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Thalassiosira pseudonana and *Skeletonema costatum* biomass optimization: Cultivation, harvesting, extraction of oils and biodiesel and pelletization of the residue

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ABSTRACT

Microalgae are one of the most promising feedstocks for biofuel production that can solve the energy crisis, climate change, and the depletion of fossil fuels. Biorefineries have production capacity bottlenecks that prevent them from being economically profitable, without leaving aside the environmental safety of by-products. This research aims to analyze critical stages such as harvesting or lipid extraction from two microalgae species currently unknown, such as *Thalassiosira pseudonana* and *Skeletonema costatum*. Inorganic flocculation with a low concentration of iron or aluminum salts ($FeCl_3$ and $Al_2(SO_4)_3$) was achieved to recover >60% biomass in just 20 min in both cases. Lipids extractions through chloroform: methanol (solvent ratio 2:1) obtained low performance due to the ionic strength medium. The fatty acid composition of the algae extracts showed that stearic acid (C18:0) and palmitoleic acid (C16:1) were predominant in both species. In addition, residues from the lipid extraction process were used for the manufacture of pellets. The data collected showed that these solid biofuels should be combined with other biomass typologies if the end-use are biomass boilers. The development of these studies provides new information on different microalgae species and their potential to use their biomass through an integrated utilization.

1. Introduction

The development of sustainable pathways for substituting petroleum-based fuels and products is now a global priority to simultaneously combat the energy crisis, pollution, and climate change (Khoshnevisan et al., 2021). Consequently, the restrictions surrounding carbon dioxide emissions have caused many countries to have established environmental policies to support bio-based alternatives for replacing fossil sources in energy production (Panoutsou et al., 2021; Moncada et al., 2016). Previously, research focused on harnessing conventional natural energy sources, such as solar, wind, hydrothermal, geothermal and hydropower (Koyande et al., 2021). However, these renewable energy plants are capital-intensive, requiring large expenditure on equipment maintenance (Sreeharsha and Mohan, 2021).

In this context, introducing the biorefinery concept offers the possibility to exploit the potential of biomass for conversion into a wide range of marketable products such as biofuels, electricity, heat, chemicals, and other products reoriented to a sustainable bio-based approach (Mehmood et al., 2021; Philippini et al., 2020). On the other hand, biorefineries allow feedstocks that could be widely available at low cost and sustainable for production (Giraldo-Calderón et al., 2018), such as feedstock from microalgae. Microalgae are considered a potential feedstock for biofuels and bioproducts production (Gifuni et al., 2019). They provide high and rapid biomass production, generating 10–20 times more oil than any terrestrial oil crop plant (Akubude et al., 2019; Wicker et al., 2021). In addition, these tiny photosynthetic microorganisms show great productivity and adaptability to the growing medium (Fuad et al., 2018). The requirements for microalgae cultivation are undemanding, and although requirements may vary from different species, almost all species need light, essential nutrients, a source of organic or inorganic carbon, nitrogen, phosphorus, and iron (Khan et al., 2018). However, microalgae still have a high cost to bring microalgae biofuels to the market, mainly due to the costs of photobioreactors and the addition of nutrients to the culture media (Khan et al., 2022b). Along

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these lines, strategies have been adopted to reduce costs, such as the use of more economical high-productivity reactors; the use of nutrients from wastewater (rich in nitrogen and phosphorus), which in turn reduces an environmental problem; and also with the maximum valorization of the biomass (integral use)(Linares et al., 2017; Sipaúba-Tavares et al., 2017). Microalgae contain carbohydrates, lipids, and proteins. Thus, extracted lipids can serve for biodiesel production and microalgal carbohydrates as a carbon source (Sati et al., 2019). Solvent extraction is one of the most commonly used methods for lipid extraction for biodiesel production, although it is not the only way (Zewdie and Ali, 2020). However, solvent extraction is highly efficient in lipid recovery and low cost, in addition, with equipment such as Soxhlet it is possible to extract >98% of the lipids from microalgae cells (Patel et al., 2018; Ranjith Kumar et al., 2015).

Currently, there are three main routes for the production of liquid biofuels from microalgae: (1) biodiesel by extraction or transesterification, (2) bio-oil by pyrolysis, and (3) biocrude oil by hydrothermal liquefaction (HTL) (Tian et al., 2017). In this study, the method used was extraction, followed by transesterification for biodiesel production. Biodiesel is a mixture of fatty acid methyl esters (FAME) synthesized from vegetable fats (Torres et al., 2017). In the transesterification process, the triglycerides of the oils are converted into methyl (or ethyl) esters. The alcohol reacts with the oil causing the separation of the glycerol and methyl ester layers (biodiesel) after the response time (Sivaprakasam and Saravanan, 2007). This method has some advantages, especially for industrial use, since low temperatures and pressures are required; the conversion obtained is high, and there are no difficulties in having the necessary material (Sivaprakasam and Saravanan, 2007).

That said, the present work has primarily investigated the bottlenecks in microalgae biorefineries for simultaneous liquid and solid biofuel production (Gifuni et al., 2019; Sivaprakasam and Saravanan, 2007). This paper evaluates unitary operation as harvesting, oil extraction, and pellets manufacturing from the waste generated (Mourya et al., 2022). And secondarily, the conversion into final products such as biodiesel and glycerol from basic catalysis transesterification. Glycerol also belongs to one of the essential and unavoidable byproducts in biodiesel formation, widely used in the industry (Yang et al., 2012). To carry out these studies, two marine microalgae species, whose integral biomass exploitation has not been explored to date, have been selected: *Thalassiosira pseudonana* diatom and one of the primary algae responsible for red tides, the diatom *Skeletonema costatum*, both used in aquaculture feed (Wu et al., 2009; Zhang et al., 2017).

2. Materials and methods

2.1. Microalgae cultivation

The two microalgae were grown in polyethylene 45 L capacity photobioreactors with a Guillard culture medium (Guillard, 1975). in the Marine Science Station of Toralla (E.C.I.M.A.T.). The cultivation time of *Thalassiosira pseudonana* was 6 days at 21 ± 1 °C, pH 8.57 and a cell accounting at the end of culture was 2700 cell/μL. On the other hand, the *Skeletonema costatum* cultivation time was 8 days under the same temperature, pH 8.73, and a final cell accounting of 1300 cell/μl. The culture media were L1 and Walne, mixed with seawater.

2.2. Harvesting

One of the most critical operations in obtaining algal biomass is microalgae harvesting. This process can represent between 20 and 30% of the total production cost (Fuad et al., 2018; Khan et al., 2022a). In this research, chemical flocculation was the selected method to separate *Thalassiosira pseudonana* and *Skeletonema costatum* biomass from their growing medium. Chemical flocculation is one of the most efficient and inexpensive techniques to harvest algal biomass, with yields of up to 90% recovery of microalgal cells in culture (Branyikova et al., 2018). Microalgae have a negatively charged cell surface. Therefore, this mechanism could be described as follows: the neutralization of the negative charge surface of the microalgal cells by the influence of the multivalent cations of the chemical flocculants, which bind the cells together to facilitate flocculation (Chatsungnoen and Chisti, 2019; Demir et al., 2020). Flocculation may be affected by different parameters that can influence the efficiency, such as cell surface properties, the number of cells per unit volume, medium conditions (e.g., pH of the growth medium), type and concentration of flocculant, ionic strength of the culture solution and flocculation time (Branyikova et al., 2018; Martínez Gutiérrez, 2016). In this context, the effect of seven different flocculants has been studied: iron chloride (III), iron sulfate (II), aluminum sulfate, copper sulfate (II), calcium chloride, potassium alum, and aluminum chloride. All tests in a concentration of 50 mg/L. This preliminary study allowed to determine the flocculant with the highest yield, finally applying it to the whole crop and extrapolating the doses of the selected flocculant.

The procedure started incorporating coagulant to the medium, followed by four minutes of stirring at 200 rpm to ensure homogeneous distribution and then one minute at 50 rpm on a HANNA HI 190 M Magnetic Mini-Stirrer facilitate the formation of flocs.

All flocculation experiments were tested in a 1 L beaker, and flocculation efficiency estimation was determined by absorbance at 680 nm by Labomed Spectro 22 spectrophotometer. Samples were pipetted from a height of two-thirds from the bottom every 5 min up to 90 min in order to measure the absorbance. Flocculation efficiency was calculated according to the following equation:

$$E = \frac{A_0 - A_t}{A_0} * 100.$$

where E is the flocculation efficiency (%), A_0 is the initial optical density, and A_t is the optical density of the sample.

2.3. Dewatering

After removing the most significant quantity of water as possible, following the flocculation process, centrifugation was carried out. This method was chosen for dehydration to know the amount of water in each sample of the harvested microalgae. The process was executed in the Selecta Mixtasel centrifuge at 4000 rpm and 10–12 min. Therefore, two phases (flocculation and centrifugation) were necessary for total separation with the medium at the end of the process. As a final step, the algae were dried in a Selecta Conterm 2,000,208 oven at 105 degrees until the weight stabilized.

2.4. Lipid extraction

Oil extraction from algal biomass was conducted using the Soxhlet method. The extraction should affect the lipids to be extracted as little as possible, as well as the extraction efficiency. Therefore the choice of solvent is decisive. In this context, the use of solvent mixtures such as chloroform and methanol could speed up the procedure and improve the efficiency of oil extraction (Gorgich et al., 2020). On the one hand, chloroform is one of the most widely used solvents for lipid extraction due to its low dielectric constant (non-polar).

On the other hand, methanol extracts structural lipids that are associated with polar lipids (phospholipids) and proteins (Supaporn and Yeom, 2016). Therefore, the solvent mixture chloroform/methanol in a 2:1 ratio was considered to be the most suitable for the extraction. The mixing was carried out in a 500 mL round bottom flask, where a total of 375 mL of the solvent mixture was deposited for each test. In the Table 1 is a summary of the experiment details:

After the extractions period, the samples were introduced into the

Table 1
Characteristics of the different extraction tests carried out.

Samples	Wet microalga	Extraction time
Thalassiosira pseudonana (1)	19.82 g	4 h 35 min
Thalassiosira pseudonana (2)	22.47 g	4 h 50 min
Skeletonema costatum (1)	18.42 g	7 h 45 min
Skeletonema costatum (2)	16.83 g	4 h 50 min

oven at 65 °C to ensure solvent evaporation. To determine the lipid content, the analytical method of gas chromatography (GC) samples was carried out. GC provides a detailed and quantitative measure of the lipid composition of the microalgae (Jones et al., 2012). That is a crucial step to be carried out at laboratory scale for subsequent transfer to industrial scale, mainly for selecting the correct microalgae species for the production of biofuels and high-value compounds (Yao et al., 2015). The GC analysis was performed at the Scientific and Technological Support Centre for Research (CACTI) of the Ourense campus of the University of Vigo. The analysis carried out at CACTI calculates the content of FAME. The analysis procedure involved a transformation of the fatty acids in the sample into their corresponding methyl esters by transesterification with trimethylsulfonium hydroxide (TMSH) according to the UNE-EN ISO 12966-3 standard. Subsequently, the sample was injected in gaseous phase in a gas chromatograph and a chromatogram was obtained (annex I), in which the amount of each FAME was determined and, consequently, the amount of transesterifiable fatty acids according to the peaks that were observed. Each ester was detected at a certain time and, based on the pattern used (methyl ester of nonaic acid), it was determined which compound it was. The standard used in the determination of fatty acid methyl esters was UNE-EN 14103.

2.5. Transesterification reaction

The direct transesterification process was used to reach the final biodiesel production according to Wahlen et al. (2011). This reaction was carried out in all samples with basic catalysis, mixing sodium hydroxide (NaOH) at 2% (g NaOH/g oil), as an alkaline catalyst, and methanol in a 1:12 ratio (g oil to ml methanol). Except sample number one of *Thalassiosira pseudonana*, with a ratio of 150:1 M and a NaOH content equivalent to 14.29% by weight of dry algae. Once the exact amounts of methanol and NaOH needed to make the methoxide were determined, the transesterification was performed. In a 100 mL Erlenmeyer flask, the oil and methoxide samples were mixed under a shaking incubator with a temperature of 62 °C and a rotational speed of 150 rpm for 3 h. Subsequently, they were poured into decanters and left for 4 days. At the end of the reaction, the final product has two separate phases, biodiesel (FAME) in the upper layer and glycerol, the heavier one, in the lower layer. Theoretical fatty acid analysis of the extracted microalgae oils was carried out according to EN 14214–03 ("UNE-EN 14214:2013 V2+A2:2019, 2019). For this, the minimum content of esters that biodiesel must report, which falls within the parameters of the European standard EN 14214–03. Therefore, 96.5% was considered as a conversion percentage. Table 2 shows the conversion both in individual percentages of conversion of each fatty acid and in the total proportion of each algae in the sample.

2.6. Pelletization

This residue is based on the microalgal biomass after extraction. Therefore, the residue is wet. Therefore, the primary step before the analysis of the biomass is to dry it at temperatures around 100 °C to remove all the water present in the mass. Once the drying is completed, waste from the lipid extraction process was analyzed in terms of pelletization. The characteristics studied were humidity content, volatile matter, ash, and fixed carbon according to UNE-EN 18134–2:2015 (UNE-EN ISO 18134-2:2017, 2017. *Solid biofuels - Determination of moisture content - Oven dry method - Part 2: Total moisture - Simplified method (ISO 18134-2:2017)*, n.d.), UNE-EN 18123:2016 (UNE-EN ISO 18123:2016, 2016. *Solid biofuels - Determination of the content of volatile matter (ISO 18123:2015)*, n.d.), UNE-EN 18122:2016 (UNE-EN ISO 18122:2016, 2016. *Solid biofuels - Determination of ash content (ISO 18122:2015)*, n.d.) respectively, while fixed carbon was calculated by difference. The volatile content was determined in a muffle at 900 °C (± 2 °C) in a closed crucible for 7 min (± 5 s). To determine volatile compounds, samples were weighed before and after the process. The ash content was determined in an oven at 250 °C for one hour, then at 550 °C for the next 5 h. The calculation, as for the volatile content, was given by mass difference. Finally, the heat of combustion of the samples was determined according to UNE-EN 18125:2018 (UNE-EN ISO 18125:2018, 2018. *Solid biofuels - Determination of calorific value (ISO 18125:2017)*, n.d.) using the bomb calorimeter A Parr 1261. The heat generated was calculated from the temperature rise due to combustion.

Table 2
Proportion of biodiesel according to EN 14214 standard.

Thalassiosira pseudonana

Fatty acid	Proportion (%)		Conversion rate (%)	Biodiesel (%)	
	Sample 1	Sample 2		Sample 1	Sample 2
C14:0	11.4	9.3	96.5	11.0	9.0
C16:0	13.9	29.5		13.4	28.5
C16:1	37.5	25.6		36.2	24.7
C18:0	37.2	35.6		35.9	34.4
Total in sample	0.1	0.4	Theoretical total in sample	0.097	0.386
Total percentage respect to dry algae	0.29	3.33	Total theoretical percentage with respect to dry algae	0.28	3.21

Skeletonema costatum

Fatty acid	Proportion (%)		Conversion rate (%)	Biodiesel (%)	
	Sample 1	Sample 2		Sample 1	Sample 2
C14:0	24.5	16.0	96.5	23.6	15.4
C16:0	17.2	17.5		16.6	16.9
C16:1	33.2	21.1		32.0	20.4
C18:0	25.1	45.4		24.2	43.8
Total in sample	0.2	0.3	Theoretical total in sample	0.193	0.29
Total percentage respect to dry algae	1.41	3.23	Total theoretical percentage with respect to dry algae	1.36	3.12

3. Results and discussion

3.1. Harvesting

The performance of the seven inorganic coagulants was assessed and compared in terms of biomass recovery and time. Results presented in Fig. 1 show the tested coagulants for the microalga *Thalassiosira pseudonana.* In this case, the most promising biomass recovery was obtained using 50 mg/L of $FeCl_3$, reaching values above 87% in just 15 min. FeSO4 was less effective for its part, achieving maximum values of 51% microalgal biomass harvested after an hour and a half. Contrary to expectations, aluminum salt such as $AlCl_3$ achieved low flocculation efficiency, 21% at 10 min, going up to 36% 80 min after. Lower biomass recoveries were detected with the other flocculants tested. According to other authors, ferric chloride was also promising for *Nannochloropsis* or *Tetraselmis* biomass recovery, attaining values >90% in both species

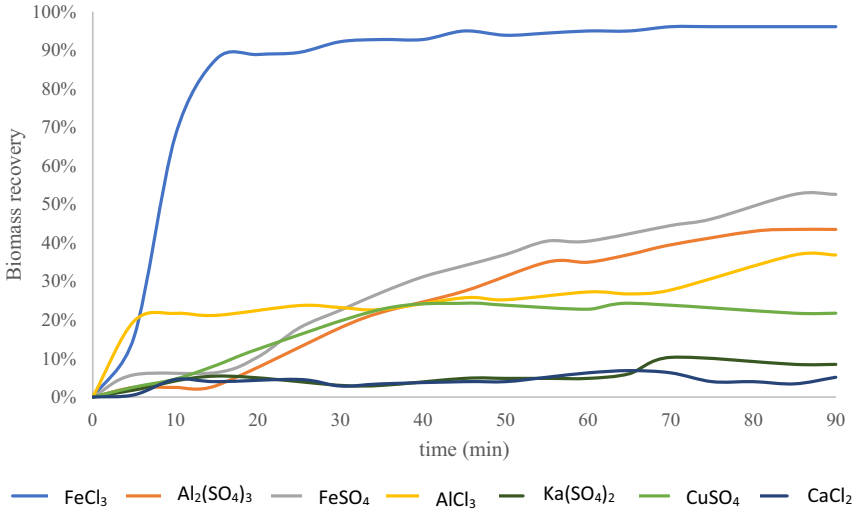

Fig. 1. Biomass recovery of *Thalassiosira pseudonana* during 90 min.

(Lama et al., 2016). The enhanced performance of iron chloride could be related to the formation of hydroxide precipitate. This fact may be due to the ferric hydroxide precipitates create links between the algae cells that connect them in flocs (Ummalyma et al., 2016). Furthermore, the flocculation mechanism is influenced by the nature of the cells and the charge of the flocculant agent. Therefore, a low concentration of flocculants used in this research (50 mg/L) seems insufficient, except for FeCl3, to effectively harvest the biomass from the *Thalassiosira pseudonana* microalgae.

Regarding the microalga *Skeletonema costatum*, Fig. 2 shows the percentage of biomass recovered over 90 min. It can be observed that aluminum sulfate had a higher flocculation efficiency than ferric chloride. These two flocculants produced the best biomass recoveries and the final results, at the end of the interval period analyzed, were very similar. Thus, $Al_2(SO_4)_3$ reached a flocculation efficiency of 64% at 20 min, while ferric chloride supported a slightly lower efficiency (53%). After 90 min, $Al_2(SO_4)_3$ resulted in a percentage biomass separation more significant than 80%. The results achieved for this aluminum salt suggest that the cation Al^{3+} has a high surface charge density. A higher charge makes it possible to improve bridge cells and neutralize the surface charge (Chatsungnoen and Chisti, 2016).

This research aims to reuse the byproducts generated throughout the process and reintroduce them into a circular economy. Therefore, it is anticipated that, in the following sections, we discuss the problems generated from the flocculants used, affecting the extraction and also the residual biomass. According to Chatsungnoen and Chisti (2019), flocculants, to be applied on an industrial scale, microalgae recovery from the culture medium must have the following criteria: be practical and cheap, environmentally benign and readily available (Chatsungnoen and Chisti, 2019). Only aluminum sulfate and ferric chloride meet these criteria (Chatsungnoen and Chisti, 2019), both with the best results for *S. costatum* and *T. pseudonana,* respectively. However, the problems caused by these flocculants during the process cannot be ignored, and it is necessary to look for biodegradable and non-toxic alternatives. This would also generate a more sustainable and economical process that, in addition to not contaminating the biomass, allows the culture medium to be reused (Singh and Patidar, 2018). Future experiments should focus on searching for and analyzing renewable, naturally degrading, and cost-effective organic alternatives. Although many effective organic flocculants have been developed, they are more expensive than inorganic flocculants, and therefore less attractive to industry.

3.2. Oil extraction

Oil extraction was conducted by the Soxhlet method using two solvents: chloroform and methanol. Experimental results indicated that a similar oil extraction efficiency was attained for the two microalgal species analyzed, and poor performance was obtained (3.33% for

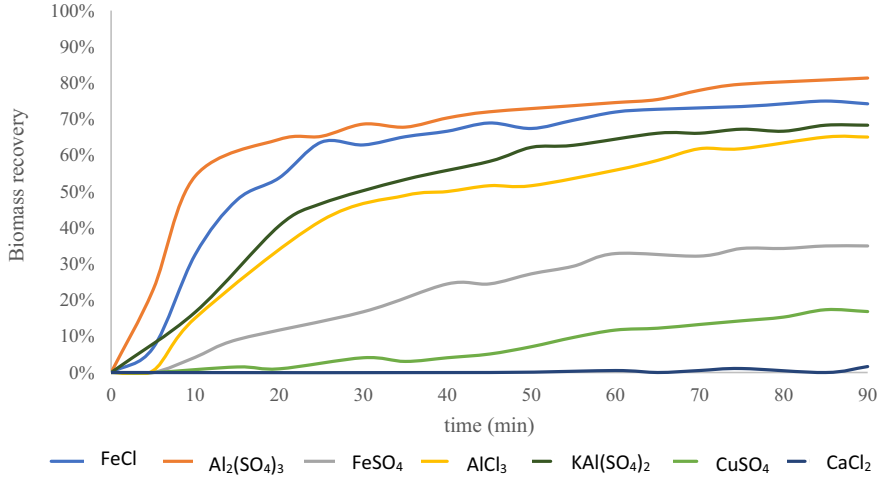

Fig. 2. Biomass recovery of *Skeletonema costatum* during 90 min.

Thalassiosira pseudonana and 3.23% for *Skeletonema costatum*), which could be attributed to the high ionic strength of the medium (seawater). Low values if they are compared with 20% of other studies (Bolognesi et al., 2022). One of the fundamental factors that can affect the biochemical composition (proteins, lipids, carbohydrates, and pigments) and microalgae growth is the composition of the culture medium (Sánchez et al., 2000). In addition, the use of wet biomass could also have conditioned the extraction. The water contained in the microalgae interferes with the solvents, making them less permeable to the cells. Even so, there is an interest in carrying out oil extractions with wet biomass, mainly to avoid drying processes before extraction, which involves energy and economic costs, and even more so on an industrial scale (Patel and Kannan, 2021). In this line, our results could be improved by applying some method of cell disruption, such as microwaves, altering the cell wall, and consequently improving the extraction yield (Sivaramakrishnan and Incharoensakdi, 2018; Zhang et al., 2014).

On the other hand, we cannot conclude that this low efficiency is due to solvents choice and combination. Numerous studies dealing with various solvent systems have been shown with promising results for this combination throughout the literature. Ramola et al. (2019) reported that the 2:1 chloroform:methanol combination had the highest extraction efficiency (14%); followed by extraction with hexane as only solvent (12.5%) (Ramola et al., 2019). It would be interesting to repeat the extractions with hexane, alone and to obtain a comparison. Hexane, having a lower polarity than chloroform, could extract neutral lipids with a higher affinity (Gorte et al., 2020).

3.3. Fatty acid profile determined by GC–MS

After oil extraction, the fatty acids composition profile of *Thalassiosira pseudonana* and *Skeletonema costatum* was determined by gas chromatography, whose composition can be observed in Fig. 3. In the two microalgae tested, stearic acid (C18:0) and palmitoleic acid (C16:1) were the predominant in both species. Saturated and unsaturated fatty acids composition varies between microalgae species; however, in this case, the two microalgae selected showed very similar values (Fig. 3). These results are consistent with data presented in different studies, which report that palmitic acid (16:0), palmitoleic acid (16:1), stearic acid (18:0), and oleic acid (18:1) keep a high proportion in algal oil (Nascimento et al., 2013; Wang et al., 2013; Zhang et al., 2014). Sharmin et al. (Sharmin et al., 2016) observed a very similar fatty acid profile of extracted lipid using $ChCl_3$:MeOH 2:1 *v*/v ratio as solvent from *Skeletonema costatum* microalgae. On the other hand, Fig. 3 shows that 68% of the oil extracted is composed of saturated fatty acids,

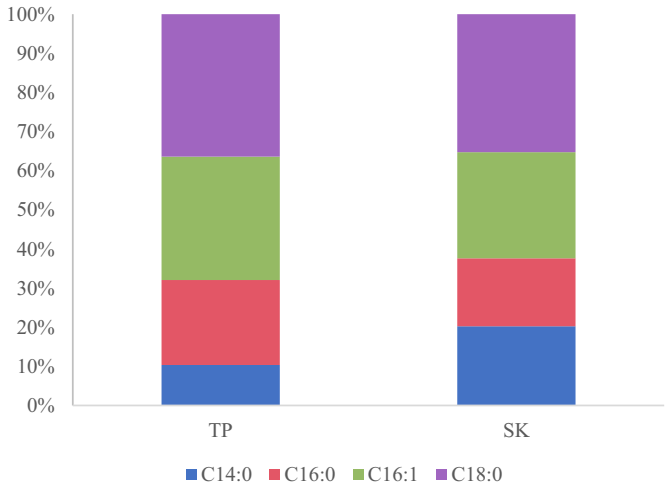

Fig. 3. Fatty acid composition of *Thalassiosira pseudonana* and *Skeletonema costatum*.

particularly stearic acid (36.40% *T. pseudonana* and 35.25% *S. costatum*). This composition could be suitable to produce biodiesel with better oxidation stability, which means that biodiesel could be stored longer (Guldhe et al., 2014).

3.4. Characterization of waste biomass for pellets

Chemical analyses were carried out to characterize the combustion power of the microalgal biomass pellets. The analyses were evaluated according to the requirements stipulated in the UNE-EN ISO 17225-2:2014 standard (Guldhe et al., 2014), which establishes the following criteria: moisture content $\leq$10%, ash content $\leq$0.7, and low calorific value (LCV) $\geq$ 16.5. Table 3 summarises the results of the analysis of each biomass sample.

Based on the results obtained, only the moisture content verifies the regulations. Both species showed a calorific value considerably lower than the required and an ash content more significant than that indicated in current legislation. The flocculants employed could be responsible for this high ash content, just as it happened in the tests carried out by Cancela et al. (Cancela et al., 2016) when cations such as Cu^{2+} and Al^{3+} were using. Other studies have presented comparisons of different commercial coagulants, in which it was demonstrated that, depending on the coagulant used, changes in the properties of the microalgal biomass could occur and thus impact the energy recovery process (Soares et al., 2020). Soares et al. (2020) demonstrated with their experiments how inorganic coagulants, namely iron, and aluminum coagulants, significantly decreased the calorific value and increased the ash content. However, the data obtained in this research indicate that the pellets obtained from the algae residues of the two selected species would not be suitable for direct use in biomass boilers (Leong et al., 2019).

In general, the exclusive use of algal biomass for pellet production does not achieve the necessary specifications for commercialization (Picchio et al., 2020). However, it is known that the combination of microalgae biomass residue in conjunction with the main pellet manufacturing sources, byproducts of primary wood processing in sawmills (bark, sawdust, chips, and shavings), could help to decrease the energy required in the pelletization process and increase the physical properties of the pellets. That said, the residue of such species could lead to mixtures with other types of biomass and ensure compliance with standards, in addition to contributing to a current problem caused by the high demand for pellets and the limited supply of sawmill residues, requiring new residues from other biomass sources (Alcaraz et al., 2021).

3.5. Transesterification by-products: biodiesel and raw glycerol

Theoretical analysis of the maximum amounts of fatty acids that could be obtained from the extracted oils was carried out, providing an economic estimate of biodiesel production. The calculations took into account the minimum content of esters that biodiesel has to report

Table 3
Analysis from the algal waste pellet of *Thalassiosira pseudonana* and *Skeletonema costatum* microalgae.

Parameter	Unit	Regulation value	*Thalassiosira pseudonana*	*Skeletonema costatum*
HHV	kJ/kg	–	10,702.76	10,120.89
Ash	%	$\leq$ 0,7	53.44	34.45
Moisture	%	$\leq$ 10	0	0
LHV	kJ/kg	$\geq$ 16,500	9380.30	8798.43
Fixed carbon	%	–	3.19	0.93
Volatile matter	%	–	43.37	64.42

according to EN 14214–03, namely 96.5%. The results were similar to those obtained for the oil with a percentage by dry weight of the biomass of 3.21% for *T.pseudonana* and 3.12% for *S.costatum*, suggesting that the amount of biodiesel obtained is due to the high storage of lipids in the oil composition in the selected diatoms (Maeda et al., 2017). Other authors have claimed that these species are suitable for biodiesel production, being promising feedstocks that may replace diesel in the future, provided that the cultivation and extraction conditions are appropriate (Sharmin et al., 2016). Another byproduct, glycerol, is formed in this step after the transfer reaction and biodiesel production to make full use of the biodiesel. To produce zero waste biodiesel, it is necessary to develop processes that valorize the crude glycerol for marketing and make the process more economically profitable (Abdul Raman et al., 2019).

4. Conclusions

Results showed that *Thalassiosira pseudonana* and *Skeletonema costatum* microalgae could be effectively harvested by chemical flocculation using low concentrations of inorganic flocculants (50 mg/L) $FeCl_3$ or $Al_2(SO_4)_3$, thus reducing costs while maximising efficiency. On the other hand, the use of more traditional methods such as Soxhlet extraction turned out not to be an effective alternative for recovering algal oil from the two selected species. Analysis of the oil extracted in this study showed the identification of saturated and unsaturated fatty acids, with stearic acid (C18:0) and palmitoleic acid (C16:1) being predominant in both species. In addition, the low percentage of polyunsaturates in oils is an optimal indicator, suggesting biodiesel quality in terms of corrosivity. Pellets manufactured with the resulting algal waste lack quality (they do not verify standards) to be used directly as a solid biofuel in biomass boilers. Nevertheless, its combination with other biomasses (e.g., pinewood) could be an alternative. In general, in view of the collection techniques and an assessment from an environmental and reuse point of view, this study suggests that alternatives to the inorganic flocculants used should be looked for a less toxic option. Finally, this study provides an innovation in the use and recycling of waste from microalgae species and their potential to take advantage of their biomass through integrated use.

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CRediT authorship contribution statement

S. Iglesias: Methodology, Investigation, Data curation, Writing – original draft. **C. Míguez:** Validation, Formal analysis, Writing – review & editing. **A. Sánchez:** Conceptualization, Resources, Project administration, Funding acquisition. **A. Cancela:** Methodology, Investigation, Supervision, Funding acquisition. **X. Álvarez:** Methodology, Investigation, Data curation.

Declaration of Competing Interest

The authors report no commercial or proprietary interest in any product or concept discussed in this article.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seares.2022.102243.

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