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Microwave Assisted Alkaline Pretreatment of Algae Waste in the Production of Cellulosic Bioethanol

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Abstract: Biomass pretreatment has an important role in the production of cellulosic bioethanol. In this study, the effectiveness of microwave assisted alkaline pretreatment of algae waste was analysed. After pretreatment, the product was hydrolysed using sulphuric acid. The effects of microwave power, irradiating time, solid–liquid ratio and NaOH concentration were examined. Under the best conditions, the fermentable sugars were converted to cellulosic bioethanol using *Saccharomyces Cerevisiae* with a bioethanol yield of 1.93 ± 0.01 g/g and a fermentation efficiency of 40.4%. The reducing sugars concentration was 30% higher than that obtained from conventional hydrolysis without pretreatment. The obtained results suggest that microwave assisted alkaline pretreatment is effective in improving the production of cellulosic bioethanol of algae waste compared to that without microwave effect. Considering energy consumption, low microwave power and short microwave irradiation time are favourable for this pretreatment.

Keywords: industrial waste; microwave pretreatment; acid hydrolysis; bioethanol; energy consumption

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1. Introduction

Currently, society depends on energy resources in a very important way. World primary consumption has increased in recent decades, and it is expected that it will continue to increase in the coming years due to the need for this resource as highly populated developing countries keep improving their standard of living [1]. The depletion of fossil resources will be increased significantly, although the use of renewable resources (hydraulic, biomass, etc.) could help cushion the demand for non-renewable resources.

In order to mitigate these factors (pollution, resource depletion, etc.), renewable energy sources have been sought in recent years. The development of these energy production systems has led to the use of sources such as solar, wind or tidal, and the use of biofuels (biodiesel, bioethanol or biomass). All of these are presented as a sustainable and clean option to fossil fuels.

Bioethanol is a type of biofuel obtained after the hydrolysis and fermentation of lignocellulosic resources [2]. In this way, it is possible to obtain sustainable energy by taking advantage of the chemical energy stored in these resources as well as creating a balance between the CO_2 consumed by plants when they grow and the carbon dioxide expelled into the environment after bioethanol combustion. This cycle means bioethanol could potentially be a carbon neutral fuel source. This biofuel can also be obtained from various types of agro-industrial residues or renewable resources [3–5].

Bioethanol yield and conversion efficiency depend upon the raw material [6,7]. Among the different types of biomass used to obtain bioethanol, algae have received considerable attention because of their advantages: they are easy to be cultivated in fresh and salt water, and even in wastewater [8], which is a clean and cheap alternative for the treatment of this

kind of water; they are fed with energy provided from the sun, water, nutrients and carbon dioxide [9]; the algae have a low concentration of hemicellulose and lignin [10]. These polymers are connected strongly through covalent and hydrogen bonds. These bonds make lignocellulosic material resistant to degradation [11] and, depending on the composition of lignocellulosic material, different methods of pretreatment need to be used to convert them into free sugars [12].

Biomass pretreatment has an important role in the cost evaluation of the process, since it contributes 30–35% of total production cost [13]. The importance of this step is fundamentally due to the removal or decomposition of the lignin [14], making cellulose and hemicellulose more easily available during the hydrolysis. In the last few decades, several pretreatments (mechanical, chemical or microbiological) have been developed depending on the used raw material [13,15] and their advantages and drawbacks have been analysed by many authors [16–19]. However, most conventional methods are accompanied by the formation of toxic inhibitors [11]. Recently, it has been demonstrated that irradiation pretreatment, namely γ-ray [20,21], ultrasound [22,23], electron beam [24] or microwave heating [25,26], can be used to improve the biodegradability of lignocellulosic materials [27,28].

Microwave pretreatment is a process that has been widely accepted thanks to its high efficiency and easy use. In addition, this method allows users to change the structure of cellulose through the degradation of lignin and hemicellulose [29]. The main advantages are the short duration of the reaction and the homogeneity in the heating of the mixture. Thus, microwave pretreatment can be considered one of the most promising pretreatments to change the initial structure of cellulose. However, this process and its conditions need to be optimized [30].

Current trends in the production of bioethanol from lignocellulosic biomass include testing the suitability of new (often waste) biomass sources, developing new pretreatment methods as well as optimizing and improving existing ones. This paper analyses the effect of microwave pretreatment of industrial waste in the bioethanol production. The waste is algae from an industrial process where the alginate is extracted, but this does not affect the cellulose content, and is, therefore, a suitable raw material. The significance of this paper comes mainly from the raw material and, after that, the use of microwave pretreatment as, an alternative to conventional methods. There is not much information about the use of industrial algae waste for bioethanol production and the effect of the microwave assisted pretreatment on that process. In this research, algae waste was pretreated with microwaves in the presence of sodium hydroxide. The influence of different process parameters, such as NaOH concentration and dosage, microwave time and power based on higher reducing sugars after hydrolysis, was also evaluated.

2. Materials and Methods

2.1. Experimental Procedure

In this research, different steps were carried out to obtain the bioethanol from an algal residue of the variety *Eucheuma denticulatum spinosum*. The first part of the work consisted of the characterization and pretreatment of the dried algal residue. In order to analyse the influence on the hydrolysis of the type of radiation applied during the pretreatment, two different methods were applied; on the one hand, the pretreatment with microwaves and, on the other, a control test without any pretreatment. The resulting sample was vacuum filtered, obtaining a liquid product, which may have contained lignin and some hemicellulose, cellulose and sugars, and another solid. The extracted liquid product was characterized to estimate the percentage of glucose and, thus, be able to verify the amount that has been lost in the pretreatment and its possible reuse.

The solid, which we will call the algal sub-residue from now on, was dried and then acid hydrolysed. Subsequently, it was centrifuged to obtain the mother liquor, discarding the solid remains. The mother liquor obtained was characterized in order to determine the amount of sugars obtained in this stage. Finally, the fermentation was carried out. Next, to

separate and purify the obtained bioethanol, two successive distillations were carried out. Finally, the cellulosic bioethanol was characterized.

2.2. Raw Material

Algae waste used in this research was provided by the Compañia Española de Algas Marinas (CEAMSA, Porriño, Spain). The waste was provided from an industrial process where alginate is extracted to *Eucheuma denticulatum spinosum* algae. This waste contains a considerable amount of water (about 35%); therefore, the samples were dried at room temperature until they reached a level of humidity of 5 ± 1%. Some parameters of dried algae waste (such as organic matter, total Kjeldahl nitrogen, ammonium nitrogen, total organic carbon and minerals) were analysed according to standards analytical methods. A halogen moisture analyser, RADWAG MA 110.R, was used to analyse the moisture content. The amount of cellulose was obtained using the method proposed by Van Soest and Wine [31]. These parameters are collected in Table 1.

Table 1. Results of industrial waste algae.

Parameter	Value
Cellulose (%)	37.3 ± 1.9
Organic matter (%)	13.2 ± 0.5
Total Kjeldahl nitrogen (mg/kg)	1180 ± 25
Ammonium nitrogen (mg N/kg)	11 ± 1.1
Total organic carbon (%)	2.9 ± 0.23
Cd (μg/g)	1.5 ± 0.02
Ca (μg/g)	70,100 ± 72
Cr (μg/g)	3.0 ± 0.15
Cu (μg/g)	3.3 ± 0.21
Fe(μg/g)	1210 ± 35
Pb (μg/g)	1.4 ± 0.09
Mg (μg/g)	5080 ± 33

2.3. Microwave Pretreatment

Microwave pretreatment was carried out in a domestic microwave oven with a maximum power of 800 W modified by the researchers. A glass balloon of 500 mL was vertically fixed to a condenser inserted in the top hole of the microwave oven to condense the vapour generated during the pretreatment. During the experiments, 10 g of algae was mixed with different amounts of sodium hydroxide (volume NaOH/algae mass ratio of 4:1, 7:1, 10:1 v/w) at different concentrations (0.5, 1 and 1.5%). To analyse the effect of some variables on the pretreatment, some tests were conducted using the Box–Behnken experimental design. The used variables in the design were: time of pretreatment (12.5–27.5 min), NaOH/dried algae ratio (4:1–10:1 v/w) and microwave power levels (160–402 W). Table 2 shows the full characteristics of all experimental variants.

After the pretreatment, the liquid and solid phases were separated by vacuum filtration. The liquid component was analysed by dinitrosalicylic acid (DNS) method to obtain the amount of glucose lost during pretreatment, and the solids were washed with distilled water to remove any remaining NaOH residues in the sample. Then, they were introduced into an oven at a temperature of 60 °C for a minimum of 6 h, to dry the sample until reaching a moisture lower than 5%, and they were ground in a mortar, preparing the residue for acid hydrolysis.

Table 2. Experimental conditions used on microwave pretreatment.

Test	Time (min)	[NaOH] (%)	Power (W)	Ratio (V/P)
1	20.0	1	249	4:1
2	20.0	1	249	7:1
3	20.0	1	249	10:1
4	20.0	0.50	249	10:1
5	20.0	1.50	249	10:1
6	5.0	1	249	10:1
7	12.5	1	249	10:1
8	27.5	1	249	10:1
9	35.0	1	249	10:1
10	12.5	1	160	10:1
11	12.5	1	402	10:1
12	20.0	1	160	10:1
13	20.0	1	402	10:1
14	27.5	1	402	10:1
15	27.5	1	160	10:1

During the pretreatment, the energy transferred to the mixture is only due to the heat provided by the microwaves. Considering that there is no loss of energy in the closed system, the heat transferred to the system can be calculated from the microwave power and time of pretreatment as follows

$$\text{Energy (kJ)} = P \times t \quad (1)$$

where P is the microwave power in kW and t the time of pretreatment in s.

2.4. Acid Hydrolysis

The acid hydrolysis of pretreated samples was performed with 7 mL/g residue of a 9% solution of sulphuric acid in an autoclave at 121 °C for 75 min. After that, the mixture was introduced into the centrifuge at 4000 rpm for 4 min in order to separate the solid phase (residue) from the liquid. Liquid samples were withdrawn for the analysis of glucose and other reducing sugars. A control experiment without pretreatment was also carried out in the same conditions for comparison. The hydrolysate with the highest reducing sugar content was used for the subsequent fermentation assay.

2.5. Fermentation

Pretreated and hydrolysed liquid samples with the best results in terms of the reducing sugar content were selected as substrate in the fermentation with *Saccharomyces cerevisiae*. Previous to this step, the pH was adjusted to 4.75 using NaOH, since the optimal range of pH for this yeast is between, 4.4 and 5.5 [32]. Fermentation process was performed in an incubator at 30 °C for 20 h and 150 rpm. Finally, samples were filtered, distilled and analysed for ethanol content.

2.6. Analytical Methods

2.6.1. Reducing Sugars and Ethanol

Total reducing sugars on liquid samples after pretreatment and hydrolysis were quantified by DNS method at 540 nm using a UV VIS spectrophotometer SPEKOL 1500. The concentration of ethanol after fermentation process was determined using a refractometer ABBE-REF 1 and a digital densimeter Mettler Toledo Densito 30PX.

2.6.2. HPLC Analysis

The concentration of carbohydrates on the hydrolysate was determined using high-performance liquid chromatography (HPLC). The samples with the highest amount of reducing sugars were analysed by HPLC using the Ultimate 3000 (Thermo Scientific),

equipped with the column Hypersil GOLD Amino (150 × 3 mm), a refractive index detector (RID) and an autosampler. Acetonitrile/water (80:20) was used as the mobile phase at 30 °C and at a flow rate of 1 mL/min. The sugars (such as glucose, galactose, fructose, sucrose, lactose, maltose) were quantified by comparing their peak areas with known concentrations of the standards. The amount of each of the sugars was used to determine the efficiency of the fermentation.

2.6.3. Calculations

Based on the reducing sugars after hydrolysis and ethanol content after fermentation, the fermentation yield was determined as follows

$$\text{fermentation yield } (\%) = \frac{V_b(\text{L}) \times C_b\ (v/v) \times \rho_b(\text{g/L})}{V_h(\text{L}) \times [\text{sugars}](\text{g/L})} \times 100\% \quad (2)$$

where V_b represents the amount of obtained bioethanol after fermentation, C_b is the obtained bioethanol concentration, ρ_b is the value of the obtained density, V_h is the amount of obtained hydrolysate and [sugars] is the sugars' concentration obtained by DNS method.

Based on volume and concentration of obtained bioethanol, the yield was calculated according to the following equation

$$\text{bioethanol yield } (\text{g/g}) = \frac{V_b(\text{L}) \cdot C_b\ (v/v) \cdot \rho_b(\text{g/L})}{40 \text{ g algae waste}} \quad (3)$$

where V_b represents the amount of obtained bioethanol after fermentation, C_b is the obtained bioethanol concentration and ρ_b is the value of the obtained density.

The fermentation efficiency was calculated [33] as follows

$$\text{fermentation efficiency } (\%) = \frac{[\text{ethanol}]}{0.511 \cdot [\text{sugars}]} \times 100\% \quad (4)$$

where [ethanol] represents the amount of obtained bioethanol after fermentation in g/L and [sugars] is the sugars' concentration obtained by DNS method in g/L.

The energy consumption during microwave pretreatment expressed by reduced sugars yield after hydrolysis and ethanol yield after fermentation were calculated according to the following equations

$$\text{energy consumption } (\text{kJ/g reduced sugars}) = \frac{P\ (\text{kW}) \times t\ (\text{s})}{\text{highest reduced sugars hydrolysate } (\text{g})} \quad (5)$$

$$\text{energy consumption } (\text{kJ/g bioethanol}) = \frac{P\ (\text{kW}) \times t\ (\text{s})}{\text{highest obtained bioethanol } (\text{g})} \quad (6)$$

where P is the microwave power and t the time of pretreatment.

3. Results and Discussion

The study was divided into two stages. The first studied the effectiveness of microwave assisted alkaline pretreatment to obtain fermentable sugars; the second, the possibility of producing bioethanol from the obtained sugars.

3.1. Efficiency of Microwave Pretreatment on Hydrolysis

The influence of different parameters of the microwave pretreatment on the amount of reducing sugars after acid hydrolysis was analysed. The use of different combinations of the parameters during pretreatment resulted in different amount of DNS values after the hydrolysis process (Figure 1). The highest reducing sugars' value (1.02 ± 0.01 g/L) after hydrolysis was obtained at 249 W, 12.5 min and at a ratio of 10:1 with an NaOH

concentration of 1%. The comparison of different tests allowed us to determine the influence of the different parameters on the process.

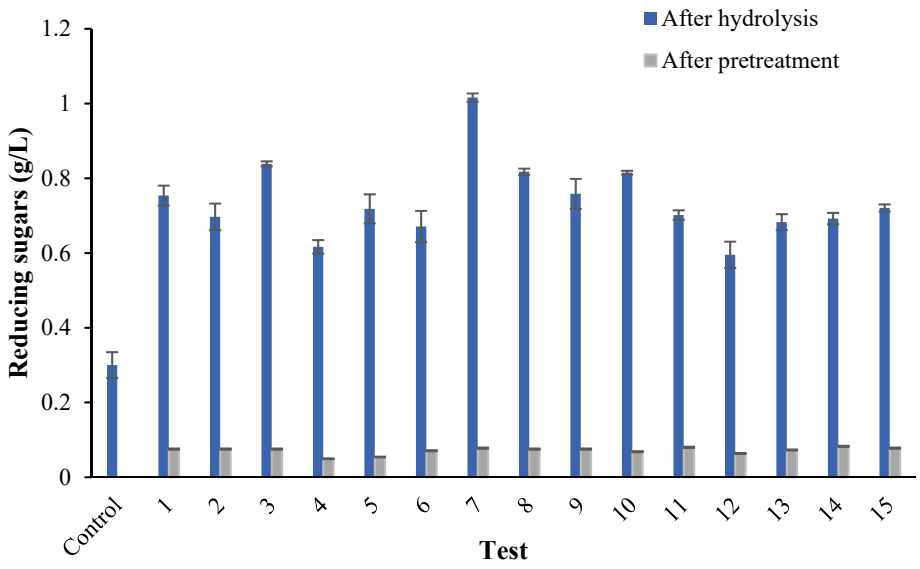

Figure 1. Reducing sugars after microwave pretreatment and after acid hydrolysis.

Figure 2 shows the influence of all studied parameters on reducing sugars. Pretreatment time and microwave power had a significant effect during pretreament by enhancing the amount of reducing sugars during hydrolysis. The highest result for the DNS value was obtained at 12.5 min and 249 W. It can be observed in Figure 2a,b that the power reached a maximum for all pretreatment times and the reducing sugars increased until at a maximum at 12.5 min. Beyond 12.5 min, the reducing sugars gradually decreased. Mikulski et at. [34] carried out microwave pretreatment of maize distillery stillage at different microwave powers and lengths of time, and observed that the use of higher power shortened the heating time but did not improve the hydrolysis efficiency. Klein et al. [35] found that the glucose yield using microwave irradiation on the pretreatment of leaves decreased beyond 8 min due to dehydration in the production of glucose. Pang et al. [36] found that lower power and shorter pretreatment times were recommended because they presented less severe conditions, energy consumption and degradation of products [28,37].

Figure 2c,d shows the influence of the concentration of the alkaline solution and the NaOH/dried algae ratio. From the obtained results, it can be mentioned that the ratio seems not to have an excessive influence on the process, showing a slight increment. The amount of reducing sugars increases with the alkaline concentration until at a maximum. Thus, the highest result was obtained with a sodium hydroxide concentration of 1% adding a dose of 10 mL of NaOH solution/g of dried algae. The literature presents different results for reducing sugars depending on the type of applied microalgae or macroalgae and pretreatment methods [35,38,39]. However, all of them show that the application of microwave irradiation or ultrasound irradiation increases the glucose yield.

With the aim of confirming that finding, the obtained results were compared with the control experiment without pretreatment where the obtained amount of reducing sugars was about 0.3 $\pm$ 0.03 g/L. This shows that a higher amount of fermentable sugars (30% more) can be obtained when the raw material is pretreated with microwave irradiation. Su et al. [40] obtained considerably higher reducing sugars yield using microwave pretreatment with sorghum liquor waste. Nowicka et al. [41] obtained a 37% g/L when using microwave heating compared to a control test without a microwave. This study confirms the usefulness of microwave pretreatment to obtain fermentable sugars. The most important parameters of this stage are the power and the length of time of the algae's exposure to microwave irradiation.

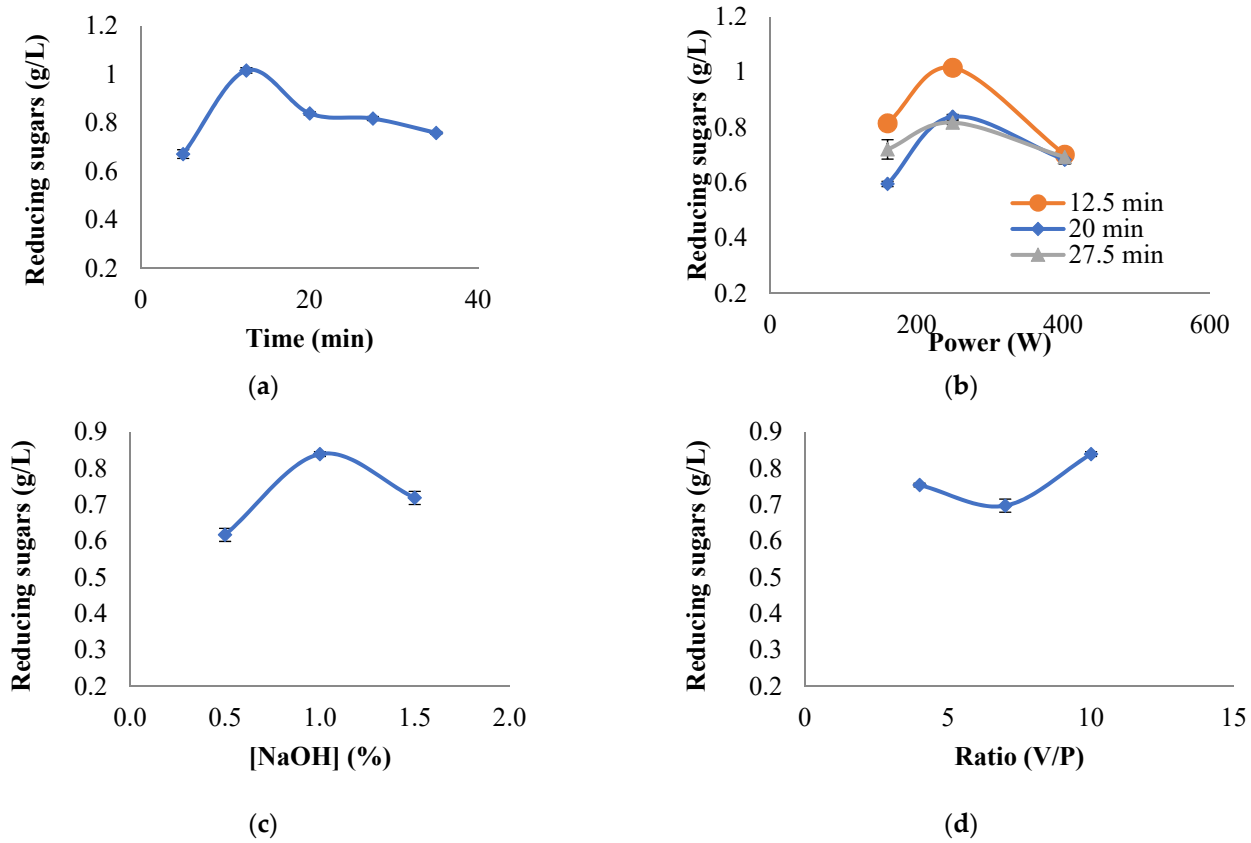

Figure 2. Influence of all studied parameters on reducing sugars: (**a**) time, (**b**) power, (**c**) concentration of NaOH, (**d**) volume NaOH/algae mass ratio.

These parameters also allow the calculation of the energy transferred to the process. Figure 3 presents the influence of energy on the amount of reducing sugars for the experiments carried out at 249 W with a sodium hydroxide concentration of 1% and ratio of 10:1 v/w. This figure shows that the amount of reducing sugars increases when the transferred energy varies from 75 to187 kJ; however, higher energy values results in sugar degradation. There are two opposite effects on these results. On the one hand, microwave irradiation can cause disintegration of the biomass structures and accelerate the breakdown of the crystalline regions, increasing the reducing sugars yield [42]. On the other hand, a higher exposure time to microwave irradiation leads to high localized overheating, resulting in sugar degradation [43].

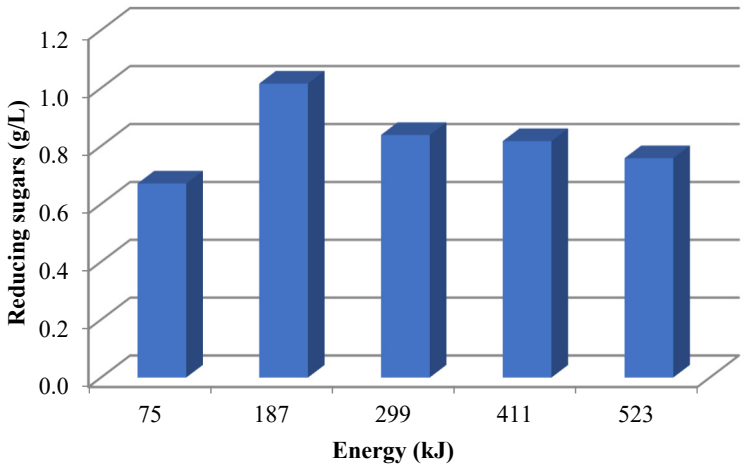

Figure 3. Influence of consumed energy on reducing sugars.

3.2. Fermentation of Sugars to Bioethanol

The fermentation and bioethanol yield were determined to analyse the effectiveness of the microwave pretreatment on the conversion of sugars to cellulosic ethanol using determined conditions. Ethanol fermentation efficiency depends on the raw material, especially on sugar content. The maximum conversion efficiency of glucose into ethanol is 51% by mass. *Saccharomyces cerevisiae*, under anaerobic conditions, uses glucose for cell growth and the synthesis of products, reducing the maximum efficiency [44]. In the present study, the fermentation efficiency, as the produced ethanol divided by the theoretical ethanol, was 40.4% fermenting 166 mL of hydrolysed liquid after microwave pretreatment for 20 h. With these conditions, the bioethanol yield was 1.93 ± 0.01 g/g of residue algae. This result was considerably higher than the unpretreated waste (0.1 ± 0.02 g/g) under the same conditions. Studies have demonstrated that the efficiency of the microwave pretreatment could increase the fermentation yield due to the increase in cellulose available for hydrolysis [45]. The aim of this research was to select the appropriate process variables for the pretreatment; thus, more research is necessary to study the variables for the intensification of the fermentation process.

3.3. Energy Consumption Calculation

In this research, the energy consumption was determined for the experiments carried out at 249 W with a sodium hydroxide concentration of 1% and ratio of 10:1 v/w. It can be observed in Table 3 that for 10 g of algae waste under the best conditions (test 7), the energy consumption was 1097.3 kJ ± 22.1 for producing 1 g of reduced sugars and 2415.6 ± 21.8 kJ for producing 1 g of bioethanol. In common with this, 1 g of ethanol can produce 27 kJ of energy; whereas 1 g of sugar can produce 16 kJ of energy. Comparing these values, it can be said that the energy input is higher than the energy output in this process. Many authors have reported that this finding is due to the energy used for the evaporation of the water during pretreatment [46,47].

Table 3. Energy consumption at different conditions.

Test	Energy Consumption (kJ/g Reduced Sugars)	Energy Consumption (kJ/g Bioethanol)
3	1755.6 ± 35.4	3864.9 ± 34.9
6	438.9 ± 8.8	966.2 ± 8.7
7	1097.3 ± 22.1	2415.6 ± 21.8
8	2414.0 ± 48.7	5314.2 ± 47.9
9	3072.3 ± 62	6763.5 ± 61.1

Microwave pretreatment has showed advantages for enhancing acid hydrolysis yields compared to methods without the microwave effect. Considering energy consumption, a low microwave power and short microwave irradiation time are favourable for this pretreatment.

4. Conclusions

This research showed the usefulness of microwave assisted alkaline pretreatment in the production of cellulosic bioethanol from algae waste using some determined conditions in the process. Moreover, low microwave power and short microwave irradiation time can be favourable for this pretreatment considering energy consumption. Furthermore, this study showed that cellulosic bioethanol production from algae waste could be a promising alternative. The influence of microwave power, irradiating time, solid–liquid ratio and NaOH concentration on the value of reducing sugars after alkaline hydrolysis was investigated. The results revealed that the best conditions were after 12.5 min, 249 W, 1% of sodium hydroxide and a dose of 10 mL of NaOH solution/g of dried algae in order to achieve 1.93 ± 0.01 g of bioethanol/g of residue algae. A prospective research is necessary to study the optimum conditions of the fermentation process, and whether the

use of ultrasound irradiation is recommended to continue with the fermentation process optimization and even for the pretreatment.

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