



Deashing macroalgae biomass by pulsed electric field treatment

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ABSTRACT

Among all biomass constituents, the ashes are major hurdles for biomass processing. Ashes currently have low market value and can make a non-negligible fraction of the biomass dry weight significantly impacting its further processing by degrading equipment, lowering process yield, inhibiting reactions and decreasing products qualities. However, most of the current treatments for deashing biomass are of poor efficiency or industrial relevance. This work is the first report on the use of Pulsed Electric Field (PEF) to enhance deashing of biomass from a high ash content green marine macroalga, *Ulva* sp., using hydraulic pressing. By inducing cell permeabilization of the fresh biomass, PEF was able to enhance the ash extraction from 18.4% (non-treated control) to 37.4% of the total ash content in average, significantly enhancing the extraction of five of the major ash elements (K, Mg, Na, P and S) compared to pressing alone.

1. Introduction

Fossil resources have driven the economy throughout the world. However, the limits of fossil-based economies are now obvious. Indeed, environmental consequences, depletion of Earth's fossil resource and unbalanced geographical repartition have been of growing concern in the last three decades and most countries are now switching to alternative sources of energy (Kircher, 2012). Among those alternatives, biomass is expected to play a key role in the transition from a fossil-based economy to sustainable ones (Scarlat et al., 2015). Numerous efforts are already deployed to build and support a biomass industry, notably through feedstock improvement, better supply chain, new applications, and products, or new processes. However, many challenges still remain in both upstream biomass processing, for example feedstock growth, transport or storage (Shastri and Ting, 2014), and downstream biomass processing, for example pre-treatment, pyrolysis, gasification, fermentation or extraction (Asadullah, 2014; Himmel et al., 2007; Jahirul et al., 2012; Jönsson et al., 2013; Wyman, 1999). Most downstream processing challenges arise from the fact that biomass constituents are difficult to fractionate. Therefore, the share of constituents of interest in any biomass feedstock for a particular process – for example, sugar or polysaccharide for ethanol fermentation – is a crucial parameter on the overall efficiency of such process.

Removing non-target constituents, which would often become feedstock of another processing unit, improves the final yield of the target product and prevents those unnecessary substances to disturb the

process. Ash content of biomass is a good example of such hurdle. “Ash” refers to the residue left after the combustion of a biomass sample at medium temperature (usually 400–600 °C) that removes organic matter (C, H, O & N). Today ash fraction has small value compared to other biomass constituents, such as sugar, protein, lipid, pigments, phenolic compounds, etc., and is also known to significantly affect various biomass-processing operations, notably thermal treatments such as pyrolysis, gasification, combustion, or hydrothermal treatment that are sensitive to ash content, which leads to equipment degradation and lower product quality (Asadieraghi and Wan Daud, 2014; Asadullah, 2014; Huang et al., 2016; Pattiya et al., 2013; Stefanidis et al., 2015; Wang et al., 2015; Yuan et al., 2017). Moreover, ash can also disturb enzymatic treatment and fermentation (He et al., 2014; Jönsson et al., 2013; Kang et al., 2011; Maiorella et al., 1984).

To address these destructing effects of ash, various deashing pre-treatments have been investigated. Washing biomass with water (Das et al., 2004; Huang et al., 2016; Liaw and Wu, 2013; Pattiya et al., 2013) or various chemical solutions, for example acid, alkali, ethanol (He et al., 2014; Pattiya et al., 2013; Pottathil et al., 2012; Stefanidis et al., 2015) have been proposed. More advanced technologies such as an ion-exchange resin (Alexandratos, 2009), ion-exchange membrane (Tanaka, 2015) or electrodialysis (Jansen and Baiada, 2007) can be used to remove ash from the liquid stream. However, their direct applications on solid biomass have not been reported as far as we know. Anyways, water washing used a considerable amount of water (several liters per kg of biomass) and has a limited efficiency (Liaw and Wu,

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2013; Pattiya et al., 2013). Therefore the use of acid or alkali solution instead of simple water has been investigated and shown to significantly increase the removal of ash (Pattiya et al., 2013) but it usually requires a subsequent neutralization step, which requires an additional amount of water and/or chemicals. Overall, considerable liquid waste streams are produced during the washing treatment and subsequent neutralization step that require being dealt with afterward, making chemical washing treatment difficult and environment-unfriendly. In addition, compared to freshwater treatments, handling of considerable volumes of strong acid and alkali solutions in industrial facilities is more expensive and requires specific installations and safety policies. Therefore, more efficient and greener alternatives for biomass deashing have yet to be found.

Although a considerable amount of ash can be found on the surface of the biomass, various biomass contains a high level of intracellular ashes that are even harder to remove by washing. We hypothesized that breaking the cell membrane barrier would help to remove recalcitrant ash elements. For that purpose, we investigated the use of pulsed electric field treatment for biomass deashing, which hasn't been reported in the literature yet. Pulsed-electric field (PEF) treatment is an emerging, non-thermal food processing technology already used for energy-efficient extraction of various molecules including water, sugar and proteins from a broad range of biomass feedstock (microalgae, macroalgae bacteria and plants, etc.) (Goettel et al., 2013; Golberg et al., 2016; Haberl et al., 2013; Polikovskiy et al., 2016; Robin and Golberg, 2016). It consists of applying short microsecond pulses of high voltage at high frequency, leading to biological tissue permeabilization (Kotnik et al., 2012). Although the exact mechanism of biological tissue permeabilization by PEF is not fully understood, PEF technology is currently used in multiple medical and biotechnological applications (Haberl et al., 2013; Kotnik et al., 2015; Yarmush et al., 2014). The current theory suggests that the membrane permeabilization is achieved through the formation of aqueous pores on the cell membrane, a phenomenon known as electroporation (Fig. 1a) (Kotnik et al., 2012). In recent years significant advances in the industrial scale PEF system have enabled the large-scale use of this process for food and biomass processing. For example, in the sugar industry, it led to saving up to 50% of the downstream energy investment in the process (Sack et al., 2010).

Encouraged by the low-energy consumption and scalability of the technology (Kotnik et al., 2015) we designed a series of experiments using PEF treatment coupled to a hydraulic press to remove ash from seaweed biomass. Seaweed biomass is an alternative to land-based biomass as a future feedstock for the production of energy, food and other valuable products (Lehahn et al., 2016). We also chose seaweed for its high ash content, much higher than most land-based biomass, which is currently a major hurdle for its use in energy production (Milledge and Harvey, 2016) or as animal feed (Makkar et al., 2016).

2. Materials & methods

2.1. Algal biomass

The biomass source used in this study corresponded to the green macroalgal species of the *Ulva*, recently identified as *U. rigida*, a seaweed of worldwide distribution and found in the intertidal and shallow waters within the Israeli Mediterranean Sea shores (Fig. 1a). For the current study, algae were taken from an outdoor seaweed collection at Israel Oceanographic & Limnological Research, Haifa, (IOLR) Israel. The seaweeds were cultivated in 600 L tanks supplied with running seawater, aeration and weekly additions of 1 mM NH_4Cl and 0.1 mM NaH_2PO_4 (Friedlander, 2008).

With each nutrient addition, the water exchange was stopped for 24 h to allow for their absorption. About 3.0 kg of fresh *U. rigida* were packed in a sealed plastic bag and delivered to Karlsruhe Institute of Technology reaching the destination point within 48 h. Upon arrival,

the seaweeds were quickly immersed for 2 days in a 400 L aquarium filled with water with a salt concentration of 3.5%.

2.2. Pulsed electric field treatment

The fresh *Ulva rigida* biomass was centrifuged using a manual kitchen centrifuge for 3 runs of 1 min each to remove the external water, so that < 1g of water has been removed during the third run. About 140g of *U. rigida* was loaded into the PEF treatment chamber with a volume of 232 cm^3 for the application of a homogeneously distributed pulsed electric field. The distance between the electrodes is 70.3 mm. Deionised water was added to the macroalgae to fill the chamber completely (< 100 mL). Pressurizing the biomass in the chamber with water allowed to avoid the formation of air bubbles that could lead to malfunction. The treatment parameters were: charging voltage (0–50 kV) and pulses numbers (0–50) delivered at 0.5 Hz. The pulse duration was measured from the voltage measurement across the chamber (see below) and was defined as the length of time between the beginning of the pulse and when the voltage reaches the half-time amplitude. Duration varied between 4 and 6 μs (Fig. 2a). The temperature was measured with a digital thermometer (TFA Type 30.1018). Current and voltage across the electrodes of the treatment chamber during each pulse were measured with a current probe (PEARSON 110 A) and a voltage divider (HILO-Test HVT 240 RCR), both connected to an oscilloscope (Tektronix TDS 640A). The resistance of the treated sample was derived from the current and voltage measurements with Ohm's law.

The total energy consumed for the PEF treatment was calculated based on the energy stored in the pulse capacitor with the following Eq. (1):

$$E_t = 0.5 \times C \times (V)^2 \times N \quad (1)$$

where E_t (J) is the total energy consumed for the treatment, C is the discharging capacitor capacitance (F), V (V) is the applied voltage and N is the total number of pulses. Additional losses of the capacitor charger have not been considered. All combinations of charging voltage and number of pulses were applied on at least two replicates.

2.3. Mechanical extraction of internal cell content with ash

The PEF-treated biomass was placed in cloth material that was folded to prevent the algae from escaping during pressing. The algae wrapped up in the fabric were placed in the mechanical press (HAPA type SPM 2.5S). A force of 45 daN cm^{-2} was applied for a determined time of 5 min using the automatic mode of the press that keeps the pressure applied to the piston constant. Extracted juice (called "juice") from pressing was collected in a 2L beaker and weighted at the end of the pressing. The pressed material (called: "press-cake") was taken out of the press and weighted. Juices were collected and kept at -20°C . Cakes were spread on a plate and dried at 40°C during 24 h, then kept at 5°C . Aliquot of the dried samples were then ground using liquid nitrogen, mortar, and pestle and then kept at -20°C until analysis.

2.4. Dry matter and ash content measurements

Juices samples (15 ± 0.5 mL) and press-cake (0.5 ± 0.01 g) press-cake powder were weighted (m1 = mass sample + crucible) and then dried at 105°C using the conventional oven for 24 h in pre-weighted clean crucibles (m2 = mass crucible). The crucibles were cooled down in a desiccator, weighted (m3), and ignited at 550°C for 3 h in a muffle furnace (Thermolyne muffle furnace, Thermo Scientific) and then cooled down to 105°C . The crucibles were finally removed from the furnace, kept in a desiccator to cool them down at room temperature and weighted (m4). The analysis was done in triplicate. Dry matter and ash content were calculated as shown in Eqs. (2) and (3).

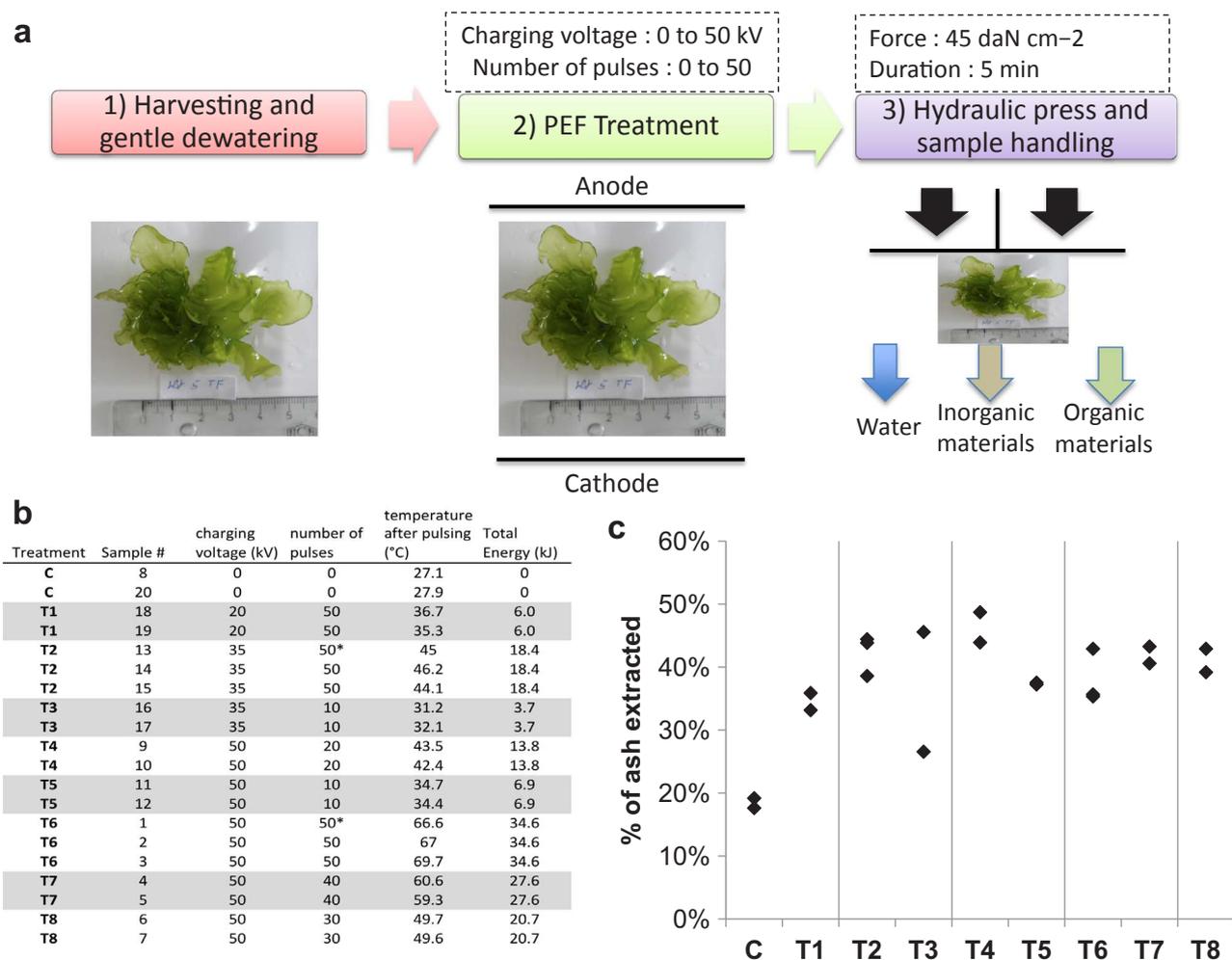


Fig. 1. a Seaweed biomass processing steps for deashing by pulsed electric treatment and hydraulic press. b. Description of the different pulsed electric field treatments applied. c. Amount of ash extracted by mechanical pressing with and without PEF pretreatment.

$$\text{dry matter content (\%)} = \frac{(m3-m2)}{(m1-m2)} \quad (2)$$

$$\text{ash content (\%)} = \frac{(m4-m2)}{(m3-m2)} \quad (3)$$

2.5. Element analysis of ash with inductively coupled plasma – Optical emission spectrometry (ICP-OES)

A batch of ash sample (100 mg) was digested in 5 mL of HNO₃ 65%. Digestion was carried out in quartz vessels using a “Discover” sample digestion system at high temperature and pressure (CEM, USA). Vessels were cooled down and the volume was made up to 14 mL of deionized water. The samples were not dissolved completely but we assumed that the concentration of the elements was not impacted.

Element concentration was measured in the clear solutions using an axial ICP-OES model ‘ARCOS’ from Spectro GMBH, Germany. Measurements were calibrated with standards for ICP from Merck, Germany. Element concentrations that exceeded the linear dynamic range were diluted and reanalyzed. Dilution was made using calibrated pipettes. The continuing calibration verification standard was measured to check the instrument stability. Analyses were performed in the Interdepartmental Equipment Unit of the Hebrew University of Jerusalem in Rehovot, Israel.

2.6. Chlorine analyses Cl analyzer

The aliquot of each press cake sample (about 250 mg) was mixed with 25 mL of deionized water in the polypropylene flask and then shaken at 250 rpm during 30 min. Then the sample was centrifuged (5000 rpm, 5 min) and Cl ion concentration was determined by “MK II Chloride Analyzer 926”, Sherwood, UK. Liquid juices sample were directly analyzed after appropriate dilution. Analyses were performed in the Interdepartmental Equipment Unit of the Hebrew University of Jerusalem in Rehovot, Israel.

2.7. Statistics

The results were analyzed by one-way analysis of variance (ANOVA) with significance level $\alpha = 0.05$ followed, when applicable, with Tukey’s multiple comparison tests. In some cases, Student’s *t*-test was used instead of ANOVA when comparing only two treatments for each variable.

3. Results & discussion

3.1. PEF ash extraction process parameters

To assess the effect of PEF on the deashing of seaweed biomass, the following steps were applied to the fresh seaweed biomass stored in salt water in an aquarium: harvesting, gentle dewatering, PEF treatment, hydraulic pressing and storage of the liquid extracts and the residual

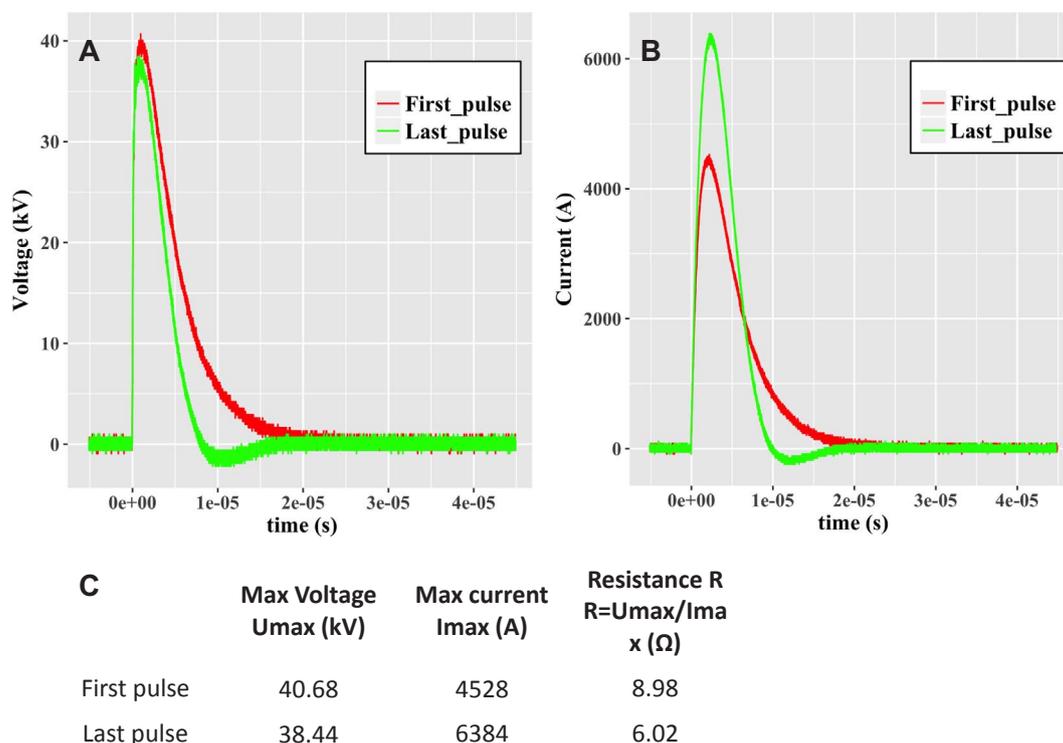


Fig. 2. a. Variation of the voltage during first and last pulses across the chamber. (T1, 50 pulses of charging voltage 50 kV). b. Variation of the current during first and last pulses across the chamber. c. Evolution of the maximum voltage, current, and resistance of the chamber between the first and the last pulses.

biomass (Fig. 1a). Samples only differed by the intensity of the PEF treatment applied in the chamber. In total, 8 different treatments were applied to different combinations of charging voltage (10, 20, 35 or 50 kV) and a number of pulses (10, 20, 30, 40 and 50). A 9th treatment where no electric field was applied (0 kV, 0 pulses) served as control. Each treatment was replicated at least twice (Fig. 1b). As the control experiments were also put in deionized water, the osmotic shock that occurred was taken into account and, therefore, we could attribute any difference only to the PEF treatment.

First differences between treated samples and controls were observed during handling as they were hotter, sticky, and the juice obtained after pressing had a more intense greenish color. The initial temperature was around 27–28 °C and the final temperature was higher for all PEF treatments (31–70 °C, Fig. 1b). Part of the energy applied during PEF treatment is known to be released as heat under the Joule effect (Raso et al., 2016). The intensity of the Joule effect is obviously directly linked to the severity of the treatment, the higher the applied voltage and the number of pulses, the higher the energy released by Joule effect and thus the higher the final temperature. This, however, needs to be kept in mind while analyzing results, as the temperature is a key parameter of many physicochemical phenomena such as molecule diffusion in a liquid matrix (Vorobiev and Lebovka, 2009).

The shapes of pulses obtained from voltage measurement at the chamber were mainly due to the design of the pulse generator (sparkgap, etc.). However, a decrease in measured voltage, as well as slight differences in pulse shape, were monitored throughout the treatments. Those differences were maximum between the first and last pulses of each treatment as seen in Fig. 2a. In parallel with the voltage slightly decreasing, an increase in the current in the chamber was observed as well (Fig. 2c). This phenomenon is linked to the decreasing of the resistance inside the chamber as intracellular components such as ions are released during electroporation leading to decrease of the resistance inside the chamber. This observation is commonly observed during electroporation (Bluhm and Sack, 2008; Polikovskiy et al., 2016). Drops between initial and final resistances for all treatments were in the range of 2–7 Ω (Fig. 2c). The voltage amplitudes measured at the

chamber were smaller than the charging voltage set on the pulse generator, this is caused by the inductance of the capacitor discharging circuit and is a common characteristic of capacitor discharge circuits and is due to the design of the pulse generator as well as the resistance of the chamber (Raso et al., 2016).

At all applied electric fields (from 2 to 6 kV/cm), the biomass was permeabilized, which is confirmed by resistance decreasing during the treatment. Electroporation of seaweed cell in such condition is expected as the threshold to induce permeabilization of the membrane is linked to the cell size with the usual following correlation: the bigger the cell, the lower the voltage threshold (Kotnik et al., 2012). In the conditions of our experiments, we were above that threshold, and thus this could expect the relatively similar results obtained for most applied treatments. For the more severe treatments (higher number of pulses and/or higher charging voltage), the extra-heating from the Joule effect could have led to differences between the 8 treatments, notably due to thermo-induced damages or the quicker diffusion of molecule out of the seaweed matrix, however, the hydraulic press step would have made this observation hard to monitor.

3.2. Dry matter and ash contents analysis of liquid PEF extracts and residual biomass

The average dry matter content of liquid extracts (reflecting the amount of materials extracted) was 3.8% (min = 2.9% and max = 5.7% for T2 (35 pulses of 50 kV) and T6 (50 pulses of 50 kV) respectively, Table 1), of the juice weight for PEF-treated samples compared to 2% for the control samples (min = 1.7%, max = 2.3%, Table 1). Of this dry matter, a considerable amount was found to be ashes (82.5 ± 3.0% for controls and 72.1 ± 1.2% for PEF-treated samples, the difference was not significant). The ash contents per mass of juice were 2.76 (min = 2.07%; max = 3.94%, for T2 (35 pulses of 50 kV) and T6 (50 pulses of 50 kV, respectively)) and 1.65% (min = 1.48%, max = 1.82) for PEF-treated samples and control respectively (see Table 1). ANOVA analysis showed that there were significant differences in both dry matter and ash content (ANOVA P-

Table 1

Dry matter and ash contents of liquid extract and residual biomass of the different samples and treatments. (FW = fresh weight, DM = dry matter).

Sample n°	Treatment n°	Liquid extract			Residual biomass
		DM	Ash (%FW)	Ash (%DM)	Ash (%DM)
1	T6	5.75%	3.94%	68.50%	19.17%
2	T6	3.75%	2.66%	70.81%	21.74%
3	T6	4.46%	3.15%	70.78%	23.17%
4	T7	2.93%	2.11%	71.98%	17.81%
5	T7	3.74%	2.71%	72.55%	18.87%
6	T8	3.38%	2.49%	73.77%	20.26%
7	T8	3.24%	2.32%	71.69%	21.23%
8	C	2.26%	1.82%	80.38%	25.75%
9	T4	3.58%	2.60%	72.45%	20.57%
10	T4	3.69%	2.66%	72.12%	18.75%
11	T5	4.27%	3.12%	73.13%	20.41%
12	T5	4.04%	2.87%	71.17%	22.82%
13	T2	3.77%	2.73%	72.36%	20.41%
14	T2	2.88%	2.10%	72.93%	18.68%
15	T2	2.87%	2.07%	72.20%	18.80%
16	T3	3.46%	2.51%	72.43%	19.58%
17	T3	4.99%	3.57%	71.47%	24.88%
18	T1	4.46%	3.25%	72.98%	22.81%
19	T1	3.94%	2.86%	72.41%	21.36%
20	C	1.74%	1.48%	84.69%	26.45%

value = 0.028 and 0.00004 respectively). Subsequent post hoc tests (pair-wise test) demonstrate that most PEF treatments had significantly different dry matter and ash contents (but 3 treatments: T2, T7 (40 pulses of 50 kV) and T8 (30 pulses of 50 kV)) compared to control experiments. Interestingly, PEF treatments were not statistically different from each other for both dry and ash content with the exception of the results of treatment T6, which had significantly different dry matter and ash contents compared to the treatments T2, T7, and T8. In other words, the PEF-assisted extraction process extracted more ash into the liquid juice compared to pressing alone in a significant manner in 5 over 8 treatments. And if a relatively wide range of results was found between the dry matter and the ash content of liquid extracts of the different PEF-treated samples (Table 1), only a few were significantly different from others.

This was confirmed by the analysis of the ash remaining in the residual biomass. The average ash contents of the residual biomass were 20.63% (min = 17.81, max = 24.88, for T7 (40 pulses of 50 kV) and T3 (10 pulses of 35 kV), respectively) for the PEF-treated samples and 26.1% (min = 25.75, max = 26.45) for the control (see Table 1). Significant differences among the different treatments were found following ANOVA analysis on the ash content of the residual biomass (p-value = 0.023). The following pair-wise comparison confirms that the ash content of residual biomass of all PEF-treated groups was significantly different from the ash content of the residual biomass of the control group. Once again, no significant differences were found between the ash content of the residual biomass of the different PEF-treated samples (but for T3 and T1 (50 pulses of 20 kV) with T7).

Fig. 3a and c show the effect of a different number of pulses applied with the same charging voltage (50 kV) on dry matter and ash content of the liquid extract (3a) and the ash remaining in the residual biomass (3c). Fig. 4b and d show dry matter and ash content of the liquid extract (4b) and the ash remaining in the residual biomass (4d) for treatments of 50 pulses of the different charging voltage. There was no visible trend showing an improvement of ash extraction with treatment severity (increase in a number of pulses and/or charging voltage). Moreover, the profile of the ash contents of the liquid extracts was not visibly correlated with the profile of the amount of ash in the residual biomass of the same samples, we, therefore, believe that this difference between treatments could be attributed to the process conditions, the recovery and the storage of samples.

Total ash content of the initial materials was calculated from the

sum of ash extracted plus the remaining ash, the average value was $29 \pm 0.1\%$ of the dry matter. From those values, the fractions of ash extracted through the applied process were showed in Fig. 1c. For the control experiment, < 20% of the initial ash was extracted ($18.4 \pm 1.1\%$), whereas, for PEF-treated samples, between 27 and 49% of the initial ash were extracted with an average value of $39.7 \pm 5.3\%$, which is a twofold increase.

We showed, for the first time, that PEF could enhance ash extraction directly from freshly harvested biomass when coupled with mechanical pressing. When an electric field was applied, permeabilization of the cell helped the extraction of ash by hydraulic pressing leading to a twofold increase compared to pressing alone as mentioned above. No major differences were observed between the different PEF treatment conditions thus we advise using the treatment with the lowest energy expenditure (Fig. 1b) in order to reduce energy expenditure. Future work would be needed to assess the minimum treatment allowing the permeabilization of seaweed cell, as working in such conditions would save energy, reduce processing cost and thermal damage to biomass constituents. Nevertheless, parameters of minimum required treatments such as voltage threshold highly depend on the process conditions and the type of the biomass (Haberl et al., 2013; Kotnik et al., 2012) and should be tuned accordingly, on a case by case basis.

Removal of around 40% of the initial ash content could be a key step to enhance the value of the biomass for energy, animal feed, human consumption or other uses as described in the introduction. Notably, analysis of the liquid extract shows that the major party of the extracted dry matter was ash (> 70%, see Table 1), thus only a small amount of organic compounds was extracted. This selectivity toward ash could be explained by the small size of salts that could easily diffuse through the pore of the membrane (Haberl et al., 2013; Kotnik et al., 2012). However, one needs to keep in mind that a fraction of the ash content could also be included in organic molecules such as protein or polysaccharide. In *Ulva* sp. for example, the polysaccharide ulvan is the major constituent of the cell wall (Robic et al., 2009). This polysaccharide is a sulfated polysaccharide thus containing an appreciable amount of ash bond to its organic skeleton as a sulfated group. Thus, extraction of valuable organic molecules containing metals or other ash elements could still occur. In any case, the ratio of ash over an organic matter of 70/30 makes the loss of valuable organic molecules such as protein or polysaccharide negligible. In addition, low severity PEF-treatment lead to a temperature increased of the biomass of only few degree Celsius while most of the treatment applied in this work did not warm the biomass over 50 °C (Fig. 1b), thus preserving most of its thermo-sensitive constituents.

3.3. Element analysis of liquid extracts and residual biomass

In order to get a more detail insight of the ash extraction process by PEF treatment followed by the hydraulic press, we analyzed the ash composition of both the liquid extract and the residual biomass of selected experiments. The two controls were therefore chosen to be compared with experiment 1 and 2, which were subject to the most severe treatment (T1, 50 pulses of charging voltage 50 kV, Fig. 1b).

Results from ICP-OES analysis show that the main elements in the seaweed ash were S, Cl, Mg, Na, K, Ca, P, in decreasing order, which account for $25.3 \pm 1.15\%$, $21.37 \pm 1.12\%$, $18.51 \pm 0.96\%$, $13.32 \pm 1.13\%$, $11.47 \pm 0.91\%$, $8.66 \pm 2.00\%$, $1.08 \pm 0.05\%$ of the crude ash, respectively, which is a common profile for such seaweed (Bikker et al., 2016). Other trace elements were also monitored such as Al, Cd, Co, Cr, Ni, Ti, Zn, Fe, Cu, Mn, and Si. Some elements were not found or their concentration was below the equipment threshold (such as Pb, As, Bi, Hg, Mo, Se, Sb, W, and Au), therefore, they were not displayed. Concentrations of major (> 1% of total ash) and traces (< 1% of total ash) were then correlated with the mass of liquid extracts and residual biomass obtained after pressing (data not shown) to determine the fraction of each element that was extracted and the

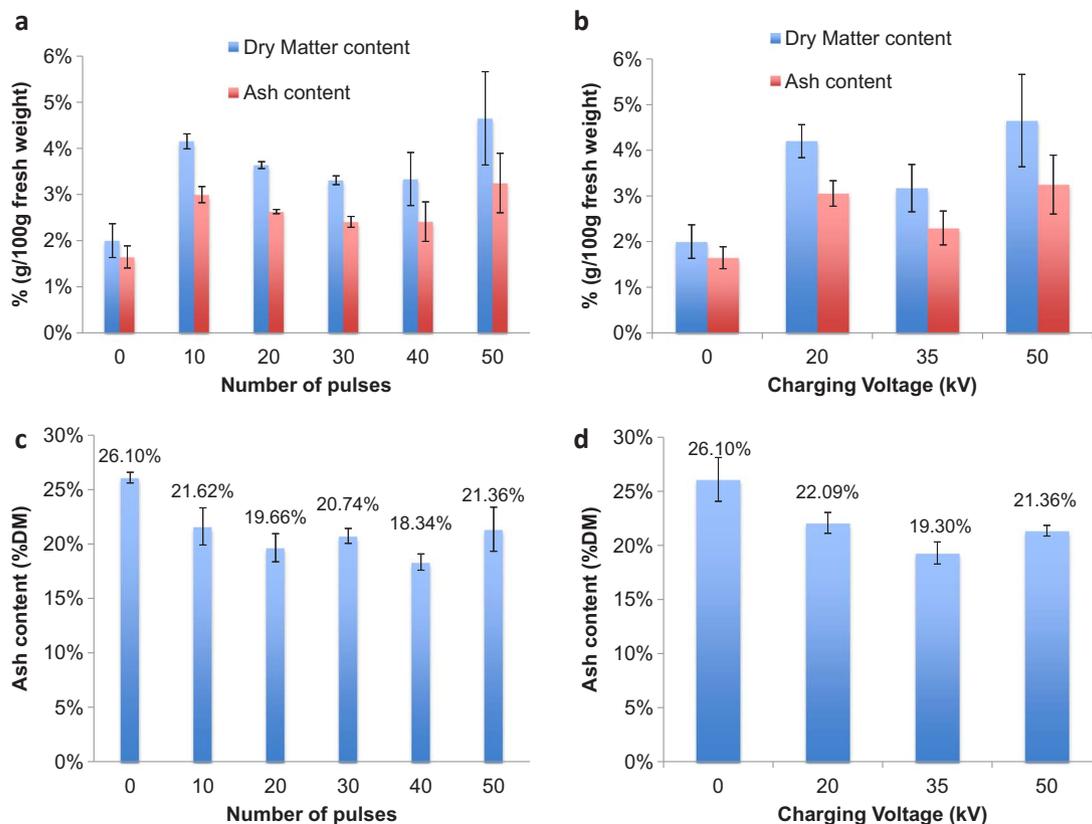


Fig. 3. Effect of pulse number and charging voltage on dry matter content and ash content of the liquid extract (a and b), and the ash content of the residual biomass (c and d). Results are expressed as mean of duplicates while error bar show standard deviation. Analyses of the variance were performed and show pulse number and charging voltage had an effect on those three parameters (dry matter and ash content of liquid extracts, ash content of residual biomass) (p-value < 0.05).

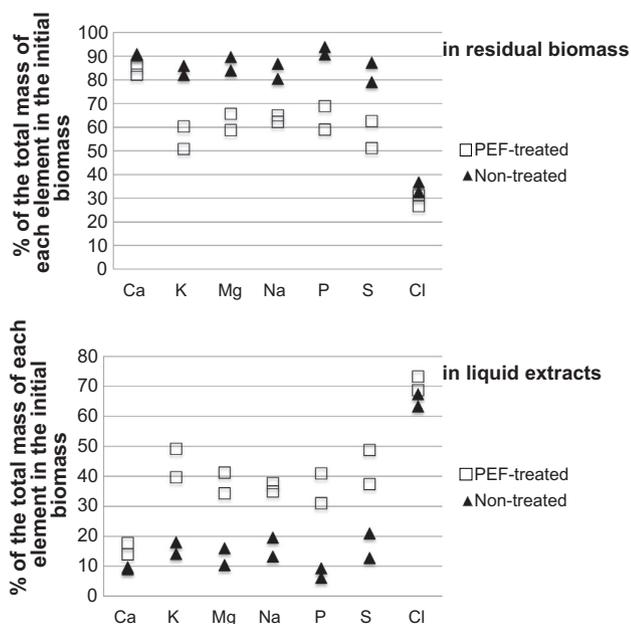


Fig. 4. Fractions of the main ash elements (defined as elements that made 1% or more of the total ash content) extracted in the liquid extracts (A) or remaining in treated and non-treated seaweed residues (B) after hydraulic press, as% of the mass of the element in the initial biomass.

fraction that remains in the residue. The results are shown in Table 2. Traces elements such as Cd, Cu, Fe or Cr had a very high standard deviation between the two duplicates for each experiment, which was attributed to their very low value (< 1% of the total ash content).

Osmotic shock followed by mechanical pressing was able to extract an average value of $18.2 \pm 14.0\%$ of each element; with a maximum value of 65.3% for Cl and a minimum value of 4.7% for Zn (Table 2). PEF-assisted deashing was able to extract an average value of $29.3 \pm 17.8\%$ for each element; with a maximum value of 71.0% for Cl and a minimum value of 4.7% for Fe (Table 2).

Among all elements presented in Table 2, only K ($44.43 \pm 6.72\%$ vs $16.04 \pm 2.75\%$), Mg ($37.8 \pm 4.89\%$ vs $13.22 \pm 3.97\%$), Na ($36.37 \pm 1.96\%$ vs $16.4 \pm 4.44\%$), Ni ($22.8 \pm 3.8\%$ vs $10.01 \pm 2.99\%$), P ($36.04 \pm 7.03\%$ vs $7.72 \pm 2.25\%$), S ($43.11 \pm 8.04\%$ vs $16.84 \pm 5.8\%$) and Zn ($7.38 \pm 0.79\%$ vs $4.74 \pm 0.71\%$) were significantly affected by the PEF-pretreatment. Those seven elements were extracted in a higher amount for the PEF-treated samples compared to the control, showing certain selectivity in the ash extraction compared to simple mechanical pressing. Moreover, among those seven elements, 5 of them (K, Mg, Na, P, and S) were considered as a major element (> 1% of total ash) supporting the use of PEF to successfully enhance major ash element extraction from biomass.

Interestingly, pressing alone was sufficient to remove > 60% of the Cl initial content, and PEF did not significantly influence the extraction of this major element although it slightly improved it (see Table 2). Cl ions are therefore easily extractable from seaweed biomass with water washing and mechanical pressing, which is due to the fact that their presence is mainly due to residual seawater containing a high level of NaCl. In an opposite fashion, Ca, was the only major element that was hardly extracted with PEF-assisted mechanical pressing ($15.87 \pm 2.72\%$ extracted) and with mechanical pressing alone ($9.37 \pm 0.42\%$ extracted). We make the hypothesis that those Ca ions (as well as Zn and Fe, that were also poorly extracted) might be trapped by ionic or weak interactions with charged molecules such as a sulfated polysaccharide (Chiellini and Morelli, 2011).

Table 2

Amount of the main elements in the ash of the residual biomass and the liquid extract obtain by hydraulic-pressing of PEF-treated (PEF, Pulsed Electric Field) and non-treated (CTRL = control who was osmotic shock treated only) seaweed samples. Results are expressed as mean (%) of duplicate \pm stdev. P-values were obtained from Tukey *t*-test between the treated and non-treated sample, bold values are the significant values (< 0.05).

Element	Residual biomass		Liquid extract		p value
	PEF	CTRL	PEF	CTRL	
Al	80.88 \pm 12.05	81.97 \pm 2.29	19.13 \pm 12.05	18.04 \pm 2.29	0.46
Ca	84.14 \pm 2.72	90.64 \pm 0.42	15.87 \pm 2.72	9.37 \pm 0.42	0.09
Cd	53.93 \pm 29.71	71.68 \pm 23.06	46.08 \pm 29.71	28.33 \pm 23.06	0.29
Cr	85.73 \pm 2.32	86.51 \pm 12.89	14.28 \pm 2.32	13.5 \pm 12.89	0.48
Cu	53.27 \pm 6.1	67.14 \pm 32.97	46.74 \pm 6.1	32.87 \pm 32.97	0.33
Fe	95.34 \pm 0.3	87.33 \pm 13.2	4.67 \pm 0.3	12.68 \pm 13.2	0.28
K	55.58 \pm 6.72	83.97 \pm 2.75	44.43 \pm 6.72	16.04 \pm 2.75	0.04
Mg	62.21 \pm 4.89	86.79 \pm 3.97	37.8 \pm 4.89	13.22 \pm 3.97	0.02
Mn	75.01 \pm 2.01	89.07 \pm 8.47	25 \pm 2.01	10.94 \pm 8.47	0.13
Na	63.64 \pm 1.96	83.61 \pm 4.44	36.37 \pm 1.96	16.4 \pm 4.44	0.04
Ni	77.21 \pm 3.8	90 \pm 2.99	22.8 \pm 3.8	10.01 \pm 2.99	0.04
P	63.97 \pm 7.03	92.29 \pm 2.25	36.04 \pm 7.03	7.72 \pm 2.25	0.05
S	56.9 \pm 8.04	83.17 \pm 5.8	43.11 \pm 8.04	16.84 \pm 5.8	0.04
Si	83.09 \pm 3.1	79.69 \pm 0.52	16.92 \pm 3.1	20.32 \pm 0.52	0.18
Ti	88.18 \pm 1.16	86.51 \pm 12.89	11.83 \pm 1.16	13.5 \pm 12.89	0.45
Zn	92.63 \pm 0.79	95.27 \pm 0.71	7.38 \pm 0.79	4.74 \pm 0.71	0.04
Cl	28.99 \pm 3.23	34.67 \pm 2.89	71.02 \pm 3.23	65.34 \pm 2.89	0.11

Only three elements were extracted in lower amount when PEF-treatment was applied, this is the case for Fe, Ti, and Si, but there were no significant differences between control and PEF-treated samples (see Table 2). More precise work would be needed to show if PEF specifically inhibits the extraction of some compounds.

Finally, there are few missing elements that could not be monitored using CI-analyzer and ICP-OES and whom fate could be of major importance. It is the case for nitrogen and iodine. The first one, N, has a key role as a major nutrient for plants (Carey et al., 2016), whereas I am of crucial interest for its impact (positive or negative) on human health (Zimmermann, 2009). Additional work incorporating those two elements as well as others could fill such gap and contribute to the understanding and the application of PEF-assisted deashing step.

3.4. Comparisons with previous works to enhance biofuel productions by biomass deashing

We showed that PEF was improving the extraction of almost all elements, notably most of the major seaweed ash elements (K, Mg, P, Na, and S) when using to pre-treat biomass prior to mechanical pressing. Not only PEF was able to improve the ash extraction, but our proposed process can be directly applied on freshly harvested biomass. Thus, it could be easily integrated, as a first unitary operation, in biomass processing lines or directly on the harvesting site.

This is critical, because if the deashing operation prior to processing was shown to improve biomass uses to produce energy and fuel by thermochemical or biological means (Chen et al., 2014; He et al., 2014; Hu et al., 2017; Huang et al., 2016; Kang et al., 2011; Pattiya et al., 2013; Stefanidis et al., 2015), the industrial and environmental relevance of such operation are not always taken into account. For example, deashing of corn stover as described in (He et al., 2014), showed that an ash reduction of 51% (from 9.60 to 4.98% ash), improved the hydrolysis yield 1.6 time (from 43.30 to 70.99%) and the ethanol yield 1.4 time (from 51.74 to 73.52%) (He et al., 2014). However, the results mentioned above were the maximum results obtained after the biomass was washed with an amount of water corresponding to 100 times the weight of biomass. In another work, a similar deashing strategy was used on seaweed biomass (washing with a ratio of 1:100 w/w of water during 12 h) and decreasing in K (78%), Ca (5%), Na (74%) and Mg (54%) were reported (Hu et al., 2017). These values are higher than the ones obtained in this work (except for Ca, confirming the difficulty of its removal from seaweed biomass with water alone) however, one need to

note that using such amount of water and time is not easily integrated with biomass processing, and potential microbial growth that can occur during that lapse of time and the tremendous amounts of water that need to be handled afterward. In this same study, different acids treatments (with HCl, H₂SO₄, and H₃PO₄) were also investigated. In almost all cases, the ash removal was almost complete ($< 90\%$ reduction in K, Ca, Na and Mg in most case), but the usage of strong acids (7 or 10% solution) with a solid to volume ratio of 1:100 is not likely to be easily integrated in biorefineries, notably when considering that the biomass was then washed again with water to remove the acid. Moreover, loss of organic compounds was reported, and although it was not clear how much of the organic content in the biomass was lost, it is obvious that strong acid treatment for such time will degrade a considerable amount of sensitive materials and should not be considered when targeted products are acid sensitive. Nevertheless, this study shows significant removal of Ca using a strong acid, which confirms the presence of weak interactions between Ca and other biomass components, and give a viable option for Ca removal.

Another study by Chen and Al (Chen et al., 2014) focus on deashing microalgae from wastewater using water and centrifugation. A maximum of 35% ash reduction was obtained (from 28.6% to 18.6% which are similar values than in this work) and yield of crude bio-oil obtained from deashing algal biomass was improved. But the scalability and the integration of the suggested deashing process can be questioned again considering its complexity as the fresh biomass they used was washed a first time with water (1h), dried, grinded, divided by screening, suspended in water (5% w/v ratio), centrifuged, re-suspended (5% w/v ratio) and re-centrifuged for a total processing time superior to 1 h. In our work, we obtained similar results on seaweed biomass by removal of surface water, PEF-treatment, and mechanical pressing, which were quicker (< 10 min) and used less water than all studies mentioned above (< 100 mL of water was added to 140g of fresh seaweed biomass containing about 26g of dry matter). Additionally, if a batch process was used in our study, continuous processing can be considered, as continuous PEF devices are already available at industrial scale (Bluhm and Sack, 2008).

PEF-assisted deashing has therefore considerable advantages that when put into balance with current solutions can make it a promising technology for deashing fresh biomass as it is easily scalable (Bluhm and Sack, 2008), it is a food grade technology (Mahnič-Kalamiza et al., 2014), mild thermal (Kotnik et al., 2015; Mahnič-Kalamiza et al., 2014), do not use any chemical addition (Kotnik et al., 2015; Mahnič-

Kalamiza et al., 2014), quick (few minute in this work) (Bluhm and Sack, 2008; Golberg et al., 2016), and with an energy and water consumptions relatively low (Golberg et al., 2016; Sack et al., 2010). Optimization of the process itself such as applying a second washing and pressing step and tuning the PEF-treatment could further improve the ash extraction yield and reduce the energy consumption, respectively.

3.5. Relevance for other biomass processing

The ash content of biomass such as seaweed can be detrimental to their uses as feed for livestock; thus, deashing biomass could improve their nutritional value (Makkar et al., 2016). Notably, high ash content limits their inclusion into the diet of common livestock as it reduces the feed energy value and can provide some mineral in excess (Evans and Critchley, 2014; Makkar et al., 2016).

Ash extracts are usually of less value than the residual biomass, yet, it still contains valuable nutrients that are essential to biomass productions and the key to the development of sustainable biorefineries (Carey et al., 2016). Thus the recycling of part of the nutrients such as P or K is of major interests for the development of future biorefineries as it can be recycled for biomass production. In our case, such nutrients can be re-used in seagrass, but also for traditional land agriculture where seaweed and seaweed extract have been used as fertilizer for a century (Craigie, 2011) and are currently a booming sector (Craigie, 2011).

Finally, the removal of ash, as well as water, would improve the supply chain for future biorefineries, as “useless” weight would be removed before transport to the processing plant, saving the precious amount of transport fuels. Moreover, lower ash content means higher organic content in the residual biomass (higher carbohydrate, higher protein etc.), increasing the value of the residual biomass and improving subsequent processing steps in general.

4. Conclusions

Pulsed Electric Field treatment (10–50 pulses of 10–50 kV) followed by hydraulic pressing was able to drastically increase the extraction yield of ash from fresh seaweed biomass leading to the extraction of $39.7 \pm 5.3\%$ of the initial ash content compared to $18.4 \pm 1.1\%$ for hydraulic pressing alone. Pulsed electric field treatment could, therefore, be used as a quick, water efficient, energy efficient, scalable, continuous or discontinuous, mild thermal and non-chemical pretreatment for deashing biomass without significantly impacting its valuable constituents. The proposed pretreatment is particularly promising for ash removal, nutrient recovery and dewatering of biorefinery feedstock, notably of the marine source.

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