

Levulinic Acid Production from Macroalgae: Production and Promising Potential in Industry

by Pak Fpik Unsoed

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Review

Levulinic Acid Production from Macroalgae: Production and Promising Potential in Industry

Maria Dyah Nur Meinita ^{1,2,*}, Amron Amron ¹, Agus Trianto ³, Dicky Harwanto ³, Wahyu Caesarendra ^{4,*}, Gwi-Taek Jeong ⁵ and Jae-Suk Choi ^{6,7}

¹ Faculty of Fisheries and Marine Science, Jenderal Soedirman University, Purwokerto 53123, Indonesia; amron@unsoed.ac.id

² Center for Maritime Bioscience Studies, Jenderal Soedirman University, Purwokerto 53123, Indonesia

³ Faculty of Fisheries and Marine Science, Diponegoro University, Semarang 50275, Indonesia; agustrianto.undip@gmail.com (A.T.); dickyharwanto@lecturer.undip.ac.id (D.H.)

⁴ Faculty of Integrated Technologies, Universiti Brunei Darussalam, Gadong, Bandar Seri Begawan 1410, Brunei

⁵ Department of Biotechnology, Pukyong National University, Busan 48513, Korea; gjeong@pknu.ac.kr

⁶ Seafood Research Center, Industry Academy Cooperation Foundation (IACF), Silla University, Busan 49277, Korea; jsc1008@silla.ac.kr

⁷ Department of Food Biotechnology, College of Medical and Life Sciences, Silla University, Busan 46958, Korea

* Correspondence: maria.meinita@unsoed.ac.id (M.D.N.M.); wahyu.caesarendra@ubd.edu.bn (W.C.)



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Abstract: The development of macroalgal biorefinery products as an alternative source of renewable fuels is an opportunity to solve the dependence on fossil fuels. Macroalgae is a potential biomass that can be developed as a raw material for producing platform chemicals such as levulinic acid (LA). In the industrial sector, LA is among the top 12 biomass-derived feedstocks designated by the U.S. Department of Energy as a high-value chemical. Several studies have been conducted on the production of LA from terrestrial-based biomass, however, there is still limited information on its production from macroalgae. The advantages of macroalgae over terrestrial and other biomasses include high carbohydrate and biomass production, less cultivation cost, and low lignin content. Therefore, this study aims to investigate the potential and challenge of producing LA from macroalgae in the industrial sector and determine its advantages and disadvantages compared with terrestrial biomass in LA production. In this study, various literature sources were examined using the preferred reporting items for systematic reviews and meta-analyses (PRISMA) method to identify, screen, and analyze the data of the published paper. Despite its advantages, there are some challenges in making the production of levulinic acid from macroalgae feasible for development at the industrial scale. Some challenges such as sustainability of macroalgae, the efficiency of pretreatment, and hydrolysis technology are often encountered during the production of levulinic acid from macroalgae on an industrial scale.

Keywords: levulinic acid; macroalgae; platform chemical; biomass; polysaccharide

1. Introduction

Sustainable economic growth requires secure resource management and long-term investments in ecology and industry. Currently, most of energy use comes from petroleum and natural gas [1]. Meanwhile, the depletion of the availability of fossil resources has caused an increase in petrochemical prices, while its use has affected the environment [2]. The carbon dioxide produced from the combustion residue accumulates in the air and in high quantities disturbs the climate and the balance of nature. Therefore, the search for alternative sources of renewable fuels becomes important to reduce the consumption of fossil resources. This has led to the discovery of the conversion of biomass into sustainable bioenergy from bio-based products, specifically those derived from biomass. Among the promising biomass feedstocks, macroalgae have attracted global attention, which appears to produce various high-value products [3].

The development of basic biorefinery products is the key to access integrated production in industries, such as chemical, biotechnology, and also biomass-based fuel generation [4]. Biomass is a renewable energy source, which is currently developing on a larger scale. In the early 19th and 20th centuries, the use of biomass was focused on pulp production, paper making from wood, fat preservation, sugar refining, and protein separation [2]. Recently, biomass is also used in industrial biotechnology processes to produce biofuels and high-value chemical products such as furfural, levulinic acid (LA), starch, ethanol, acetic, lactic, and citric acids. Among the platform chemicals produced from the biomass, LA is emerging as one of the most environmentally friendly platform chemicals derived from biomass such as macroalgae.

According to the U.S. Department of Energy, National Renewable Energy Laboratory (NREL, Golden, CO, USA), and the Pacific Northwest National Laboratory (PNNL, Richland, WA, USA), LA is classified as one of 12 top high-value chemicals from biomass for the production of environmentally friendly chemicals [5]. It is a distinctive potential building block chemical that efficiently synthesizes various value-added energy compounds from commercial biomass. Furthermore, it is among the 12 biomass-derived raw materials that can be produced from C5 (pentose) and C6 (hexose) carbohydrates through dehydration and cellulose hydrolysis reactions [2,6]. Hexose sugar comes from starch or lignocellulose which is formed from carbohydrates by acid treatment, while pentose comes from hemicelluloses such as xylose and arabinose with the addition of a reduction step after acid treatment [5]. LA compounds are obtained directly through the conversion of plant biomass and agricultural byproducts as environmentally friendly raw materials [7].

Currently, LA is being developed as a substitute for fossil fuels as environmentally friendly and renewable biofuels [1], which are used as resins, coatings, plasticizers, anti-freezing agents, solvents, chemical intermediates, and biorefinery fuels. It is very important because, among various platform chemicals, it has potential in the synthesis of versatile chemicals [8]. Meanwhile, LA can be produced through high-temperature acid hydrolysis of carbohydrates such as glucose, galactose, sucrose, fructose, chitose, and also biomer or polymeric materials such as starch (lignocellulose), wood, agricultural waste, and macroalgae [8,9].

Since its introduction in the 18th century, LA production is underdeveloped due to the high availability of raw materials in high quantities, lack of equipment, and conversion technology. Although macroalga biomass has received global attention as a promising biomass resource in LA production, its commercial production is still facing some challenges. Therefore, this study aims to fill the gap based on the newest published papers which focus on LA production from macroalgae. The advantages of macroalgae compared with terrestrial biomass include high polysaccharide content, ease of culture, low lignin content, and high productivity [10–12]. However, some factors need to be considered in the development of macroalgae for producing LA at the industrial scale to make its production feasible and sustainable.

2. Materials and Methods

In this study, a systematic literature review method based on the preferred reporting items for systematic reviews and meta-analyses (PRISMA) statement was used to review, systematically and explicitly, in selecting, identifying, and assessing relevant articles [13]. This systematic review formulated, collected, processed, and analyzed literature data that fulfills the review criteria, which include the type of article, topic, year of publication, and the quality of the research article. The targeted articles were articles categorized as research articles which study LA production from macroalgae and were published during 2012–2020. Scientific databases, namely Google Scholar, Science Direct, Springer Link, Wiley Online Library, and MDPI were used to collect the articles. Furthermore, meta-analysis and statistical methods were used for summarizing, analyzing, and integrating results. The main keywords used for the collection and screening of the articles included “macroalgae” and “levulinic acid”, “seaweed”, “polysaccharide”, “physiochemical”, “biomass”, “chemi-

cal", "waste", "pretreatment", "acid", and "enzymatic hydrolysis". The four systematic stages used to collect the article were identification, screening, eligibility, and inclusion process. In the identification stage, some keywords were used to collect the relevant articles, followed by screening, which was conducted by checking the title, keyword, abstract, and content of the articles. Furthermore, in-depth observation of full content was carried out in the eligibility stage, while in the inclusion stage, articles that did not fulfill the criteria were excluded.

3. Results and Discussion

The literature search based on the PRISMA statement obtained 72 articles, meanwhile, after the identification, screening, eligibility, and inclusion process, 18 articles were selected to be extracted and reviewed. The total number of articles on LA production from macroalgae based on the class and the hydrolysis method are shown in Figure 1. Among the 18 articles, study on LA production was mostly conducted using red macroalgae (77.8%) and green macroalgae (16.7%), and carrageenan waste (5.6%). Based on the type of hydrolysis method, most studies on LA production from macroalgae were conducted using acid hydrolysis (94.4%) and a combination of acid and enzymatic hydrolysis (5.4%).

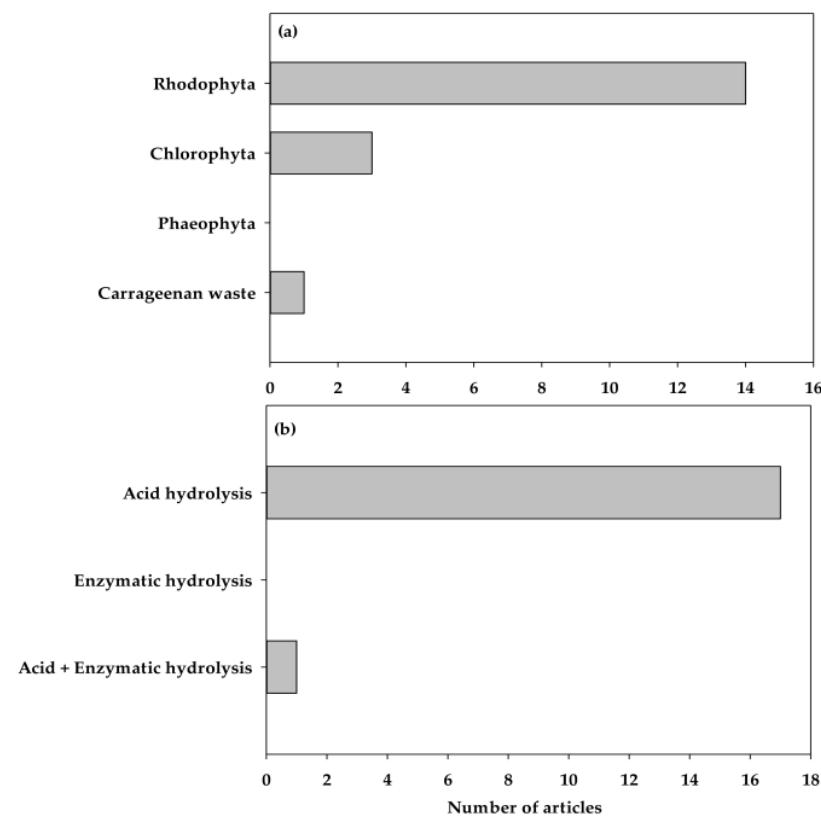


Figure 1. Number of literature on the production of LA from macroalgae based on (a) class division of macroalgae and (b) type of hydrolysis method.

3.1. The Use of Levulinic Acid in Industry

Levulinic acid (LA) was first introduced in the 1840s by a Dutch professor, G.J. Mulder, who also introduced the term protein. Professor Mulder synthesized LA by heating fructose

with HCl [14], however, it was not widely used commercially after its first introduction. In the 1940s, LA was produced commercially by A.E. Staley and considered as a platform chemical with a high potency of cellulose products in 1956 [2].

LA is among the top 12 important chemicals due to the presence of a bifunctional acid carboxyl and a carbonyl ketone group. The reactive nature of LA's bifunctionality makes it a broad synthetic potential for the production of various energy-rich chemical products [15]. Moreover, it is currently used in various industrial fields such as biofuel and versatile high-value chemicals, and also several chemical applications as a precursor for the synthesis of fuels and as a solvent [7]. Various important industries that use LA include the pharmaceutical, food, polymer/plastic, resin, textile, pesticides, fuel/energy, and organic synthesis industries. These industries are interested in developing new commercial technologies to produce LA from biomass and raw waste materials using a biorefinery approach [15]. This showed that LA is a versatile material, which can be used to produce chemical products derived as shown in Figure 2 [4,16,17].

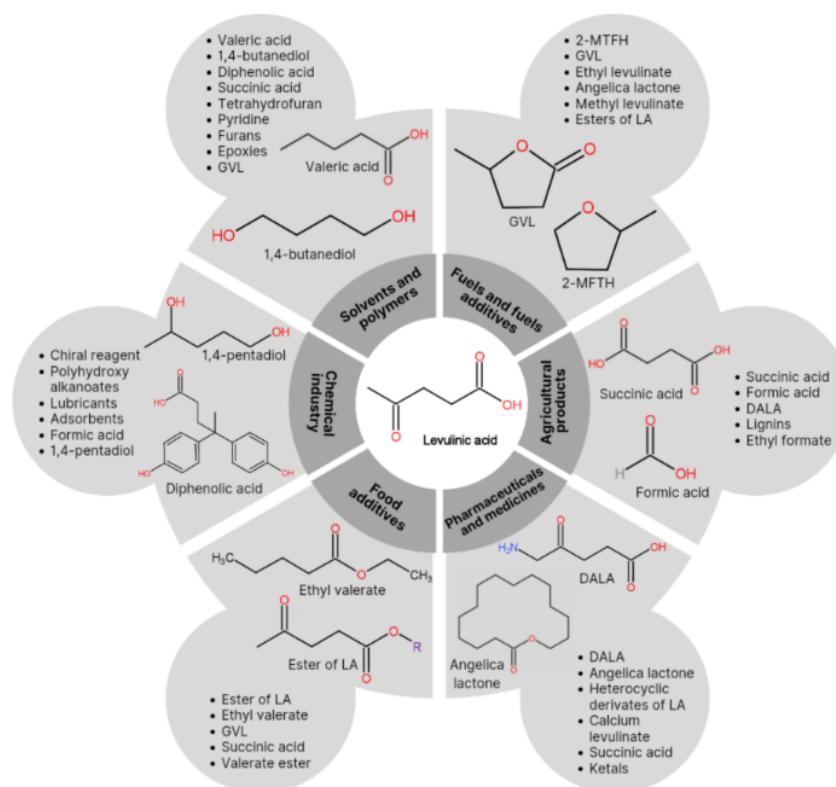


Figure 2. The use and application of levulinic acid in industrial scale.

Biofuel and chemicals are produced from biomass such as sugar, starch, lignocellulose, and algae through biological and thermochemical processes [16]. Previous studies discovered that LA can be converted into methyltetrahydrofuran (MTHF), solvent, fuel extender, d-aminolevulinic acid (DALA), and diphenolic acid (DPA) through a catalytic hydrogenation process [17]. Meanwhile, new LA-derived products that are currently being developed include dilevulinic and pentanoic acids, 1,4-butanediol, 1,4-pentadiol, 1,4-pentadiene, bromolevulinates, angelica lactone, adipic acid, 2-oxoglutaric acid, ethyl levulinate, methylethyl ketone, methylvinyl ketone, n-alkylpyrrolidones, acetylacrylic acid, aminolevulinic acid and other useful compounds [18].

According to Silva et al. (2018), the application of LA and its derivatives in the industry is grouped into several parts, namely, chemical industry, fuels and their additives, pharmaceuticals and medicines, food additives, agricultural products, as well as solvents and polymers [4]. The chemical industry group includes chiral reagents, polyhydroxy alkanoates, lubricants, adsorbents, and formic acid. Meanwhile, the group of fuels and their additives includes EL, MTHF, GVL, angelica lactone, methyl levulinate, and other esters. The pharmaceuticals and medicines group includes DALA, calcium levulinate, heterocyclic derivatives of LA, angelica lactone, ketals, tetrapyrroles, and succinic acid, while the food additives group includes GVL, ethyl valerate, succinic acid, and valerate esters. Furthermore, the agricultural products group includes DALA, formic acid, lignins, and ethyl formate, while the solvent and polymers groups are diphenolic acids, succinic acid, pyridine, furans, epoxies, 1, 4-butanediol, THF, NMP, and GBL.

3.2. Chemical Structure of Levulinic Acid (LA)

LA is a complex organic compound with the chemical formula $C_5H_8O_3$ that contains a keto group and a carboxylic acid group with the IUPAC name 4-oxopentanoic acid. It is a keto acid from the conversion of lignocellulose, sugar, to waste material biomass. Furthermore, it is a versatile platform chemical that is very attractive and has one carbonyl, one carboxyl, and α -H structure, with a short chain and non-volatile fatty acids [18]. The chemical structure of LA is shown in Figure 3.

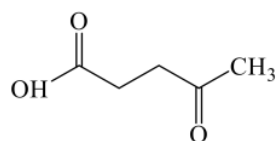


Figure 3. Chemical structure of LA.

The carboxylate and carbonyl groups in LA chemical structure are electrophilic centers that are highly reactive to nucleophiles. Due to the presence of the carbonyl group, LA can be isomerized into enol isomers. The physical properties of LA include a molecular weight of 116.2 g/mol, a melting point of 306–308 K, a boiling point of 518–519 K, a density of 1140 kg·m^{−3}, and a refractive index of 1.4796 [18]. Based on the source of lignocellulosic biomass, it is produced from raw materials such as cellulose or hemicellulose. Cellulose material has a semicrystalline structure, while hemicellulose is a group of polysaccharides with a heterogeneous, amorphous, and branched structure [19].

LA can be produced cost-effectively with high yields through the use of renewable raw materials such as agricultural waste biomass, lignocellulosic, sugar, and macroalgae. The abundance and advantages of macroalgae materials make it a promising raw material for production. The main component of macroalgae biomass is organic carbon content which contributes greatly to LA formation mechanism. Moreover, the direct transformation of polysaccharides to LA is a complex process of various biomass substrates which is complemented by the formation of several important intermediates [15].

3.3. Levulinic Acid Production from Macroalgae

Macroalgae contain polysaccharides as the carbohydrate of primary metabolite production in the form of agar, carrageenan, alginate, ulvan, cellulose, and other polysaccharides. Carbohydrates from macroalgae are easily degraded into potential resources for the production of biofuels and chemicals such as ethanol, butanol, methane, 5-HMF, LA, formic acid, and furfural [16].

The reaction for the conversion of carbohydrate-containing biomass into LA is divided into two processes, namely, dilute acid treatment at high temperature with a pressurized atmosphere and high concentrated acid at lower temperature with normal pressure. Meanwhile, the complete process of LA formation from hemicellulose includes three steps which

include hydrolysis of hemicellulose polymers under an acid catalyst and dehydration of the C5 sugar to furfural, which is released into furfural alcohol by hydrogenation, and hydrolysis of furfural alcohol to LA [18].

LA can be produced through several different process routes, namely, a five-step route process from petrochemical intermediate maleic anhydride, which shows good results but is not economically sustainable on a large scale. Secondly, the hydrolysis process of complex carbohydrates from biomass such as starch, cellulose, and hemicellulose involves the acid treatments of C6 sugar such as glucose, fructose, mannose, and galactose. Furthermore, the synthesis of LA from the direct conversion of furfural alcohol from furfural hydrogenation [18]. In the production of LA from macroalgae, polysaccharides or carbohydrates are hydrolyzed into monosaccharides which are further converted to LA and the reaction process is the synthesis of cellulose, which is hydrolyzed into glucose. Subsequently, glucose is dehydrated to 5-hydroxymethylfurfural and hydrolyzed again to become LA. Meanwhile, all stages involved in the formation of LA are shown in Figure 4.

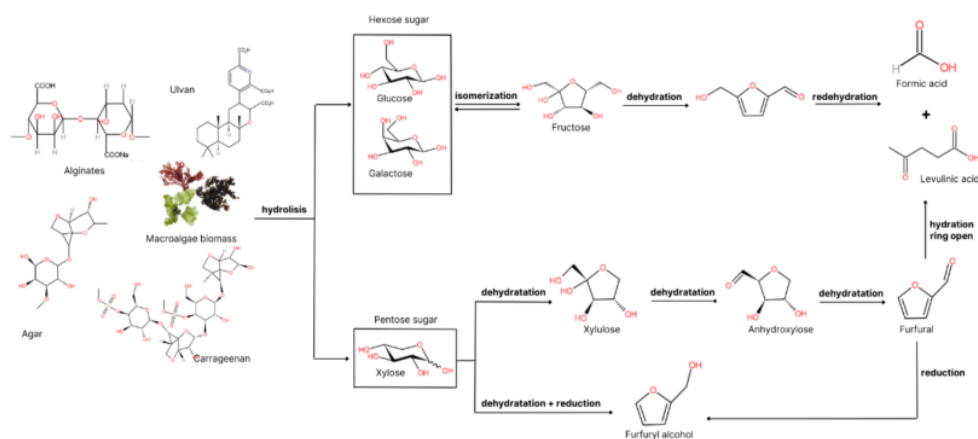


Figure 4. LA formation reaction process using macroalgae biomass.

Hydrolysis occurs to form glucose, which begins with biomass, followed by isomerization of glucose to fructose, dehydration of fructose to product 5-HMF from acid-catalyzed dehydration of C6 sugar [18]. Subsequently, the 5-HMF is hydrolyzed once more to form LA. According to Antonetti et al. [18], the hydration of 5-HMF starts by adding a water molecule to the olefinic bond of the furan ring, which leads to an unstable tricarbonyl intermediate that decomposes to LA and formic acid. Therefore, the temperature is increased in the reaction step of the aqueous environment, which causes the dehydration reaction to being thermodynamically more favorable.

3.4. Biochemical of Potential Macroalgae Candidate

Several studies have been carried out on terrestrial plants in the term of the production of LA, however, only a few are on the production from macroalgae. Macroalgae have the huge potential to produce LA because of their abundant and high polysaccharide content, which are primary metabolism products that were produced by macroalgae. These macroalgae polysaccharides are a broad group of compounds with various important dietary fiber biological functions. This dietary fiber is divided into two fractions, namely, soluble and insoluble fibers. The red, green, and brown macroalgae have soluble fiber fractions, including pectin, xyloglucans, galactomannan hemicelluloses, gums, and waxes. Meanwhile, the insoluble fiber fraction consist of celluloses, arabinoxylan hemicelluloses, and lignin [20].

Polysaccharides are groups of compounds with varying compositions for each type of macroalgae species, which are distinguished based on their function and chemical structure into storage and structural polysaccharides. The main storage polysaccharide in green macroalgae is starch, while it is floridean starch in red macroalgae, and laminaran in brown macroalgae. The cell wall of macroalgae differs from the presence of structural polysaccharides. Green macroalgae cell walls are formed from ulvans, xylan, mannan, and cellulose, red macroalgae is formed by sulfated galactan (carrageenan and agar), cellulose, some found xylan, and mannan, while brown macroalgae mostly consist of fucoidans, cellulose, and alginate [20].

The polysaccharides in the red macroalgae group include carrageenan and agar. Meanwhile, carrageenan is formed from $\alpha(1\rightarrow4)$ -anhydro-D-galactose and $\beta(1\rightarrow3)$ -D-galactose, while agar is formed from the repeating unit of agarobiose or agarose, which is a disaccharide composed of D-galactose and 3,6-anhydro-L-galactopyranose. Based on previous studies, the polysaccharide content of *Kappaphycus alvarezii* was $32.95 \pm 1.43\%$ [12] while *Eucheuma denticulatum* ranged from 17.8 to 35.5% [21]. According to the extraction method, the yield of polysaccharides from *Gracilaria verrucosa* ranged from 0.135 to 35.11%, while *Gracilaria lemaneiformis* and *Gelidium amansii* had 64.80 and 58.60%, respectively [22,23].

The green macroalgae in the majority have polysaccharides in form of ulvan. Moreover, *Ulva lactuca* contains $1\rightarrow$, $1\rightarrow6$, $1\rightarrow2$, $1\rightarrow2$, 6, or unoxidized glycosidic bonds which differ from other species. The results showed that the higher polysaccharide yield was 17.57%, with monosaccharides such as rhamnose, xylose, glucose, glucuronic acid, and sulfate [24]. Furthermore, water-soluble sulfated polysaccharides of *Enteromorpha intestinalis* are composed of $(1\rightarrow2)$ -linked rhamnose and $(1\rightarrow2)$ -linked glucose residues with polysaccharide yield of 11.38 to 59.1% [25,26]. Previous study showed that *Ulva pertusa* produced a polysaccharide content of 18.30% [27].

Brown macroalgae have a polysaccharide type in the form of alginate, where the contents of *Sargassum polycystum* are 15.85% and consist of $(1\rightarrow4)$ -linked β -D-mannuronate (M) and α -L-guluronate (G) [28]. *Hizikia fusiforme* contains 63.56% sulfated polysaccharides, which are made of glucose, xylose, galactose, and fucose [29]. Based on the high polysaccharide content, macroalgae can be used as raw materials for LA since polysaccharides are the starting material in the production of LA. The polysaccharide content in macroalgae and their types are shown in Table 1.

Table 1. Type, chemical structure, and content of polysaccharides of red, green, and brown macroalgae.

Macroalgae	Types of Polysaccharide	Chemical Structure	Polysaccharide Content (%)	Ref.
Red Macroalgae				
<i>Kappaphycus alvarezii</i>	Carrageenan	$\alpha(1\rightarrow4)$ -anhydro-D-galactose and $\beta(1\rightarrow3)$ -D-galactose	32.95 ± 1.43	[12]
<i>Eucheuma denticulatum</i>	Carrageenan		17.8 to 35.5	[21]
<i>Gracilaria verrucosa</i>	Agar	D-galactose and 3,6-anhydro-L-galactopyranose	0.135 to 35.11	[30]
<i>Gracilaria lemaneiformis</i>	Agar		64.80	[22]
<i>Gelidium amansii</i>	Agar	D-galactose and 3,6-anhydro-L-galactose	58.60	[23]
Green Macroalgae				
<i>Enteromorpha intestinalis</i>	Water-soluble sulfated polysaccharides	$(1\rightarrow2)$ -linked rhamnose and $(1\rightarrow2)$ -linked glucose residues	11.38 to 59.1	[25,26]
<i>Ulva lactuca</i>	Ulvan	$1\rightarrow$, $1\rightarrow6$, $1\rightarrow2$, $1\rightarrow2$, 6, or unoxidized glycosidic bonds	17.57	[24]
<i>Ulva pertusa</i>	Crude polysaccharide		18.30	[27]
Brown Macroalgae				
<i>Sargassum polycystum</i>	Alginate	$(1\rightarrow4)$ -linked β -D-mannuronate (M) and α -L-guluronate (G)	15.85	[28]
<i>Hizikia fusiforme</i>	Alginate		63.56 ± 0.32	[29]

Macroalgae contain a high nutritional value which contributes to human nutrients. There are several articles on the proximate composition of macroalgae, specifically the carbohydrate content is the important material in LA production. The proximate compositions for biomass of various macroalgae species have been widely reported in previous studies (Table 2).

Table 2. Proximate composition of red, green, and brown macroalgae biomass.

Biomass	Proximate Composition (g/100 g Dry Weight)				Ref.
	Carbohydrate	Protein	Lipid	Ash	
Red Macroalgae					
<i>Kappaphycus alvarezii</i>	67.8 ± 10.40	3.60 ± 0.00	0.60 ± 0.00	18.40 ± 0.50	[16]
<i>Euchema denticulatum</i>	64.70 ^(a)	4.50 ^(a) , 5.06 ^(b)	0.20 ^(a) , 1.78 ^(b)	30.6 ^(a) , 27.13 ^(b)	[31] ^(a) , [32] ^(b)
<i>Gracilaria verrucosa</i>	66.95	9.4	0.65	7.42	[33]
<i>Gracilaria verrucosa</i>	38.38 to 60.81	6.64 to 9.86	0.80 to 0.58	13.85 to 12.51	[34]
<i>Gracilaria lemaneiformis</i>	71.5	9.30	0.92	18.2	[22]
<i>Gracilaria gigas</i>	47.31 to 64.71	8.14 to 12.63	0.60 to 1.31	17.86 to 19.59	[34]
<i>Gelidium amansii</i>	66.0 to 75.2	18.5 to 20.5	0.20 to 0.60	5.70 to 13.3	[35]
<i>Carpopeltis cornea</i>	60.7	23.4	0.4	15.6	[36]
<i>Chondrus crispus</i>	65.7	8.1	0.9	25.2	[37]
Green Macroalgae					
<i>Enteromorpha intestinalis</i>	42.8	31.6	1.30	24.3	[38]
<i>Ulva lactuca</i>	50.4	26.8	0.60	22.2	[35]
<i>Ulva pertusa</i>	52.3	25.1	0.10	22.5	[35]
Brown Macroalgae					
<i>Sargassum polycystum</i>	46.6	6.00	0.30	47.1	[35]
<i>Hizikia fusiforme</i>	47.5	9.80	1.20	41.5	[35]
<i>Undaria pinnatifida</i>	43.2	23.80	3.50	29.5	[35]
Non-Macroalgae					
Corn cob	10.4 ^(a)	7.10 ^(a)	-	3.00 ^(b)	[39] ^(a) , [40] ^(b)
Rice straw	58.3 ^(a)	-	-	8.20 ± 0.10 ^(b)	[41] ^(a) , [42] ^(b)
Corn stover	71.7 ^(a)	-	-	1.50 ± 0.10 ^(b)	[19] ^(a) , [42] ^(b)
Sweet sorghum bagasse	7.70 ^(a)	5.40 ^(a)	-	1.00 ± 0.10 ^(b)	[39] ^(a) , [42] ^(b)
Miscanthus	65.4 ^(a)	3.2 ^(a)	-	2.10 ± 0.30 ^(b)	[43] ^(a) , [42] ^(b)

Note: A superscript symbol ^(a) and ^(b) shows a reference source in each row. Data with the same superscript symbol in the same row represents the same reference.

The carbohydrate compound in red macroalgae ranged from 38.38 g to 71.5 g/100 g dw. The results showed that *Gracilaria lemaneiformis* had the highest carbohydrate content among red macroalgae species, while *Carpopeltis cornea* showed highest content of protein 23.4 g/100 g dw. Furthermore, the lipid content of red macroalgae value was less than 1 g/100 g dw and the ash content ranged from 5.70 g to 30.6 g/100 g dw. The green macroalgae had a carbohydrate value that ranged from 42.8 g to 52.3 g/100 g dw and also exhibited high protein content compared with other macroalgae. *Ulva pertusa* gave a low lipid content (0.10 g/100 g dw) than other species in the same group [35]. The ash content of green algae ranged from 22.2 g to 24.3 g/100 g dw. Among the brown macroalgae with a value ranging from 43.2 g to 47.5 g/100 g dw, *Hizikia fusiforme* showed the highest carbohydrate content of 47.5 g/100 g dw. Meanwhile, *Sargassum polycystum* contained the lowest protein which was 6.00 g/100 g dw, and lipid content 0.30 g/100 g dw, while the highest ash content was 47.1 g/100 g dw [35]. The main material in LA production is a carbohydrate, which is hydrolyzed to monosaccharide and converted to LA. In this study, red macroalgae contained the highest carbohydrate compared with green and brown macroalgae. The carbohydrate of red macroalgae can be hydrolyzed into glucose and galactose, therefore, it is the most investigated among the other class of macroalgae.

Several biomasses were also derived from terrestrial plants such as corn cob, rice straw, corn stover, sweet sorghum bagasse, and Miscanthus with potential as LA producers. Corn cob has a proximate composition in form of carbohydrates, protein, and ash by 10.40 g, 7.10 g, and 3.00 g/100 g dw, respectively [39,40], while rice straw biomass

has 58.30 g/100 g dw and the crude ash content of $8.20 \text{ g} \pm 0.10 \text{ g}/100 \text{ g dw}$ [41,42]. Meanwhile, the highest carbohydrate content of land plants is corn stover biomass with 71.70 g/100 g dw [19,42], followed by Miscanthus with 65.4 g/100 g dw [42,43]. The use of terrestrial plants has some problems such as the competition with the food demand, high lignin content, and land. Compared with terrestrial plants, macroalgae contained higher carbohydrate content, even some macroalgae species.

LA is the target product that is produced from the conversion of sugars through hydrolysis and thermochemical reaction. Studies of hydrolysis and thermochemical reaction optimization have been conducted to obtain the optimum sugar and high LA (Table 3).

Table 3. Sugar, HMF, and levulinic acid composition of macroalgae and other biomass after hydrolysis.

Biomass	Sugar, HMF and Levulinic Acid after Hydrolysis		Ref.
Red Macroalgae			
Kappaphycus alvarezii	Glucose	0.215 g/L	[16]
	Galactose	1.447 g/L	
	5-HMF	0.302 g/L	
Gracilaria verrucosa	Glucose	0.27%	[8]
	Galactose	1.23%	
	5-HMF	0.47%	
Gracilaria verrucosa	Glucose	4.29 g/L	[33]
	Galactose	18.38 g/L	
	5-HMF	3.74 g/L	
Gracilaria verrucosa	Glucose	18.17 g/L	[44]
	Galactose	10.83 g/L	
	Gelidium amansii	Glucose	3.76 g/100 g
Galactose		1.36 g/100 g	
Glucose		8.4 g/L	
Gelidium amansii	Galactose	20.3 g/L	[46]
	5-HMF	3.8 g/L	
	Formic acid	1.6 g/L	
Gelidium latifolium	Glucose	2.4 g/L	[47]
	Galactose	34.43 g/L	
	5-HMF	5.7 g/L	
Gracilaria fisheri	Glucose	7.86 g/L	[48]
	Galactose	8.37 g/L	
	5-HMF	1.55 g/L	
Gracilaria tenuistipitata	Glucose	3.15 g/L	[48]
	Galactose	5.75 g/L	
	5-HMF	1.42 g/L	
Green Macroalgae			
Enteromorpha intestinalis	Glucose	10.42%	[38]
	Xylose-mannose-galactose (XMG)	18.08%	
	Total reducing sugar (TRS)	28.61%	
	5-HMF	1.71%	
	Furfural	2.03%	
Other biomasses			
Glucosamine	Formic acid	50.80%	[49]
	Lignin	27.00%	
	Glucan	28.20%	
Corn stover	Xylan	21.60%	[50]
	Arabinan	2.50%	
	Others	14.20%	
	Cellulose	60.70 g/L	
	Lignin	31.40 g/L	
Corn cob	Hemicellulose	2.70 g/L	[40]
	Others	2.20 g/L	

Table 3. Cont.

Biomass	Sugar, HMF and Levulinic Acid after Hydrolysis		Ref.
Rice straw	Glucan	36.30 ± 0.10 wt%	[42]
	Xylan	14.00 ± 1.00 wt%	
	Arabinan	3.70 ± 0.00 wt%	
	Acid-insoluble lignin (AIL)	15.00 ± 0.70 wt%	
	Acid-soluble lignin (ASL)	2.10 ± 0.40 wt%	
Corn stover	Glucan	33.00 ± 0.90 wt%	[42]
	Xylan	18.40 ± 0.70 wt%	
	Arabinan	5.30 ± 0.10 wt%	
	Acid-insoluble lignin (AIL)	15.20 ± 0.30 wt%	
	Acid-soluble lignin (ASL)	2.20 ± 0.10 wt%	
Sweet sorghum bagasse	Glucan	41.30 ± 0.20 wt%	[42]
	Xylan	11.70 ± 0.00 wt%	
	Arabinan	3.10 ± 0.10 wt%	
	Acid-insoluble lignin (AIL)	12.00 ± 0.30 wt%	
	Acid-soluble lignin (ASL)	1.30 ± 0.10 wt%	
Miscanthus	Glucan	44.30 ± 0.30 wt%	[42]
	Xylan	18.40 ± 0.10 wt%	
	Arabinan	3.50 ± 0.00 wt%	
	Acid-insoluble lignin (AIL)	18.90 ± 0.30 wt%	
	Acid-soluble lignin (ASL)	0.70 ± 0.00 wt%	
Glucose	Formic acid	50.79%	[51]
	Glucose	99.80%	
	5-HMF	0.06%	

The chemical composition from the hydrolysis process also includes glucose, galactose, 5-HMF, and formic acid. Based on the results, red macroalgae, *Gelidium amansii*, produced the highest galactose (34.43 g/L), glucose (8.4 g/L), and 5-HMF (5.7 g/L) [46,47] compared with other species in the same group. However, all species of red macroalgae had lower glucose production than galactose.

There was only one literature source on sugar and byproduct production from green macroalgae. A study by Kim et al. [38] showed that *Enteromorpha intestinalis* produce glucose, xylose-mannose-galactose (XMG), total reducing sugar (TRS), 5-HMF, and furfural of 10.42%, 18.08%, 28.61%, 1.71%, and 2.03%, respectively. This showed that TRS has the highest percentage among other chemicals. There were no articles on the products from the hydrolysis process on brown seaweed. Therefore, further study on platform chemicals as raw materials for bioenergy from green and brown macroalgae is recommended.

This study also observed the sugar composition from other sources of biomass such as land plants and waste material, where glucosamine produced 50.80% formic acid [49]. Other materials produced from the delignification and hydrolysis process are lignin, glucan, xylan, arabinan, cellulose, hemicellulose, acid-insoluble lignin (AIL), and acid-soluble lignin (ASL). Furthermore, glucose produced 50.79% of formic acid, 99.80% of glucose conversion, and 0.06% of 5-HMF through hydrolysis [51].

The comparison of macroalgae biomass and other biomasses, specifically terrestrial plants, showed that macroalgae can be used as raw materials in LA production. The product value obtained by macroalgae is similar to other biomasses that were previously developed. These results illustrated the potential of macroalgae in the bioenergy industry due to their natural abundance and cultivation. As shown in Table 2, the carbohydrate content of macroalgae is comparable or even higher than a terrestrial plant, while the sugar yield is still lower than the theoretical yield. Therefore, an optimum reaction pathway in the pretreatment and hydrolysis process needs to be developed to overcome the problem.

3.4.1. Pretreatment

The pretreatment process is an important step to obtain the optimum LA production and can affect the recovery of LA. Meanwhile, it is necessary to conduct an initial

pretreatment before achieving the desired results. The aim of pretreatment on biomass is to increase the efficiency of catalysts and enzymes in synthesizing a compound. The pretreatment of macroalgae and other biomasses to produce LA is shown in Table 4.

Table 4. Pretreatment biomass as raw material in LA production.

No.	Raw Material	Pretreatment	Ref.
1	<i>Kappaphycus alvarezii</i> (macroalgae)	1. The biomass sample was rinsed in distilled water 2. Dry at 60 °C to constant 3. Dry biomass was milled and filtered with a screen up to >100 µm 4. Stored in a sealed bag at room temperature	[16]
2	<i>Gracilaria verrucosa</i> (macroalgae)	1. Samples were washed with distilled water 3 times 2. Lyophilized (freeze-drying) for 3 days 3. Milled and filtered to a particle size below 100 µm 4. Stored in a sealed bag	[8]
3	<i>Gracilaria verrucosa</i> (macroalgae)	1. Washing in distilled water 3 times to remove salt was carried out for 2 days at 60 °C 2. Dry biomass was ground and screened with a net to sizes below 100 µm 3. Stored in a sealed bag at room temperature	[33]
4	<i>Gracilaria lemaneiformis</i> (macroalgae)	1. Samples were washed with deionized water 2. Dried in the oven at 60 °C for 48 h 3. The dry sample was ground and filtered using a 0.5 mm sieve and stored in a closed container at 4 °C	[22]
5	<i>Gelidium amansii</i> (macroalgae)	1. Samples were washed with distilled water 2. Dried for 2 days at 60 °C 3. Milled and sieved to a size of 20–40 mesh	[52]
6	<i>Gelidium amansii</i> (macroalgae)	1. The sample was ground and filtered with a pore screen of 2 mm 2. α-selulosa was used as control	[45]
7	<i>Enteromorpha intestinalis</i> (macroalgae)	1. Biomass was dried, milled, and filtered through a screen with sizes below 200 µm 2. Stored in a sealed bag at room temperature	[38]
8	<i>Chaetomorpha linum</i> (macroalgae)	1. Samples were dried at room temperature for 15 days 2. The biomass was pretreated at room temperature and stirred for 2 h before the hydrolysis reaction was carried out to facilitate contact between the catalyst and the inner biomass fiber	[53]
9	<i>Valonia aegagropila</i> (macroalgae)	1. Samples were dried at room temperature for 15 days 2. The biomass was pretreated at room temperature and stirred for 2 h before the hydrolysis reaction was carried out to facilitate contact between the catalyst and the inner biomass fiber	[53]
10	<i>Scenedesmus obliquus</i> (microalgae)	1. Microalgae were cultured mixotrophically for 14 days 2. Microalgae lipid extracts were prepared by lipid extraction using organic solvents 3. Total carbohydrates extracted lipids were $29.36 \pm 0.29\%$ based on dry weight determined by standard procedure	[54]
11	Corn stover	1. Conducted hydrothermal treatments during pretreatment with reactor pressure maintained using a backpressure regulator and pressure gauge 2. The flow rate was maintained during the heating, reaction, and cooling phases 3. Liquid hydrolyzate was collected and mpH measured	[50]
12	Corn cob	1. Corn cob was given a solution of sulfuric acid at a temperature of 373.15 K with a ratio of 1:10 for 3 h to remove hemicellulose from corn cob 2. The hydrolyzate was filtered and the remaining corn cob was washed with deionized water 3 times 3. Leftover corn cob was dried in the oven	[40]
13	Rice straw	1. The biomass was ground and sieved using a sieve with a size of 10–35 mesh (0.5–2.0 mm) 2. The pretreatment process was carried out by giving Na ₂ CO ₃ and Na ₂ S reagents dissolved in deionized water 3. The solution was titrated and continued in the delignification process	[42]
14	Corn stover	1. The biomass was ground and sieved using a sieve with a size of 10–35 mesh (0.5–2.0 mm) 2. The pretreatment process was carried out by giving Na ₂ CO ₃ and Na ₂ S reagents dissolved in deionized water 3. The solution was titrated and continued in the delignification process	[42]

Table 4. Cont.

No.	Raw Material	Pretreatment	Ref.
15	Sweet sorghum bagasse	<ol style="list-style-type: none"> 1. The biomass was ground and sieved using a sieve with a size of 10–35 mesh (0.5–2.0 mm) 2. The pretreatment process was carried out by giving Na_2CO_3 and Na_2S reagents dissolved in deionized water 3. The solution was titrated and continued in the delignification process 	[42]
16	Miscanthus	<ol style="list-style-type: none"> 1. The biomass was ground and sieved using a sieve with a size of 10–35 mesh (0.5–2.0 mm) 2. The pretreatment process was carried out by giving Na_2CO_3 and Na_2S reagents dissolved in deionized water 3. The solution was titrated and continued in the delignification process 	[42]

There are several pretreatment methods used in lignocellulosic biomass to remove lignin and acetyl groups from hemicelluloses, increase the porosity of the material, and reduce the cellulose crystallinity [55]. These methods include: (i) physical pretreatment methods, which through the use of milling or chipping reduce the size of the biomass and reduce crystallinity; (ii) physicochemical pretreatments, which use a combination of physical and chemical treatment in the form of evaporation, using liquid ammonia or carbon dioxide to degrade hemicellulose and lignin through explosive decompression; and (iii) chemical pretreatments, using solvents, oxidants, acids, or bases. Meanwhile, raw materials such as macroalgae and other biomasses need physical pretreatment to reduce the size and increase the surface area of the sample for easy extraction. In some macroalgae studies, freeze-drying methods were applied as part of the pretreatment method.

According to a study by Pulidindi and Kim [42], the conversion of LA from several biomasses such as rice straw, corn stover, sweet sorghum bagasse, and miscanthus were carried out using acid-catalyzed hydrothermal as a pretreatment method. In addition, the biomass delignification process was conducted using simulated green liquor (SGL) in the form of Na_2CO_3 - Na_2S , 20% total titratable alkali (TTA), 40% sulfidity to remove lignin [42]. Furthermore, the hydrothermal pretreatment for lignocellulosic biomass increased LA production because water under high pressure and temperature can penetrate the biomass and increase the surface area. This makes it more accessible to catalysts and hydrolytic enzymes which hydrate the cellulose, remove hemicellulose, and partially remove lignin [56].

3.4.2. Hydrolysis

The main process of converting sugar to LA is hydrolysis, which is carried out using chemical and enzymatic techniques. Chemical hydrolysis is typically catalyzed by acids, while the enzymatic technique is catalyzed by enzymes that break down polysaccharides into monosaccharides [57].

Acid Hydrolysis

Acid hydrolysis involves the use of an acid catalyst to speed up the thermochemical reaction. It is carried out by adjusting the reaction conditions using acid as a catalyst to increase the target product [58]. Acid hydrolysis is conducted by adding a certain concentration of acid catalyst to the biomass through hydrothermal heating at a specific temperature and time. Each hydrolyzed material requires different optimum conditions, which depend on the content of the biomass. The optimal conditions in the acid hydrolysis reaction for the production of LA from various biomass are shown in Table 5.

Table 5. Optimal reaction condition of biomass by acid hydrolysis.

Biomass	Hydrolysis	Temperature (°C)	Reaction Condition Time (min)	Catalyst Concentration	Yield of LA	Ref.
Red Macroalgae						
<i>Kappaphycus alvarezii</i>	Acid	178.2	39.3	2.87% H ₂ SO ₄	1.17 g/L	[16]
<i>Kappaphycus alvarezii</i>	Acid	130	15	0.2 M HCl	2.8 g/L	[57]
<i>Kappaphycus alvarezii</i>	Acid	130	15	0.2 M H ₂ SO ₄	1.07 g/L	[59]
<i>Kappaphycus alvarezii</i>	Acid + enzyme	120	15	0.2 M H ₂ SO ₄	2.11:2.02% g/g	[12]
<i>Kappaphycus alvarezii</i>	Acid	130	15	H ₂ SO ₄	0.96 g/L	[60]
<i>Gracilaria verrucosa</i>	Acid	180	20	0.5 M MSA	22.02%	[8]
<i>Gracilaria verrucosa</i>	Acid	180.9	50	2.85% H ₂ SO ₄	1.47 g/L	[33]
<i>Gracilaria lemaneiformis</i>	Acid	180	20	0.2 M H ₂ SO ₄	16.30 wt%	[22]
<i>Gelidium amansii</i>	Acid	160	43.1	3% H ₂ SO ₄	9.74 g/L	[52]
<i>Gelidium amansii</i>	Acid	180	48.22	3% H ₂ SO ₄	42.88%	[45]
<i>Gelidium amansii</i>	Acid	142.6	11	358.3 mM H ₂ SO ₄	6.3 g/L	[46]
<i>Gelidium amansii</i>	Acid	180	20	H ₃ PO ₄ :HNO ₃ = 5:5, mM	7.87 g/L	[61]
<i>Gracilaria fisheri</i>	Acid	96	150	1 M H ₂ SO ₄	3.66 g/L	[48]
<i>Gracilaria tenuistipitata</i>	Acid	96	150	1 M H ₂ SO ₄	6.12 g/L	[48]
<i>Gracilariopsis chorda</i>	Acid	130	15	0.2 M H ₂ SO ₄	0.42 g/L	[62]
<i>Gelidium latifolium</i>	Acid	130	15	H ₂ SO ₄ and HCl	3.45 g/L and 1.88 g/L	[47]
Green Macroalgae						
<i>Enteromorpha intestinalis</i>	Acid	175	35	3.7% H ₂ SO ₄	4.00%	[38]
<i>Chaetomorpha linum</i>	Acid	190	45	4.7% H ₂ SO ₄	19 wt%	[53]
<i>Valonia aegagropila</i>	Acid	200	45	4.7% H ₂ SO ₄	16 wt%	[53]
<i>Codium fragile</i>	Acid	160.7	39.1	3.9% H ₂ SO ₄	4.26 g/L	[63]
Other Biomasses						
<i>Scenedesmus obliquus</i> (microalgae)	Acid	180	10	0.85 M HCl	45.63 wt%	[54]
Glucosamin (crustacean shell chitosan monomer from food waste)	Acid	200	20	15 mol% ZrOCl ₂	21.29 mol%	[64]
Glucosamin (crustacean chitosan)	Acid	188	49	4% H ₂ SO ₄	30.30 g/L	[65]
Glucosamin (chitin/chitosan monomer)	Acid	200	30	0.5 M MSA	49.90%	[49]
Corn stover	Acid	190	5	2% H ₂ SO ₄	10–35 wt%	[50]
Corn cob	Acid	180	50	0.5 mol/L H ₂ SO ₄	107.93 g/L	[40]
Rice straw	Acid	150	300 (5 h)	1 M HCl	60.20 wt%	[42]
Corn stover	Acid	150	300 (5 h)	1 M HCl	75.10 wt%	[42]
Sweet sorghum bagasse	Acid	150	300 (5 h)	1 M HCl	78.50 wt%	[42]
Miscanthus	Acid	150	300 (5 h)	1 M HCl	61.70 wt%	[42]
Glucose	Acid	181.2	44.4	0.35 M MSA	48.95%	[51]

In this study, the hydrolysis of macroalgae and other biomasses was dominated by acid hydrolysis. Furthermore, temperature, time, and catalyst concentration significantly affected LA production. The optimum temperature and reaction time catalyst concentration to produce LA ranged from 96–180.9 °C and 11–150 min, respectively, while the most catalyst used was H₂SO₄ with varying concentrations. In addition, *Gelidium amansii* possessed the highest LA yield of 9.74 g/L, where the optimum temperature (°C), time (min), and catalyst concentration are 160 °C, 43.1, and 3% H₂SO₄, respectively [61].

The most dominant red macroalgae biomass used in LA production is *Kappaphycus alvarezii*, with *Gelidium amansii* as the second highest. The optimum reaction conditions for *Kappaphycus alvarezii* were at a temperature of 178.2 °C for 39.3 min using a catalyst concentration of 2.87% H₂SO₄, which produced 1.17 g/L of LA [16]. Furthermore, Meinita et al. [60] also discovered LA as a byproduct in bioethanol production from *Kappaphycus alvarezii*, *Gelidium*, *Gracilaria* *Gracilariopsis*, carrageenan waste and agar waste. Detoxification of these byproducts was done to minimize its inhibition effect on ethanol

fermentation [60]. *Gracilaria verrucosa* produced LA in the optimum conditions at 180 °C for 20 min in a catalyst concentration of 0.5 M MSA (methanesulfonic acid) with a yield of 22.02% [8]. Meanwhile, a study by Jeong et al. [33] reported that *Gracilaria verrucosa* produced LA of 1.47 g/L in the optimum conditions at a temperature of 180.9 °C for 50 min with a catalyst concentration of 2.85% H₂SO₄, while *Gracilaria lemaneiformis* obtained a LA yield of 16.30 wt% at 180 °C for 20 min using 0.2 M H₂SO₄ as the catalyst.

According to Kim et al. [38], the use of *Enteromorpha intestinalis* gave LA of 4.00% with optimum conditions at a temperature of 175 °C for 35 min using 3.7% of H₂SO₄ as a catalyst. Galletti et al. [53] also synthesized LA from *Chaetomorpha linum* and *Valonia aegagropila* to obtain LA yields of 19 wt% and 16 wt%, respectively at a temperature of 190 °C and 200 °C for 45 min with 4.7 % of H₂SO₄ as a catalyst.

Several studies were carried out on LA production from other biomasses. These include the study of Jeong and Kim [54] that synthesized LA from the microalgae *Scenedesmus obliquus* with a yield of 45.63 wt% at optimum conditions of 180 °C for 10 min using 0.85 M of HCl as catalyst. It was also produced from waste biomass of crustacean shells in the form of glucosamine as stated by Park et al. [64], where a yield of 21.29 mol% was obtained with a reaction temperature of 200 °C for 20 min using 15 mol% of ZrOCl₂ as a catalyst. Meanwhile, the glucosamine biomass synthesized by Jeong et al. [65] produced a LA of 30.30 g/L at 188 °C for 49 min with 4% H₂SO₄ as catalysts. The glucosamine produced 49.90% of LA at optimum conditions of 200 °C for 30 min with a catalyst concentration of 0.5 M H₂SO₄ [49]. The use of biomass derived from agricultural raw materials such as corn stover conducted by Thakkar et al. [50] produced LA of 10–35% at a temperature of 190 °C for 5 min with 2% H₂SO₄ as catalysts. According to Liang et al. [40], corn cob produced the maximum 107.93 g/L of LA at a temperature of 453.15 °C for 50 min with 0.5 mol/L H₂SO₄ as a catalyst. In a study by Pulidindi and Kim [42], several biomasses such as rice straw, corn stover, sweet sorghum bagasse, and Miscanthus were synthesized with LA yields of 60.20 wt%, 75.10 wt%, 78.50 wt%, and 61.70 wt%, respectively, at an optimum condition of 150 °C for 5 h using 1 M HCl as catalysts. Kim et al. [51] also conducted a study using glucose and obtained a LA yield of 48.95% at optimum conditions of 181.2 °C for 44.4 min using 0.35 M MSA (methanesulfonic acid) as catalysts. Since macroalgae contain low levels of glucan, it shows that glucan conversion alone is not sufficient to produce high concentrations of LA. Therefore, it is necessary to produce LA from specific carbohydrate compounds such as sulfated polysaccharides, mannitol, alginate, agar, and carrageenan in macroalgae [66].

Based on this study, the acid hydrolysis method is the most common thermochemical pretreatment in LA production and is widely used due to its low cost and simplicity. However, it has some disadvantages such as the release of byproduct compounds, the residue of lignin, and the formation of humin during the carbohydrate conversion from lignocellulosic feedstock. This drawback can interfere with the LA formation and decrease its yield. Since macroalgae contain low lignin, the humin formation can be minimized by determining the optimum reaction pathways.

Enzymatic Hydrolysis

Enzymatic hydrolysis involves the use of enzymes to accelerate the reaction process which is more efficient and easier than acid hydrolysis. The effectiveness of the enzymatic hydrolysis of cellulose can be increased by heating the raw material to be hydrolyzed using water vapor at high temperatures [67] to degrade the hemicellulose into pentose. It is affected by several factors such as temperature, reaction time, mixing, catalyst concentration, starch suspension levels [68], and requires a higher cost than acid hydrolysis. The results showed that there is no study on enzymatic hydrolysis in the production of LA from macroalgae. Therefore, further study is recommended to focus on LA production using enzymatic hydrolysis.

4. Future Needs and Challenges

The demand for LA and its chemical derivatives in the industry is increasing steadily, therefore, a sustainable feedstock is needed to fulfill the global demand. Studies show that macroalgae have a promising feedstock for the industrial production of LA due to their advantages compared with terrestrial and non-macroalgae biomass. The high biomass productivity, high degradable carbohydrate content, and low lignin content of macroalgae, which make it a potential feedstock for producing LA. Macroalgae is mainly composed of polysaccharide and carbohydrate that can be hydrolyzed into monosaccharide. This study discovered that macroalgae contained similar or higher carbohydrate content compared with terrestrial or other biomasses. However, the LA yield produced from macroalgae is still lower than the theoretical yield due to low levels of glucan, making it insufficient to produce high concentrations of LA. Therefore, it is necessary to produce LA from specific carbohydrate components such as sulfated polysaccharides, mannitol, alginate, agar, and carrageenan in macroalgae [66], while some factors need to be considered to make macroalgae feasible to apply on an industrial scale.

4.1. Technology to Optimize the Conversion of Carbohydrates into Monosugar

The optimization of thermochemical pretreatments including hydrothermal, dilute acid, organic solvents, and hydrolysis of macroalgae biomass need to be developed. The pretreatment mainly plays an important role in the depolymerization of the polysaccharide matrix surrounding the cell wall of macroalgae. The efficient pretreatment and hydrolysis lead to optimum LA production. Furthermore, efficient reaction pathways need to be discovered to optimize LA yield and production.

4.2. Cultivation Technology

The sustainability of biomass is an important factor in the biorefinery concept. Meanwhile, one of the benefits of using macroalgae in LA production is its high production. Globally, macroalgae production is increasing to over 30 million tons [69], however, the cultivation technology still needs improvement. Furthermore, production of genetically and developmentally modified seaweeds through somatic variants, artificial hybrids, and mutant development is also needed.

4.3. The Drawback of LA Production

The drawback during the conversion of carbohydrates in LA production includes the formation of undesirable byproducts such as humin, lignin, and other compounds which affect LA yield. This can be minimized by optimizing reaction conditions, pathways, and reusing the byproduct compounds.

5. Conclusions

Levulinic acid (LA) is one of the top 12 biomass-derived raw materials that have important potential applications in various industries. Meanwhile, macroalgae have attracted attention as a promising raw material for LA production due to their degradable carbohydrate which can be converted into LA and high-value chemical platforms. This study showed the gaps and challenges in the production of LA from macroalgae based on the industrial scale, which can be overcome by cultivation technology. Meanwhile, the low yield in LA production from algae is solved by developing the synthesis pathways reaction for optimal reaction conditions to minimize undesirable byproduct compounds. Since macroalgae have been cultivated by coastal communities, their use in LA production can empower and increase the income of such communities. The degradable carbohydrate of macroalgae can be converted into several valuable products, therefore, an integrated low-cost biorefinery industry from macroalgae can be achieved.

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