary mixtures resulted in an increase and influence of the pH on the biogas and substrate production. The study showed that the amount of biogas produced in a range of pH 6.93 to 7.53 could promote the development of methanogenic bacteria in mixtures M3, M4, M6, M7, M8, M9, and M10, which were also higher compared to the amount produced from a pH between 6.08 to 6.43 for mixtures M1, M2, and M5. The pH value between pH 6.93 to 7.53 that allowed for a significant production of biogas is in agreement with those mentioned in the literature whose favorable range is between 6.5 and 7.6 [24]. There is also a significant increase in the C/N ratio between 18.21 and 25.09%; also, mixture 7 with the highest value of 25.09% was able to produce a significant amount of biogas. The obtained value of C/N of the best biogas producer is in agreement with those reported in the literature for anaerobic treatment and biogas production [25,26]. Table 3 presents the physicochemical and nutritional characterization of all digestates generated from the 10 mixtures inoculated or not by fungi. The results in Table 3 clearly show that the fungal inoculum has a significant impact on the anaerobic digestion of agro-industrial waste, both in terms of biogas production and the lowering of the organic load of the substrate. The pH of the digestate (control) ranged from 5.32 to 7.22, whereas that of SC ranged from 6.51 to 7.78 and AN ranged from 6.55 to 8.06, implying that inoculation with SC and AN indicates the higher stability in the AN process. The conductivity of these mixtures in Table 3 varies between 2.24 and 6.52. It should be noted that plants root better in a substrate containing few nutrients [27]. It can be seen that the conductivity values show a difference in values between the control and those inoculated with AN and SC. The OM was substantially more evolved (decreased) in the AN and SC than in the control, implying that the AN and SC biomethanizing agents cause significant organic matter degradation. The most significant components in AD are the key plant nutrients, such as nitrogen, phosphorus, and potassium. A large part of the organic nitrogen is transformed into ammonium by nitrogen mineralization during the anaerobic digestion process, which allows an overall increase of ammonium in the final digestate for all mixtures; these results are confirmed by Moller et al. [28]. In this study, we see that the final total nitrogen is considered equal to that added, and the agronomic azote potential of digestates obtained by anaerobic digestion for all mixtures is below the legal limits set by the standard NF U 44-051 for agricultural use (N content of less than 3%). Anaerobic digestion does not cause phosphorus losses via biogas but changes the form of phosphorus [29]. The increase in phosphorus in the mixtures M1, M2, M4, M6, M7, M9, and M10 inoculated by AN is explained by the change from organic to precipitated mineral forms. It is also noticed that the mixtures M3, M5, and M8 with low mineral content could not have an increase in phosphorus. And this is in agreement with the results of [30,31]. We also see that as the pH of the

digestate rises, the phosphorus converts to additional phosphates in different forms. We also find that they develop more easily in alkaline conditions, particularly for mixtures 7 inoculated by AN, which has a pH of 8.06. As well, the transformation of K in liquid form allowed a better bioavailability of this element, especially for mixture 7 inoculated by AN, which could have a great evolution (increase) that is confirmed by [32]. Also, this augmentation perhaps leads to an ideal characterization of the pH (8.06) for the culture. As a result, the anaerobic digestion C/N ratio can be used to determine a link between carbon and nitrogen contents. The drop in the C/N ratio was detected in all combinations [33], and this shift was primarily due to the degradation of organic matter and carbon content in the treated waste, which is a frequent process observed in biological treatment [34]. Digestates with a C/N ratio of 15 to 20 are regarded as ideal for use on agricultural land. The combinations inoculated by AN with a value of 17.06 and SC with a value of 15.16 in this study might display significant values between 15 and 20; these are the mixtures characterized by a pH between 7.40 and 8.06 with optimal biogas production. The biogas yields obtained are comparable to other studies [35-38] (Table 4) from the general C/N criteria. The results obtained also meet the criteria required by the ISO NF U 44-051 standard (C/N > 8) for the amendment of agricultural soils. The advantage of this study is that the proposed solution does not require any pre-treatment or the use of a specific additive; therefore, it is inexpensive. It only takes advantage of the effect of the formulation and the use of fungal inoculum.

3.3. Determination of ions and heavy metals and microbiological test

The microbiological test confirms the presence of bacteria for combinations M3 and M5, as well as Cu, Ni, and Pb values that do not match the criteria of standard NF U 44-051 for the control (Table 5). The results of the microbiological analysis show that the process used can result in safe and hygienic products. These studies also show that S. cerevisiae and A. niger have an inhibitory effect on E. coli growth. Indeed, in the case of some unfavorable mixtures, specifically M5 and M3, inoculation with the two molds allows them to be microbiologically comparable to the control. This may be explained generally by the probiotic activity created by the production of organic acids by the fungal strains, which prevent the growth of spoilage/alteration flora (proteolytic and lipolytic), so ensuring the microbiological quality of the mixtures [18]. For the same mixes (M3 and M5), the control of *S. aureus* was only positive for the control. Aside from A. niger in the case of M5, the effect of inoculation of mixtures by fungal shows that the latter can inhibit the growth of bacteria indicators of spoilage/alteration. Taking into account the M5 data, in particular, it appears that S. cerevisiae probiotic activity is more powerful than that of A. niger. These findings agree with the work of Taiek et al. [10], which demonstrated the significance of S. cerevisiae and confirmed that its addition to fish waste allowed for effective biotransformation and inhibition of pathogenic spoilage/alteration bacteria.

3.4. Statistical analysis

Figures 4a and 4b show the biplot and circle of correlation from the principal component analysis (PCA). These figures show the following:

1. Both the formulation and the nature of the inoculum affect the quality of anaerobic biodigestion in terms of biogas production and the quality of generated residues.
2. The inoculation by fungal strains allows a better production of biogas. In fact, the group of uninoculated cases are anticorrelated with the volume of biogas produced.
3. Generally, M7: 66% SW;17% PP;17% PW is the best formula that allows the maximum generation of biogas, probably due to its high content of C/N.
4. Inoculation with A. niger makes it possible to generate a residue rich in chemical elements for the growth of plant crops (nitrogen, phosphorus, and potassium).

Table 3. Characterization of digestate.

Parameter	Inoculum	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
pH	Control	6.46 (± 0.01)	5.32 (± 0.03)	6.12 (±0.00)	6.87 (± 0.02)	6.22 (± 0.01)	6.41 (± 0.00)	6.24 (± 0.01)	7.04 (± 0.01)	7.1 (± 0.03)	7.22 (± 0.01)
	SC	6.81 (± 0.30)	6.51 (± 0.30)	6.65 (±0.10)	7.34 (± 0.20)	6.85 (± 0.16)	7.01 (± 0.15)	6.80 (± 0.09)	7.40 (± 0.20)	7.71 (± 0.30)	7.78 (± 0.04)
	AN	6.55 (±0.13)	6.22 (± 0.20)	6.78 (± .15)	7.32 (± 0.10)	6.64 (± 0.03)	7.03 (± 0.15)	8.06 (± 0.24)	7.37 (± 0.15)	7.41 (± 0.20)	7.33 (± 0.35)
Cond. (mS/cm)	Control	3.11 (± 0.13)	2.24 (± 0.02)	3.6 (±0.00)	2.33 (± 0.01)	3.56 (± 0.01)	2.88 (± 0.10)	3.32 (± 0.00)	4.24 (± 0.15)	4.45 (± 0.01)	3.87 (± 0.00)
	SC	5.13 (± 0.02)	3.56 (± 0.11)	6.07 (±0.03)	4.24 (± 0.01)	6.03 (± 0.12)	4.57 (± 0.00)	6.14 (± 0.23)	4.01 (± 0.00)	5.87 (± 0.00)	5.43 (± 0.00)
	AN	3.25 (± 0.00)	4.02 (± 0.16)	6.32 (±0.27)	4.45 (± 0.02)	6.52 (± 0.01)	4.19 (± 0.03)	5.37 (± 0.14)	4.58 (± 0.01)	5.78 (± 0.01)	5.35 (± 0.02)
DM (%)	Control	40.23 (± 1.19)	25.51 (± 3.02)	44.44 (± 2.19)	32.16 (± 1.72)	37.46 (± 0.66)	37 .39 (± 2.28)	33.12 (± 1.31)	32.22 (± 0.11)	41.1 (± 2.24)	37.81 (± 1.01)
	SC	37.33 (± 4.30)	27.61 (± 0.32)	43.54 (± 0.91	29.74 (± 2.62)	38.33 (± 1.33)	35.87 (± 3.33)	30.23 (± 0.25)	32.11 (± 5.44)	37.56 (± 2.02)	34.18 (± 0.33)
	AN	35.26 (± 0.56)	24.58 (± 3.01)	40.47 (±1.09)	29.89 (± 3.01)	39.47 (± 2.07)	32.57 (± 2.01)	30.56 (± 0.71)	31.89 (± 0.19)	39.41 (± 0.05)	34.45 (± 0.01)
OM (%)	Control	36.67 (± 1.06)	22.02 (± 1.56)	40.26 (±0.50)	29.72 (± 3.16)	34 .12 (± 0.06)	33.57 (± 0.12)	29.18 (± 0.71)	28.33 (± 0.06)	38.24 (±0.66)	34.76 (±0.89)
	SC	33.10 (± 1.62)	23.41 (± 0.06)	40.98 (±2.10)	25.57 (± .08)	35.30 (± 0.18)	31.70 (± 6.04)	26.03 (± 0.38)	28.91 (± 0.04)	33.56 (± 0.88)	31.02 (± 0.78)
	AN	31.2 (± 0.51)	20.41 (± 0.07)	38.03 (±1.04)	26.46 (±0.13)	36.42 (± 0.22)	28.64 (± 0.41)	26.27 (± 0.05)	28.80 (± 0.66)	35.40 (± 0.26)	30.02 (± 0.13)
N (%)	Control	1.86 (± 0.02)	0.95 (± 0.00)	1.72 (±0.01)	1.46 (±0.00)	1.37 (± 0.00)	1.62 (± 0.00)	1.28 (± 0.02)	1.2 (± 0.03)	1.64 (± 0.00)	1.37 (± 0.01)
	SC	1.89 (± 0.04)	0.90 (± 0.04)	1.68 (±0.01)	1.05 (±0.00)	1.14 ± (0.05)	1.23 (± 0.12)	0.94 (± 0.01)	1.12 (±0.03)	1.27 (± 0.05)	1.30 (± 0.08)
	AN	2.22 (± 0.03)	0.70 (± 0.01)	1.53 (±0.03)	1.55 (± 0.00)	1.62 (± 0.06)	1.33 (± 0.01)	1.30 (± 0.00)	1.28 (± 0.00)	1.63 (± 0.01)	1.22 (± 0.01)
P (%)	Control	0.38 (± 0.00)	0.22 (± 0.00)	0.34 (±0.00)	0.42 (± 0.01)	0.45 (± 0.00)	0.33 (± 0.00)	0.42 (± 0.00)	0.31 (± 0.00)	0.38 (± 0.00)	0.28 (± 0.00)
	SC	0.41 (± 0.03)	0.27 (± 0.02)	0.30 (±0.01)	0.38 (± 0.01)	0.48 (± 0.00)	0.36 (± 0.01)	0.47 (± 0.06)	0.38 (± 0.00)	0.46 (± 0.01)	0.35 (± 0.01)
	AN	1.12 (± 0.00)	0.78 (± 0.00)	0.38 (±0.00)	1.52 (± 0.00)	0.65 (± 0.00)	0.88 (± 0.00)	1.72 (± 0.00)	0.58 (± 0.00)	0.98 (± 0.00)	1.52 (± 0.00)
K (%)	Control	0.3 (± 0.00)	0.22 (± 0.00)	0.1 (±0.00)	0.38 (± 0.00)	0.45 (± 0.00)	0.31 (± 0.00)	0.77 (± 0.00)	0.43 (± 0.00)	0.36 (± 0.00)	0.28 (± 0.00)
	SC	0.32 (± 0.03)	0.20 (± 0.01)	0.07 (±0.01)	0.40 (± 0.06)	0.23 (± 0.03)	0.22 (± 0.02)	0.26 (± 0.01)	0.52 (± 0.01)	0.18 (± 0.01)	0.300 (± 0.00)
	AN	0.65 (± 0.00)	0.42 (± 0.00)	0.36 (±0.00)	1.44 (± 0.00)	1.47 (± 0.00)	0.40 (± 0.00)	2.13 (± 0.00)	1.50 (± 0.00)	1.23 (± 0.00)	1.28 (± 0.00)
C/N	Control	10.87	12.68	12.48	11.10	12.74	10.77	11.11	13.78	12.20	13.89
	SC	10.02	11.38	12.75	13.61	14.78	13.55	14.73	15.16	15.01	13.59
	AN	8.85	14.31	15.09	10.23	13.45	13.28	17.06	13.66	12.97	14.55

C: Control; SC: *Saccharomyces cerevisiae*; AN: *Aspergillus niger*.

Table 4. Comparison of the results obtained with other works.

Nature of Substrate	Substrate	Process	Inoculum	C/N	Biogas	Reference
Marine Waste	Microalgae	Mesophilic AD	Sawarage sludge	13	227ml	[35]
Food waste	Cooked bone: 2.6%, cooked vegetable: 24.3%, pasta/rice: 27.7%, fruit peeling: 20.9%, and cooked eggshell: 1.3%,	Mesophilic AD With thermal pretreatment in different duration	Seed sludge	14.4	~1000 ml	[36]
Agro-food waste	Chicken manure	Mesophilic AD	dairy manure	10	425 ml	[37]
Agro-food waste	Animal waste	Mesophilic AD	none	8.21	288 ml	[38]
Agro-food waste	Sardine 16.67% potatoes peel 16.67% poultry waste 66.67%	Mesophilic AD	*S. cerevisiae*	14.73	~1200 ml	This study
	Sardine 16.67% potatoes peel 16.67% poultry waste 66.67%	Mesophilic AD	*A. niger*	17.06	~1200 ml	This study

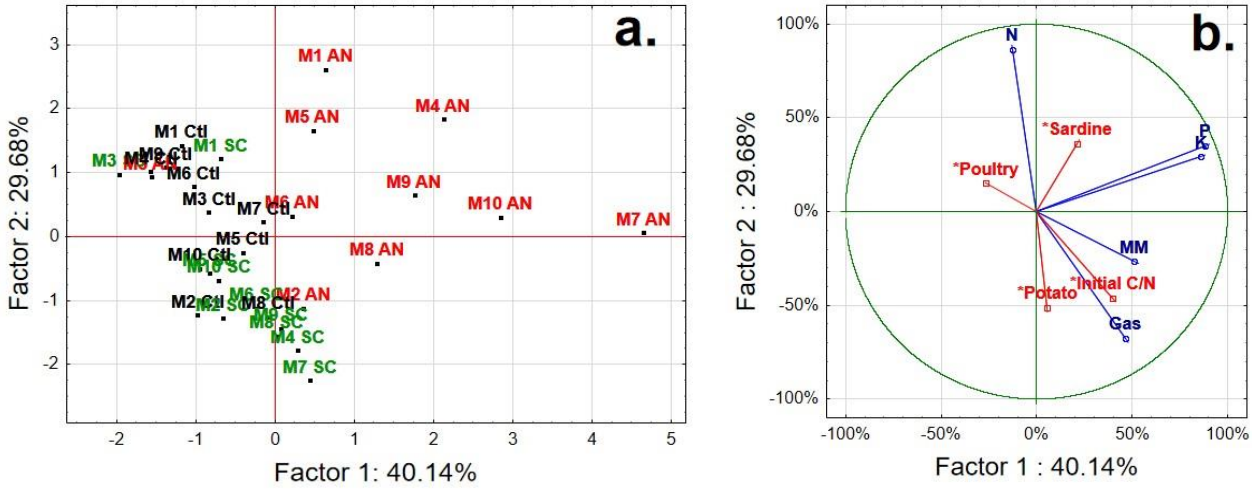

Fig. 4. The Principal Component Analysis (PCA) in two-dimensional patterns explained almost 100% of the total variance. (a) The Biplot consisting of projection on PC1 (40.14%) and PC2 (29.68%), (b) Correlation circle between variables and principal components.

Table 5. The control of effectiveness, chemical and microbiological harmlessness criteria.

Microbiological criteria control

	Standard NF U 44-051	*inoculum*	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
Staph.	-	Ctl	nd	nd	d	nd	d	nd	nd	nd	Nd	nd
		SC	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd
		AN	nd	nd	nd	nd	d	nd	nd	nd	Nd	nd
E. coli	$< 10^2$ CFU g^{-1}	Ctl	nd	nd	d*	nd	d*	nd	nd	nd	Nd	nd
		SC	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd
		AN	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd
Salm.	nd in 25g	Ctl	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd
		SC	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd
		AN	nd	nd	nd	nd	nd	nd	nd	nd	Nd	nd

Chemical criteria control

	Standard NF U 44-051	*inoculum*	M1	M2	M3	M4	M5	M6	M7	M8	M9	M10
Cu mg/kg	< 300	Ctl	186.34 (±2.09)	52.23 (±1.66)	422.11 (±5.41) *	213.37 (± 7.11)	378.87 (± 4.53)*	97.55 (± 2.04)	196.67 (±3.20)	198.13 (±5.77)	522.10 (± 1.19)*	122.78 (±0.66)
		SC	201.8 (±0.29)	68.26 (±0.40)	134.8 (±2.75)	111.6 (±1.76)	312.9 (± 3.16)*	81.92 (±0.41)*	295.2 (±1.75)	185.6 (±2.83)	157.0 (± 0.94)	162.06 (± 1.18)
		AN	103.6 (±3.29)	56.16 (±4.53)	167.4 (±1.87)	115.3 (±0.16)	411.4 (±3.02)*	88.65 (±0.55)	90.66 (±2.06)	168.3 (±4.41)	172.5 (± 0.90)	133.2 (±6.28)
Ni mg/kg	< 60	Ctl	23.44 (±0.22)	48.56 (±1.92)	98.28 (± 1.73)*	34.36 (±2.00)	76.03 (±1.47)*	54.20 (±0.99)	49.08 (± 1.16)	56.77 (± 1.33)	71.74 (±0.85)*	34.22 (± 0.29)
		SC	13.29 (±0.50)	26.58 (± 1.12)	12.34 (±0.21)	15.5 (±0.05)	8.54 (±0.12)	78.70 (±1.09)*	15.19 (±0.07)	22.78 (±0.13)	10.44 (±0.12)	9.49 (±0.14)
		AN	9.33 (±0.03)	29.38 (±0.00)	10.54 (±1.11)	17.4 (±0.02)	78.4 (±1.02)*	58.09 (±0.23)	26.22 (±0.02)	43.47 (±0.05)	18.46 (±0.15)	12.96 (±0.11)
Pb mg/kg	< 180	Ctl	74.08 (±1.92)	58.61 (±2.16)	112.10 (±3.01)	70.23 (±0.68)	92.82 (±4.33)	133.22 (±2.03)	104.87 (±1.11)	143.09 (±3.44)	82.55 (±2.63)	176.93 (±1.06)
		SC	56.34 (±1.02)	24.73 (±0.24)	59.35 (± 1.19)	34.62 (±0.07)	74.18 (± 0.11)	69.24 (± 1.06)	84.08 (±2.00)	98.91 (±1.04)	62.24 (±0.33)	194.3 (±2.09)*
		AN	34.78 (±1.12)	32.87 (±1.52)	89.15 (±1.19)	55.12 (±0.91)	67.23 (±3.02)	72.47 (±2.21)	89.55 (±3.10)	112.67 (±1.62)	46.60 (±0.14)	170.7 (±3.22)
Cd mg/kg	< 3	Ctl	0.23 (±0.00	1.34 (±0.00)	0.56 (±0.00)	1.12 (±0.00)	1.55 (±0.00)	0.72 (±0.00)	0.65 (±0.00)	0.44 (±0.00)	0.13 (±0.00)	0.26 (±0.00)
		SC	0.04 (±0.00)	0.21 (±0.00)	0.09 (±0.00)	0.30 (±0.00)	0.16 (±0.00)	0.25 (± 0.01)	0.26 (±0.01)	0.06 (±0.00)	0.16 (±0.00)	0.03 (±0.00)
		AN	0.03 (±0.00)	0.55 (±0.00)	0.07 (±0.00)	0.78 (±0.00)	0.89 (±0.00)	0.33 (±0.00)	0.04 (±0.00)	0.05 (±0.00)	0.22 (±0.00)	0.15 (±0.00)

C: Control; SC: *Saccharomyces cerevisiae;* AN: *Aspergillus niger;* nd : not detected; d : detected; *non-compliant.

3.5. Test of germination

The germination tests show that the M7AN has no inhibiting effect on germination and that about 80% of the seeds germinated in 168 h; similarly, M7 SC has no inhibiting effect on germination either, since 70% of the seeds germinated in 192 h. Table 6 shows the characterization of the fertilizers and fertilization test. According to the results of the fertilization tests, M7 AN and M7 SC seem to be the most suitable for bell pepper cultivation. Also, M7 AN has a better performance

in vegetative growth due to the high content of potassium, resulting in a more robust plant that is more adaptable to nutritional and environmental changes [39]. The liquid fraction seems to be more effective as a biofertilizer than the solid fraction, probably due to the high presence of bioassimilable nutrients in this part of the product.

Table 6. Characterization of the fertilizers.

	Biofertilizer from	Soil only	Solid fraction	Liquid fraction	Commercial
Characterization of the fertilizers					
pH	M7 Ctl	6.42 (± 0.01)	6.12 (±0.01)	7.44 (±0.01)	6.78 (± 0.01)
	M7 SC		6.33 (± 0.02)	8.23 (± 0.02)	
	M7 AN		7.23 (± 0.00)	8.03 (± 0.00)	
N (%)	M7 Ctl	0.72 (± 0.00)	0.10 (± 0.01)	1.15 (± 0.01)	12.35 (± 0.01)
	M7 SC		0.12 (± 0.04)	1.03 (± 0.01)	
	M7 AN		0.21 (± 0.02)	1.43 (± 0.00)	
P (%)	M7 Ctl	0.30 (± 0.03)	0.30 (± 0.02)	0.06 (± 0.04)	6.65 (0.03)
	M7 SC		0.35 (± 0.01)	0.08 (± 0.03)	
	M7 AN		0.28 (± 0.00)	0.05 (± 0.01)	
K (%)	M7 Ctl	0.91 (± 0.01)	0.18 (0.00)	0.06 (± 0.02)	8.46 (± 0.00)
	M7 SC		0.20 (± 0.02)	0.08 (± 0.05)	
	M7 AN		1.41 (± 0.00)	0.32 (± 0.01)	
Fertilization test on the bell pepper crop					
Root (cm)	M7 Ctl	5.0 (± 0.01)	10.2 (± 0.02)	11.0 (± 0.02)	7.4 (± 0.00)
	M7 SC	6.1 (± 0.01)	12.1 (± 0.01)	12.8 (± 0.01)	9.3 (± 0.01)
	M7 AN	5.4 (± 0.05)	15.6 (± 0.01)	14.3 (± 0.03)	10.2 (± 0.02)
Stem (cm)	M7 Ctl	2.9 (± 0.01)	10.1 (± 0.00)	9.8 (± 0.01)	4.8 (± 0.01)
	M7 SC	4.5 (± 0.02)	10.4 (± 0.00)	11.4 (± 0.03)	7.7 (± 0.02)
	M7 AN	4.8 (± 0.04)	12.3 (±0.00)	13.8 (± 0.01)	6.5 (± 0.01)
Leaf (cm)	M7 Ctl	2.6 (± 0.03)	3.8 (± 0.01)	4.5 (± 0.01)	2.2 (± 0.02)
	M7 SC	2.9 (± 0.04)	4.3 (± 0.03)	4.1 (± 0.04)	3.8 (± 0.03)
	M7 AN	3.1 (± 0.06)	4.0 (± 0.01)	4.6 (± 0.01)	3.3 (± 0.00)

M7 SC: M7 with *Saccharomyces cerevisiae*; M7 AN: M7 with *Aspergillus niger*; M7 Ctl : M7 (control).

4. Conclusions

The simultaneous use of three types of agro-industrial waste allowed us to produce an important quantity of biogas. The potential of biogas production was increased by the use of the two fungal strains, *Aspergillus niger* and *Saccharomyces cerevisiae*. But the digestate produced in the use case of Aspergillus niger presented better doubly recovery of the studied organic wastes. The resulting digestion residues demonstrated an optimal performance in vegetative growth. It meets the chemical and microbiological harmlessness criteria of the international standards NF U 44-051.

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References

[1] Shi, C.,Wang, K., Zheng, M., Liu, Y., Ma, J., Li, K. (2021). The efficiencies and capacities of carbon conversion in fruit and vegetable waste two-phase anaerobic digestion: Ethanol-path vs. butyrate-path. *Waste management, 126*, 737-746.

[2] Yu, Q., Yang, Y., Wang, M., Zhu, Y., Sun, C., Zhang, Y., Zhao, Z. (2021). Enhancing anaerobic digestion of kitchen wastes via combining ethanol-type fermentation with magnetite: Potential for stimulating secretion of extracellular polymeric substances. *Waste management, 127*, 10-17.

[3] Mayer, F., Bhandari, R., Gäth, S. A. (2021). Life cycle assessment on the treatment of organic waste streams by anaerobic digestion, hydrothermal carbonization and incineration. *Waste management, 130*, 93-106.

[4] Yan, B., Yan, J., Li, Y., Qin, Y., Yang, L. (2021). Spatial distribution of biogas potential, utilization ratio and development potential of biogas from agricultural waste in China. *Journal of cleaner production, 292*, 126077.

[5] Yaqoob, H., Teoh, Y. H., Ud Din, Z., Sabah, N. U., Jamil, M. A., Mujtaba, M. A., Abid, A. (2021). The potential of sustainable biogas production from biomass waste for power generation in Pakistan. *Journal of cleaner production, 307*, 127250.

[6] United Nations environment program (2021). Food waste index report 2021. Nairobi.

[7] Maldaner, L., Wagner-Riddle, C., VanderZaag, A. C., Gordon, R., Duke, C. (2018). Methane emissions from storage of digestate at a dairy manure biogas facility. *Agricultural and forest meteorology*, *258*, 96-107.

[8] Vergote, T., Vanrolleghem, W., Van der Heyden, C., De Dobberlaere, A., Buysse, J., Meers, E., Volcke, E.I.P. (2019). Volcke Model-based analysis of greenhouse gas emission reduction potential through farm-scale digestion. *Biosystems engineering, 181*, 157-172.

[9] Tait, S., Harris, P. W., McCabe, B. K. (2021). Biogas recovery by anaerobic digestion of Australian agro-industry waste: A review. *Journal of cleaner production,* 299, 126876

[10] Taiek, T., Boutaleb, N., Bahlaouan, B., El Jaafari, A., Khrouz, H., Safi, A., El Antri, S. (2014). Valorisation de déchets de poisson alliés à des rejets brassicoles en vue d'obtenir un biofertilisant. *Déchets sciences and techniques, 68*, 24-30.

[11] Lakhal, D., Boutaleb, N., Bahlaouan, B., Taeik, T., Fathi, A., Mekouar, M., Abouakil, N., Lazar, S., Elantri, S. (2017). Mixture Experimental Design in the Development of a Bio Fertilizer from Fish Waste, Molasses and Scum.*Int. International journal of engineering research and technology,* 6, 588-94.

[12] Li, F., Cheng, S., Yu, H., Yang, D. (2016). Waste from livestock and poultry breeding and its potential assessment of biogas energy in rural China. *Journal of cleaner production, 126*, 451–460.

[13] Kazemi-Bonchenari, M., Alizadeh, A., Javadi, L., Zohrevand, M., Odongo, N. E., Salem, A. Z. M. (2017). Use of poultry pre-cooked slaughterhouse waste as ruminant feed to prevent environmental pollution. *Journal of cleaner production, 145*, 151-156,

[14] Gohil, A., Budholiya, S., Mohan, C. G., Prakash, R. (2021).Utilization of poultry waste as a source of biogas production. *Materials today: Proceedings, 45*, 783-787.

[15] Chohan, N. A., Aruwajoye, G. S., Sewsynker-Sukai, Y., Gueguim Kana, E. B. (2019). Valorisation of potato peel wastes for bioethanol production using simultaneous saccharification and fermentation: Process optimization and kinetic assessment. *Renewable energy, 146*, 1031-1040.

[16] Shekhar, C., Jaiswal, A., Ji, G., Prakash, R. (2021). Ethanol extract of waste potato peels for corrosion inhibition of low carbon steel in chloride medium. *Materials today: proceedings, 44*, 2267-2272.

[17] Fathi, A., Boutaleb, N., Bahlaouan, B., Bennani, M., Lazar, S., El Antri, S. (2021). Filamentous fungi and natural supports as a carrier in moving bed biofilmreactors for ecological treatment of halieutic industrial effluent. *Desalination and water treatment*, *237*, 37-44.

[18] Taiek, T., Boutaleb, B., Bahlaouan, B., EL Jaafari, A, Letilly, V., Sire, O., EL antri, S. (2014a). Biotransformation de déchets halieutiques au Maroc: Essais de production d'un fertilisant biologique. *Techniques sciences methodes*, *11*, 158-171.

[19] Hadidi, M., Bahlaouan, B., Assaba, S., Ozi, F. Z., Fathi, A., El Antri, S., Boutaleb, N. (2020). Optimisation de la production du biogaz par les plans de mélanges de déchets agro-industriels et biofertilisation par les résidus de codigestion. *Techniques sciences methodes, 10*, 53-66.

[20] Redel-Macías, M. D., Pinzi. S., Leiva-Candia, D. E., López, I., Dorado, M. P. (2017). Ternary blends of diesel fuel oxygenated with ethanol and castor oil for diesel engines. *Energy procedia, 142*, 855-60.

[21] Kim, J. K., Dao, V. T., Kong, I. S., Lee, H. H. (2010). Identification and characterization of microorganisms from earthworm viscera for the conversion of fish wastes into liquid

fertilizer. *Bioresource technology, 101*, 5131-5136.

[22] Gao, M., Zhang, S., Ma, X., Guan, W., Song, N., Wang, Q., Wu, C. (2020). Effect of yeast addition on the biogas production performance of a food waste anaerobic digestion system. *Royal society open science, 7*, 200443.

[23] Battista, F., Fino, D., Erriquens, F., Mancini, G., Ruggeri, B. (2015). Scaled-up experimental biogas production from two agro-food waste mixtures having high inhibitory compound concentrations. *Renewable energy, 81*, 71-77.

[24] Helenas Perin, J. K., Biesdorf Borth, P. L., Torrecilhas, A. R., Santana da Cunha, L., Kuroda, E. K., Fernandes, F. (2020). Optimization of methane production parameters during anaerobic co-digestion of food waste and garden waste. *Journal of cleaner production, 272,* 123130.

[25] Matheri, A. N., Ndiweni, S. N., Belaid, M., Muzenda, E., Hubert. R. (2017). Optimising biogas production from anaerobic co-digestion of chicken manure and organic fraction of municipal solid waste. *Renewable* and *sustainable energy reviews, 80*, 756-764.

[26] Wang, X., Lu, X., Li, F., Yang, G. (2014). Effects of temperature and Carbon-Nitrogen (C/N) ratio on the performance of anaerobic co-digestion of dairy manure, chicken manure and rice straw: focusing on ammonia inhibition. *PloS ONE, 9*(5), e97265.

[27] Waltham, B., Örmeci, B. (2020). Fluorescence intensity, conductivity, and UV–vis absorbance as surrogate parameters for real-time monitoring of anaerobic digestion of wastewater sludge. *Journal of water process engineering, 37*, 101395.

[28] Moller, K., Stinner, W., Deuker, A., Leithold, G. (2008). Effects of different manuring systems with and without biogas digestion on nitrogen cycle and crop yield in mixed organic dairy farming systems. *Nutrient cycling in agroecosystems, 82*(3), 209-232.

[29] Möller, K., Müller, T. (2012). Effects of anaerobic digestion on digestate nutrient availability and crop growth: A review. *Engineering in life sciences, 12*(3), 242–257.

[30] Bachmann, S., Wentzel, S., Eichler-Löbermann, B. (2011). Codigested dairy slurry as a phosphorus and nitrogen source for Zea mays L. and Amaranthus cruentus L. *Journal of plant nutrition and soil science*, *174*(6), 908-915.

[31] Alburquerque, J. A., de la Fuente, C., Ferrer-Costa, A., Carrasco, L., Cegarra, J., Abad, M., Pilar Bernal, M. (2012). Assessment of the fertiliser potential of digestates from farm and agroindustrial residues. *Biomass and bioenergy, 40*, 181-189.

[32] Möller, K., Stinner, W. (2010). Effects of organic wastes digestion for biogas production on mineral nutrient availability of biogas effluents. *Nutrient cycling in agroecosystems, 87*(3), 395–413.

[33] Hartmann, H., Angelidaki, I., Ahring, B. K. (2001). Anaerobic digestion of the organic fraction of municipal solid waste with recirculation of process water. *9th world congress of anaerobic digestion antwerpen Belgium. September*, 301-303.

[34] Jędrczak, A., Suchowska-Kisielewicz, M. (2018). A comparison of waste stability indices for mechanical-biological waste treatment and composting plant. *International journal of environmental research and public health, 15*(11), 2585.

[35] Pardilhó, S., Boaventura, R., Almeida, M., MaiaDias, J. (2022). Marine macroalgae waste: A potential feedstock for biogas production. *Journal of environmental management*, *304*, 114309.

[36] Li, Y., Jin, Y., Li, J., Li, H., Yu, Z. (2016). Effects of thermal pretreatment on the biomethane yield and hydrolysis rate of kitchen waste. *Applied energy*, *172*, 47-58.

[37] Kafle, G. K., Chen, L. (2016). Comparison on batch anaerobic digestion of five different livestock manures and prediction of biochemical methane potential (BMP) using different statistical models. *Waste management*, *48*, 492-502.

[38] Afilal, M. E., Elasri, O., Merzak, Z. (2014). Caractérisations des Déchets Organiques et évaluation du Potentiel Biogaz (Organic Waste Characterization and Evaluation of Its

Potential Biogas). *Journal of materials and environmental science*, *5*(4), 1160-1169.

[39] Fernández-Rodríguez, M. J., Mancilla-Leytón, J. M., Jiménez-Rodríguez, A., Borja, R., Rincón, B. (2021). Reuse of the digestate obtained from the biomethanization of olive mill solid waste (OMSW) as soil amendment or fertilizer for the cultivation of forage grass (Lolium rigidum var. Wimmera). *Science of the total environment, 792*, 148465.