



Exploring macroalgae biorefinery: Extraction of bioactive compounds and production of volatile fatty acids

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ABSTRACT

Macroalgae have gained significant attention in recent research owing to their potential as novel food source and their noteworthy nutritional properties. However, a substantial amount of these macroalgae accumulates along the coast without being utilized, highlighting the need for proper treatment and disposal methods to mitigate secondary pollution effects. Previous studies on macroalgae have primarily focused on extracting bioactive compounds or anaerobic digestion processes to produce methane or volatile fatty acids (VFA), with observed improvements following different pre-treatments. In this study, three biorefinery options for macroalgae have been compared. Additionally, the extraction of bioactive compounds followed by VFA production is proposed as a promising new valorization strategy. Milled macroalgae exhibited a low methane production yield (138 ± 17 NmL CH₄-g volatile solid⁻¹), corresponding to 31 ± 4 % biodegradability, while the acidification percentage was higher (45 ± 1 %). Among the three solvents applied (water, ethanol and acetone), ethanol (80%) at 25 °C was the most effective in recovering bioactive compounds, such as chlorophylls, sugars, and phenolic compounds with antioxidant activity. The extraction of chlorophylls and phenolic compounds was not influenced by particle size reduction. However, a more efficient extraction of sugars was observed with lower particle size. Moreover, ethanol treatment demonstrated the good efficiency in VFA production, reaching up to 3.6 ± 0.2 g VFA-(chemical oxygen demand, COD)-L⁻¹, with a VFA spectrum (in COD basis) consisting of 51% acetic acid, 29% propionic acid, 5% i-butyric acid, 7% butyric acid, and 7% i-valeric acid. These findings highlight the potential of ethanol for efficient compound recovery and VFA production from macroalgae.

1. Introduction

Macroalgae are organisms that can be found in all aquatic habitats, including oceans, rivers, ponds, and wastewater, although they can also be cultivated in large areas along the coast (Sultana et al., 2023). These organisms use the natural nutrients present in seawater for their growth, and their primary metabolites consist of lipids, proteins, carbohydrates, and water. Additionally, macroalgae contain chlorophylls, cytochromes, nucleotides, and other compounds involved in various metabolic reactions (Xu et al., 2023). Due to their unique biochemical composition, macroalgae are suitable for producing a wide range of compounds, including carotenoids, phenolic compounds, and vitamins (Babich et al., 2022; Pardilhó et al., 2022). The content of primary metabolites varies depending on the type of macroalgae and the conditions in which they are cultivated. Macroalgae also produce secondary metabolites, which

are specific to one or more species and are not involved in primary metabolism. These secondary metabolites include agar, alginate, fucoïdan, ulvan, laminarin, starch, cellulose, pectin, β-D-mannuronic, α-L-guluronic substances, and carrageenan (Babich et al., 2022; Xu et al., 2023). These metabolites possess biological activity and have antiviral, antitumor, anti-inflammatory, plant growth-stimulating, and antioxidant properties (Babich et al., 2022).

Despite their benefits, uncontrolled macroalgae growth can have negative environmental and ecological impacts on coastal areas and affect fishing and shellfish industries. Nevertheless, the utilization of kelp biomass holds immense promise as a valuable resource for biorefinery operations, particularly in the production of biofuels and chemicals through biological methods, such as anaerobic digestion and ethanol fermentation (Casoni et al., 2020; Pardilhó et al., 2023; Sharmila et al., 2022).

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To recover high added value components from macroalgae, a previous stage is required to break the physical and chemical barriers of its complex structural framework. With a previous pre-treatment, the accessibility to these compounds is increased (Pardilhó et al., 2022). Yet, macroalgae pre-treatment involves several challenges, such as enhancing economic viability, ensuring environmental compatibility, reducing extraction time, addressing scalability issues, improving process robustness, and, of course, preserving the biological activity of the compounds (Narayanan, 2024; Sadeghi et al., 2024; Ummat et al., 2021). There exist different pre-treatment methods, such as thermal treatments, that have been widely studied, but they present the disadvantage of the possible degradation of some thermosensitive compounds (Rodríguez-Gutiérrez et al., 2019). Regarding the extraction, traditional methods, such as Soxhlet or thermal, offer several advantages like low cost, reproducibility, and simplicity, but they also have significant drawbacks, including longer extraction times, higher solvent requirements, potential risk to biological activity, and lower efficiency (Sadeghi et al., 2024). Therefore, novel and environmentally sustainable studies are underway (Quit et al., 2022), incorporating advanced technologies, such as supercritical fluid extraction, pressurized liquid extraction, ultrasound-assisted extraction, microwave-assisted extraction, enzyme-assisted extraction, ultrasound-microwave-assisted extraction, and liquefied gas extraction.

However, the recovery of these compounds of interest does not eliminate the problem regarding macroalgae management, since there is still residual biomass after the recovery step. The most effective process for organic waste treatment is anaerobic digestion, where the organic matter is transformed mainly into biogas, a mixture of CH₄ (60–70%) and CO₂ (30–40%). However, recently, the production of volatile fatty acids (VFA) is gaining interest as VFA are key components for the industry due to their role as basic molecules for obtaining bioplastics, chemical products with high added value or for biofuels production (Cubero-Cardoso et al., 2022; Liang et al., 2024). Due to this wide range of applications, VFA have a larger market than energy, with prices ranging between 400 and 800 €·t⁻¹ for acetic acid (C2) and 2000–2500 €·t⁻¹ for propionic acid (C3) (Atasoy et al., 2018; Calt, 2015). Producing VFA through microbial processes is considered a very interesting alternative to its production from fossil sources, due to its renewable, biodegradable and sustainable nature (Bevilacqua et al., 2021; Bhatia and Yang, 2017). However, VFA production from by-products or residues is not yet widespread at an industrial level. More specifically, studies about VFA production from macroalgae are limited (Banu et al., 2020; Pham et al., 2012, 2013), with the majority of studies focusing on *Ulva fasciata* and *Laminaria japonica* macroalgae and employing chemo-thermal, microwave, and alkaline pre-treatment methods. These studies underscore the necessity for further research aimed at enhancing bioconversion yields to VFA by exploring more efficient pre-treatment methods. For this reason, we propose a pre-treatment approach that synergistically combines the extraction of bioactive compounds with enhanced bioconversion to VFA.

The general objective of this study was to develop a biorefinery process for macroalgae valorization, including the extraction of compounds with high added value followed by the anaerobic fermentation of the residual biomass for VFA production. This holistic valorization strategy was compared to the single anaerobic digestion and anaerobic fermentation of macroalgae.

2. Materials and methods

2.1. Macroalgae conditioning

Macroalgae were manually harvested from the coast of Illa de Arousa (Galicia, Spain) in January 2023. This particular macroalgae contained a considerable amount of sand and shells and predominantly featured *Ulva lactuca* macroalgae. This raw macroalgae without any conditioning was designated as “untreated” (U). Macroalgae was conditioned with

tap water in order to remove the majority of sand and shells and this was termed “washed” (W). Lastly, the particle size of washed macroalgae was reduced to sizes smaller than 1 cm using a coffee grinder (Orbegozo, MO-3250), resulting in the substrate named “milled” (M). After conditioning, macroalgae were stored at –20 °C to avoid the uncontrolled fermentation of the substrate. The three types of macroalgae were characterised in terms of solids (total and volatile) and organic matter content.

2.2. Extraction of bioactive compounds from macroalgae

Water, ethanol, and acetone are among the commonly used organic solvents for the conventional extraction of bioactive compounds (Raghunath et al., 2023; Ummat et al., 2021). Therefore, these three solvents were tested in triplicate: acetone 80% for pigments recovery, such as chlorophylls; ethanol 80% for phenolic compounds recovery; and water for sugars recovery. The extractions took place in 500 mL screw-top bottles, where 50 g of washed (W) or milled (M) macroalgae and 250 mL of solvent were added. Acetone treatment involved 2 h of magnetic agitation in amber bottles at room temperature (25 °C). The ethanol and water extractions were conducted in a water bath at 70 °C for 2 h. After each pre-treatment, the liquid phase (extract), containing the bioactive compounds, was separated from the solid phase by centrifugation. The solid phases (SP) of the triplicates were mixed and dried together for 24 h at 105 °C, for their subsequent use as a substrate in the anaerobic fermentation tests. For the liquid phase (LP), the COD, TS and VS, total phenolic content (TPC), antioxidant activity, and total sugars were determined. Liquid phase samples with organic solvent were dried with a nitrogen stream (TurboVap LV Concentration Evaporator) prior to analytical determinations. Furthermore, 10 mL of the LP extracted was evaporated to dryness using a vacuum rotary evaporator (Büchi, vacuum controller V-800) and weighted to determine the yield of the extraction, which represents the dry weight of the extract (DWE) per liter of LP.

2.3. Methane production from macroalgae

Methane production was carried out with milled (M) macroalgae through biochemical methane potential test at pH 7.5 and 37 °C. The test was conducted in triplicate using a reactor with a capacity of 2 L, coupled with an AMPTS II equipment (Bioprocess Control) (Taboada-Santos et al., 2020). The study employed an inoculum to substrate ratio of 2, in terms of VS, and an inoculum concentration of 5 g VS·L⁻¹. The flocculent inoculum for the study was sourced from the sludge digester of the wastewater treatment plant located in Lugo (Spain). The main characteristics of the inoculum were: pH: 7.4 ± 0.1; alkalinity: 7800 ± 500 mg CaCO₃·L⁻¹; total COD (tCOD): 15900 ± 400 mg L⁻¹; soluble COD (sCOD): 500 ± 70 mg L⁻¹; TS: 23270 ± 12 mg L⁻¹; and VS: 13914 ± 2 mg L⁻¹. Methane production was monitored daily over a 23-day period, and the mixed liquor of the bottles was analyzed at the end of the assay. The results were expressed in milliliters of methane under normal conditions (0 °C and 1 atm) per gram of volatile solids of the substrate (NmL CH₄·g VS⁻¹). Physico-chemical characterization at the beginning and end of the BMP test is shown in Table S1.

2.4. Anaerobic fermentation of macroalgae

Batch anaerobic fermentation tests were conducted at pH 7.5 and 37 °C, allowing for comparison with batch anaerobic digestion (Z. Wang et al., 2023). Moreover, mesophilic range and neutral pH were reported as a good conditions to maximize VFA production from organic substrates (Sun et al., 2021). In the first test, macroalgae before and after conditioning, namely U, W, and M, was used as substrate. In the second test, the residual macroalgae after the extractions with the different solvents, was used. For each test, blanks (only the inoculum) were incorporated to account for the background sCOD and VFA

contributions from the inoculum (Fig. S1). The batch tests were conducted in triplicate using 0.5 L (0.4 L of working volume) screw-top bottles with plastic septum. The bottles underwent incubation at 37 °C inside an Optic Ivymen Systems Shaking Incubator, with a rotation speed of 75 rpm. The inoculum to substrate ratio was 1, in terms of VS, using an inoculum concentration of 10 g VS·L⁻¹. The flocculent inoculum for the study was sourced from the sludge digester of the wastewater treatment plant located in Vigo (Spain). The main characteristics of the inoculum were: pH: 8.1 ± 0.1; alkalinity: 10360 ± 60 mg CaCO₃·L⁻¹; tCOD: 48600 ± 850 mg L⁻¹; sCOD: 5150 ± 10 mg L⁻¹; TS: 56170 ± 260 mg L⁻¹; and VS: 28502 ± 14 mg L⁻¹. The methanogenic inhibitor 2-bromoethanesulfonate was dosed at the beginning of each test with a concentration of 0.5 g g VS_{substrate}⁻¹. The assays were monitored by measuring the pH, sCOD, VFA and intermediates of a 10 mL sample extracted on days 1, 3, 7, 14, 21, and 28. Physico-chemical characterization at the beginning and end of the batch fermentation tests is shown in Tables S2 and S3.

2.5. Analytical methods

The determination of the pH, alkalinity, TS, VS, tCOD and sCOD was carried out by following standard methods (APHA, 2017).

Volatile fatty acids, ranging from acetic acid (C2) to heptanoic acid (C7), were quantified using gas chromatography. The analysis was performed using an Agilent 6850 gas chromatograph equipped with a flame ionization detector (FID). A DB-Wax column from Agilent Technologies (30 m × 0.250 mm × 0.25 μm) was utilized. The injector temperature was set at 250 °C, while the detector temperature was set at 280 °C. Nitrogen was used as the carrier gas. Prior to analysis, the samples were filtered (0.45 μm) and acidified with 10 μM concentrated H₃PO₄ (85%) (Bevilacqua et al., 2021). Biogas composition was determined using a Hewlett-Packard (HP) 5890 Series II gas chromatograph.

The total phenolic content (TPC) was determined by the Folin-Ciocalteu spectrophotometric method, using visible spectrophotometry in a Powerwave XS2 spectrophotometer (BioTek Instruments), and expressed as milligrams of gallic acid equivalents (GAE) per kilogram of washed or milled macroalgae (Singleton and Rossi, 1965). Anthrone colorimetric method was used to determine the total sugar content (Mokrasch, 1954). The absorbance of samples was taken at 630 nm by visible spectrophotometry in a Powerwave XS2 spectrophotometer (BioTek Instruments). A calibration curve with known glucose concentrations was made, and the results were expressed as milligrams of D-glucose equivalents per kilogram of washed or milled macroalgae.

The antioxidant activity of the extracts was assessed using two spectrophotometric methods commonly applied: DPPH (2,2-diphenyl-1-picrylhydrazyl) method, as described by Brand-Williams et al. (1995), and ABTS radical (2,2-azinobis-(3-ethyl-benzothiazoline-6-sulfonate), as described by Re et al. (1999). The results were quantified as milligrams of Trolox per kilogram of washed or milled macroalgae.

Content in chlorophyll *a*, *b* and *c* was determined according to Jeffrey and Humphrey (1975) using the following equations (Eqs. (1)–(3)) and expressed in milligrams per kilogram of washed or milled macroalgae.

$$\text{Chlorophyll } a = (11.85 \cdot (A_{664} - A_{750})) - (1.54 \cdot (A_{647} - A_{750})) - (0.08 \cdot (A_{630} - A_{750})) \quad (1)$$

$$\text{Chlorophyll } b = (-5.47 \cdot (A_{664} - A_{750})) + (21.03 \cdot (A_{647} - A_{750})) - (2.66 \cdot (A_{630} - A_{750})) \quad (2)$$

$$\text{Chlorophyll } c = (-1.67 \cdot (A_{664} - A_{750})) - (7.60 \cdot (A_{647} - A_{750})) + (24.52 \cdot (A_{630} - A_{750})) \quad (3)$$

The spectrophotometric measurements were performed using a UV-visible spectrophotometer (Shimadzu UV-1800, Shimadzu). Results were expressed as mean values ± standard deviations. To assess the differences between samples, a comparison was performed using the software Sigmaplot 14.5. One-way analysis of variance (ANOVA)

followed by Tukey's comparison test was performed. Results were considered statistically significant for $p < 0.05$.

3. Results and discussion

3.1. Influence of macroalgae conditioning on VFA production

The characteristics of untreated, washed and milled macroalgae are shown in Table 1. The conditioned macroalgae exhibit a notable decrease in TS and an increase in VS and tCOD as a result of the removal of a substantial amount of sand and shells through washing. Particle size reduction only affected TS, probably due to a decrease in moisture content caused by the friction in the coffee grinder which resulted in increased TS.

Batch fermentation tests were conducted to assess macroalgae fermentation yield and product spectra, comparing the impact of macroalgae conditioning (untreated, washed, and milled). pH remained in the neutral range (6.9–7.5) throughout all the experiments (data not shown). A gradual increase in VFA concentration was observed over time (Fig. 1). In parallel, sCOD concentration also increased over time, but it is important to note that, during the first days of experiment, around 60% of the sCOD comprises soluble compounds other than VFA, which remain unidentified at present. Nevertheless, this unidentified proportion decreased over time, with the majority of the measured sCOD eventually being attributed to VFA at the end of the experiment. During the final days of the assay, the sCOD of untreated and milled macroalgae decreased due to biogas production, specifically hydrogen, methane, carbon dioxide, and hydrogen sulfide. At the end of the test, VFA production accounted for 4.0 ± 0.1 g COD·L⁻¹, 3.2 ± 0.1 g COD·L⁻¹ and 4.6 ± 0.1 g COD·L⁻¹ for untreated, washed and milled macroalgae, respectively (Fig. 1). These values correspond to acidification percentages of 36 ± 1%, 27 ± 1%, and 39 ± 1%, respectively.

Particle size reduction may alter the crystalline index of cellulose, thereby enhancing the release of soluble compounds, as evidenced by comparing the exponential growth in sCOD between untreated and milled macroalgae (Dharmaraja et al., 2023). As a consequence, the overall efficiency of the biorefinery process increases by 44% when macroalgae are milled after washing. The fermentation of mechanically pre-treated *Ulva reticulata* demonstrated a notable VFA increase from 33 mg L⁻¹ to 135 mg L⁻¹ (Kumar et al., 2018), which highlights the significant impact of size reduction on the fermentation process. In contrast, macroalgae conditioning did not have a significant effect on product spectra. Acetic acid was the major VFA component in all tests, accounting for more than 50% (in COD basis), followed by propionic acid (20%), and lastly butyric and valeric acids, each accounting for less than 10% of the total VFA content (Table S4). This VFA composition closely resembled findings from a previous work (Banu et al., 2020).

3.2. Methane vs. volatile fatty acid production

The BMP test was conducted to assess the methane production potential of macroalgae. Milled macroalgae was selected for this study based on prior observations and the results of previous studies, which demonstrated that particle size reduction enhances substrate bioavailability for microorganisms (Tedesco et al., 2013; Trujillo-Reyes et al., 2022). The methane yield of milled macroalgae was 138 ± 17 NmL CH₄·g VS⁻¹ (Fig. S2), which is similar to the values obtained in other

Table 1
Characterization of the conditioned of macroalgae.

Macroalgae	Total solids (g·kg ⁻¹)	Volatile solids (g·kg ⁻¹)	tCOD (g·kg ⁻¹)
Untreated (U)	309 ± 12	84 ± 2	95 ± 10
Washed (W)	164 ± 11	123 ± 12	138 ± 2
Milled (M)	191 ± 15	116 ± 1	134 ± 2

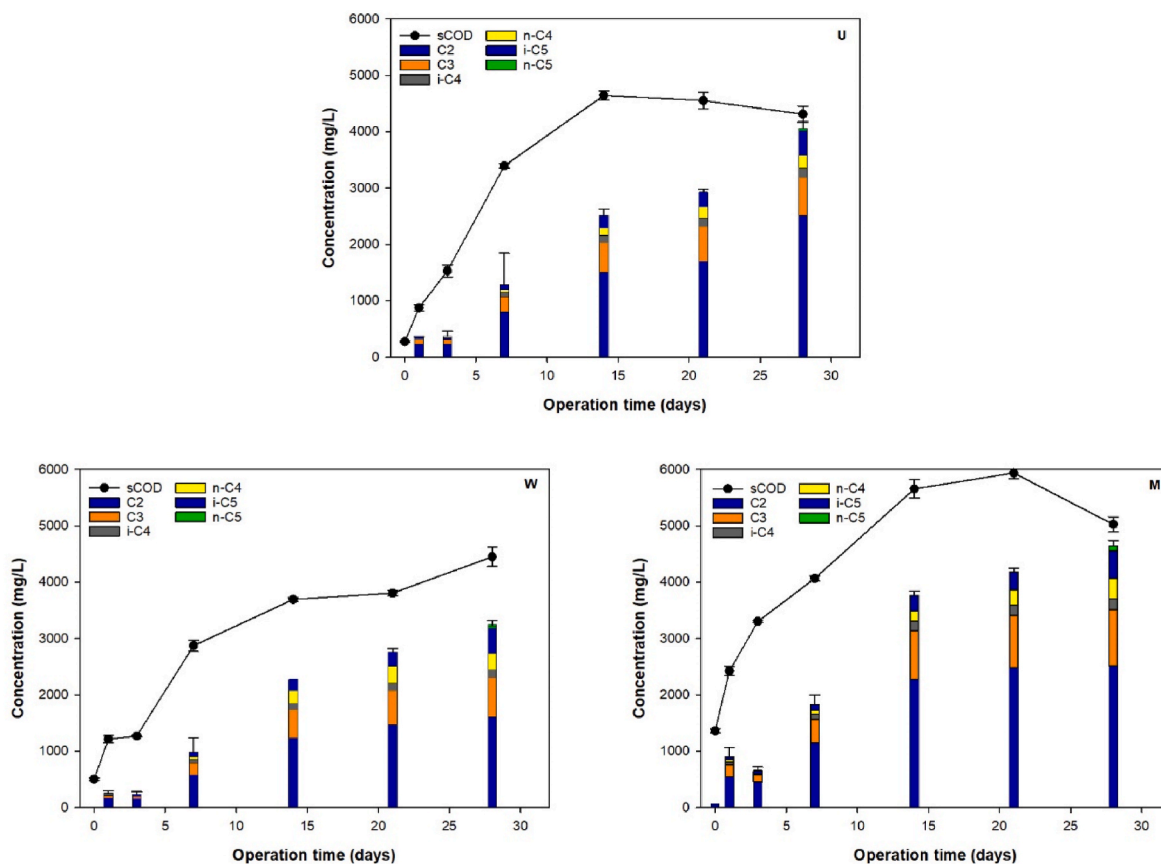


Fig. 1. Evolution of VFA (COD basis) and sCOD over time in the batch fermentation tests with untreated (a), washed (b) and milled (c) macroalgae.

recent studies that used untreated *Ulva lactuca* (Tabassum et al., 2017). This yield corresponds to an anaerobic biodegradability of $31 \pm 4 \%$ (COD basis), which is lower than the $45 \pm 1 \%$ observed for VFA production. In a comparative study on methane and VFA production from food waste, results showed methane yields of $435 \text{ mL CH}_4 \cdot \text{g VSfed}^{-1}$ and VFA yields of $434 \text{ mg VFA} \cdot \text{g VSfed}^{-1}$ (Tampio et al., 2019). In contrast, experiments with milled macroalgae produced $138 \pm 17 \text{ NmL CH}_4 \cdot \text{g VSfed}^{-1}$ and $460 \text{ mg VFA} \cdot \text{g VSfed}^{-1}$. Therefore, the efficiency of bioconversion processes varies depending on the substrate, with macroalgae producing lower methane but higher VFA yields compared to food waste. Ganesh Saratale et al. (2018) studied the anaerobic digestion of various species of macroalgae (red, green, and brown) and the values obtained ranged from 110 to $310 \text{ mL CH}_4 \cdot \text{g VS}^{-1}$. Municipal sludge and food waste are common industrial waste products used for methane production, with median methane yields of 144.5 and $429 \text{ mL CH}_4 \cdot \text{g VS}$ in BMP tests, respectively (Liu et al., 2024). Recent research has shown that producing VFA from waste through anaerobic fermentation results in lower life-cycle environmental impacts compared to conventional anaerobic digestion for bioenergy production (Gálvez-Martos et al., 2021). VFA offer additional advantages as they can be utilized as platform chemicals or substrates for biotechnologically relevant microorganisms, enabling the production of biofuels, biochemicals, and biomaterials (Tomás-Pejó et al., 2023). Nevertheless, methane remains a vital end product, primarily for bioenergy generation.

3.3. Bioactive compounds extraction from macroalgae

Table 2 presents the physico-chemical characterization of the liquid and solid phases after the extraction processes using washed and milled macroalgae. The solvent that allowed the greatest solubilization of organic matter was ethanol (Table 2), which is evidenced by the decrease in organic matter in the solid phase and the increase in liquid

Table 2

Physico-chemical characterization of solid (SP) and liquid phases (LP) after solvent extraction.

	Solid phase		Liquid phase	
	VS ($\text{g} \cdot \text{kg}_{\text{dry}}^{-1}$ SP)	tCOD ($\text{g} \cdot \text{kg}_{\text{dry}}^{-1}$ SP)	Yield ($\text{g DWE} \cdot \text{L}_{\text{LP}}^{-1}$)	sCOD ($\text{g} \cdot \text{L}_{\text{LP}}^{-1}$)
Acetone (80%)				
Washed (W)	809 ± 19	735 ± 20	1.3 ± 0.5	1.2 ± 0.1
Milled (M)	660 ± 8	674 ± 27	2.4 ± 0.4	2.7 ± 0.1
Ethanol (80%)				
Washed (W)	748 ± 9	693 ± 5	5.6 ± 0.1	3.5 ± 0.1
Milled (M)	676 ± 14	629 ± 25	5.3 ± 0.4	3.3 ± 0.1
Water				
Washed (W)	768 ± 7	777 ± 32	5.0 ± 2.1	1.2 ± 0.1
Milled (M)	780 ± 10	785 ± 21	5.5 ± 1.6	1.8 ± 0.2

phase. Water extraction had the lowest sCOD levels in the liquid phase, for both washed and milled macroalgae, and acetone extraction showed different results between washed and milled macroalgae. The extraction yield was significantly lower with acetone than with ethanol and water (Table 2). The extraction yield is influenced by various parameters, including particle size, solvent type, solvent-to-solid ratio, and temperature (Quit et al., 2022). The difference in extraction yields between washed and milled macroalgae was most evident with acetone extraction, likely due to the greater variability in measurements associated with efficiently transferring components from the longer macroalgae and the selectivity of compounds. Conversely, in the case of water and ethanol, the temperature effect causes the macroalgae to move with vapor bubbles, resulting in a more effective homogenization process.

The extraction of chlorophylls was carried out due to their utility as pigments in the food industry and their potential applications in the pharmaceutical field (X. Wang et al., 2023). As expected, a higher concentration of chlorophylls was obtained using acetone (Fig. 2a), particularly with the milled macroalgae (225 ± 12 mg chlorophyll a \cdot kg⁻¹, 96 ± 4 mg chlorophyll b \cdot kg⁻¹, and 296 ± 2 mg chlorophyll c \cdot kg⁻¹). This could be attributed to the treatment being conducted at room temperature with agitation using a magnetic stir bar, facilitating enhanced material transfer with smaller particle sizes. However, lower amounts of chlorophyll were extracted with water, regardless the type of macroalgae used (washed or milled). Conventional extraction of phenolic compounds, secondary metabolite in macroalgae, is generally carried out using ethanol, methanol, or acetone (Gil-Martín et al., 2022; Sasaki and Yoshikuni, 2022). In this study, ethanol was the most adequate solvent, reaching 260 ± 50 mg GAE \cdot kg⁻¹ and 210 ± 20 mg GAE \cdot kg⁻¹ for the W and M macroalgae, respectively (Fig. 2b). These results were higher than those obtained in another study with *Ulva lactuca* and *fasciata* macroalgae with values of 80 mg GAE \cdot g kg⁻¹ (Egodavitharana et al., 2023). Concerning antioxidant activity (Fig. 2c), ethanol extraction was the most efficient, with values of 120 ± 10 mg DPPH-Trolox \cdot kg⁻¹ and 380 ± 30 mg ABTS-Trolox \cdot kg⁻¹ for washed macroalgae, which are consistent with previous studies (Loganayaki et al., 2013). The assessment of total sugars content in the extracts was conducted, as macroalgae are abundant in diverse carbohydrates, including alginate, fucoidans, and ulvans (Sasaki and Yoshikuni, 2022). The highest value was achieved for milled macroalgae with water as

solvent (2.1 ± 0.1 g D-glucose \cdot kg⁻¹, Fig. 2d).

3.4. Effect of the extraction of bioactive compounds on the anaerobic fermentation of macroalgae

The anaerobic fermentation of residual macroalgae (solid phase) after bioactive compound extraction has been assessed through a batch-mode experiment, comparing the impact of three solvents (80% acetone, 80% ethanol, and water) and two types of macroalgae (washed and milled) on the fermentation process (Fig. 3). The highest acidification percentages were achieved after extraction with acetone (around $40 \pm 2\%$), followed by the extraction with ethanol ($38 \pm 1\%$) and lastly the water extraction ($27 \pm 3\%$). No significant differences were observed between the two types of macroalgae.

VFA spectrum was similar for all experiments with around 50% acetic acid (Fig. 3). Interestingly, after solvent extraction, propionic acid percentages increased compared to non-extracted macroalgae (Table S4). For instance, in the case of milled macroalgae, propionic acid increased from 22% to 29% after ethanol extraction. Conversely, a decrease in the production of valeric acid is observed when macroalgae were treated with different solvents. These results confirm that the extraction of bioactive compounds does not negatively impact the bioprocess, as the VFA profile and production remained similar than untreated biomass. Additionally, after ensuring the thorough removal of organic solvents to prevent any detrimental effects on the bioprocess, other valuable alternatives following bioactive compound extraction

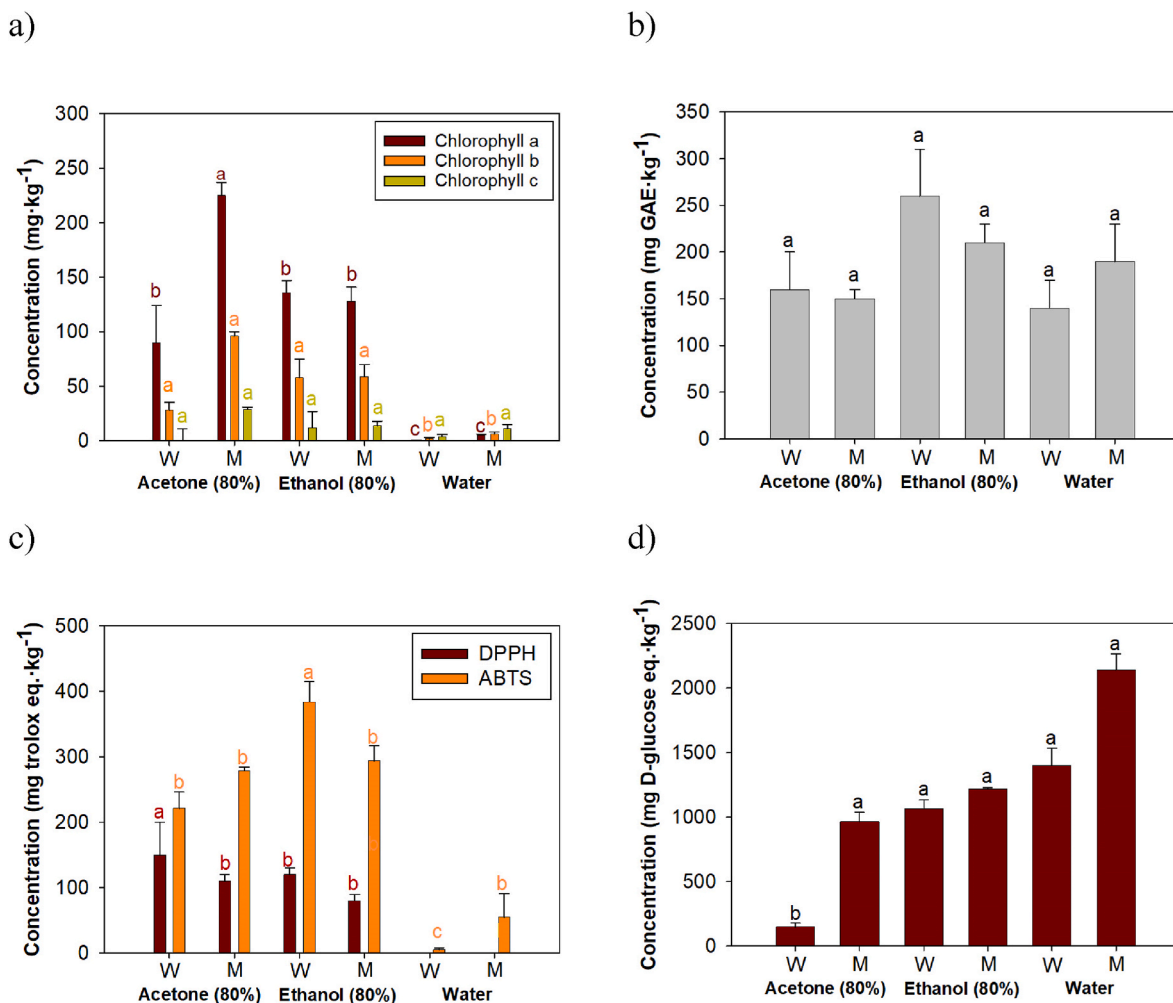
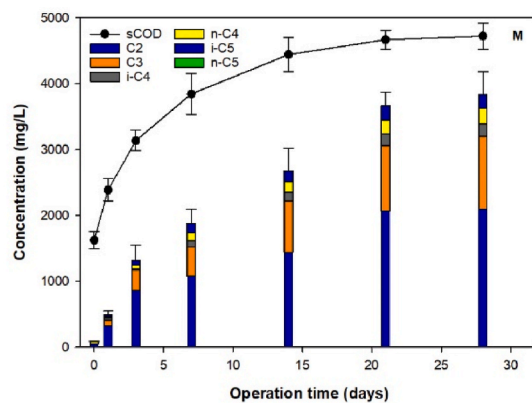
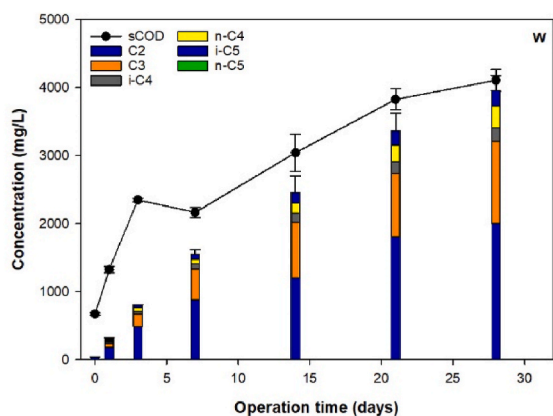
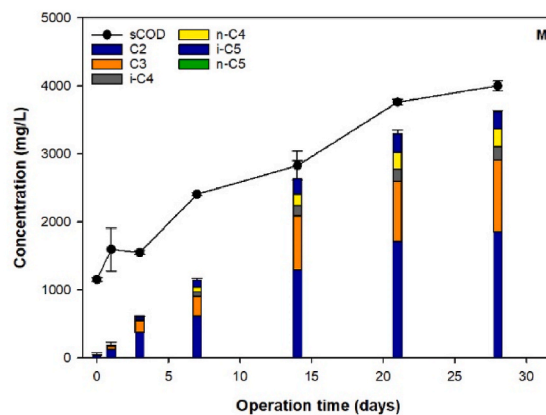
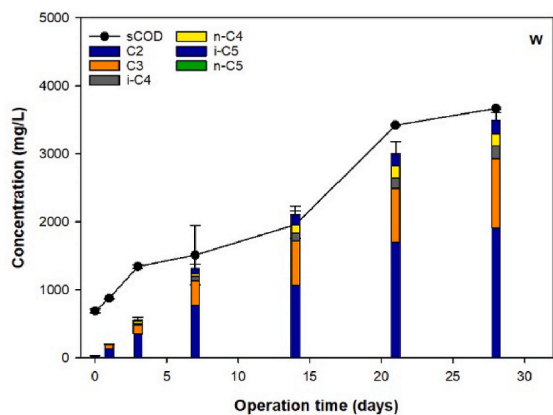


Fig. 2. Concentration of chlorophyll (a), total phenolic compounds (b), antioxidant activity (c) and total sugars (d) extracted from washed and milled macroalgae using different solvents. Data given per kg of macroalgae.

Acetone (80%)



Ethanol (80%)



Water

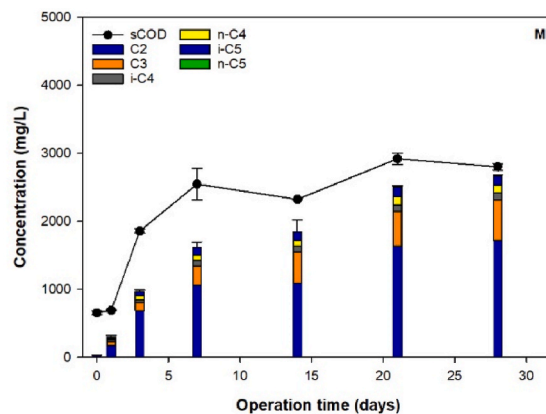
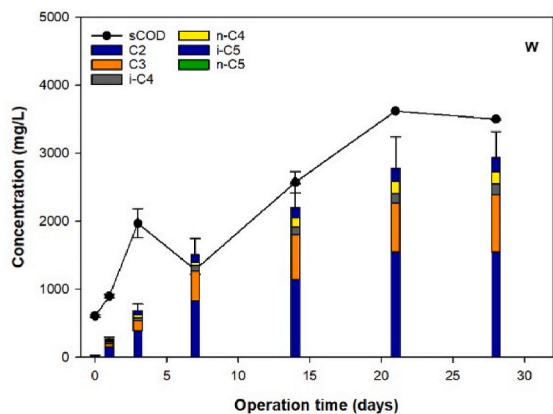


Fig. 3. Evolution of VFA (COD basis) and sCOD over time during the batch fermentation of residual macroalgae after bioactive compound extraction with three different solvents (acetone, ethanol and water).

could include the production of biohydrogen, bio-oil, or bioethanol (Kammler et al., 2024).

3.5. Comparative material balance analysis of macroalgae biorefinery processes

Three valorization options have been evaluated for 1 kg of milled

macroalgae (100 g of organic matter) (Fig. 4). The first option involves anaerobic digestion to produce methane, yielding 15 L of CH₄ per kilogram of macroalgae. The second option is similar, but it focuses on anaerobic fermentation to produce VFA, specifically 27 g of acetic acid and 8 g of propionic acid per kilogram of macroalgae. The third option involves a pre-treatment step to extract various bioactive compounds from the macroalgae (Fig. S3). The ethanol solvent treatment

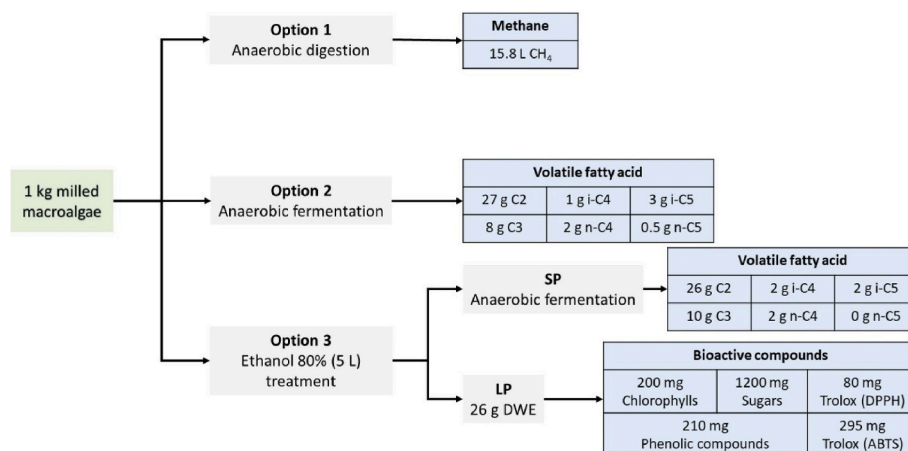


Fig. 4. Comparison among three valorization options for 1 kg of macroalgae (100 g of organic matter): 1) anaerobic digestion for methane production, 2) anaerobic fermentation for VFA production, and 3) extraction of bioactive compounds using ethanol (80%), followed by anaerobic fermentation for VFA production.

demonstrated better performance, yielding 200 mg of chlorophyll, 210 mg of phenolic compounds, and 1200 mg of sugars per kilogram of macroalgae. Additionally, 26 g of acetic acid and 10 g of propionic acid were produced per kilogram of macroalgae. This valorization option resulted in the conversion of over 50% of the organic matter into a product of industrial interest. However, this option has certain drawbacks, including the use of a significant amount of organic solvent that could be reclaimed, and the need for a drying process, which increases operational costs. Further research is recommended to explore alternative, more sustainable, and environmentally friendly extraction processes that eliminate the use of organic solvents.

4. Conclusions

This study demonstrates that the extraction to obtain bioactive compounds followed by VFA production from the residual biomass is a more attractive valorization option for macroalgae than only methane or VFA production. Particle size reduction is crucial to increase the yields. Yet, further research is recommended to explore alternative, more sustainable, and environmentally friendly extraction processes that eliminate the use of organic solvents.

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CRedit authorship contribution statement

Juan Cubero-Cardoso: Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Gemma Eibes:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization. **Marta Carballa:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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Appendix A. Supplementary data

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References

- APHA, 2017. Standard methods for examination of water and wastewater. Am. Public Heal. Assoc. Washington, DC, USA, 21. ISBN9780875532356.
- Atasoy, M., Owusu-Agyeman, I., Plaza, E., Cetecioglu, Z., 2018. Bio-based volatile fatty acid production and recovery from waste streams: current status and future challenges. *Bioresour. Technol.* 268, 773–786. <https://doi.org/10.1016/j.biortech.2018.07.042>.
- Babich, O., Sukhikh, S., Larina, V., Kalashnikova, O., Kashirskikh, E., Prosekov, A., Noskova, S., Ivanova, S., Fendri, I., Smaoui, S., Abdelfafi, S., Michaud, P., Dolganyuk, V., 2022. Algae: study of edible and biologically active fractions, their properties and applications. *Plants* 11, 228–234. <https://doi.org/10.3390/plants11060780>.
- Banu, J.R., Kavitha, S., Kannah, R.Y., Usman, T.M.M., Kumar, G., 2020. Application of chemo thermal coupled sonic homogenization of marine macroalgal biomass for energy efficient volatile fatty acid recovery. *Bioresour. Technol.* 303, 122951 <https://doi.org/10.1016/j.biortech.2020.122951>.
- Bevilacqua, R., Regueira, A., Mauricio-Iglesias, M., Lema, J.M., Carballa, M., 2021. Steering the conversion of protein residues to volatile fatty acids by adjusting pH. *Bioresour. Technol.* 320, 124315 <https://doi.org/10.1016/j.biortech.2020.124315>.
- Bhatia, S.K., Yang, Y.H., 2017. Microbial production of volatile fatty acids: current status and future perspectives. *Rev. Environ. Sci. Biotechnol.* 16, 327–345. <https://doi.org/10.1007/s11157-017-9431-4>.
- Brand-Williams, W., Cuvelier, M.E., Berset, C., 1995. Use of a free radical method to evaluate antioxidant activity. *LWT - Food Sci. Technol.* 28, 25–30. [https://doi.org/10.1016/S0023-6438\(95\)80008-5](https://doi.org/10.1016/S0023-6438(95)80008-5).
- Calt, E.A., 2015. Products produced from organic waste using managed ecosystem fermentation. *J. Sustain. Dev.* 8 <https://doi.org/10.5539/jstd.v8n3p43>.
- Casoni, I., Ramos, F.D., Estrada, V., Diaz, M.S., 2020. Sustainable and economic analysis of marine macroalgae based chemicals production - process design and optimization. *J. Clean. Prod.* 276, 122792 <https://doi.org/10.1016/j.jclepro.2020.122792>.
- Cubero-Cardoso, J., Russo, E., Serrano, A., Trujillo-Reyes, Á., Villa-Gomez, D., Esposito, G., Feroso, F.G., 2022. Enhancing the recovery of volatile fatty acids from

- strawberry extrudate through anaerobic fermentation at different pH values. *Environ. Technol. Innov.* 28, 102587 <https://doi.org/10.1016/j.eti.2022.102587>.
- Dharmaraja, J., Shobana, S., Arvindnarayan, S., Francis, R.R., Jeyakumar, R.B., Saratale, R.G., Ashokkumar, V., Bhatia, S.K., Kumar, V., Kumar, G., 2023. Lignocellulosic biomass conversion via greener pretreatment methods towards biorefinery applications. *Bioresour. Technol.* 369, 128328 <https://doi.org/10.1016/J.BIORTECH.2022.128328>.
- Egodavitharana, D.I., Manori Bambaranda, B.V.A.S., Mudannayake, D.C., 2023. Phytochemical composition of two green seaweeds (*Ulva lactuca* and *Ulva fasciata*) and their utilization as a functional ingredient in crackers. *J. Aquat. Food Prod. Technol.* 32, 158–174. <https://doi.org/10.1080/10498850.2023.2174394>.
- Gálvez-Martos, J.L., Greses, S., Magdalena, J.A., Iribarren, D., Tomás-Pejó, E., González-Fernández, C., 2021. Life cycle assessment of volatile fatty acids production from protein- and carbohydrate-rich organic wastes. *Bioresour. Technol.* 321, 124528 <https://doi.org/10.1016/J.BIORTECH.2020.124528>.
- Ganesh Saratale, R., Kumar, G., Banu, R., Xia, A., Periyasamy, S., Dattatraya Saratale, G., 2018. A critical review on anaerobic digestion of microalgae and macroalgae and co-digestion of biomass for enhanced methane generation. *Bioresour. Technol.* 262, 319–332. <https://doi.org/10.1016/J.BIORTECH.2018.03.030>.
- Gil-Martín, E., Forbes-Hernández, T., Romero, A., Cianciosi, D., Giampieri, F., Battino, M., 2022. Influence of the extraction method on the recovery of bioactive phenolic compounds from food industry by-products. *Food Chem.* 378, 308–8146. <https://doi.org/10.1016/j.foodchem.2021.131918>.
- Jeffrey, S.W., Humphrey, G.F., 1975. New spectrophotometric equations for determining chlorophylls a, b, c1 and c2 in higher plants, algae and natural phytoplankton. *Biochem. Physiol. Pflanz. (BPP)* 167, 191–194. [https://doi.org/10.1016/S0015-3796\(17\)30778-3](https://doi.org/10.1016/S0015-3796(17)30778-3).
- Kammler, S., Malvis Romero, A., Burkhardt, C., Baruth, L., Antranikian, G., Liese, A., Kaltschmitt, M., 2024. Macroalgae valorization for the production of polymers, chemicals, and energy. *Biomass Bioenergy* 183, 107105. <https://doi.org/10.1016/J.BIOMBOE.2024.107105>.
- Kumar, M.D., Tamilarasan, K., Kaliappan, S., Banu, J.R., Rajkumar, M., Kim, S.H., 2018. Surfactant assisted disperser pretreatment on the liquefaction of *Ulva reticulata* and evaluation of biodegradability for energy efficient biofuel production through nonlinear regression modelling. *Bioresour. Technol.* 255, 116–122. <https://doi.org/10.1016/j.biortech.2018.01.116>.
- Liang, J., Zhang, P., Zhang, R., Chang, J., Chen, L., Zhang, G., Wang, A., 2024. Bioconversion of volatile fatty acids from organic wastes to produce high-value products by photosynthetic bacteria: a review. *Environ. Res.* 242, 117796 <https://doi.org/10.1016/j.envres.2023.117796>.
- Liu, Y., He, P., Peng, W., Zhang, H., Lü, F., 2024. Biochemical methane potential database: a public platform. *Bioresour. Technol.* 393, 130111 <https://doi.org/10.1016/J.BIORTECH.2023.130111>.
- Loganayaki, N., Siddhuraju, P., Manian, S., 2013. Antioxidant activity and free radical scavenging capacity of phenolic extracts from *Helicteres isora* L. and *Ceiba pentandra* L. *J. Food Sci. Technol.* 50, 687–695. <https://doi.org/10.1007/s13197-011-0389-x>.
- Mokrasch, L.C., 1954. Analysis of hexose phosphates and sugar mixtures with the anthrone reagent. *J. Biol. Chem.* 208, 55–59. [https://doi.org/10.1016/S0021-9258\(18\)65623-6](https://doi.org/10.1016/S0021-9258(18)65623-6).
- Narayanan, M., 2024. Promising biorefinery products from marine macro and microalgal biomass: a review. *Renew. Sustain. Energy Rev.* 190, 114081 <https://doi.org/10.1016/J.RSER.2023.114081>.
- Pardilh, S., Pereira, L., Figueirinha, A., Maia, J., 2023. Valorisation of marine macroalgae waste using a cascade biorefinery approach : exploratory study. *J. Clean. Prod.* 385, 135672 <https://doi.org/10.1016/j.jclepro.2022.135672>.
- Pardilhó, S., Cotas, J., Pereira, L., Oliveira, M.B., Dias, J.M., 2022. Marine macroalgae in a circular economy context: a comprehensive analysis focused on residual biomass. *Biotechnol. Adv.* 60, 107987 <https://doi.org/10.1016/j.biotechadv.2022.107987>.
- Pham, T.N., Nam, W.J., Jeon, Y.J., Yoon, H.H., 2012. Volatile fatty acids production from marine macroalgae by anaerobic fermentation. *Bioresour. Technol.* 124, 500–503. <https://doi.org/10.1016/j.biortech.2012.08.081>.
- Pham, T.N., Um, Y., Yoon, H.H., 2013. Pretreatment of macroalgae for volatile fatty acid production. *Bioresour. Technol.* 146, 754–757. <https://doi.org/10.1016/j.biortech.2013.07.080>.
- Quit, E., Grosso, C., Ferraz, R., Delerue-matos, C., 2022. A critical comparison of the advanced extraction techniques applied to obtain health-promoting compounds from seaweeds. *Mar. Drugs* 20, 677.
- Raghunath, S., Budaraju, S., Gharibzadeh, S.M.T., Koubaa, M., Roohinejad, S., Mallikarjunan, K., 2023. Processing technologies for the extraction of value-added bioactive compounds from tea. *Food Eng. Rev.* 152 (15), 276–308. <https://doi.org/10.1007/S12393-023-09338-2>.
- Re, R., Pellegrini, N., Proteggente, A., Pannala, A., Yang, M., Rice-Evans, C., 1999. Antioxidant activity applying an improved ABTS radical cation decolorization assay. *Free Radic. Biol. Med.* 26, 1231–1237. [https://doi.org/10.1016/S0891-5849\(98\)00315-3](https://doi.org/10.1016/S0891-5849(98)00315-3).
- Rodríguez-Gutiérrez, G., Cubero Cardoso, J., Rubio-Senent, F., Serrano, A., Borja, R., Fernández-Bolaños, J., Feroso, F.G., 2019. Thermally-treated strawberry extrudate: a rich source of antioxidant phenols and sugars. *Innov. Food Sci. Emerg. Technol.* 51, 186–193. <https://doi.org/10.1016/j.ifset.2018.05.017>.
- Sadeghi, A., Rajabiyan, A., Meygoli Nezhad, N., Nabizade, N., Alvani, A., Zarei-Ahmady, A., 2024. A review on Persian Gulf brown algae as potential source for anticancer drugs. *Algal Res.* 79, 103446 <https://doi.org/10.1016/J.ALGAL.2024.103446>.
- Sasaki, Y., Yoshikuni, Y., 2022. Metabolic engineering for valorization of macroalgae biomass. *Metab. Eng.* 71, 42–61. <https://doi.org/10.1016/j.ymben.2022.01.005>.
- Sharmila, V.G., Rajesh Banu, J., Dinesh Kumar, M., Adish Kumar, S., Kumar, G., 2022. Algal biorefinery towards decarbonization: economic and environmental consideration. *Bioresour. Technol.* 364, 128103 <https://doi.org/10.1016/J.BIORTECH.2022.128103>.
- Singleton, V.L.V.L., Rossi, J.A.J.A., 1965. Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid reagents. *Am. J. Enol. Vitic.* 16, 144–158.
- Sultana, F., Wahab, M.A., Nahiduzzaman, M., Mohiuddin, M., Iqbal, M.Z., Shakil, A., Mamun, A. Al, Khan, M.S.R., Wong, L.L., Asaduzzaman, M., 2023. Seaweed farming for food and nutritional security, climate change mitigation and adaptation, and women empowerment: a review. *Aquac. Fish.* 8, 463–480. <https://doi.org/10.1016/j.aaf.2022.09.001>.
- Sun, J., Zhang, L., Loh, K.C., 2021. Review and perspectives of enhanced volatile fatty acids production from acidogenic fermentation of lignocellulosic biomass wastes. *Bioresour. Bioprocess.* 8, 1–21. <https://doi.org/10.1186/s40643-021-00420-3>.
- Tabassum, M.R., Xia, A., Murphy, J.D., 2017. Potential of seaweed as a feedstock for renewable gaseous fuel production in Ireland. *Renew. Sustain. Energy Rev.* 68, 136–146. <https://doi.org/10.1016/j.rser.2016.09.111>.
- Taboada-Santos, A., Rivadulla, E., Paredes, L., Carballa, M., Romalde, J., Lema, J.M., 2020. Comprehensive comparison of chemically enhanced primary treatment and high-rate activated sludge in novel wastewater treatment plant configurations. *Water Res.* 169, 115258 <https://doi.org/10.1016/j.watres.2019.115258>.
- Tampio, E.A., Blasco, L., Vainio, M.M., Kahala, M.M., Rasi, S.E., 2019. Volatile fatty acids (VFAs) and methane from food waste and cow slurry: comparison of biogas and VFA fermentation processes. *GCB Bioenergy* 11, 72–84. <https://doi.org/10.1111/GCBB.12556>.
- Tedesco, S., Benyounis, K.Y., Olabi, A.G., 2013. Mechanical pretreatment effects on macroalgae-derived biogas production in co-digestion with sludge in Ireland. *Energy* 61, 27–33. <https://doi.org/10.1016/j.energy.2013.01.071>.
- Tomás-Pejó, E., González-Fernández, C., Greses, S., Kennes, C., Otero-Logilde, N., Veiga, M.C., Bolzonella, D., Müller, B., Passoth, V., 2023. Production of short-chain fatty acids (SCFAs) as chemicals or substrates for microbes to obtain biochemicals. *Biotechnol. Biofuels Bioprod* 161 16, 1–17. <https://doi.org/10.1186/S13068-023-02349-5>.
- Trujillo-Reyes, Á., Serrano, A., Cubero-Cardoso, J., Fernández-Prior, Á., Feroso, F.G., 2022. Does seasonality of feedstock affect anaerobic digestion? *Biomass Convers. Biorefinery* 1, 1–10. <https://doi.org/10.1007/S13399-022-03336-W/FIGURES/2>.
- Ummat, V., Sivagnanam, S.P., Rajauria, G., O'Donnell, C., Tiwari, B.K., 2021. Advances in pre-treatment techniques and green extraction technologies for bioactives from seaweeds. *Trends Food Sci. Technol.* 110, 90–106. <https://doi.org/10.1016/J.TIFS.2021.01.018>.
- Wang, X., Zhang, Y., Xia, C., Alqahtani, A., Sharma, A., Pugazhendhi, A., 2023a. A review on optimistic biorefinery products: biofuel and bioproducts from algae biomass. *Fuel* 338, 127378. <https://doi.org/10.1016/j.fuel.2022.127378>.
- Wang, Z., Hu, Y., Wang, S., Wu, G., Zhan, X., 2023b. A critical review on dry anaerobic digestion of organic waste: characteristics, operational conditions, and improvement strategies. *Renew. Sustain. Energy Rev.* 176, 113208 <https://doi.org/10.1016/J.RSER.2023.113208>.
- Xu, J., Liao, W., Liu, Y., Guo, Y., Jiang, S., Zhao, C., 2023. An overview on the nutritional and bioactive components of green seaweeds. *Food Prod. Process. Nutr.* 5, 18. <https://doi.org/10.1186/s43014-023-00132-5>.