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Posidonia oceanica Balls (Egagropili) from Kefalonia Island Evaluated as Alternative Biomass Source for Green Energy

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Abstract: Research attempts on biomass use constitute a response to the growing demand for sustainable and low-cost energy from renewable sources. Hence, the sustainable use of *Posidonia oceanica* (PO) waste as a material for biomass to produce green energy is being considered in many countries in the Mediterranean region. PO meadows are considered as the main type of sea flora in the Greek coasts. PO can extract biomass from nearby ecosystems of the coastal zone, either directly through the transportation of disposed non-living leaves or indirectly via benthic organisms. The aim of this study is to investigate the use of PO waste derived from Kefalonia Island (Greece) as a biomass source. PO samples were collected around the island, and they were mineralogically and microstructurally analyzed. In addition, physicochemical, chemical, and thermogenic tests were performed in order to obtain the optimum and most completed characterization of the material. Based on the results, cellulose seems to be the main structural component of PO, which also seems to determine their behavior. PO presents microscopic similarities to other lignocellulosic materials which composition is made of carbonates, lignin, extractives, and minerals. Ash and moisture content constitute the two critical parameters that are responsible for the energy differences of each biomass. The outcome of this study shows the potential use of PO wastes as an interesting source for energy production.

Keywords: *Posidonia oceanica*; Egagropili; green energy; marine biomass; Kefalonia Island

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1. Introduction

In Greek mythology, the term Posidonia comes from Poseidon, the God of the seas and oceans. Posidonia is also well known as "The grass of Poseidon" and belongs to the wide category of phanerogamic angiosperm plants. Its distribution is dynamically affected by various environmental factors, such as climate, bathymetry (shallow zones and shallow coastal waters), chemical composition of the sea, availability of nutrients, and geological characteristics (e.g., sedimentary features at the seabed) [1,2]. In Greece, the most common species of seagrass are *Posidonia oceanica* (L.) Delile, Cymodocea no-dosa (Ucria) Ascherson, Zostera nolti Hornemann, and Halophila stipulacea [3].

The endemic fields (meadows) of *Posidonia oceanica* (PO) are considered the main type of sea flora in the coastal region of Greece and generally in the Mediterranean littoral area [1]. This kind of seagrass consists of roots, rhizomes, and long leaves [4]. Biomass of PO can be detected in two forms: Posidonian spheroids and fallen dead leaves. Round shaped-conglomerations, also known as Egagropiles (EG), are spherical or oval balls with a felt-like texture and light brown color; they are generated by the progressive disintegration of fibrous material sourced from its foliage and are transported to nearby coastal areas by waves. The latter derives from living leaves [5]. During their development, they form a layer called "matte", which includes entangled remnants of roots, rhizomes, and leaves, and are able to trap sediment and organic carbon as well as create stable substrates [1]. Despite its growth rate, PO forms spacious, homogenous, and thick meadows, which are extended in waters with a vertical distribution of 5 to 40 m in depth [6]. The export of *Posidonia oceanica* organs from such a meadow is presented in Figure 1.

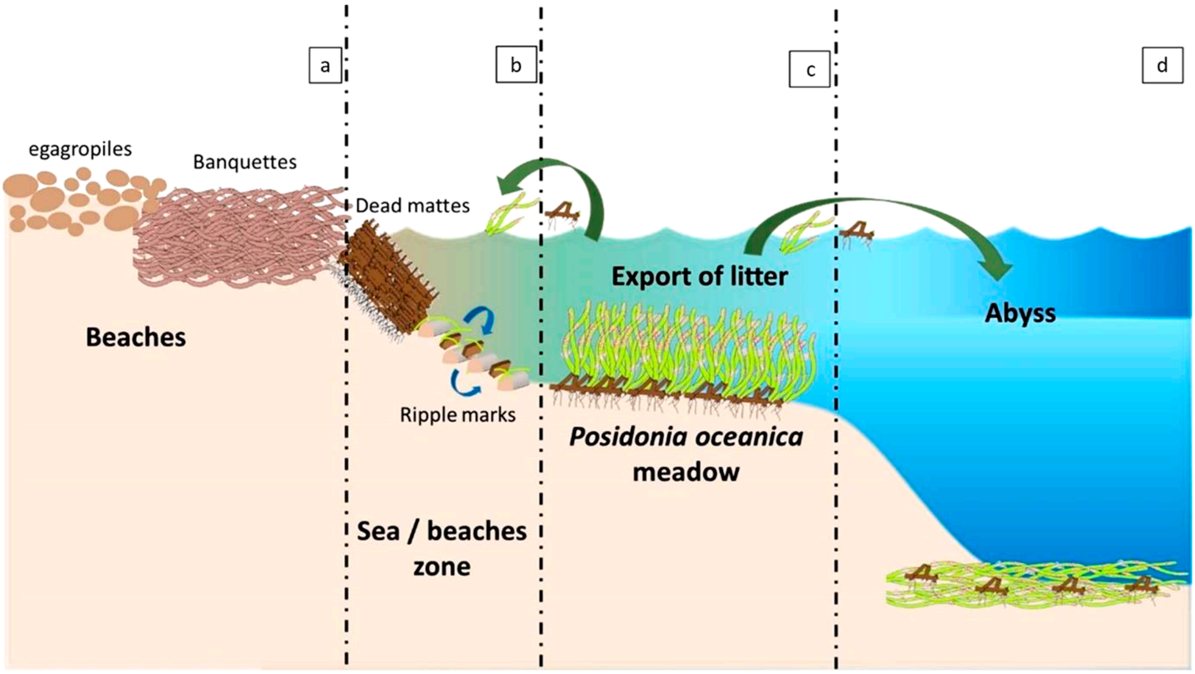

Figure 1. Meadow of *Posidonia oceanica* organs: (**a**) beaches with Egagropiles and banquettes; (**b**) beach zone with dead mattes and meadow organs in ripple marks; (**c**) *Posidonia oceanica* meadow with its coastline being exported to the abyss; and (**d**) the abyss with clusters of various organs of the meadow [7].

PO represents one of the most productive Mediterranean ecosystems. It is noteworthy that PO is frequently used as a suitable biological indicator for the evaluation of water quality and of the environmental situation in a marine habitat [8]. According to formal data, PO had spread across 8% of the Greek coastline until recently, while its distribution was associated with particular regions of the Natura 2000 network [9]. However, based on the latest updated findings, meadows of PO are located in the greatest part of the Greek coastline and shoreline, which is estimated to be around 2619 square kilometers [10].

The ecological value of PO is of paramount importance because it provides a variety of marine environment-related services. Initially, this kind of seaweed constitutes a significant source of diet for plenty of herbivorous species of fish and invertebrates. Additionally, it has the ability to extract biomass from nearby ecosystems of the sub-coastal and peri-coastal zone, either directly through the transportation of disposed non-living leaves or indirectly via benthic organisms [11]. Furthermore, fields of PO are considered a shelter, a reproduction site, and a rich habitat for diverse organisms belonging to the marine flora and fauna [11]. Its leaves discarded and gathered on shores, which are known as "banquettes", function as a natural mooring area, protecting beaches from erosion and minimizing the energy of waves as a result of the increased presence of sand [8]. Another element supporting its contribution to the equilibrium of the ecosystem is its potential to absorb great amounts of carbon dioxide, helping to decrease carbon dioxide levels in the atmosphere and, thereby, combat climate change [12].

Regarding the applicability of PO's biomass, there are studies in recent years that have pointed out how priceless PO is, either as a primary source for the manufacturing of industrial materials or as a substrate for energy production. Posidonian balls, a type of biomass waste of PO, have been successfully used for the isolation of α-cellulose, which can further produce, through chemical reaction, cellulose acetate (CA) and glycidyl methacrylate-grafted cellulose (GMA-C) materials [13]. The former is a synthetic fiber that can be utilized for the production of different commercial products, such as textiles, cigarette filters, eyeglasses, and thermoplastics. It has been found to be a suitable material for the making of membranes used in the filtration of wastewater [13]. Dead seagrass material washed ashore has been tested for its capability to function as a fuel for anaerobic digestion leading to biogas. The results are promising because, in this way, greenhouse gases, such as methane, may become less abundant. The results of a study, which was held in the Istrian beaches of Croatia, show that dead seagrass material washed ashore is a promising source of energy generation via combustion due to its high heating value and its contents of sulfur, nitrogen, and carbon. However, this biomass has two disadvantages as a potential fuel, which, according to the authors, are its high content of ash and its low content of carbon [14]. The revalorization of PO's biomass waste has also been performed since it is considered a good source of low-cost biochar through pyrolysis. Therefore, tons of PO's waste, which are normally displaced every year from beaches, can be employed for the production of biochar, which in turn can give renewable energy and participate in carbon sequestration [15].

Posidonian balls, as a marine waste, are washed away, especially at the shores of the Mediterranean Sea, and remain systematically unexploited until recently despite their high lignin content and the opportunity that they present for an alternative production of bioenergy. Moreover, the mapping of this natural material is considered as not fully characterized, especially in the coastline of Mediterranean islands. The aim of this study is to analyze and visualize the spatial distribution of PO balls (Egagropili) in the Greek Island of Kefalonia, which is among the biggest European islands and is the richest habitat of this particular seagrass. In this study, samples of PO collected from various places of Kefalonia were examined as a candidate origin of biofuel in the context of promising and sustainable green energy solutions. A secondary aim is to provide insights into PO to motivate future research efforts focusing on several issues, such as the definition of available biomass quantities in the Mediterranean Sea, and the evaluation of biogas yield of PO balls on an industrial scale.

2. Research Methods

In the present study, the chemical and thermochemical properties of PO, the possible ways to exploit its energy content using thermochemical methods, and the total cost of such methods were considered and analyzed. PO balls from Kefalonia Island were used as the raw material. The sampling took place during the autumn of 2022.

The methodology consisted of three distinct steps after the initial sampling. In total, 20 kg of samples were collected from various coastal areas of Kefalonia Island (Figure 2). The first step was the quantitative mapping of PO occurrences and the development of a thematic map using ArcGIS Pro v3.0.3.

The second step of the methodology consisted of a mineralogical and microstructural analysis of the samples at the University of Patras. Polished blocks were prepared according to the ISO 7404-2 [16]. The blocks were examined by using a Leica DMRX coal-petrography microscope (under white light and fluorescence mode), with oil-immersion X50 objective and total magnification of X500. The analytical methods were carried out in the Laboratories of the Centre of Research and Technology Hellas (CERTH) in Thessaloniki, which are accredited with ISO 17025.

Figure 2. (**a**,**b**) *Posidonia oceanica* and drying ball samples as the raw material, and (**c**) the samples after washing.

The identification of the mineralogy of the studied samples was performed using X-ray diffraction (XRD) analyses. To prepare the random powder mounts, the samples were pressed gently into the cavity holder. The area that was scanned for bulk mineralogy covered the 2θ intervals ranging from 2 to 70°, and using the DIFFRACplus EVA 12® software (Bruker-AXS, Gmbtl, Karlsruhe, Germany), the mineral phases were determined based on the ICDD Powder Diffraction File of PDF-2 2006.

The third step included physicochemical tests and chemical analysis of the samples. The initial stage of processing of the PO balls was the washing of the samples in order to remove any salts, sand, and other impurities. For the washing, a perforated basket of 1 to 2.5 kg of raw material was weighed before being placed in a container filled with water and stirred for 15 min. Each basket was washed thrice, with additional washing to ensure the efficiency of the process. The next stage of processing was the drying of the samples at 65 °C for 24 h. For the pH measurements, a Crison Basic 20 pH meter (Mettler Toledo, Columbus, OH, USA) was used. This parameter was estimated according to the international norm ISO 10390:2005 and following the European Biochar Certificate [17]. The first method used for the analysis was the measurement of the moisture content of unprocessed raw material at the University of Patras. Four samples were dried at 105 °C for 24 h according to the ASTM D3173-03 standard [18]. For the measurement of the ash content, the dried balls were placed in an oven for 24 h at 700 °C, according to the ASTM D3174-12 standard [19]. During the chemical analysis of the PO balls, a variety of liquids was used for the assessment of the extracts according to commonly used standards. In particular, the following standards were used: hot and cold water (T207 cm-08, 2008), 1% NaOH solubility (T 212 om-07, 2007), and ethanol–tuloene (T204 cm-07, 2007). Sulphur, nitrogen, total carbon, and hydrogen were determined at the same time using dry combustion (Vario Macro CHNS analyzer—Elementar Analysensysteme GmbH, Langenselbold, Germany), following the protocols for determining carbon, hydrogen, nitrogen [20], and sulphur [21]. Then, the concentration of oxygen was estimated by subtracting the sum of the other four elements from 100. The ash content (T211 om-07, 2007) was determined, followed by an analysis of the metal fraction. After drying the PO balls, the raw material was crushed and sieved, thus dividing it into fractions of different grain sizes. Four different sieve sizes were used: $L > 700$ μm, 500 μm $< L <$ 700 μm, 125 μm $< L <$ 500 μm, and $L <$ 125 μm in order to keep the size of $500 > L > 125$ μm. The ash content of each fraction was determined on a dry basis according to the ASTM D3174-12 standard [19], in same way as the case of the raw material. Subsequently, thermogravimetric analysis (TGA) was performed, during which the mass loss of the samples was measured as a function of increasing temperature in a controlled environment. After that, the calorific value of the PO balls was determined according to the standard of the ASTM D240-19 [22], and the volatile content of the samples was determined according to the ASTM D1735-21 [23] standard. It should be noticed that after the test performance, the remaining samples of this study were used as soil conditioners in the courtyard of the institute.

3. Sampling Area

The sampling area was Kefalonia Island, which belongs to the Ionian Islands and lies at the external edge of the Hellenides fold-and-thrust system created in response to the Cenozoic continental collision following the closure of the Tethys Ocean [24–26]. The following geomorphological map illustrates the spatial distribution of PO in Kefalonia Island's coastal region (Figure 3). Specifically, the white line (central part) represents coastal regions with a low distribution, while the yellow (northern and eastern sides) and red lines (southern and western areas) depict regions with a medium and high distribution of *Posidonia oceanica* balls, respectively.

PO waste is usually rejected by the sea as balls or leaves, accumulating in certain and important amounts on the coasts every summer. PO balls are free floating and known as Egagropili, which wash up to nearby shorelines. The regions where increased amounts of PO balls were observed after recording the perimeter of the island and are capable of being an important source of harvest are presented on the map below.

Egagropiles can be found in the sand on the beaches of Kefalonia Island as balls of brown fibers, as shown in Figure 4. To avoid any bias in the sampling procedure that might cause damage to PO by human activity, all samples were selected on a morning in autumn, a day after a storm. Furthermore, only the balls which were found in a distance less than 20 m from the sea were collected, indicating that the ones located farther away from the sea might have been released by a precedent storm.

After the initial mapping of the appearances of PO balls, as shown in the above figures (Figures 3 and 4), particular emphasis was given in the present study to optical observation by utilizing the Earth observation data in order to identify the distribution of meadows where the raw material of Egagropiles is practically suitable for alternative use in the production of biomass.

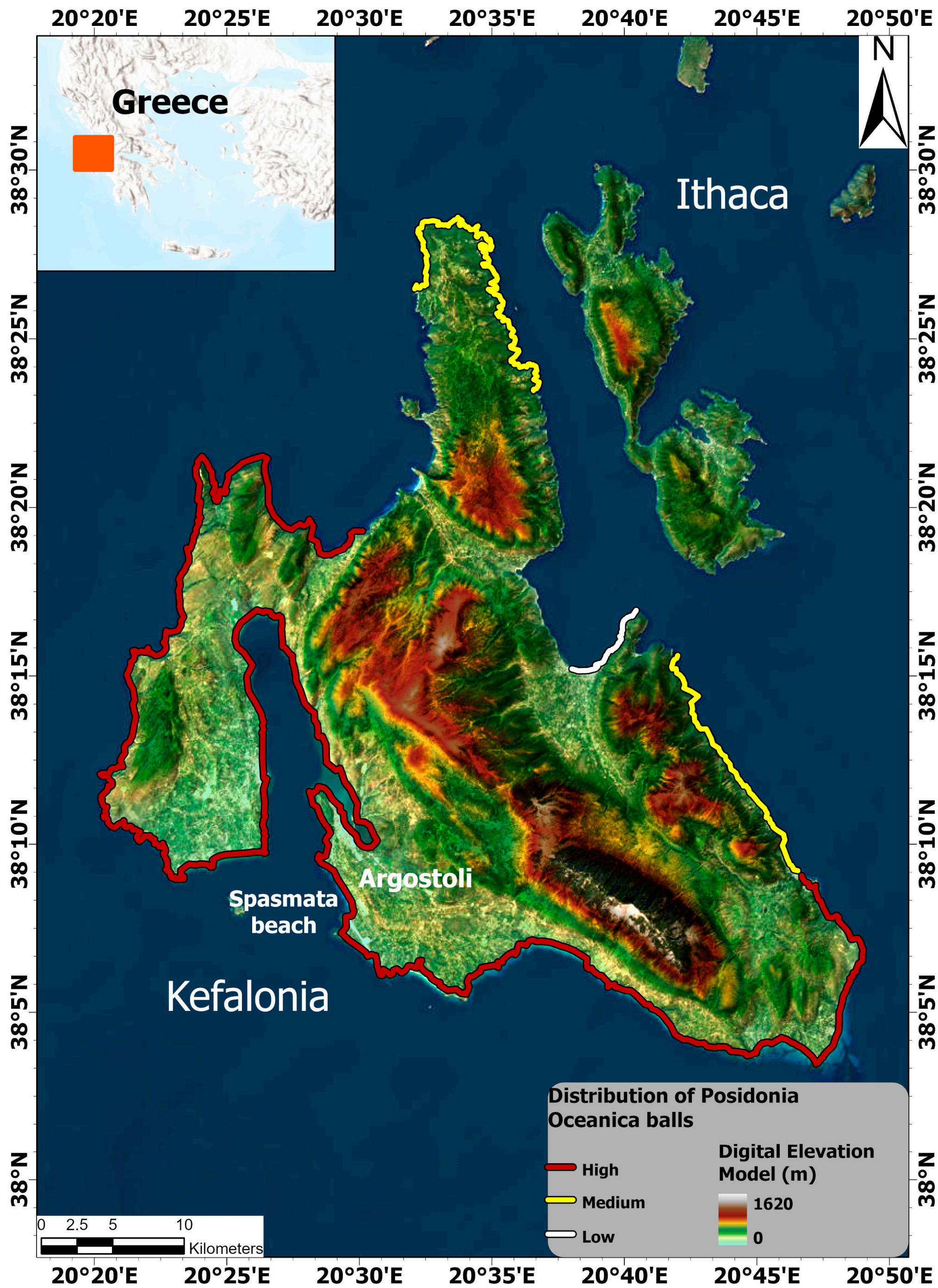

Figure 3. Spatial distribution of *Posidonia oceanica* balls in Kefalonia Island after fieldwork. Source base map: ESRI world imagery.

Figure 4. (**a**–**c**) *Posidonia oceanica* balls in Spasmata Beach (southwest part of Kefalonia Island).

4. Results and Discussion

The use of PO balls (Egagropili) for energy production is under consideration in several countries of the Mediterranean region [14]; however, the region of Greece does not seem to have been sufficiently studied, either in terms of coverage where Egagropili appear, or in terms of the exact qualitative and microscopic characteristics of these balls that are rich in lignin. This is exactly the research gap that is covered by the present study, which is based on the island of Kefalonia, where it studied the basic quality characteristics of Posidonia balls. More specifically, in this study, a detailed characterization of PO balls as potential biomass materials was carried out. The island of Kefalonia was strategically chosen as the study area as it is the largest island of the Ionian Sea and one of the largest islands of the Mediterranean Sea, with a particularly long coastline and particularly strong currents from the Mediterranean Sea, where they support the deposition of PO balls on the shores. Therefore, future exploitation of this material as an eco-biofuel becomes possible as a sustainable solution. For this reason, different fractions of Egagropili were obtained by preliminary washing, de-waxing, heating, and grinding of the starting material. The fact that the present study used Kefalonia Island as a case study does not mean that its results cannot be generalized to other islands of the Mediterranean Sea as it can be seen from the literature data that all Egagropili show similar characteristics but a different distribution, which exactly determines their potential use. Although PO is generally considered as the most important and well-studied seagrass species of the Mediterranean Sea, to date, there has been a limited effort to combine all the available spatial information and provide a complete distribution of meadows across the basin so that it is possible to realize a strategic and sustainable use of this material for energy production. These balls can be considered a renewable resource as they are made of lignocellulosic fibers. As shown in Figure 3, on the largest island of the Ionian Sea, which is also one of the largest islands of Greece, large occurrences are found on the island's coastline, which are capable of being collected in an easy and environmentally friendly way to be utilized as materials that are rich in lignin for various applications, including energy applications. However, after the island mapping, it appears that the southern and eastern parts of the island show an increased concentration compared to the northern and western parts of the island, probably due to the special weather conditions and currents that occur in the area and affect the development or not of PO meadows. In general, the environmental conditions that occur in the Ionian Sea seem to favor the establishment, maintenance, and expansion of Posidonia meadows in coastal areas as they seem to prevail in the southern and western parts of the island of Kefalonia, where sufficient light penetration seems to play an important role, and where there is an appropriate structure of the bottom sediment in relation to the geological background of the island (Figure 3). Moreover, possibly on the northern side of the island, the increased turbidity of the water, as mapped, seems to have a significant effect on the growth of Posidonia meadows and, therefore, on the absence of PO balls on the coasts. The turbidity in the water may also be a result of reckless disposal of urban, agricultural, and industrial wastewater, as well as any other human activity that directly or indirectly increases the turbidity of the water (e.g., fish farms and coastal projects).

4.1. Microstructure of Egagropili Fibers and Nanocrystalline Cellulose

Recent advances in optical, X-ray, and electron imaging tools provide new opportunities for the study of cell walls and other materials used as biomass. This work focused on the study of cellulose's microstructure to determine the final material's performance. According to the combination of the results from the optical microscopic, electron microscopic, and X-ray diffractometry analyses, the structural characteristics of the PO samples can be determined. As it can be seen from Figure 5, the PO balls viewed in parallel to the PO fibers show visible cell structure and strong internal reflections and fluorescence of cellulose and semi-cellulose remnants. Generally, as del Rio et al. [27] has already reported, lignin structure is mainly consisted of three aromatic alcohol monomers, namely pcoumaryl, coniferyl, and sinapyl alcohols, and cellulose is the primary structural component responsible for much of the mechanical strength of the cell wall. The above finding is in accordance with our observations. The distribution as well as the orientation of cellulose microfibrils within the cell wall contribute to the control of cell growth and, hence, to its further behavior as a potential raw material for biomass [14]. Cellulose is an important and most abundant raw material for carbohydrate on Earth, and it also constitutes the most promising source for renewable energy [14]. The important structural properties of cellulose include crystallite shape and size, and crystallinity can be seen in the figures shown below (Figures 5 and 6). Various analytical techniques have been employed to study the structure and assembly of cellulose microfibrils in cell walls, just like in the present study with a combination of optical and electron microscopy. Yet, a comprehensive understanding over multiple length scales remains elusive. Generally, the combination of various techniques to characterize the organization of cell wall components opens the door to the examination of interactions between cellulose and other cell wall polysaccharides, potentially revealing various aspects of cell wall assembly [28]. Regarding the results of this study, as it can be observed from Figure 5, Egagropili does not differ from other lignocellulosic materials which composition is made of carbohydrates, lignin, extractives, and minerals. The three main components, including native cellulose, hemicellulose, and lignin, are always present in different compositions, depending on the natural origin of the material [29].

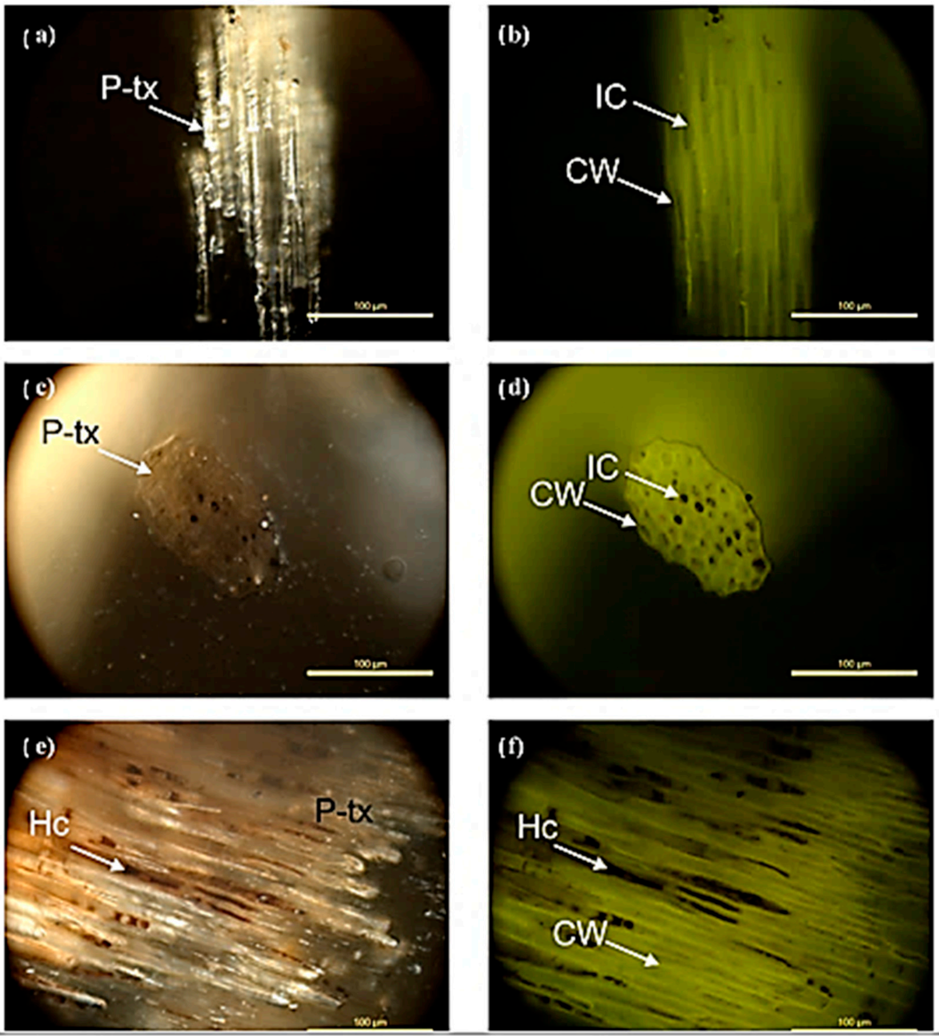

Figure 5. Photomicrographs of Posidonia Oceanic balls taken under white incident light (**a**,**c**,**e**) and blue-light excitation (**b**,**d**,**f**) in oil immersion and total magnification of ×500: (**a**,**b**) view in parallel to the PO fibers showing visible cell structure and strong internal reflections and fluorescence of cellulose and semi-cellulose remnants; (**c**,**d**) view in perpendicular to the PO fibers; and (**e**,**f**) view in parallel to the PO fibers showing visible infilling of the intracellular space with humified non-fluorescing colloids, which have probably originated from the protoplasm (CW: cell-wall, Hc: humic colloids, IC: intracellular space, P-tx: pre-textinite).

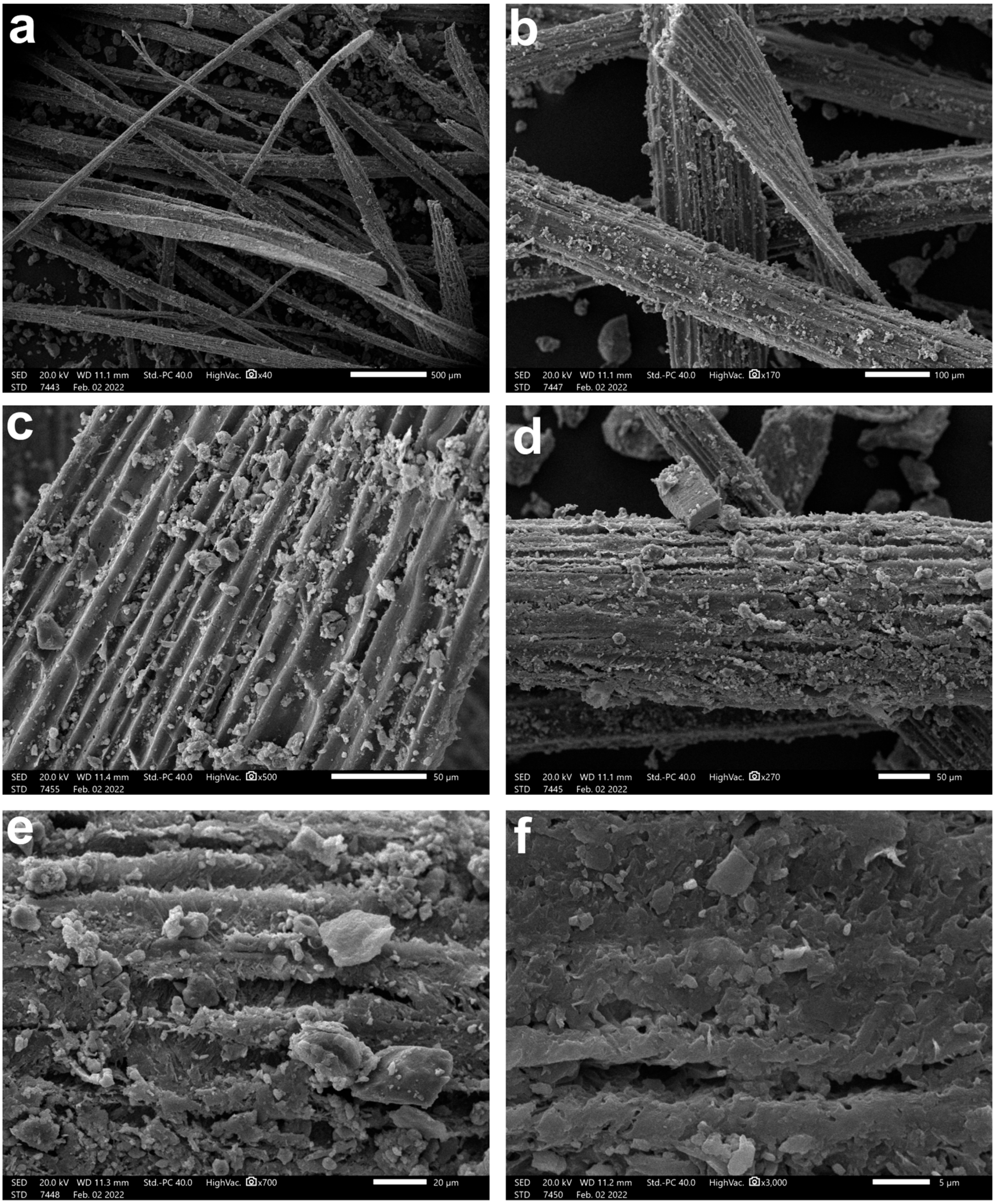

Figure 6. (**a**–**f**) Back-scattered electron images of Posidonia Oceanic balls' morphological aspect.

The SEM method was chosen to describe the structural features and degradation of biomass surfaces at the cellular and nano-resolution levels. In this study, fibers of Egagropili were observed using SEM, as it is shown in Figure 6. The connectivity and mechanical response of the PO balls are relative to the nature of the fiber components. For this reason, we visually analyzed Posidonia fibers using electron microscopy. A characteristic example is presented in Figure 6b, where it can be seen that the fibers are slender objects and have a typical cross section of 100 μm in width. We notice that these fibers are mainly resided at the surface, and their damage might be caused by the action of the surrounding environment. The SEM images showing the surface microstructure of the grinded Egagropili fibers indicate that Egagropili have a typical lignocellulosic fibrous structure, and the determined fiber length is between 1 and 2 mm (Figure 6). The observed agglomeration of some fibers of Egagropili is probably due to, as previously reported, the hydrophilic characteristics of cellulose (Figure 6) [30].

Regarding the study of the XRD patterns (Figure 7), the main minerals, which were identified in the samples, are calcite (Cc) and quartz (Qz), with an amount of 77.8% and 22.2%, respectively. It can be deduced from this figure that all the samples show three main peaks at 2θ = 16.5°, 22.6°, and 34.5°, corresponding to the (110), (200), and (004) crystalline diffraction planes of cellulose type I, respectively. The appearance of the peaks at 2θ = 16.5° and 2θ = 22.6° in all XRD patterns confirms that the cellulose present is of type I. Silicate minerals seem to be responsible for maintaining the rigidity of plant tissues and, nevertheless, are considered to enter into feedstock through endocytosis during the growth stage [31]. The percentages are semi-quantified on the crystalline phases of the *Posidonia oceanica* balls after being grinded in an agate mortar. In the crystalline phase, the cellulose fibers are ordered by inter- and intramolecular hydrogen bonds, while in the non-crystalline phase, the network does not have an organized structure [32]. The differences in the structures determine the different accessibilities of hydroxyl groups during the synthesis of cellulose acetate. The amorphous part consists of random orientation of cellulose chains.

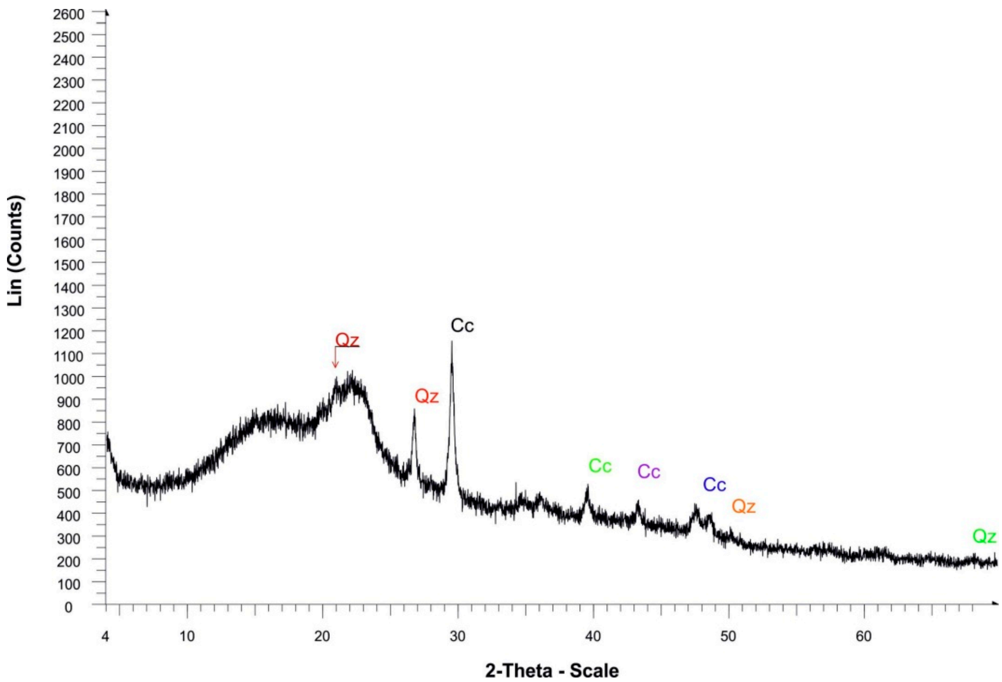

Figure 7. XRD patterns of the Egagropili samples from Kefalonia Island.

The proximate analysis, which typically involves a determination of the moisture content, volatile matter, fixed carbon, and ash content, together with pH, represents the most frequently used method for biomass characterization [33]. The chemical composition of the *Posidonia oceanica* balls derived from Kefalonia Island is presented in Table 1.

Regarding the results of Table 1, similar characteristics are observed among the samples derived from different sampling areas, through which the homogeneity of the materials is indicated. As we can see, in cold and hot water, the quantity of extractives for PO from Kefalonia Island is higher than those found in hardwood and softwood [34], which is particularly encouraging for the use of this material as a sustainable alternative material that is rich in cellulose and, until today, does not find any use; for most people, it is considered a waste, and before the start of each tourist season, it is thrown away without thinking.

Table 1. Chemical composition of *Posidonia oceanica* balls.

	Samples			
Amounts in % (*w*/*w* with Respect to Oven-Dried Raw Materials)	**PO.1**	**PO.2**	**PO.3**	**PO.4**
Cold water extractives	7.0	7.2	7.3	7.2
Hot water extractives	12.0	12.3	12.3	12.4
1% NaOH extractives	15.0	15.2	15.7	16.0
Ethanol–toluene extractives	11.5	11.2	10.9	11.3
Ash	13.0	12.8	12.6	13.3
Lignin	28.0	28.6	28.4	29.0
Holocellulose	60.0	60.2	60.7	61.0
Cellulose	42.0	40.0	40.8	41.6

The 1% NaOH extractives are similar to those of wood sources, i.e., less than 15%, but are lower than those of annual plants. Finally, the number of extractives in ethanol–toluene (≈11.5%) for the raw materials under investigation is relatively high, although in the same order of magnitude as those observed for other annual plants or agricultural crops. Lignin constitutes a unique and quite complex component of wood with a particular structure. It has been reported that softwood species include 27–32% of lignin, hardwood species contain 21–31% of lignin, and in herbaceous plants, it ranges between 0 to 40%. Regarding our results, lignin indirectly presents values that are similar to the aforementioned findings [35–39]. In a similar way, the percentages of holocellulose and cellulose for *Posidonia oceanica* balls were found to be similar to those found in biomass of woody origin, but without the need for any additional energy expenditure as Posidonia balls are deposited in the coastal parts, especially of the Mediterranean Sea, where the temperate climate strongly helps in the production of these Posidonia meadows. Regarding the ash content of the examined samples, it is quite high for *Posidonia oceanica* balls and comparable to many other known woody materials, which present particularly high percentages of ash and have already found use in various energy production applications as biomass. Similar results have been observed in analyses from Tunisia as reported by Khiari et al. [34], which indicates that PO balls in the Mediterranean Sea present similar characteristics to those of this study but they may differ in pH values; this may happen due to variable water quality, which is directly depending on human activities. The energy value of a specific type of biomass depends entirely on the chemical and physical properties of the molecules from which it is composed. The chemical composition of *Posidonia oceanica* balls shows, on the one hand, materials that present homogeneity and, on the other hand, materials of high amount of cellulose, which justifies their valorization in cellulose derivatives or as a source to produce green energy.

Figure 8 below shows the elemental analyses of the *Posidonia oceanica* balls used in this study, as analyzed through a scanning electron microscope (SEM). As it can be determined from the previous analyses of the representative samples, the last images display typical characteristics, and they are rich in organic materials. It is also obvious that silicon and calcium prevail secondarily in the *Posidonia oceanica* balls of Kefalonia. These percentages of silicon and calcium are likely to adversely affect the process of future chemical recovery and, therefore, could be a serious disadvantage. Nevertheless, it should be noted that possible contamination of the raw material with sand is likely to be responsible for this fact as the coastal areas of Kefalonia present mineral raw materials, and such materials, which are rich in calcium and silicon, will produce their corrosion. In Table 2, the pH values, as they have been calculated in the dried samples of PO balls, are given.

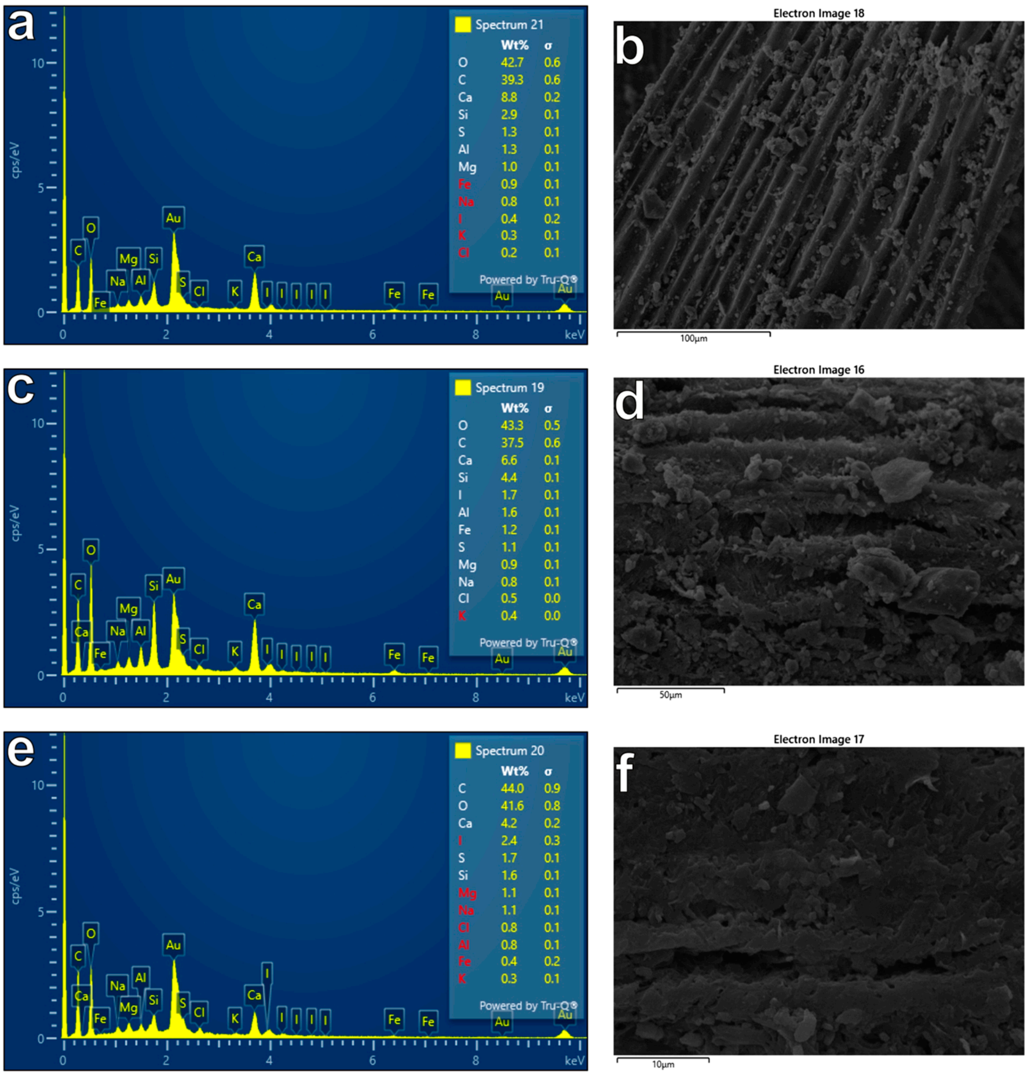

Figure 8. (**a**–**f**) Elemental analyses and back-scattered images (BSEI) of the investigated *Posidonia oceanica* balls.

Table 2. Elemental analyses of *Posidonia oceanica* balls.

N. Analysis/Elements (wt%)	Spectrum 19	Spectrum 20	Spectrum 21	Average
Si	4.4	1.6	2.9	3.0
Ca	6.6	4.2	8.8	6.5
I	1.7	2.4	0.4	1.5
S	1.1	1.7	1.3	1.4
Mg	0.9	1.1	1.0	1.0
Na	0.8	1.1	0.8	0.9
Cl	0.5	0.8	0.2	0.5
Al	1.6	0.8	1.3	1.2
Fe	1.2	0.4	0.9	0.8
K	0.4	0.3	0.3	0.3
C	37.5	44.0	39.3	40.3
O	43.3	41.6	42.7	42.5

The determined pH values in these investigations present a range from 7.38 up to 7.50 in the dried samples. Similar pH values were found by Cocozza et al. [40], who reported a range of 7.9 to 8.3 for PO waste that was collected from the beaches in Southern Italy. The most anaerobic digestion processes operate at a neutral pH. The insignificant fluctuation in pH values also indicates that the raw materials are characterized by high homogeneity and by an absence of contaminations.

The elemental analyses of the PO balls display C, O, Ca, and Si as the main elements (average 40.3 wt%, 42.5 wt%, 6.5 wt%, and 3.0 wt%, respectively) and S, Al, and Mg in minor amounts (average 1.4 wt%, 1.2 wt%, and 1.0 wt%, respectively). Because they occur in a marine environment, they also present I, Na, and K in minor amounts (Table 2).

4.2. Physicochemical Tests, Chemical Analysis, and Thermogenic Tests

Variations from this region are observed perhaps due to the accumulation of basic metabolic products. The optimum value for combined actions is a pH of 6.8–7.5, and if the pH value drops below 6.60, the operation of methanogens is hindered, resulting in the accumulated produced acids not being consumed, thereby further reducing the pH of the reactor where they participate in [41]. Regarding the physical characteristics of the tested PO ball samples, a loss after heating is observed. This loss varies around 50% of the initial volume of the samples (Table 3). The increased percentage of moisture by almost half of their volume is expected precisely because this plant (PO) grows in a marine environment, where it is submerged during its whole life. However, this fact, which shows approximately 50% of moisture content by weight, seems to potentially present a very crucial role in the energy conversion of biomass as the moisture content of a biomass is likely to affect the final calorific value of the material so that it produces thermal energy during its combustion. The result of this increased moisture content is likely to be its lower calorific value, and it is greatly reduced because water absorbs heat energy in order to pass into the gas phase. Based on this property alone, which the PO balls have, it seems that it is not an ideal possible fuel and, thus, its energy utilization only through combustion is not indicated.

Table 3. Cumulative table showing the laboratory tests of the investigated *Posidonia oceanica* balls and dry ash calculation.

Samples	PO.1	PO.2	PO.3	PO.4
pH content	7.50	7.45	7.38	7.40
Moisture content (%)	43.0	45.0	40.0	41.0
Dry ash calculation	10.95	11.20	11.40	11.35

However, with regard to the biochemical processes of biomass production through anaerobic digestion, material rich in lignin with particularly high moisture content is required in order to achieve their efficient conversion into energy; this is in contrast to thermochemical processes (combustion) where high humidity has a negative effect on their energy performance. Gasification, although classified as a thermochemical process, requires some moisture from the raw material as this increases its hydrogen content in the final product (synthesis gas). From the above findings, the investigation was conducted such that the moisture content of the raw material was only half of the amount originally collected. Subsequently, the ash of the now dried raw material was measured in an oven at 700 °C for 24 h. Thus, the percentage of non-combustible substances contained in PO was determined. These results are shown in Table 3.

The ash content measured in the dried raw material ranges between 10.95% and 11.40%. This high percentage of ash is due to the chemical composition of the aquatic environment in which PO grows and possibly the adhesion of sand, even if it had been thoroughly washed before the measurement. The main reason why less ash is desirable has to do with its effect on the energy content of biomass. Regarding moisture, as ash is a way of estimating non-combustible inorganic matter of biomass, it significantly affects its energy content. Mainly, these two parameters are mostly responsible for the energy differences of each biomass: if ash and moisture are not taken into account, then most biomass sources will have similar energy content.

Based on the results of the previous laboratory tests and analyses, the sample PO.1 was considered as the most effective for sieving and thermogenetic measurement, as it is presented below (Table 4). This choice was made to avoid the wasting of both energy and material. In a further stage, the sieving of grounded PO was performed, and the intermediate sample at 500 > L > 125 µm was kept as the most representative sample for its further study regarding ash and calorific value, and its comparison with the homogenized non-sieved sample was also performed.

Table 4. Ash after the sieving of *Posidonia oceanica*.

Grain Size	Sample	Dry Ash
500 > L > 125 µm	PO.1	9.30
	PO.2	10.41
	PO.3	10.39
	PO.4	10.10

Carbon, nitrogen, and oxygen are the main components of solid fuels. Carbon and oxygen react during combustion in an exothermic reaction, generating CO_2 and H_2O; thus, they contribute in a positive way to the fuel's HHV and the combustion process itself. Table 5 below shows the ultimate analysis of the investigated PO biomass.

Table 5. Ultimate analysis of PO biomass.

Sample	C (% db)	H (% db)	S (% db)	N (% db)	O (% db)
Sample before sieving	28.33	3.81	1.13	1.72	58.21
500 > L > 125 µm	30.45	4.02	1.16	1.90	59.84

In the next stage, measurements were carried out using the TGA method on the PO samples. Figure 9 below shows the mass loss (TG) that occurred during the pyrolysis of PO in an inert atmosphere and at a heating rate of 5 °C/min. The diagram shows well-separated areas for the release of moisture content, the agglomeration stage, and the char formation stage. In particular, the PO DTG shows an elevation peak at low temperatures. Its composition, as mentioned above through the microscopic and chemical analyses, is in agreement with the DTG curve, where the main thermal degradation corresponds to the decomposition of cellulose.

Regarding combustion (Figure 9), there is a first stage of weight loss even in the sieved or non-sieved sample, which corresponds to the sample's moisture loss. The thermal stability of the different extracts was evaluated by means of TGA, and the results are shown in Figure 9. In the TGA diagram, the peaks detected within the range of 100 to 260 °C correspond to the dehydration processes and are extracts, given their more hydrophilic character. In this phase, the organic macromolecular chains are broken, leading to the formation of smaller molecules. When in contact with oxygen at a temperature above their flash point, volatilized gases rapidly oxidize and burn. A sharper rise is observed at 120 °C, while a broad peak is located at ~260 °C, which may arise from the degradation of carbohydrates in both the samples before and after sieving. This is similar to the report by Izquierdo et al. [42]. Moreover, it can be observed that up to 500 °C, there is a smoother and almost linear decrease in mass as a function of temperature. Above 500 °C, the resulting mass fraction corresponds to the oxidation of the carbonaceous residues formed or the biochar. The difference in grain size between samples S1 and S2 is also reflected in the diagram of Figure 9 and appears to play a decisive role in the rate of mass loss per time as a function of temperature. However, after 200 °C, the samples show a similar trend in mass loss. The biomass pyrolysis results are in accordance with the results of the other laboratory tests, which have characterized the tested PO as biomass. It should also be mentioned that it is difficult to identify lignin decomposition as it decomposes over a wide range of temperatures.

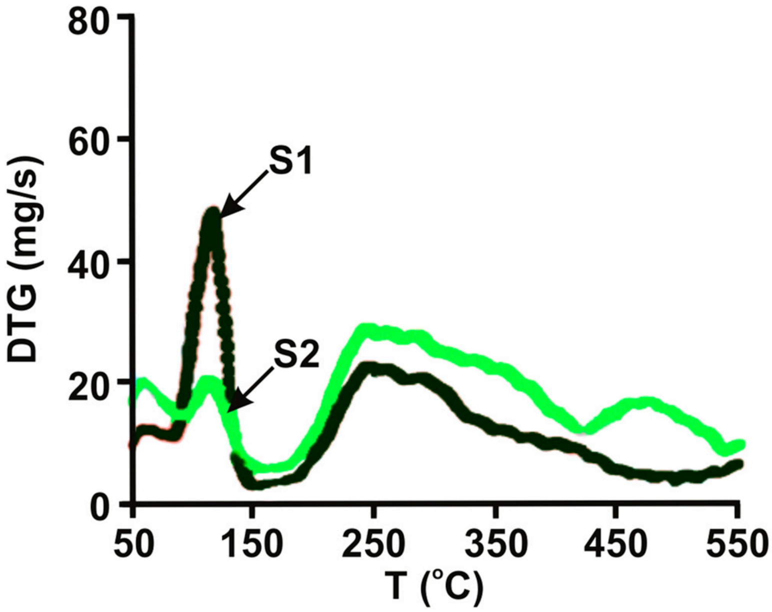

Figure 9. Thermogravimetric TGA analysis of Posidonia samples of various fractions: single sample before and after sieving (500 > L > 125 μm).

In fact, a previous study reported that hemicelluloses and celluloses contained in PO biomass show degradation processes, with their maximum located at 260 °C and 334 °C, respectively [43]. According to Órfão et al. [44], the phenomenon of mass loss in the first phase was mainly caused by the combination of the total decomposition of hemicellulose and partially by the decomposition of lignin, while in the second phase, the mass loss was a consequence of the decomposition of the remaining lignin and the ignition of carbon residues [34,45]. As for the use of this material as a biomass material, even before and after sieving, it is considered to be suitable for use as a biomass.

The results of the measurement of the calorific value and volatile samples of PO of different fractions are listed in Table 6 below.

Table 6. Thermogenic measurement of *Posedonia oceanica*.

Sample	GCV (cal/g)	Volatile (wt%)
Sample before sieving	3532.22	81.02
500 > L > 125 μm	3438.65	79.01

According to the measurements, the lower calorific value of PO ranges from 3438 to 3532 cal/g for the sample before and after sieving, and it is comparable to many other types of biomasses. The obtained results show that the heating value of the dried samples is high, while there are no significant variations by location. The heating values for the onshore PO biomass samples are within the range of main agricultural and forest biomasses. Some examples of heating values are as follows: heating value of plant residues is 5.8–16.7 MJ/kg, the value of wood is 8.2–18.7 MJ/kg, the value of rice husk is 14.850 MJ/kg, the value of rice straw is 14.693 MJ/kg, the value of bagasse is 13.356 MJ/kg, the value of sweet sorghum bagasse is 13.305 MJ/kg, and the value of bamboo dust is 14.728 MJ/kg [46]; these findings demonstrate that the heating value of PO leaves is within the range of lower average values of some biomasses. The lower calorific value of Posidonia, according to the measurements carried out, was found to be approximately equal to 3530 cal/g. This is a satisfactory and acceptable value that is at the same level as the calorific value of several other types of biomasses. Even though any thermochemical method could be used to produce energy by *Posidonia oceanica* balls, its high potential volatile content makes it suitable for gasification since most of the fuel's energy is rapidly released in the gas phase. To summarize, it can be said that from the point of view of thermochemical and chemical characteristics, Posidonia is a very interesting case of biomass which possible exploitation would provide many benefits. So far, there have been no significant steps in this direction, while at the same time, the study of the properties of this seagrass has not been studied/determined in depth. It is noteworthy that unlike many other types of biomasses, the literature on *Posidonia oceanica* is scarce. Further study and experimental measurements would be desirable in order to be able to draw safer and more complete conclusions. This fact highlights the important role of this "waste" of the coasts as an alternative sustainable source of energy. Just as olive core is used in many places, mainly in the Mediterranean region, PO balls can be an important attractive alternative source of energy in western Greece and especially in Kefalonia Island, where there are increased concentrations. More specifically, the physical, chemical, and microscopic parameters show that the fibrous part of PO residues can be used as biofuel due to its lower calorific value, which ranges between 3438 and 3732 cal/g. PO has been recognized by other scientists as a valuable feedstock for thermo-chemical processes, including combustion, gasification, and pyrolysis [47]. Based on our primarily results, the sustainable use of PO balls as an alternative source for energy production could be proposed, even though extended study is necessary for the validation of their use.

5. Conclusions

The most crucial findings of this eco-friendly research to find a pathway for a potential alternative energy source can be summarized by the following points:

- The southern and western parts of the island show a higher concentration in terms of *Posidonia oceanica* balls than the northern and eastern parts, probably due to the turbidity of water that occurs in Kefalonia Island.
- Based on the microscopical study, cellulose is the characteristic structural component responsible for the increased mechanical behavior of the cell wall observed.
- Egagropili present similarities in microscopic characteristics to other lignocellulosic materials which composition is made of carbonates, lignin, extractives, and minerals.
- All of the samples of *Posidonia oceanica* balls of the island show similar chemical characteristics, a fact that indicates a homogeneous material.
- The ash content of the Kefalonia samples is similar to that of others in the Mediterranean Sea region.
- The insignificant fluctuation in pH values indicates homogeneous raw materials, which are also characterized by an absence of contaminations.
- Ash and moisture content constitute the two critical parameters that are responsible for the energy differences of each biomass.
- According to the findings of this research and mainly according to the thermogenetic analysis, the tested *Posidonia oceanica* balls derived from Kefalonia island are considered a potential alternative energy source.

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