# Assessment of macroalgal biomass potential in Tunisia for bioethanol production

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# Abstract

The valorization of marine macroalgal biomass is among the most promising international programs focusing on the sustainable exploitation of marine environment. However, macro-algal biomass remains unexploited in Tunisia even though it represents a renewable and highly abundant biomass. This causes several environmental disorders due to the excessive proliferation of several algal species.

The objective of this work is to study the potential use of macroalgae as biofuels feedstock in the Mediterranean region and particularly in Tunisia. This study focuses on certain macroalgal species collected from Bizerte and Tunis lagoons. It reports the biochemical characterization of the studied macroalgae in order to determine their possible valorization areas and demonstrate their richness in high value molecules. In fact, our experiments show that the maximum protein content of 16.22%±0.270 was obtained with *G. verrucosa* and the maximum carbohydrates content of 58.46%±1.52 was obtained with *Dictyopteris polypodoies*. This leads us to believe that macroalgae constitute a potential source of fiber and proteins. After a sulfuric acid pretreatment, the macroalgae's solid fractions were hydrolyzed using cellic Ctec2 enzymatic extract leading to a maximum reducing sugars yield of 324.6 mg/gDM. Monomeric sugars from the studied macroalgae hydrolyzates were fermented, leading to maximum ethanol yield of 0.24g/gDM.


# 1. Introduction

The rising world population, the increasing massive energy consumption, the decrease of oil resources and the atmospheric pollution by toxic gases from fossil resources combustion are the main driving factors for developing renewable energies [1, 2, 3, 4, 5]. In this context, biofuels as biodiesel and bioethanol are considered as potential alternative biofuel sources due to their renewable nature, sustainability and low carbon emissions [6, 7]. However, first and second generations bioenergy feedstocks compete with other crops for land and water. They require large agricultural land and water reserves for their cultivation which limits their sustainability [8, 9]. Hence, to overcome those feedstocks' limitations, fast-growing and renewable biomass sources such as marine algae, have attracted attention as an alternative for fuels [10]. In fact, algal biomass and particularly macroalgae could be a promising source for third generation biofuels and additional high added value products such as proteins, vitamins, or trace elements. Macroalgae are rich in carbohydrates, which compose up to 76% of macroalgae's dry weight [3]. This high carbohydrate content is critical for conversion into bio-based products. Some carbohydrate components are common among macroalgae and terrestrial crops. Other carbohydrate components are made up of monosaccharides found only in specific macroalgae species [3]. Moreover, macroalgae have a wide spectrum of bioactive compounds (e.g. vitamins, minerals, pigments, proteins, lipids and polyphenols), making them very attractive for feedstock, fermentation and biorefinery in general that can be used in several fields like food, cosmetics or pharmacy industries [11]. Besides, some Macroalgae can produce energy with higher efficiency compared to traditional biofuels due to their high biomass productivity and important prolific growth in fouled beaches and coastal waterways [12].

Macroalgae are classified into three types based on pigment synthesis, namely, Chlorophyceae (green algae: 1200 species), Phaeophyceae (Brown algae: 1800 species) and Rhodophyceae (Red algae: 6000 species) [13, 14].

The main green algae pigments are chlorophylls a and b [15]. The main constituents of green macroalgae's carbohydrates are sulfated and/or carboxylated polysaccharides, glucans and floridean starch (e.g amylopectin) [16]. They contain between 14% and 40% carbohydrates dry weight [15]. According to their uronic-acid content richness, two major green algae categories are distinguished. A group of limited uronic-acid, mainly composed of sulfated galactans, arabinopyranans and mannans and a group of rich uronic-acid mainly composed of ulvans [17].

Red macroalgae have r-phycoerythrin as pigment and are mainly composed of sulfated galactans (e.g. carrageenan, agar and porphyrin), storage carbohydrates (e.g. α-1,4-glucan and floridean starch) and structural polysaccharides (e.g. cellulose, mannans and xylans) [17].

Brown macroalgae is distinguished by the predominance of xanthophyll pigments [18]. They are composed of polysaccharides such as laminarin, mannitol, cellulose, alginate and fucoidan [19].

The use of macroalgae biomass is an interesting sustainable energy and presents several advantages including high biomass production and high photosynthetic efficiency compared to terrestrial crops [20]. Macroalgal biomass is easily cultivated since it doesn't require agricultural additives such as fertilizers and pesticides, has low cost of collection without environmental damage [21] and does not require arable land or freshwater resources [22]. Its chemical composition characterized by the absence or the very low lignin content and the high carbohydrates level which makes it suitable for bioethanol production [23, 24].

Bioethanol production from macroalgae involves various pretreatment steps followed by an enzymatic hydrolysis to get the monomeric sugars and a microbial fermentation process by yeast or bacteria [25, 26].

Several fermentation methods may be used to transform reducing sugar produced from macroalgae into bioethanol. The processes are denoted as follows: (i) separate hydrolysis and fermentation (SHF); (ii) simultaneous saccharification and fermentation (SSF) and (iii) simultaneous saccharification and co-fermentation (SSCF) [27].

Various treatments have been used during hydrolysis of seaweed for bioethanol production. These treatments include dilute acid thermal [28, 29], dilute alkaline thermal [30], enzymatic [31, 32], thermal [33, 34], ball milling [35], hydrothermal (HTT) [35] and ultrasound [36].

However, the dilute acid method is the most widely used treatment in seaweed bioethanol due to its relatively low cost, ease of use [37] and high efficiency [38]. Indeed, chemical properties have an important role in the hydrolysis of seaweed's polysaccharides. The high solubilization of seaweed and the subsequent high concentrations of reducing sugars have been achieved as a result of using different chemicals and thermochemical methods [39, 40, 41]. The chemical pretreatment hydrolyzes cellulose,

hemicellulose and other storage carbohydrates (e.g. laminarin) [40, 42]. However, this pretreatment causes sugar degradation leading to toxic compounds formation that inhibit the fermentation step. Moreover, the dilute acid pretreatment wastes constitute a significant environmental problem [38].

Tunisia is committed to international conventions to limit pollution and increase the share of renewable energies in its energy mix. However, the promising potential of energy production from algal biomass available in Tunisia is still less valued than other algal or lignocellulosic biomasses at industrial scale due to the lack of collection and absence of optimal recovery processes. Indeed, according to Shili et al (2002), The macroalgae communities observed in the south lagoon of Tunis are characterized by the predominance of nitrophilous algae considered as important biomass: Ulva, Cladophora and Enteromorpha [43].

In this context, we propose a new sustainable source of bioenergy, and we suggest a solution for the macroalgae proliferation. We performed a comparative study of several macroalgae issued from Tunisian lagoons (Mediterranean region) to determine their potential in bioenergy production. The macroalgae's biochemical composition was determined to maximize its valorization. In order to achieve this objective, a three steps process were performed. First, the macroalgal biomass was pretreated by dilute sulfuric acid. Then, an enzymatic saccharification was performed to produce fermentable sugars. Finally, the obtained sugars were converted into bioethanol using *Saccharomyces cerevisiae* yeast.

# 2. Material And Methods

# 2.1. Macroalgae

Different species of macroalgae were collected from the north of Tunisia. *Chaetomorpha linum*, *Enteromorpha intestinalis* and *Gracillaria gracillis* were collected in April 2019 from the lagoon of Tunis (GPS: 36°48'56.3"**N**, 10°12'52.9"**E**,), *Ulva rigida* in January 2020 and *Gracillaria verrucosa* in March 2020 from lagoon of Bizert and *Cystoseira brachycarpa*, *Laurencia obtusa*, C*ystoseira sedoides* and *Dictyopteris polypodioides* in June 2020 from lagoon of Bizert (GPS: 37° 11' 48" N, 9° 51' 23). Samples were washed to dilute the salts concentration and eliminate sand and other impurities collected with the seaweed when removed from the lagoon. Then, they were dried at ambient temperature under the sun for two weeks to remove water that speeds up algae decay and ensure therefore long-term storage of the biomass. Once dried, algae were finally ground with a blender to obtain 15mm length and stored until use.

Cellic CTec2 enzyme (0.43 pNPG U/mL, 61.25 CMC U/ml) from Novozymes (Denmark) was used for the algal biomass enzymatic hydrolysis. The endoglucanase activity is standardized based on its activity on carboxymethylcellulose (CMC). A CMC activity unit liberates 1 µmol of reducing sugars in 1 min under specific assay conditions of 50°C and pH 5. The β-glucosidase activity is standardized on the basis of *p*NPglucoside hydrolysis. One *p*NPG unit denotes 1 µmole of Nitrophenol liberated from para-nitrophenyl-β-D-glucopyranoside per minute at 55° C and pH 5.

A strain of yeast *Saccharomyces cerevisiae* was selected as a model strain for fermentation of simple sugars. Fermentation was achieved using fresh commercial baker's yeast *S. cerevisiae* (Tunisian Society of yeasts) purchased from local market) [44].

## 2.2. Biomass productivity determination

In order to estimate the algal biomass productivity, the quadra sampling method was performed. Three quadrats of 50x50 centimeters were taken at random in Tunis lagoon with maximum of 1m of depth. The macroalgae samples were collected, dried in the air and then weighed.

Diluted Acid pretreatment of macroalgae

10g of the biomasses were suspended in 200 ml of sulfuric acid $H_2SO_4$ (1%) for 40 min at 121 ° C for 30 min. The pretreated macroalgae biomasses were filtered and the solid residue washed three times by distilled water. Afterwards, the residue was dried at room temperature and used as substrate for enzymatic saccharification [45].

## 2.3. Enzymatic saccharification

After algal biomass pretreatment with diluted acid, enzymatic saccharification was performed for 48 h at 55°C and pH5 adjusted with 5 mM sodium acetate buffer in presence of a Cellic CTec2 enzymatic preparation (1 mg of enzyme/g dry substrate of macroalgae) as developed in previous work [46]. These experiments were replicated three times. The estimation of total reducing sugar in the biomass's enzymatic hydrolysate was done by the DNS method [47]. The reducing sugars yield was determined according to the following Eq. 1:

$$\text{Reducing sugars yield (\%)} = \frac{Reducingsugars\,(g)}{Pretreatedbiomss\,(g)} * 100$$

## 2.4. Yeast cultivation

*Saccharomyces cerevisiae* was used as an ethanol fermentation strain. The yeast strain was maintained on agar plates made from 5 g/L yeast extract, 5 g/L of peptone, 20 g/L of *D*-glucose and 20 g/L of agar. Inoculation flasks were prepared by autoclaving 100 mL of 50 g/L glucose, 1 g/L of $KH_2PO_4$,1 g/L of $MgSO_4$ $7H_2O$, 5 g/L of peptone and 5 g/L of yeast extract. The medium was incubated for 24 h at 30°C and shaken at 150 rpm prior to use.

## 2.5. Fermentation

Batch fermentation experiments have been carried out in 100 mL flasks under anaerobic conditions with working volumes of 20 mL. The fermentation medium had a pH of 5.5. The solid fraction hydrolysates (15 mL) resulting from macroalgae pretreatment was used as substrates with 2 mL of YPX10 (200 g/L of yeast extract and 400 g/L of peptone) and 2 mL of the yeast suspension. The fermentation medium was incubated for 24 h at 37° C with shaking (100 rpm) [48]. These experiments were replicated three times.

## 2.6. Analytic methods

The estimation of total reducing sugar in the enzymatic hydrolysate of biomass was done by DNS method [41]. Dry matter and ash content were determined according to the AOAC standard 950.46 and 920.153 respectively, by drying the macroalgae biomass at 105°C (24 h) followed by incineration at 550°C (5 h) [49]. Total fiber contents were determined according to the analytical method described by Van Soest that gives not only an estimation of total cell wall content in the biomass (NDF, Neutral detergent fiber), but also its main constituents of (ADF: acid detergent fiber or lignocellulose fraction and Acid detergent lignin: lignin fraction) [50, 51]. The lipid contents were determined using the protocol described by Folch et al (1957) [52]. Kjeldahl nitrogen (TKN) was carried out using the normalized Kjeldahl method 928.08 [49]. The conversion factor 6.25 was used to estimate the crude protein according to the following Eq. 2.

$$\%P = (V \times N \times 14 \times 6,25 \times 100)/m$$

With:

- V: volume (ml) of hydrochloric acid (0.1N) used for titration.
- 6. 25: Kjeldahl factor.
- N: normality of hydrochloric acid.
- m: mass (mg) of sample.

An Agilent gas chromatograph was used with a capillary column type (CP-Wax-57, 50 m × 0.32 mm chrompack). Oven initial temperature was 50 ° C and raised after injection to 180 ° C at a rate of 10 ° C/min. The total analysis time was around 13 min. The carrier gas (He) had a flow rate of 1 mL/min. The injector's temperature was 220 ° C and that of the flame ionization detector was 280°C. The ethanol yield was determined according to the following Eq. 3:

$$\text{Ethanol yield (\%)} = \frac{Producedethanol(g)}{Pretreatedbiomss(g)} * 100$$

# 3. Results And Discussion

# 3.1. Biochemical composition

The results of the organic, mineral, lipid, fiber and protein contents of macroalgae species collected from Tunisian lagoons (Table 1).

Carbohydrate content was determined according to the Van Soest and Wine method. This method is based on the successive extraction of the macroalgae's main constituents by detergents. After each extraction, the product retained on the filter was dried and weighed.

Macroalgae with high carbohydrate contents are promising candidates for bioethanol production as shown in Table 1, such as: *D. polpodoides* (58.46 ± 1.52%), *C. brachycarpa* (42.94 ± 0.28%), *C. linum* (41.21 ± 2%), *E. intestinalis*, *U. rigida*, *C. sedoides* and *L. obtusa* (up to 40%). Macroalgae carbohydrate

contents vary widely among species and cultivar. The present results agree with previous studies on various locations where macroalgae demonstrated great potential as fibers source [53]. In fact, *Laminaria Japonica, Entermorpha intestinalis* and *Palmaria palmata* contain 54.5%, 42.8% and 39.4% of carbohydrate respectively [54, 55, 56]. Therefore, these biomasses could be considered as adequate substrates for ethanol production.

The crude protein (CP) content of macroalgae was determined using the “kjeldahl method” by measuring the nitrogen they contain. According to the results enumerated in Table 1, the macroalgae species collected from Tunisian lagoons show considerably high crude protein contents (> 9% relative to the dry matter) [57]. Indeed, for green macroalgae, the crude protein contents were 19.03%, 9.84% and 11.28 ± 0.3%, for *E. intestinalis, C. linum*, and *U. rigida* respectively. Protein contents of 16.22 ± 0.27%, 10.17% and 9.19 ± 0.05% were observed in red macroalgae *G. verrucosa*, *L. obtusa* and *G. gracillis* biomasses respectively. For Brown macroalgae, the protein contents range from 3 to 15% for *C. brachycarpa* (5.35 ± 1.06%) *and C. sedoides* (3.55 ± 1.09%). According to the literature, the protein fraction in brown macroalgae is low (from 3–15% relative to the dry matter) when compared to the green and red macroalgae (from 10–47%) which confirms our obtained results [58]. It is reported that the protein content of marine macroalgae varies significantly between species and depends on seasons and environmental conditions [59]. These contents can sometimes exceed those of fabaceae which can make it an interesting source for human and animal food [57, 60].

The lipid contents of the studied macroalgae species are lower than 2% relative to the dry matter. This agrees with the results of Ivanova et al. (2013) asserting that the lipid content in macroalgae varies between 1% and 5% [61]. It was reported that macroalgae have low lipid contents, but their polyunsaturated fatty acids can be as high as those of terrestrial plants, which explains their dietary and even pharmacological benefits [62]. It is important to note that macroalgae’s chemical composition presents a great variability related to several abiotic factors, mainly environmental ones such as salinity, water, temperature, light, and seasonal variation of nutrients [53]. Commonly, these ecological parameters fluctuate with reference to the locality and the seasonal effects. Additionally, the tidal periods can also indirectly affect the macroalgal biomass availability and biochemical composition [53].

Finally, the obtained results demonstrate that the studied Macroalgae from Tunisia having high carbohydrates and proteins contents, are promising candidates for bioethanol production and animal feeds industries.

Table 1
Biochemical composition of studied macroalgae (%) collected from Tunisian saline environment.

| Macroalgae | Classification | Humidity content (%) | lipid content (%) | Protein content (%) | carbohydrates content (%) |
|---|---|---|---|---|---|
| *C. linum* | Green | 82.18 ± 0.84 | 0.59 ± 0.02 | 9.84 ± 0 | 41.21 ± 2 |
| *E. intestinalis* | Green | 81.42 ± 0.56 | 0.1 ± 0.01 | 19 ± 0.62 | 39.9 ± 0.8 |
| *G. gracillis* | Red | 90.44 ± 0.26 | 0.21 ± 0.01 | 9.19 ± 0.59 | 32.06 ± 0.47 |
| *U. rigida* | Green | 85.2 ± 0.34 | 0.35 ± 0.02 | 11.29 ± 0.03 | 38.57 ± 0.17 |
| *G. verrucosa* | Red | 92.2 ± 0.32 | 0.35 ± 0.03 | 16.22 ± 0.27 | 25.56 ± 0.2 |
| *D. polypodoides* | Brown | 92.38 ± 0.16 | 0.37 ± 0.04 | 4.43 ± 0.64 | 58.46 ± 1.52 |
| *L. obtuse* | Red | 96.4 ± 0.2 | 0.27 ± 0.09 | 10.17 ± 0.0 | 37.24 ± 3.38 |
| *C. sedoides* | Brown | 85.2 ± 0.34 | 0.25 ± 0.15 | 3.55 ± 1.09 | 38.88 ± 1.26 |
| *C. brachycarpa* | Brown | 93.05 ± 0.07 | 0.34 ± 0.05 | 5.34 ± 1.06 | 42.94 ± 0.28 |

# 3.2. Bioethanol production from Tunisian macroalgae biomass

## 3.2.1. Enzymatic saccharification of pretreated macroalgae biomass

The solid residue obtained after dilute acid pretreatment was utilized as a substrate for the enzymatic saccharification in order to recover fermentable sugars. Enzymatic hydrolysis was carried out under mild conditions (55°C and pH 5) for 48 h using a commercial enzymatic preparation (Cellic C-Tec2). Figure 1 shows reducing sugars yields obtained after an enzymatic saccharification of macoralgae collected from Tunisia northern lagoon and pretreated by dilute acid.

The results in Fig. 1 show that maximum reducing sugars yields obtained after an enzymatic hydrolysis were 325 ± 0.2 mg/g and 280.8 ± 0.35 mg/g dry biomass from *C. linum and E. intestinalis* respectively. We obtained a reducing sugars yield of 170.9 ± 0.02 mg/g after an enzymatic hydrolysis of pretreated *Ulva rigida*. These results were compared with the results described by korzen et al (2015). In fact, the authors obtained 196. ±2.5 mg/g db after hydrolysis catalyzed by Amylo-glucosidase, alpha-amylase and cellulase enzymes of *U. rigida* biomass pretreated by milling and sonication (40kHz frequency and power 120 W) [63].

In previous work, enzymatic hydrolysis of *U. lactuca* biomass pretreated by liquid hot water or Air-drying, size reduction using centrifugal and vibratory ball mill gave respectively 97.5 mg/g db and 131 mg/g of

reducing sugars yields [64, 65]. Moreover, a yield of 345mg/g was obtained following an enzymatic hydrolysis with CMCase enzyme of 3% NaOH pretreated *Ulva sp* [66]. For red macroalgae, our results demonstrate that maximum reducing sugars yields were obtained with *Gracillaria gracillis* (277.2 ± 0.06 mg/g) and *Gracillaria verrucosa* (175.2 ± 0.05 mg/g) respectively. Other studies found lower sugars yields for the same kind of Gracillaria, as Saravanan et al (2018) that observed 140.6 mg/g of yield following an enzymatic hydrolysis using Cellulase and beta-glucosidase of *Gracillaria sp* biomass pretreated by 4% of $H_2SO_4$ [67].

When using brown macroalgae as raw material, the maximum sugars yield was obtained with *C. Sedoides* biomass at 175 ± 0.12 mg/g. According to data reported by literature, Borines et al (2013) found 120 mg/g yield with *Sargassum sp* pretreated by sulfuric acid and hydrolyzed by cellulase and cellobiase [68]. In addition, yields of 127 ± 0.05 mg/g and 44 ± 0.05 mg/g were obtained with *Ascophyllum nodosum* biomass pretreated by microwave assisted acid hydrolysis or microwave assisted thermo chemical treatment [69]. In the present work, reducing sugars yields after pretreatment followed by saccharification are comparable and even higher than yields reported in literature. This can be explained on the one hand by the efficiency of the applied chemical pretreatment with sulfuric acid and on the other hand by the richness of our macroalgae in carbohydrates as depicted in Table 1. In fact, the chemical pretreatment method with sulfuric acid is widely accepted and involves various steps such as cellulose de-polymerization, hemicellulose-solvation and structural modification using mild alkali or dilute-acid treatment [56]. Dilute-acid pretreatment demonstrated its efficiency and is mainly used in several macroalgae biomass pretreatment [56]. When followed by enzymatic hydrolysis, it seems to be economically viable method for bioethanol production. Therefore, through dilute-acid treatment, the holocellulose content can be broken down into monomeric sugars (e.g., glucose and xylose, etc.) while further saccharification efficiency can be increased using enzymatic hydrolysis.

# 3.2.2. Ethanol production from Tunisian macroalgae

The hydrolysate obtained from the enzymatic hydrolyze of dilute acid pretreated macroalgae collected from Tunisian lagoons were fermented with *Saccharomyces cerevisae* under anaerobic conditions. Figure 2 shows the obtained ethanol yields for several species of Tunisian macroalgae after a fermentation.

The maximum obtained ethanol yield for green macroalgae was 0.21g/gdw with *C. linum*. The alcoholic fermentation of *Gracillaria gracillis* gave a 0.12g/gdw. In order to avoid a sorting step for the macroalgae collected from Tunis Lagoon, a mixture was considered and used as a substrate for ethanol production. This macroalgae mixture (*C.linum* + *E. intestinalis* + *G. gracillis*) was pretreated with sulfuric acid, hydrolyzed by a commercial enzyme and converted by *Saccharomyces cerevisiae* into ethanol with a yield of 0.23%. Thus, it constitutes an advantage for the bioenergy industry since it reduces the process's cost by avoiding an expensive sorting step and by improving of the ethanol yield and therefore the biomass productivity. For *D. polypodoides, C. brachycarpa* and *C. sedoides*, the ethanol yields were reached to 0.24g/g db, 0.21g/g db and 0.19g/g db respectively.

The ethanol yields obtained in this study are comparable to previous results for first-generation biomasses which were respectively 0.25 g/g and 0.21 g /g from sorghum [70] and from sweet potato [71].

For the second-generation biomasses, the ethanol yields obtained were 0.3g/g for wheat straw and 0.3g/g for corn stover [72]. Our results are in accordance with others reported for example by Borines et al (2013). In fact, the authors obtained 0.13g/g of ethanol yield for *Sargassum sp* collected from the coastal region of Bolinao [53]. The alcoholic fermentation of *Chaetomorpha linum* that were collected from coastal region of Monastir, Tunisia, resulted in a 0.28g/g of ethanol yield [58] and was even higher than the obtained ethanol yields for *U. lactuca* (0.06g/g), *U. rigida* (0.12g/g) and *Padina tetrastromatica* (0.16g/g) using S*accharomyces cerevisiae* as fermenter microorganism [65, 73, 74].

The results show that macroalgae collected from Tunisian lagoons constitute a potential feedstock for bioenergy. This macroalgal biomass is abundant in nature and can compete favorably with other classical biomasses for biofuel production. Therefore, we believe it can be considered as a promising substrate for ethanol production.

### 3.3 Feasibility assessment of bioethanol production from marine macroalgal biomass: case study: macroalgae collected from Tunis lagoon, Tunisia.

The bioethanol production process proposed in this work, was experimented on Tunisian macroalgae in order to demonstrate its feasibility and potential. We used a macroalgae mix collected from Tunis lagoon and it composed of *G. gracillis*, *C. linum* and *E. intestinalis*. In order to achieve this objective, three steps of process were performed. First, the macroalgal biomass was treated by dilute sulfuric acid. Then, an enzymatic saccharification was performed by a commercial enzyme (Cellic C-tech2) at 50°C in order to produce fermentable sugars. Finally, the latter was converted into bioethanol using *S. cerevisiae* yeast at 37°C (Fig. 3).

The obtained results show that the estimated ethanol yield was 0.23g/g of dry matter macroalgae. The lagoon can have a maximum biomass productivity of 344 ± 48.5 g of dry matter per $m^2$ in the period from April to September. Since the total area of the lagoon is 4000 hectares, the projected estimate is 13760 Ton of dry matter for the entire lake. The estimated maximum productivity of bioethanol therefore 791.2 Kg per hectare per year.

Tunisia has several coastal regions and lagoons that can be exploited to collect larger macroalgae quantities for conversion into bioethanol. This may amplify yields and to demonstrate the project's feasibility in the Mediterranean regions.

## Conclusion

To combat the present environmental concerns and increasing fuel prices, the macroalgal biomass has shown a great potential as feedstock for biofuel production. In this study, we proposed a valorization perspective of the abundant and unexploited macroalgal biomass into third generation bioethanol. The

study focuses on macroalgae collected from Tunisian lagoons in the Mediterranean region (i.e. Bizert lagoon and north lagoon of Tunis).

The results revealed that the studied marine macroalgae, particularly the carbohydrates-rich ones, from Tunisian lagoons constitute an interesting biomass for bioenergy and high added value molecules. In our experiments, the maximum ethanol yield of 0.24g/gDM, obtained with *D. polypodoides*, demonstrates the value of our proposal leading to more sustainable bio energies without the compromises of first and second generations biomasses.

Improving the conversion and the valorization processes will be vital in establishing this emerging source of bioenergy for commercial utilization. The optimization of some steps in the proposed integrated process in addition to a complete techno-economical study are therefore necessary to scale it up. If these technologies are further optimized, the production of bioethanol and other value-added molecules from macroalgae could lead to a new sustainable industry in the near future.

# Declarations

### Statement of Novelty

Our work makes a contribution in the field of renewable energy and in marine biomass valorization. The novelty resides in highlighting the potential of an abundant, forsaken and unexploited renewable natural resources, namely macroalgae, as an alternative for bioethanol production and other value-added products in Tunisia and the Mediterranean region. The work evaluates the energetic potential of several Mediterranean macroalgae by studying their biochemical compositions and bioethanol yields. The obtained results are encouraging and may lead to a new industry in Tunisia focusing on renewable energy and biomass valorization.

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### Ethical Approval

The present article does not contain any studies with human participants or animals performed by any of the authors. Therefore, no formal consent is required.

### Consent to Participate

All authors have given consent to participate.

### Consent to Publish

All authors have given approval of the manuscript to be published.

### Competing Interests

The authors declare that there are no conflicts of interest.


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### Declaration of competing interest

The authors declare that there is no conflict of interest and give their informed consent. No financial or other interests influenced the outcome of the research.

### Authors' contributions

Conception and design of study, analysis and or interpretation of date and Drafting the manuscript were performed by N. Smichi.

Y Messaoudi participated in manuscript drafting

N Moujahed C. Messaoud and M. Gargouri participated in Analysis and/or interpretation of data.

Revising the manuscript critically for important intellectual content was performed by N. Moujahed, C. Messaoud, M. Bezzarga, H. Langar

Approval of the version of the manuscript to be published was performed by all the authors.

### Availability of date and materials

The authors confirm that all data underlying the findings are fully available without restriction. All relevant data are within the paper.

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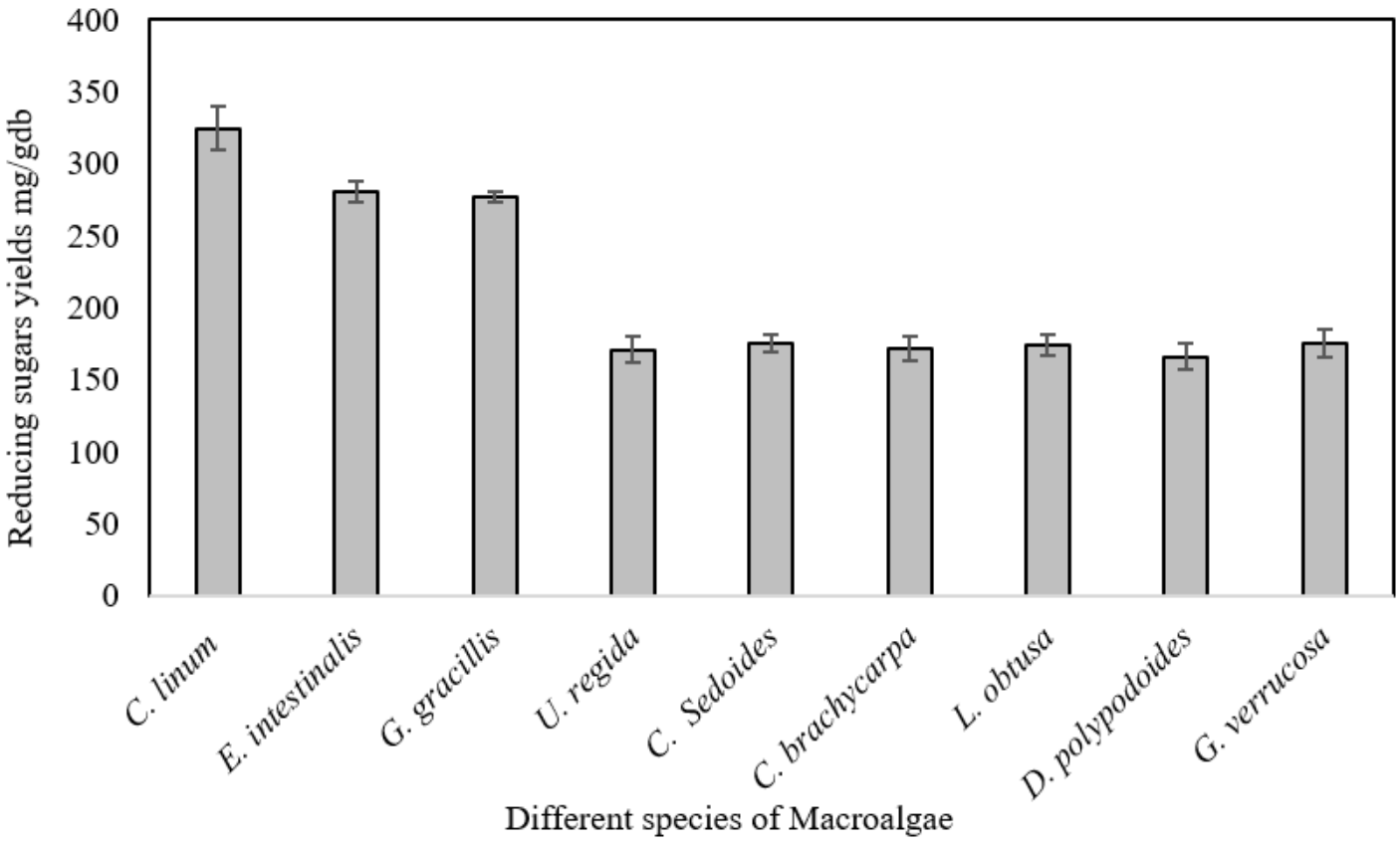
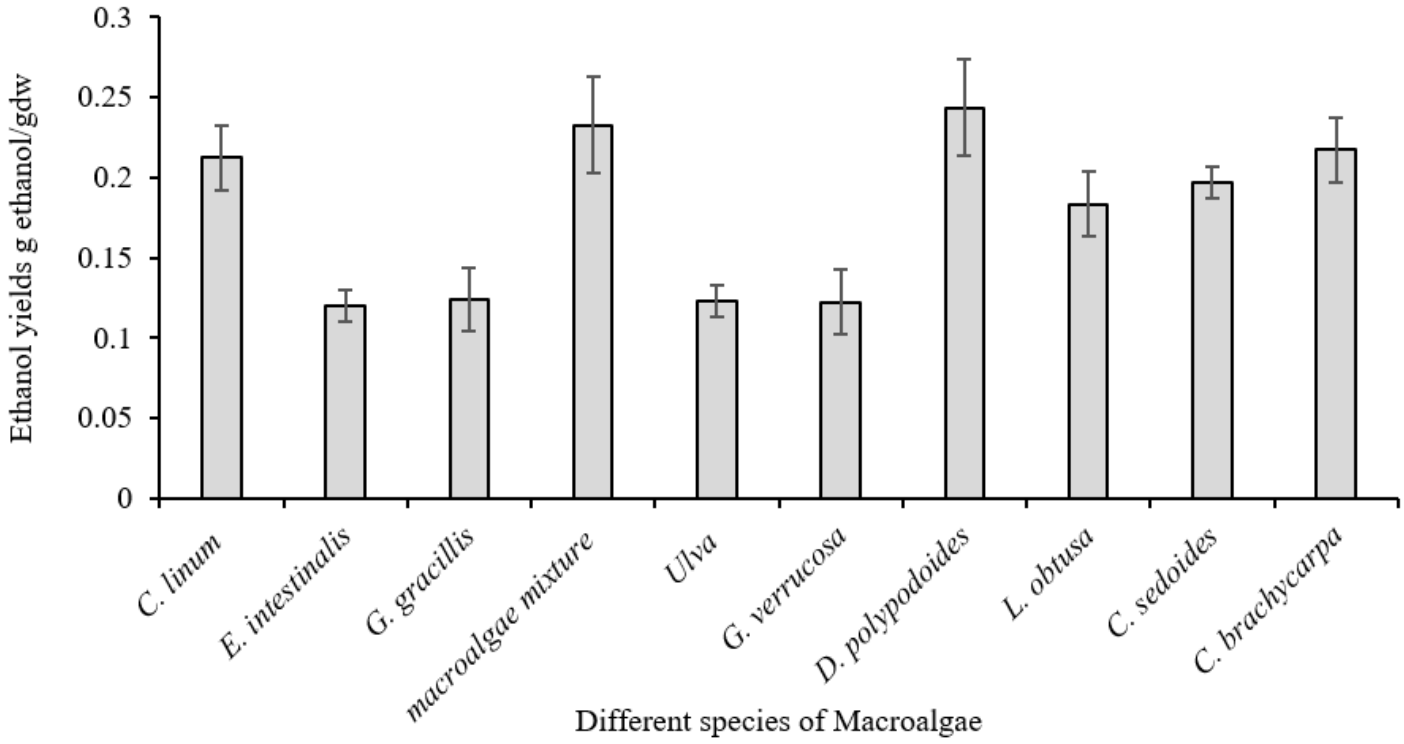
# Figures



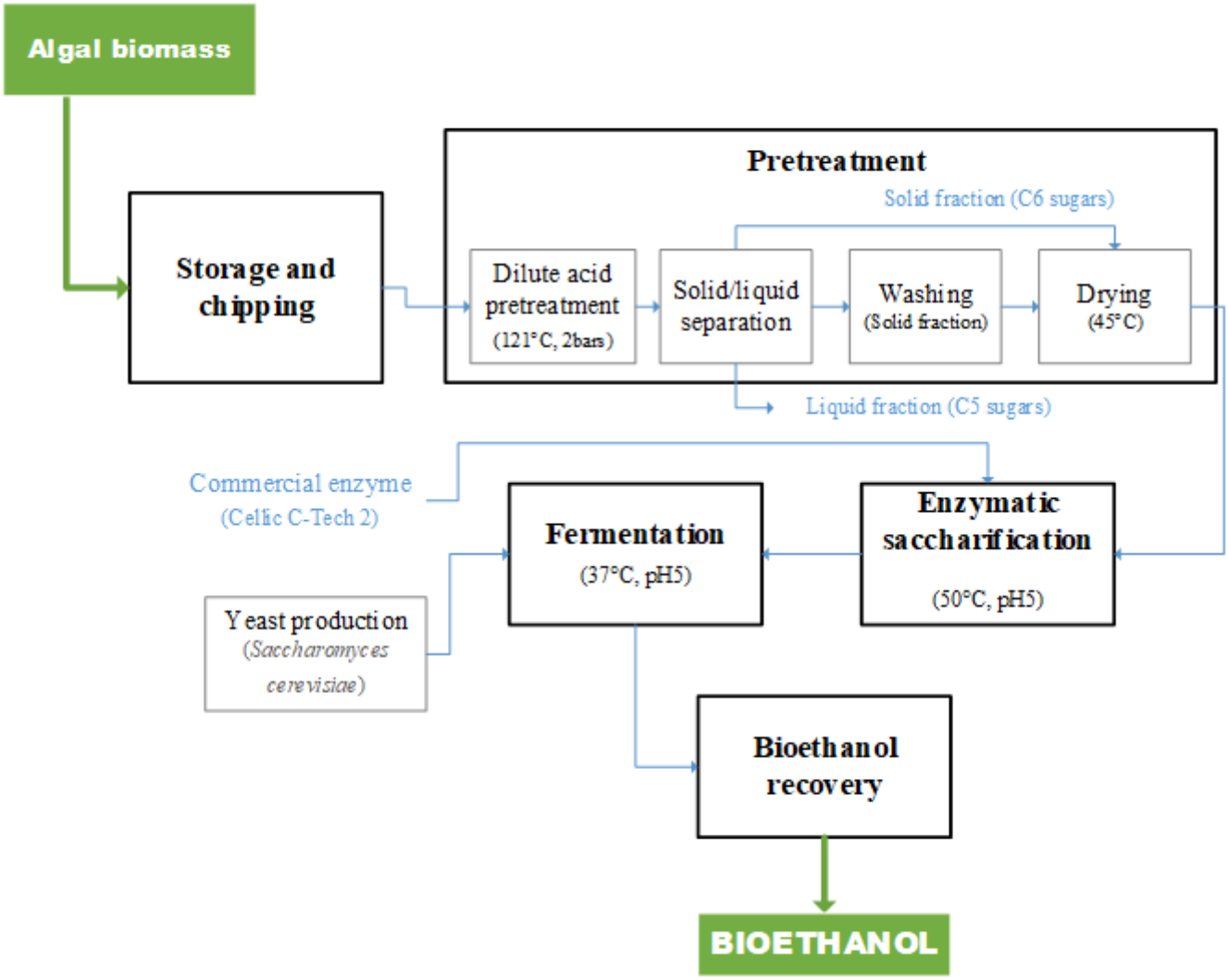
## Figure 1

Reducing sugars yields of diluted- acid pretreated macroalgae saccharification catalyzed with Cellic CTec2 enzyme at 55°C for 48h.




## Figure 2

Maximum ethanol yields from treated Tunisian macroalgae hydrolysates by *Saccharomyces cerevisiae*




## Figure 3

Block diagram of bioethanol process production from marine macroalgal biomass: case of Tunis Lake macroalgae