

# Levulinic Acid Production from the Green Macroalgae *Chaetomorpha linum* and *Valonia aegagropila* Harvested in the Orbetello Lagoon

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In recent years, the replacement of fossil resources with renewable ones has received great interest, especially as regards the production of new valuable bio-products and bio-fuels, in order to replace the traditional petroleum-based ones. In this context, the exploitation of waste biomasses into added-value bio-chemicals is strongly encouraged. Among these ones, the algae ones are attracting considerable attention, in particular macroalgae which cause eutrophication problems in estuaries and lagoons, due to the drastic reduction of dissolved oxygen during their decomposition. This is true for Orbetello lagoon (Italy), where a large amount of algal biomasses is removed every year through an expensive practice, with consequent environmentally serious disposal problems. In this work, for the first time, the acid-catalyzed conversion of two different macroalgae harvested in Orbetello lagoon, *Chaetomorpha linum* (Muller) Kutzinger and *Valonia aegagropila* C. Agardh, into levulinic acid was studied and optimized, adopting a one-pot hydrothermal treatment, under microwave heating and in the presence of aqueous diluted mineral acids, H<sub>2</sub>SO<sub>4</sub> and HCl. Levulinic acid is a versatile platform chemical, classified by the United States Department of Energy as one of the top-12 promising bio-based building blocks. The effect of the main reaction parameters to give levulinic acid was investigated and discussed, in particular the type and concentration of the acid catalyst, the temperature and the reaction time. The highest levulinic acid yields of 19 wt% for *Chaetomorpha linum* and 16 wt% for *Valonia aegagropila*, calculated respect to the weight of the starting dried biomass, were reached. The achieved results are very promising and confirm the significant potential of these green algae as renewable starting feedstocks for levulinic acid production.

## 1. Introduction

Owing to the depletion and limited supply of fossil resources, bio-energy has received significant attention as an alternative energy source from the points of view of security, sustainability and diversity. In recent years, much work has been carried out on the utilization of marine macroalgae (Seaweeds, SW) as a renewable bio-energy resource (Jiang et al., 2016). SW are able to effectively fix CO<sub>2</sub> released from burning of fossil fuels and other human activities and consequently slash emissions of principal greenhouse gas. Furthermore, SW show a rapid growth rate, high photosynthetic efficiency and do not require either arable land or fresh water resources to grow (Konda et al., 2015). On the other hand, unfortunately, SW are responsible for the eutrophication of coastal areas, especially estuaries and lagoons, which represents a global and serious environmental problem (Lotze et al., 2006). This phenomenon can cause massive blooms of opportunistic macroalgae and drastic reduction of dissolved oxygen levels due to biomass decomposition, with a consequent negative impact on aquatic ecosystems. This is true for Orbetello lagoon (Italy, Southern Tuscany), a coastal shallow basin that covers a surface of about 25 km<sup>2</sup>, characterized by a low water turnover. In order to solve eutrophication problems, a large amount of algal biomass is currently removed every year from the lagoon (about 700-1000 tons per year), but this practice is highly expensive and creates

disposal problems (Lenzi, 2015). Various efforts have been made to employ the harvested biomass but, up to date, a viable industrial application has not been still found. From a different perspective, SW are a very valuable source of easily degradable carbohydrates, thus representing a potential and very valuable feedstock for bio-fuels and bio-chemicals production, such as levulinic acid (LA) (Park et al., 2018). LA is obtained from the hydrolysis of the cellulosic component of biomass employed for the synthesis of many bio-based building blocks, showing a key role towards a post-petroleum society. In particular, LA is a versatile platform chemical and a valuable intermediate for the synthesis of many fine chemicals, such as surfactants and plasticizers (Freitas et al. 2016; Rivas et al., 2016, 2015). However, the scale-up of LA production from the pilot up to the commercial scale has still some unsolved bottlenecks, which still require insights and improvements. Among these ones, the choice of the appropriate starting feedstock is fundamental for lowering LA production costs and, for this purpose, the use of cheap or, even better, negative-value biomass, is now an essential requirement for its production on a larger industrial scale in the next future (Licursi et al., 2018; Puccini et al., 2016). Both the high cellulose content of SW and the absence of the lignin fraction, the latter which is instead typical of the terrestrial biomasses, should make SW more easily hydrolysable, and therefore a very promising feedstock for LA production. In this work, for the first time, the direct conversion of two different SW deriving from Orbetello lagoon, *Chaetomorpha linum* (Muller) Kützinger (*C. linum*) and *Valonia aegagropila* C. Agardh (*V. aegagropila*), to LA, was studied and optimized, adopting the one-pot hydrothermal treatment, in the presence of an aqueous diluted mineral acid (H<sub>2</sub>SO<sub>4</sub> or HCl). For this purpose, the effect of the main reaction parameters on LA production was investigated, in particular the type and concentration of the acid catalyst, the temperature and the reaction time. Microwave (MW) irradiation has been chosen as the heating system, taking advantage of heat generation of the reactants/solvent by dielectric and conduction losses, due to rotation of the dipolar molecules and ionic conduction of electrolytes, respectively (Antonetti et al., 2016). In the field of the hydrothermal process, MW approach has revealed acknowledged benefits, based on a fast and efficient heating of the reaction medium, thus reducing the occurrence of side-reactions of sugars to humins, if compared with the conventional conductive heating. The dielectric loss of the reaction medium is markedly dependent on microwave frequencies and temperature, and this contribute is notable mainly for the bulk solvent, e.g. water, which generally acts as a good MW susceptor. The dielectric loss of water significantly decreases with the temperature increase, as required for LA synthesis, because of the enhanced molecular mobility at elevated temperature. In this way, water becomes more transparent to microwave and the biomass can be more exposed to microwave irradiation. In addition, the ionic conduction mechanism is particularly relevant for this aqueous reaction system, thanks to the presence of i) electrolytes of algal source, ii) homogeneous catalyst, which can effectively work as both acid catalyst and microwave sensitizer, and iii) acid intermediates/products deriving from the biomass conversion. The MW-assisted exploitation of SW for LA production opens new possibilities for bio-refining waste to produce bio-fuels and chemicals in a more sustainable and efficient manner, in agreement with the principles of the European bio-economy.

## 2. Materials and Method

### 2.1 Materials

Two SW samples were used in this work, *C. linum* and *V. aegagropila*, both harvested in December 2017 from Orbetello lagoon. The starting samples were dried at room temperature for 15 days before their use. Both hydrochloric acid (ACS reagent, 37 wt%), and sulfuric acid (ACS reagent, 96 wt%) were purchased by Sigma-Aldrich. LA (purity = 98 %) and D-Glucose (purity > 99.5 %) were purchased by Sigma-Aldrich, and used as analytical standard.

### 2.2 Experimental procedure

The hydrolysis of the SW samples was carried out in a single-mode microwave reactor (CEM Discover S-class System), employing a 35 mL vessel with a Teflon stir bar. The starting biomass was pre-treated at room temperature under stirring for 2 h in order to make easier the contact between the homogeneous acid mineral catalyst with the inner biomass fibers. Then, the reactor was closed and the sealed system was irradiated up to the set-point temperature, by employing a fixed ramping time of 10 minutes. MW frequency radiation of 230 MHz and maximum pulsed-power of 150 W were used to heat the samples. During the reaction, pressure and temperature values were continuously acquired with the software and controlled with a feedback algorithm to maintain the constant temperature. At the end of each hydrolysis reaction, the reactor was rapidly cooled at room temperature by blown air and the solid-liquid slurry was recovered, filtered and analyzed by High Performance Liquid Chromatography (HPLC).

### 2.3 Analytical equipment

The hydrolysate mixture was properly diluted with water, filtered on a 0.2  $\mu\text{m}$  Whatman filter and analyzed by HPLC. A Perkin Elmer Flexar Isocratic Platform HPLC was used for this purpose, equipped with a Benson 2000-0 BP-OA (300 mm x 7.8 mm) column, which was kept at 60  $^{\circ}\text{C}$  and with a WATERS 2410 Refractive Index detector. A 0.005 M  $\text{H}_2\text{SO}_4$  solution was employed as the mobile phase, with a flow rate of 0.6 mL/min. The analysis was carried out in duplicate and the reproducibility of this analysis was within 3%. Mass yield  $Y$  (wt%) was expressed as the ratio between the amount of the selected compound in the reaction mixture (in grams,  $M$ ) and the starting dried feedstock (in grams,  $m$ ), according to Eq.(1):

$$Y \text{ (wt\%)} = \frac{M}{m} \cdot 100 \quad (1)$$

Then, the yield of LA based on theoretical yield was calculated according to Eq. (2):

$$\text{LA yield based on theoretical yield (wt\%)} = \frac{\text{LA recovered (g)}}{\text{dried biomass (g)} \cdot \text{glucan content} \cdot 0.7155} \cdot 100 \quad (2)$$

Lastly, the yield in the solid residue (wt%) was calculated according to Eq. (3):

$$\text{Yield in solid residue (wt\%)} = \frac{\text{Solid recovered after hydrolysis (g)}}{\text{dried starting biomass (g)}} \cdot 100 \quad (3)$$

FT-IR characterization of the samples was performed by a Perkin Elmer Spectrum-Two spectrophotometer, which was equipped with an attenuated total reflectance ATR apparatus. The acquisition of each spectrum has provided 12 scans in the range 4000–650  $\text{cm}^{-1}$ , with a resolution of 4  $\text{cm}^{-1}$ .

### 3. Results

The starting samples of *C. linum* and *V. aegagropila* were analyzed in order to evaluate their chemical composition, in particular the carbohydrates and the acid-insoluble lignin (Klason) content. *C. linum* resulted composed of 3.3 % galactan, 43.7 % glucan, 7.9 % mannan, 37.5 % other components and 6.6 % Klason lignin, whereas *V. aegagropila* was made up of 2.6 % galactan, 33.9 % glucan, 1.2 % xylan, 5.2 % arabisan, 44.4 % other components and 12.4 % Klason lignin, in both cases these data expressed on dry biomass weight basis. The significant high content of cellulose and the low amount of lignin ascertained for both algae make them suitable feedstocks for LA production. FT-IR spectra of both algae are reported in Figure 1.

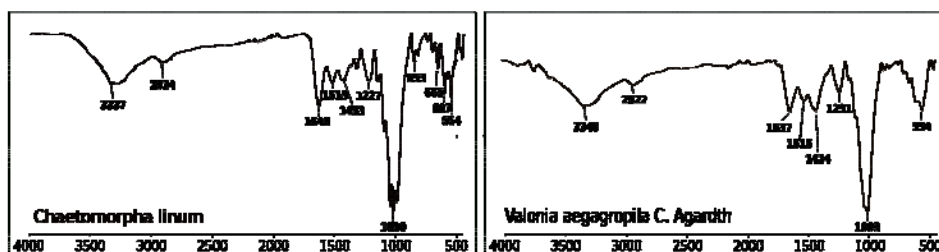


Figure 1: FT-IR spectra of *C. linum* and *V. aegagropila*.

The band at 3300–3400  $\text{cm}^{-1}$  is attributed to -OH stretching vibration of hydroxyl or carboxylic groups and it preliminarily confirms the significant presence of cellulose and hemicelluloses fractions in these kinds of biomasses. Among the main absorption bands of interest, those in the range 1200–950  $\text{cm}^{-1}$  characterize the C–O–C vibration absorption of polysaccharides (Meng et al., 2014). In addition, a significant peak is present in both cases at about 1640  $\text{cm}^{-1}$ , which is typical of the -C=O stretching vibrations of amide groups, whereas the band at 1515  $\text{cm}^{-1}$  can be attributed to -C=O asymmetric stretching of carboxylic groups, highlighting the well-known presence of the proteins in both starting biomasses (He et al., 2013). The acid-catalyzed conversion of starting algal biomasses to LA was studied and optimized adopting microwave (MW) as the only heating system. In this regard, MW heating represents a very effective tool for fast screening of the appropriate reaction conditions for the hydrothermal treatment (Antonetti et al. 2017; Rivas et al., 2015). In fact, MW significantly reduces the ramping time to reach the set-point temperature and cool the reaction mixture, thus minimizing the possible degradation of the reaction intermediates, at the same time, achieving significant energy and time savings (Galia et al., 2015; Peleterio et al., 2018). Taking into account that the most abundant algal biomass in Orbetello lagoon is *C. linum*, the acid-catalyzed hydrolysis of this algal species was first

studied and optimized. At the beginning, the type and the concentration of the catalyst were investigated, performing the reaction in the presence of one of the two most widely employed acid mineral systems, H<sub>2</sub>SO<sub>4</sub> or HCl. The hydrothermal conversion of *C. limun* mixture (biomass loading: 5 wt%) to LA was tested adopting reaction conditions already optimized by our research group for lignocellulosic and waste biomasses (Rivas et al., 2016; Licursi et al., 2017, 2016), 170 °C for 20 minutes, and the obtained results are reported in Table 1. The comparison between the two different mineral acid catalysts adopted at the concentration of 1.7 wt% (runs 1 and 2) shows that HCl promoted the hydrolysis towards LA, whereas H<sub>2</sub>SO<sub>4</sub> selectively favoured the production of glucose, therefore achieving LA yields of 10.6 and 2.7 wt%, respectively. Then, the influence of the acid catalyst concentration was investigated, keeping constant the other reaction conditions (runs 2 and 3 for HCl and runs 1 and 5 for H<sub>2</sub>SO<sub>4</sub>). Employing HCl as catalyst, the increase of its concentration from 1.7 to 2.3 wt% did not cause any improvement of LA yield, which remained almost constant (9.6 wt%), whereas a small decrease of glucose yield was observed, suggesting the significant occurrence of side-reactions, leading to a higher formation of soluble humins and/or other condensation by-products, further confirmed by the lower yield in the recovered solid residue, which decreased from 40.0 to 35.2 wt%.

Table 1: Acid-catalyzed conversion of 5 wt% *C. linum* mixture to LA in water under MW heating.

Run	Acid	Acid Concentration (wt%)	T (°C)	Time (min)	Glucose Yield (wt%)	LA Yield (wt%)	wt% on theoretical LA Yield (wt%)	Yield in solid residue (wt%)
1	H <sub>2</sub> SO <sub>4</sub>	1.7	170	20	12.1	2.7	8.6	44.7
2	HCl	1.7	170	20	4.1	10.6	33.2	40.0
3	HCl	2.3	170	20	3.5	9.6	30.1	35.2
4	HCl	1.7	170	45	2.8	10.5	33.1	38.5
5	H <sub>2</sub> SO <sub>4</sub>	6.1	170	20	5.2	8.2	25.7	38.2
6	H <sub>2</sub> SO <sub>4</sub>	3.2	180	20	4.0	7.3	23.0	41.7
7	H <sub>2</sub> SO <sub>4</sub>	4.7	190	20	4.4	14.0	44.0	51.3
8	H <sub>2</sub> SO <sub>4</sub>	4.7	200	20	0.7	16.8	53.0	18.6

Moreover, in order to improve the process toward LA with 1.7 wt% HCl, an additional run (run 4) was performed prolonging the reaction time up to 45 minutes. In this case, LA yield resulted in 10.5 wt%, with a corresponding yield in the solid residue of 38.5 wt%, both values comparable to those reached after 20 minutes, underlining as the extension of reaction time did not significantly improve the catalytic performances. On the other hand, adopting H<sub>2</sub>SO<sub>4</sub> as the acid catalyst, the increase of its concentration from 1.7 to 6.1 wt% resulted in a significant increase of LA yield which moved from 2.7 to 8.2 wt%, values corresponding to 8.6 and 25.7 wt% on the theoretical yield, respectively. On the basis of this marked enhancement of LA yield with the increase of H<sub>2</sub>SO<sub>4</sub> concentration, in order to reduce the strong acidity of the medium used in run 5, runs 6 and 7 were carried out employing intermediate acid concentrations, 3.2 and 4.7 wt% respectively, counterbalancing this reduction with the increase of temperature at 180 and 190 °C, respectively. The LA yield of 7.3 wt% was obtained at 180 °C, whereas of 14.0 wt% was ascertained at 190 °C, this last corresponding to 44.0 wt% on the theoretical yield. Finally, the effect of temperature was studied under the optimized reaction conditions, but performing the reaction at 200 °C (run 8). The increase of temperature of 10 °C allowed us to reach the LA yield of 16.8 wt% which corresponds to 53 wt% on the theoretical yield. This result is very interesting because i) it is obtained with a direct one-pot approach, ii) starting directly from the algal biomass, and iii) the best achieved LA yields are comparable with those reported in the literature for green algae, but whose conversion is mostly performed in two steps (Park et al., 2018). However, the two-step procedure, very often adopted in the literature, is disadvantageous, making the entire process less sustainable and not economic. The adopted reaction conditions are favorable for the selective synthesis of LA, whilst furfural, which derives from conversion of C5 fraction, was not detected in the liquid phase in significant amount, in agreement with our previous work (Licursi et al., 2018). Its selective synthesis should require much milder reaction conditions (acidity, temperature and time), and this possibility is not of particular interest for this biomass feedstock, because of its low content of pentoses. Once optimized the main reaction parameters, the effect of reaction time was examined and the kinetic profiles at 190 and 200 °C are shown in Figure 2, starting from 5 wt% *C. linum* mixture, in the presence of 4.7 wt% H<sub>2</sub>SO<sub>4</sub>. For the test carried out at 190 °C, the increase of the reaction time favored the production of LA up to 18.2 wt%, which corresponds to 57.3 wt% on the theoretical yield, whereas for the reaction at 200 °C, products yields at low reaction times (10-30 minutes) were determined in order to not increase excessively the severity of the process. In this case, the highest LA yield of 16.8 wt%, corresponding to 53.0 wt% on the theoretical yield, was obtained after 20 minutes but, when

the time was prolonged over that, LA yields decreased, probably due to the considerable amount of soluble humins, as reported in the literature data (Park et al., 2018).

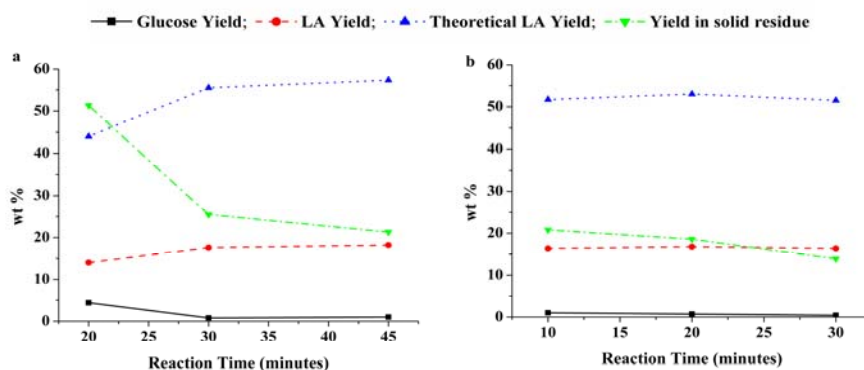


Figure 2: Kinetic profiles of the acid-catalyzed conversion of 5 wt% *C. linum* mixture to LA, in the presence of 4.7 wt%  $H_2SO_4$ , at 190 °C (a) and 200 °C (b).

Regarding the second macroalgae, *V. aegagropila*, for this preliminary study, its hydrolysis to LA was investigated adopting the reaction conditions already optimized for *C. linum* (190 °C, 45 minutes), starting again from 5 wt% algal biomass mixture, and in the presence of 4.7 wt%  $H_2SO_4$ . The achieved results are shown in Figure 3, together with the outcomes reached with the increase of temperature up to 200 °C.

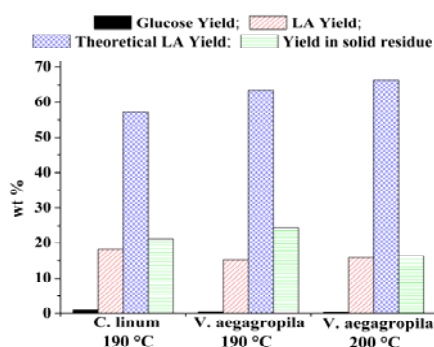


Figure 3: Acid-catalyzed conversion of 5 wt% *V. aegagropila* mixture to LA in the presence of 4.7 wt%  $H_2SO_4$  at 190 °C and 200 °C (for comparison, also the corresponding run with *C. linum* at 190 °C is shown).

The comparison between the two investigated macroalgae shows that the LA yield was slightly higher for *C. linum* rather than *V. aegagropila*, but the latter enabled us to reach the highest theoretical LA yield, equal to 63.3 wt%, indicating as, in this case, the exploitation of its cellulose fraction resulted more effective. Finally, in the case of *V. aegagropila*, the increase of the reaction temperature up to 200 °C allowed us to obtain the LA yield of 15.9 wt%, corresponding to 66.2 wt% on the theoretical yield. This result is very interesting because highlights the possibility of converting this algal biomass under reaction conditions of higher severity than those adopted for *C. linum*, making more reactive the cellulose fraction of *V. aegagropila*. As in the case of the starting untreated samples, also compositional analysis of the solid residues recovered from the best LA hydrolysis tests (Figure 3) was carried out, and the absence of residual hemicellulose and cellulose fraction was preliminary confirmed, thus assessing their “pseudo-lignin” behavior, mostly given by the presence of insoluble sugar-derived humins.

#### 4. Conclusions

The present investigation represents a very interesting example of exploitation of two macroalgae harvested in Orbetello lagoon, *C. linum* and *V. aegagropila*, which are currently removed in order to limit the eutrophication problems and therefore traditionally considered as a waste. From a different and smarter perspective, their high cellulose contents make them suitable feedstocks for the acid-catalyzed hydrothermal conversion to LA. The highest LA yields of 19 wt% for *C. linum* and 16 wt% for *V. aegagropila* were reached, working at 190 and 200 °C respectively, in both cases after 45 minutes, in the presence of 4.7 wt%  $H_2SO_4$ , and starting from 5

wt% mixtures of each algal biomass. These results, achieved adopting a direct one-pot hydrothermal treatment, are very promising and confirm the significant potential of these green algae as renewable starting feedstocks for LA production.

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