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High Saccharification, Low Lignin, and High Sustainability Potential Make Duckweeds Adequate as Bioenergy Feedstocks

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Abstract

Duckweeds are the smallest free-floating aquatic monocots. They have a unique cell wall containing pectin polymers named apiogalacturonan and xylogalacturonan. Knowing that the cell wall composition is essential for duckweeds as a bioenergy feedstock, notably ethanol production, this work reports the five duckweed species' (*Spirodela polyrhiza, Landoltia punctata, Lemna gibba, Wolffiella caudata, and Wolffia borealis*) composition and saccharification potential. Nonstructural carbohydrates were, on average, 42% of the dry weight. The cell wall comprises 20.1% pectin and glucomannan, 35.2% hemicelluloses, 30% cellulose, and 5% lignin, and the fermentable sugars in duckweed walls are glucose, galactose, and xylose. Together, these monosaccharides constitute 51.4% of the cell wall. Duckweeds displayed low recalcitrance to hydrolysis, probably due to the low lignin and cellulose contents. Furthermore, the saccharification of the duckweeds was higher than sugarcane, a primary bioethanol feedstock. Results indicate that duckweed biomass displays a high potential as a feedstock for bioethanol production.

Keywords Cell wall · Polysaccharides · Ethanol · Fermentable sugars

Introduction

Carbon emissions from fossil fuels contribute to global warming, demanding sustainable energy resources [1]. One of the leading renewable fuel options is ethanol, produced from various feedstocks [2, 3]. The first-generation (1G) ethanol is produced from starch or sucrose, whereas the second-generation (2G) ethanol is produced from biomass. Sugarcane (*Saccharum* sp.), sugar beet (*Beta vulgaris*), and maize (*Zea mays*) are by far the most employed for 1G production [3]. Each crop has its features and production capacity technologies. For Brazil, sugarcane is essential, since it is the largest producer and ethanol is the second worldwide producer with 30.3 billion/liters/year [4].

On the other hand, the lignocellulosic material used for 2G is divided into agro-industrial residues (for example, sugarcane bagasse and straw, corn stover, rice straw, wheat straw, cassava pulp, and palm residues), as well as eucalyptus, fast-growing legume trees (e.g., *Leucaena*), fast-growing grasses (e.g., elephant grass - *Pennisetum purpureum*, switchgrass - *Panicum virgatum*, poplar, willow, and *Miscanthus*), and some other crops as alfalfa, hemp, soybean, and water hyacinth [5]. However, the characteristics of the lignocellulosic material and the demand for cultivation areas are still problematic. One barrier for the 2G ethanol production is the recalcitrance, that limits cell wall cell wall polymers hydrolysis. Biomass requires a pretreatment to disrupt cell walls and access fermentable sugars for conversion [6]. The crop recalcitrance steams from the cell wall polymer (cellulose, hemicellulose, pectin, and lignin) features and interactions [5, 7, 8]. It is known that pectin structure and assembly could negatively impact the biomass saccharification due to the lack of fermentable sugars [9]. High hemicellulose levels prevent biomass saccharification due to polymers interlinking [7], especially arabinoxylans and xyloglucans, and their interaction with cellulose [8]. Moreover, cellulose microfibrils' crystallinity can be a limiting factor for cellulases penetration into biomass [7]. Thus, understanding cell wall composition and structure tends to allow more cost-effective approaches to bioethanol production.

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Cell wall polysaccharides are arranged in a complex assembly of polymers, displaying different architectural features depending on the taxonomic group [10]. The plant cell wall is encrypted as a Glycomic Code, forming a complex of an encoded mixture of proteins, phenolic compounds, and polysaccharides [11]. The plant cell wall usually displays three carbohydrate domains: cellulose, hemicelluloses, and pectins, whose proportions and composition characterize its primary wall type [10]. Many bioenergy crops display a type II cell wall, in which the pectin domain is reduced along with the predominance of the hemicellulose arabinoxylan and its cross-linkings with ferulic acids and lignin. The grasses contain another type of hemicellulose, the mixed linkage β-glucan [3], a polymer that is quite suitable for bioenergy purposes as its hydrolysis produces glucose only. Alternatively, monocotyledons may also display a type I cell wall with more pectins, xyloglucan, and show a higher hexose:pentose ratio [10].

Cellulose is composed of β-1,4-linked glucosyl residues whose polymers form microfibrils [12]. Hemicelluloses are β-1,4-linked polymers that may be decorated with different branching residues. The main hemicelluloses found in nature—particularly in bioenergy feedstocks—are xyloglucans, arabinoxylans, and (galacto- or gluco-) mannans [3, 10, 13]. Pectins are arranged as a network of complex polymers composed of a main acid chain of galacturonic acids, interspaced or not by rhamnose residues. The latter may be branched with short chains of galactans and/or arabinans displaying various glycosidic linkages [14, 15].

Duckweeds are small plants that display high productivity (up to 100-ton dry matter ha^{-1} $year^{-1}$), fast growth and starch accumulation, high contents of fermentable sugars, low levels of lignin, no land requirement, cultivation associated with wastewater treatment, and easy manipulation [16–18]. In duckweeds, xylo- or apiogalacturonans may appear as the main component of the wall in specific taxonomic groups [14].

Duckweeds are free-floating aquatic plants categorized as non-commelinid monocots into Lemnoideae (*Spirodela*, *Lemna*, *Landoltia)* and Wolffioideae (*Wolffiella* and *Wolffia*) [19, 20]. Their biomass has been chemically characterized, possessing 17–35% of carbohydrates, 16–42% of crude protein, 9–16% of crude fiber, 5–9% of lipids, and 21–38% starch [18, 21, 22]. Concerning the cell wall, duckweeds are believed to display pectin-rich walls containing relatively low hemicelluloses (xylan, xyloglucan, and glucomannan) and lignin [14, 18, 23, 24]. Differently from the bioenergy crops, the cell wall may be classified as type I.

Duckweeds are a promising source of bioenergy, especially for 1G [25]. Indeed, 1G ethanol has been produced from *Lemna minor* and *Landoltia punctata* at a low scale [21] with a fermentation efficiency of 93% [23, 26]. Besides ethanol, butanol and oil can also be produced from duckweeds carbohydrates [27, 28]. Although several duckweed species may be suitable for bioenergy purposes due to their high biomass production potential and no need for land use, there is still a lack of information about their cell walls' composition so that ways of saccharification could be better designed. This work reports a comparative study of the cell wall composition and saccharification of five duckweed species to fill this gap. The high level of nonstructural carbohydrates, together with less recalcitrant walls, indicated that duckweeds are indeed quite useful species for both 1G and 2G ethanol production. For the first time, the high levels of saccharification of duckweed's biomass are reported compared to the golden standard bioenergetic biomass of sugarcane.

Methodology

Plant Material

Three Lemnoideae species (*Spirodela polyrhiza* (9509), *Landoltia punctata* (7624), *Lemna gibba* (DWC128)) and two Wolffioideae species (*Wolffiella caudata* (9139) and *Wolffia borealis* (9144)) were obtained from the collection Rutgers Duckweed Stock Corporation (RDSC) (New Jersey, USA). The plants were cultivated in a growth chamber (EL011, ELETROlab®– São Paulo, Brazil) in 1L Erlenmeyer with 400 mL of ½ Schenk-Hildebrandt medium (pH 6.5) with 0.5% of sucrose at 25 °C with a photoperiod of 16 h of light (500 μmoles photons m^{-2} s^{-1}) and 80% humidity for 21 days. The cultivated plants were frozen in liquid nitrogen, freeze-dried, and pulverized for cell wall evaluation. Sugarcane culms (*Saccharum* spp. cv. SP80-3280) were used for comparison. The sugarcane plants were grown on the field for 12 months in Piracicaba, São Paulo, Brazil (S 22° 43′ 07.9″, W 47 °C 41′ 91.7″), manually harvested, dried, and ball-milled.

Cell Wall Isolation

One gram of pulverized biomass from duckweed was extracted five times, with 25 mL of 80% ethanol at 80 °C for 20 min. The supernatants containing soluble compounds were discarded after centrifugation at 3220*g* for 10 min at room temperature, and the alcohol insoluble residue (AIR) acquired was dried at 45 °C. AIR was treated with 120 U/mL of α-amylase (E.C. 3.2.1.1) from *Bacillus licheniformis* (Megazyme®) at 75 °C for 1 h to solubilize starch [29]. The de-starched AIR (cell wall) recovered was washed three times, with 25 mL of ethanol 80% and dried at 45 °C [15]. Five hundred milligrams of sugarcane milled-culms were extracted six times with 25 mL of 80% ethanol at 80 °C for 20 min, and the AIR was recovered and dried as described for duckweeds [30].

Cell Wall Fractionation

The duckweed cell walls were fractionated in subsequent extractions following De Souza et al. [30]. De-starched AIR was extracted four times with 25 mL of ammonium oxalate (pH 7.0) at 80 °C for 1 h with continuous stirring for pectin solubilization. The supernatants were recovered after 10-min centrifugation at 3220*g* at room temperature, and the oxalate-extracted cell wall residue was subjected to four extractions with 25 mL of 4M NaOH containing 3 mg mL^{-1} sodium borohydride for 1 h at room temperature with continuous stirring for hemicellulose solubilization. The supernatants of the extractions of 4M NaOH were neutralized with glacial acetic acid, and the residue obtained was cellulose. After the pectin and hemicellulose extractions, the remaining residue was washed with distilled water to neutral pH and freeze-dried. The acquired fractions were dialyzed to remove salts, frozen, and freeze-dried. The cell wall domain yields were obtained gravimetrically.

Uronic Acid Content Determination

The uronic acid content was determined by the m-hydroxyphenyl method, as described by Filizetti-Cozi and Carpita [31]. The cell wall fractions were hydrolyzed two times, with 1 mL of 98% sulfuric acid and 0.5 mL of deionized water for 10 min each at 4 °C under stirring (500 rpm). Samples were diluted to 10 mL and centrifuged at 3220*g* for 10 min, at room temperature. To the 400 μL aliquots, 40 μL of 4 M sulfamic acid (pH 1.6) and 2.4 mL of 75 nM sodium tetraborate in sulfuric acid were added. The reactions were incubated at 100 °C for 20 min and then cooled on ice for 10 min. Then, 80 μL of 0.15 % (w/v) m-hydroxybiphenyl in 0.5% (w/v) sodium hydroxide solution was added and vortexed and samples were read in 525 nm. The calculation was based on a standard curve of galacturonic acid with concentrations from 0.12 to 2.5 M.

Neutral Monosaccharide Analysis

Cell wall fractions were hydrolyzed with 2 M trifluoroacetic acid (TFA) for 1 h at 100 °C to analyze pectin- and hemicellulose-derived monosaccharides. The cellulose fraction was hydrolyzed with 72% sulfuric acid for 30 min at 45 °C followed by dilution to 4% and 1.5 h at 100 °C to further analyze cellulose-derived glucose. The monosaccharide solution derived from the TFA hydrolysis was dried under vacuum, resuspended in 1 mL of deionized water, and filtered through a 0.22 μm membrane (Merk Millipore ®). The neutral monosaccharides recovered from acid hydrolysis were analyzed by high-performance anion-exchange chromatography with pulsed amperometric detection (HPAEC-PAD) on a CarboPac SA10 column (ICS 5,000 system, Dionex-Thermo®) eluted with 99.2% of water and 0.8% (v/v) sodium hydroxide (1 mL min^{-1}). The monosaccharides were detected by pulsed amperometric detection after passing through a post-column containing 500 mM sodium hydroxide (0.5 mL min^{-1}). Standard curves of apiose, arabinose, fucose, galactose, glucose, mannose, rhamnose, and xylose were used to calibrate the equipment.

Lignin Quantification

Duckweed biomass (30 mg) was treated with 1 mL of water, ethanol, ethanol-chloroform (1:1 v/v), and acetone [32]. The samples were incubated for 15 min with stirring of 750 rpm at 98 °C, 76 °C, 59 °C, and 54 °C, respectively, for each reagent. The precipitated material was recovered by centrifugation for 5 min at 14,000*g* and dried at 80 °C overnight. The lignin content was determined using the acetyl bromide method [33], in which 250 μL of 25% acetyl bromide in acetic acid was added to the dried samples and incubated for 2 h at 50 °C and 1 h with stirring of 1500 rpm. The samples were cooled in an ice bath and centrifuged for 15 min at 10,000*g*. The reaction was stabilized by the addition of 400 μL of 2 M sodium hydroxide and 75 μL of 0.5 M hydroxylamine hydrochloride. After the addition of 1425 μL glacial acetic acid and 100 μL of the acetyl bromide supernatant solution, the absorbance was read at 280 nm. The lignin measurement was calculated by Bouguer-Lambert-Beer law (Eq. 1) and corrected by the cell wall amount used in the assay.

$$A = \varepsilon * c * l \tag{1}$$

where A = absorbance; $\varepsilon = 23.35$; $c = g^{-1}\ cm^{-1}$; and $l = 0.1$ cm [34]

Saccharification

For saccharification determination, 10 mg of duckweed pulverized biomass and sugarcane culm AIR was pre-treated with 0.5 mL NaOH for 30 min at 90 °C and washed 3 times with 1 mL of sodium acetate (pH 4.5). To the treated material, 1.6 mL of Cellic Ctec2 (Novozymes®) enzymatic cocktail containing 120 U mL^{-1} in 25 mM sodium acetate buffer (pH 4.3) was added, followed by incubation at 50 °C for 16 h with gentle shaking. The determination of the sugars released was performed using 3-methyl-2-benzothiazolinone hydrazone (MBTH), as described by Gomez et al. [35]. The reducing sugar was analyzed with 0.25 M NaOH, 3 mg L^{-1} MBTH, and 1 mg L^{-1} DTT. After incubation at 70 °C for 20 min by adding the oxidizing reagent (0.2% FeNH4(SO4)2, 0.2% sulfamic acid, and 0.1% HCl), the color was developed, and the absorbance was measured at 620 nm. The concentration of reducing sugars released was calculated using a standard

curve with glucose concentrations of 50, 100, and 150 nmol mL^{-1}.

Data Analysis

Statistical analysis of the evaluated sugars on the five species was performed by a one-way ANOVA test (JMP® Statistical Discovery Software, version 5.0.1, SAS Inc., Cary, NC, USA) followed to post hoc by Tukey's test with significance $p \leq 0.05$ to compare the results among the species. All the assays were conducted with five replicates ($n = 5$).

Results

Carbohydrate analyses were conducted for five duckweed species to assess the distinction among subfamilies regarding the possible use for bioenergy purposes, focusing on second-generation bioethanol.

Biomass Composition

Of the total biomass processed in this study, 42.2% were recovered as cell wall fractions. The nonstructural carbohydrates corresponded to 42.1%. The remaining 15.7% of the biomass was lost (Suppl. Table 1).

Ammonium oxalate (AmnOX) released, on average, as 20.2% of the polysaccharides (Fig. 1). Based on the neutral monosaccharide composition, the polymers possibly present in this fraction would be pectin and glucomannan. Another evidence supporting pectins' presence was the detection of uronic acids, which amounted to 271 μg mg^{-1} DW in AmnOX fraction (Table 1). The most abundant neutral monosaccharides were galactose (23%), arabinose (17.8%), and apiose (15.7%) (Suppl. Table 2). The level of apiose in the Lemnoideae (31.6 μg mg^{-1}) was approximately four times higher than in the Wolffioideae (8 μg mg^{-1}) (Table 1), suggesting the presence of more apiogalacturonan in the former. In Wolffioideae, the levels of arabinose and xylose were higher than in Lemnoideae, suggesting the presence of arabinan and xylogalacturonan. The occurrence and proportion of mannose in the AmnOX fraction could not be used to distinguish the subfamilies. It was noteworthy that *L. punctata* did not follow the pattern of the other four species.

For the five species, the tightly bound polysaccharides obtained after extraction with 4M NaOH represented, on average, 35.2% of the cell wall (Fig. 1). This fraction had on its composition xylose (34.2%), arabinose (20.8%), glucose (17.6%), galactose (13.2%), mannose (7.6%), and apiose (4.2%) as main monosaccharides (Table 1).

When the two subfamilies were compared, a higher proportion of arabinose was found in Wolffioideae (Suppl. Table 2). These results analyzed according to the conclusions of Sowinski et al. [24] seem to confirm the presence of xylan, arabinan, and xyloglucan as the main hemicelluloses in duckweeds. Furthermore, the results suggested that Wolffioideae displayed a higher proportion of xylan and arabinan. Also, *L. punctata* appeared as an outlier as it displayed a different pattern from the other Lemnoideae.

The duckweed cell wall residue fraction showed a different proportion when the studied species were compared (Fig. 1). *S. polyrhiza* (29.5%) and *W. borealis* (32.1%) had the minor representation of residue based on the cell wall constituents, while *W. caudata* (49.7%), L. *gibba* (54.5%), and *L. punctata* (57.5%) had higher residue proportions (Fig. 1). On average, the cellulose calculated on the basis of the whole dry biomass was 14.6%. For the obtention of these fractions' compositions, two acid hydrolysis methods were employed. To estimate glucose contents in the non-cellulosic polymers, trifluoroacetic acid (TFA) was used, whereas sulfuric acid was used to completely hydrolyze the residue fraction, leading to the production of glucose associated with both, hemicelluloses and cellulose. The subtraction of the glucose present in the TFA-hydrolyzed from the sulfuric acid–hydrolyzed ones led to the content of glucose corresponding to cellulose. It was found that cellulose constituted 73.2% of residue fraction (see glucose on sulfuric acid hydrolysis - 136.7 μg mg^{-1} CW) (Suppl. Table 1, Table 1). Lignin in cell walls of the five species of duckweeds represented ca. 5%, except for *W. caudata*, which presented higher (1.3-fold) lignin compared to the other species (Fig. 2A).

The use of TFA for hydrolysis of the residue fractions helped to detect the main non-cellulosic polymers that remain attached to cellulose during extraction. This is important because non-cellulosic polymers could prevent the saccharification of cellulose in duckweed biomass. The TFA hydrolysis suggested that some pectins (i.e., polymers containing apiose and rhamnose) might be associated with lignin (Table 1). Also, the remanence of pectins associated with cellulose in the residue is suggested based on the detection of uronic acids in the fraction (Table 1—residue hydrolyzed with TFA). In Lemnoideae, the apiogalacturonan was a significant component (based on the detection of apiose) compared to Wolffioideae in which arabinans, xylans, and xylogalacturonans appeared to be the most abundant wall components (Table 1 and Suppl. Table 2).

A relevant factor related to ethanol production from residual biomass (cell walls) is the percentage of pentoses in the material. The pentose:hexose ratios of five duckweed cell walls were calculated, and the de-starched biomass produced relatively high pentoses, except for *S. polyrhiza* (Fig. 2B). The polymers extracted with

Table 1 Structural sugars content in cell walls of *Spirodela polyrhiza*, *Landoltia punctata*, *Lemna gibba*, *Wolffiella caudata*, and *Wolffia borealis*. Data represented average ± standard error of the monosaccharides in μg mg^{-1} CW (cell wall)

		μg mg^{-1} CW								
		Apiose	Arabinose	Xylose	Galactose	Glucose	Mannose	Rhamnose	Fucose	Uronic acid
AmmOx	*S. polyrhiza*	14.73 ± 0.08^{a}	15.47 ± 2.17^{b}	3.53 ± 0.30^{b}	28.02 ± 4.87^{ab}	6.96 ± 1.38^{ab}	14.91 ± 2.91^{ab}	7.29 ± 0.69^{ab}	1.37 ± 0.31^{b}	255.726 ± 63.65^{a}
	L. punctata	36.63 ± 9.48^{a}	26.74 ± 5.49^{ab}	22.87 ± 7.95^{a}	36.83 ± 6.95^{a}	8.36 ± 1.83^{ab}	13.10 ± 3.40^{ab}	10.81 ± 2.28^{a}	2.61 ± 0.82^{ab}	417.97 ± 109.38^{a}
	L. gibba	43.41 ± 1.29^{a}	24.03 ± 0.8^{ab}	19.57 ± 1.21^{ab}	30.87 ± 0.41^{ab}	5.29 ± 0.62^{b}	22.45 ± 1.72^{a}	10.45 ± 0.33^{a}	2.70 ± 0.08^{ab}	253.18 ± 62.15^{a}
	W. caudate	13.01 ± 4.69^{b}	31.67 ± 4.40^{a}	15.24 ± 3.43^{ab}	34.46 ± 4.89^{ab}	17.50 ± 5.07^{ab}	17.16 ± 3.35^{ab}	8.08 ± 1.40^{ab}	3.87 ± 0.56^{a}	251.30 ± 77.87^{a}
	W. borealis	3.15 ± 0.52^{b}	17.94 ± 1.57^{ab}	29.14 ± 3.28^{a}	17.90 ± 1.58^{b}	18.74 ± 4.33^{a}	11.20 ± 0.92^{b}	4.65 ± 0.38^{b}	2.25 ± 0.32^{ab}	176.88 ± 66.61^{a}
	p value	*0.000*	*0.0194*	*0.005*	*0.057*	*0.016*	*0.006*	*0.015*	*0.028*	0.209
4M NaOH	*S. polyrhiza*	3.01 ± 0.09^{b}	9.87 ± 0.94^{b}	16.80 ± 1.62^{b}	9.61 ± 0.92^{b}	10.00 ± 1.58^{b}	4.17 ± 0.41^{b}	2.15 ± 0.25^{a}	1.80 ± 0.16^{ab}	46.23 ± 13.13^{a}
	L. punctata	7.06 ± 0.60^{a}	16.26 ± 1.98^{b}	39.02 ± 2.95^{a}	19.05 ± 1.63^{a}	15.24 ± 1.80^{ab}	6.57 ± 0.28^{ab}	1.93 ± 0.08^{a}	2.23 ± 0.25^{a}	91.30 ± 17.77^{a}
	L. gibba	5.69 ± 0.77^{a}	7.61 ± 1.34^{b}	18.34 ± 2.88^{b}	8.35 ± 1.55^{b}	9.11 ± 1.74^{b}	3.57 ± 0.73^{b}	1.27 ± 0.19^{a}	0.87 ± 0.15^{c}	114.20 ± 51.45^{a}
	W. caudate	1.26 ± 0.71^{b}	38.37 ± 7.86^{a}	29.32 ± 3.82^{ab}	10.25 ± 1.22^{b}	26.71 ± 5.89^{a}	9.21 ± 1.40^{a}	1.36 ± 0.28^{a}	1.11 ± 0.20^{bc}	251.30 ± 6.84^{a}
	W. borealis	0.69 ± 0.34^{b}	22.76 ± 3.35^{ab}	29.91 ± 5.04^{ab}	6.27 ± 0.90^{b}	16.83 ± 2.5^{ab}	9.30 ± 1.13^{a}	1.34 ± 0.38^{a}	1.14 ± 0.13^{bc}	176.88 ± 13.14^{a}
	p value	*0.000*	*0.000*	*0.001*	*0.000*	*0.006*	*0.000*	0.084	*0.000*	0.167
Residue TFA	*S. polyrhiza*	13.22 ± 0.62^{c}	17.51 ± 1.07^{c}	13.34 ± 0.44^{c}	13.17 ± 0.63^{ab}	4.59 ± 0.44^{b}	0.46 ± 0.02^{c}	5.76 ± 0.23^{a}	2.15 ± 0.10^{ab}	161.37 ± 17.36^{ab}
	L. punctata	26.69 ± 1.43^{b}	9.13 ± 0.47^{c}	47.53 ± 3.97^{b}	8.02 ± 0.39^{c}	5.09 ± 0.38^{ab}	0.87 ± 0.07^{bc}	3.84 ± 0.23^{a}	1.37 ± 0.05^{b}	119.49 ± 5.90^{b}
	L. gibba	43.39 ± 3.81^{a}	5.94 ± 0.69^{c}	68.13 ± 7.76^{a}	10.86 ± 1.35^{bc}	6.67 ± 0.84^{ab}	0.54 ± 0.13^{c}	5.61 ± 0.59^{a}	2.00 ± 0.25^{ab}	131.23 ± 36.62^{ab}
	W. caudate	16.88 ± 1.32^{c}	49.78 ± 78.18^{a}	32.35 ± 3.66^{bc}	13.42 ± 0.80^{ab}	5.56 ± 0.35^{ab}	1.34 ± 0.15^{a}	3.92 ± 0.92^{a}	1.70 ± 0.20^{b}	145.50 ± 36.62^{ab}
	W. borealis	8.69 ± 0.73^{c}	32.33 ± 2.37^{b}	35.56 ± 3.43^{b}	15.74 ± 1.81^{a}	8.23 ± 1.32^{a}	1.10 ± 0.09^{ab}	3.78 ± 0.44^{a}	2.74 ± 0.25^{a}	176.88 ± 27.67^{a}
	p value	*0.000*	*0.000*	*0.000*	*0.001*	*0.023*	*0.000*	*0.029*	*0.001*	*0.050*
Residue H_2SO_4	*S. polyrhiza*	2.24 ± 0.18^{b}	9.83 ± 3.02^{b}	11.03 ± 1.19^{b}	9.71 ± 0.68^{a}	174.02 ± 6.83^{a}	1.56 ± 0.44^{b}	2.35 ± 0.20^{a}	1.86 ± 0.06^{a}	
	L. punctata	7.84 ± 1.22^{a}	4.01 ± 0.57^{bc}	21.74 ± 3.22^{a}	4.58 ± 0.60^{b}	126.75 ± 17.18^{a}	1.66 ± 0.24^{b}	1.19 ± 0.25^{b}	1.80 ± 0.33^{a}	
	L. gibba	8.65 ± 0.35^{a}	1.58 ± 0.08^{c}	22.42 ± 0.89^{a}	4.32 ± 0.23^{b}	120.36 ± 5.87^{a}	1.37 ± 0.09^{b}	0.95 ± 0.06^{b}	1.45 ± 0.05^{a}	
	W. caudate	2.94 ± 0.42^{b}	23.55 ± 3.99^{a}	17.22 ± 1.95^{ab}	6.30 ± 0.61^{b}	127.47 ± 6.25^{a}	3.05 ± 0.44^{a}	1.31 ± 0.27^{b}	1.30 ± 0.12^{a}	
	W. borealis	1.17 ± 0.23^{b}	11.23 ± 0.62^{b}	16.49 ± 0.75^{ab}	5.74 ± 0.25^{b}	134.99 ± 34.11^{a}	2.16 ± 0.07^{ab}	1.05 ± 0.09^{b}	1.20 ± 0.08^{a}	
	p value	*0.000*	*0.000*	*0.001*	*0.000*	0.273	*0.004*	*0.000*	0.054	

p values statistically significant are presented in italic according to ANOVA one-way. Different letters are significant different according to a posteriori Tukey's test ($p < 0.05$) ($n = 5$)

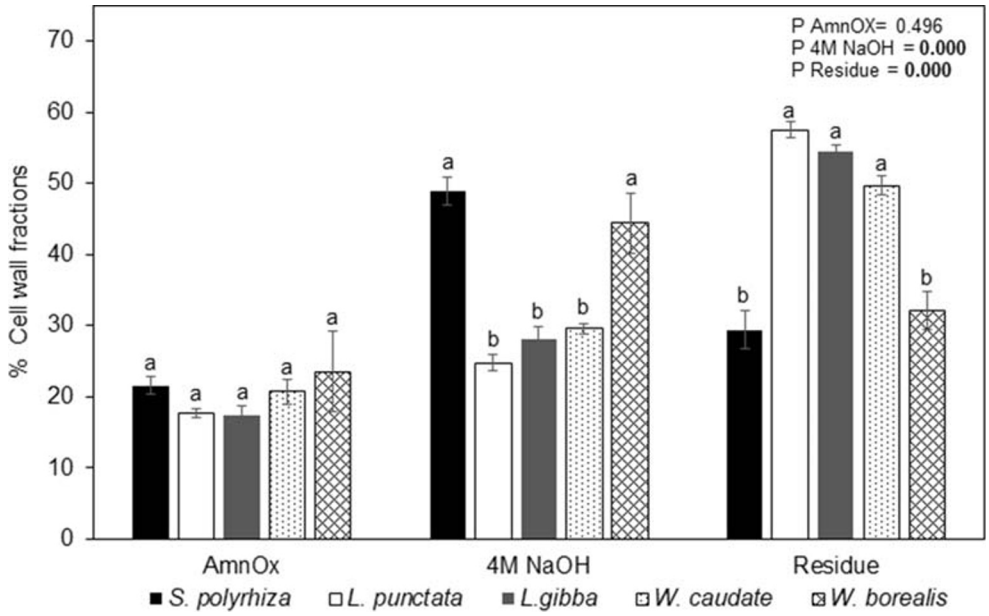

Fig. 1 Comparative cell wall fractionation yield of *Spirodela polyrhiza*, *Landoltia punctata*, *Lemna gibba*, *Wolffiella caudata*, and *Wolffia borealis*. Data are represented by percentage means ± standard errors ($n = 5$). Different letters on top of bars are statistically significant among species compared, according to Tukey's test ($p < 0.05$) in each fractionation extract

ammonium oxalate and 4M NaOH (Fig. 2B) also had a considerable amount of pentoses compared to the residue.

Saccharification

Figure 3 shows a comparison of the five species' saccharification of biomass and cell walls studied in this work. This comparison was also made using sugarcane culm biomass as a reference.

The duckweeds' whole biomass displayed higher saccharification than isolated cell walls (de-starched AIR), probably due to the nonstructural carbohydrates (starch and soluble sugars) present in the biomass. The saccharification level of the whole biomass (light bars - Fig. 3) is the same for the five species evaluated, while the isolated cell wall saccharification of the Wolffioideae species rendered less glucose equivalent residues. Compared to the saccharification of sugarcane cell walls, these data suggested that duckweeds could be more suitable for sugar production.

Discussion

Bioethanol can be produced from different biomass feedstocks, including grasses and wood species [3]. The 1G bioethanol technology uses soluble sugars (sucrose, glucose, and fructose) or starch as a raw material. Alternatively, structural carbohydrates (cell wall polymers) can also be used as sources of free sugars giving rise to 2G technologies [36]. In both cases (1G and 2G), sugars are subsequently subjected to yeast fermentation [37].

Duckweeds have been considered a promising feedstock for bioenergy production despite its biomass characteristics [18, 24]. The present work evaluated five duckweed species as candidates for 1G and 2G bioethanol production.

Duckweed biomass has been characterized as being rich in carbohydrates (17–51%), especially in pectins (20–33%) [18, 21, 38]. Carbohydrates corresponded to approximately 80% of the dry mass of the five species of duckweeds (nonstructural carbohydrates and cell wall) evaluated (Suppl. Table 1). Between 30 and 49% represented nonstructural carbohydrates (soluble sugars and starch), whereas the cell walls represented 35–52% of the five species' dry mass. In the cell walls, 20% is pectin, 35% is hemicellulose, and 45% is cellulose plus small proportions of lignin and proteins (Figs. 1 and 2B). The nonstructural carbohydrates accounted for ca. 42% of the duckweed biomass, corroborating with Duff [39], who found 48% in *Lemna minor*. The differences between the analyses and the ones in the literature can probably be attributed to the methodology used. It is well established that duckweeds can quickly accumulate high starch levels depending on growth conditions (up to 75%) [40]. Besides, there is a negative relationship between the composition of the cell wall, apiose, and starch contents [15]. Thus, saccharification was carried out in two ways: using biomass as a substrate, without any treatment, and using the isolated cell walls (AIR) as a substrate. The idea was to contemplate duckweed's potential for use in 1G and 2G ethanol production, respectively. The findings corroborate previous discoveries of Pagliuso et al. [15], in which species of the subfamily Wolffioideae, which accumulate more starch, displayed greater saccharification of non-treated biomass than the cell wall (compare light and dark bars in Fig. 3 to *W. caudata* and *W. borealis*). Therefore, *W. borealis* would be more suitable for 1G than for 2G production due to the starch content.

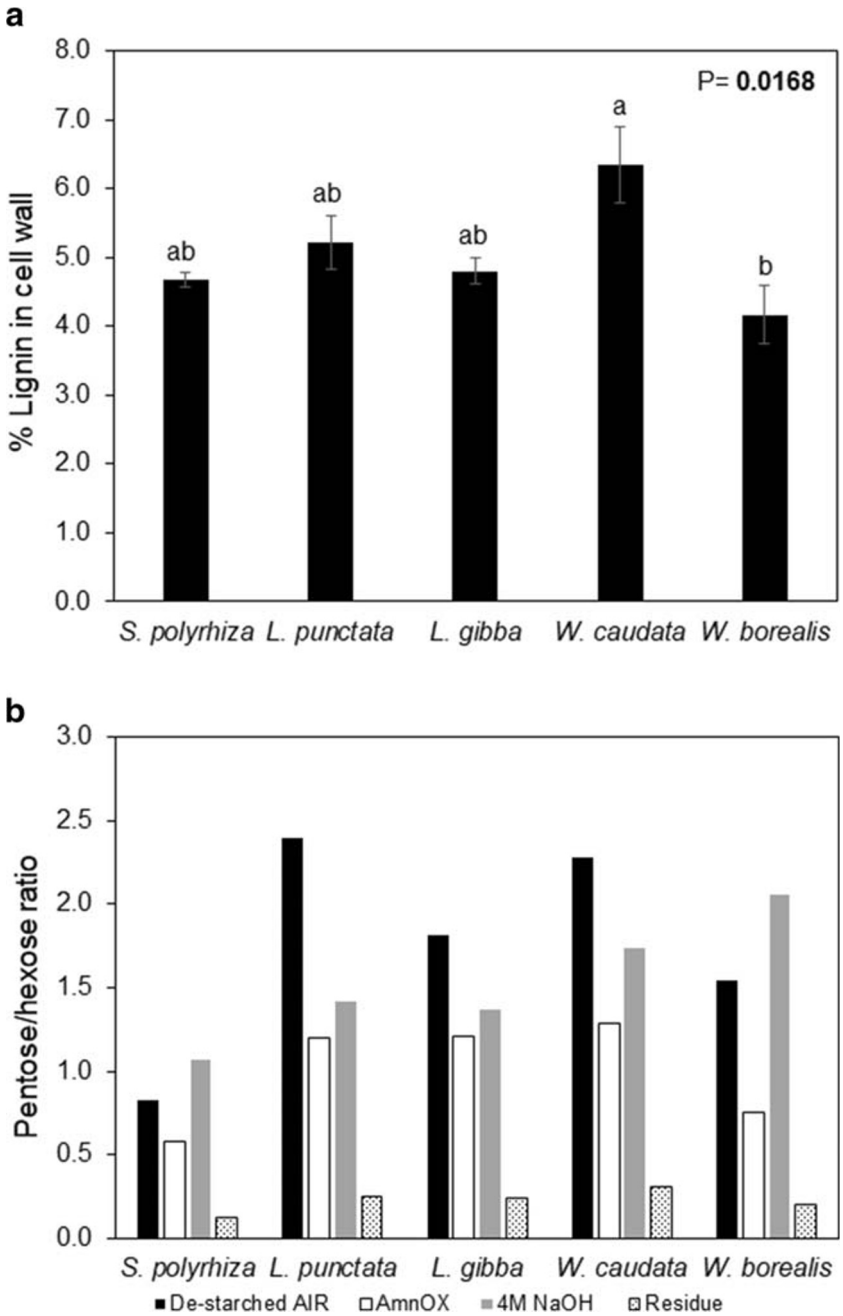

Fig. 2 Recalcitrance features in *Spirodela polyrhiza*, *Landoltia punctata*, *Lemna gibba*, *Wolffiella caudata*, and *Wolffia borealis*. (A) Lignin content. (B) Pentose:hexose ratios of the cell wall monosaccharides. Data are represented by percentage average ± standard error. Statistically, a significant *p* value was obtained by ANOVA one-way. Different letters on the top of the bars are significant, according to Tukey's test ($n = 5$)

The polysaccharides' composition, interactions, and linkages to lignin confer the biomass' recalcitrance to the hydrolysis [41]. Thus, to produce ethanol, the biomass needs pretreatment, which is a process that usually facilitates access to the cell wall polymers by hydrolases. There are several pretreatment procedures [1], which usually lead to higher energy consumption by the whole process [3]. These are consequently costly and directly impact efficiency and ethanol yield. Thus, materials containing lower lignin levels and more soluble polysaccharides—less recalcitrance—would lead to biomass more prone to saccharification implicating in lower costs for 2G ethanol production.

The findings point out that duckweed biomasses have advantages compared to other bioenergy crops since it contains high levels of fermentable sugars [18, 42] and low lignin [43, 44]. Lignin crosslinks polysaccharides and cellulose confers rigidity to the structure and recalcitrance to plant materials. The presence of lignin, as well as the composition of its subunits and degrees of ester linkages, modulates different wall architectures and levels of recalcitrance [45]. For example, lower lignin levels with higher proportions of syringyl/guaiacyl units increase the release of sugars from the wall, so that crops with these characteristics are targeted for 2G ethanol [45]. The lignin contents found for the five species

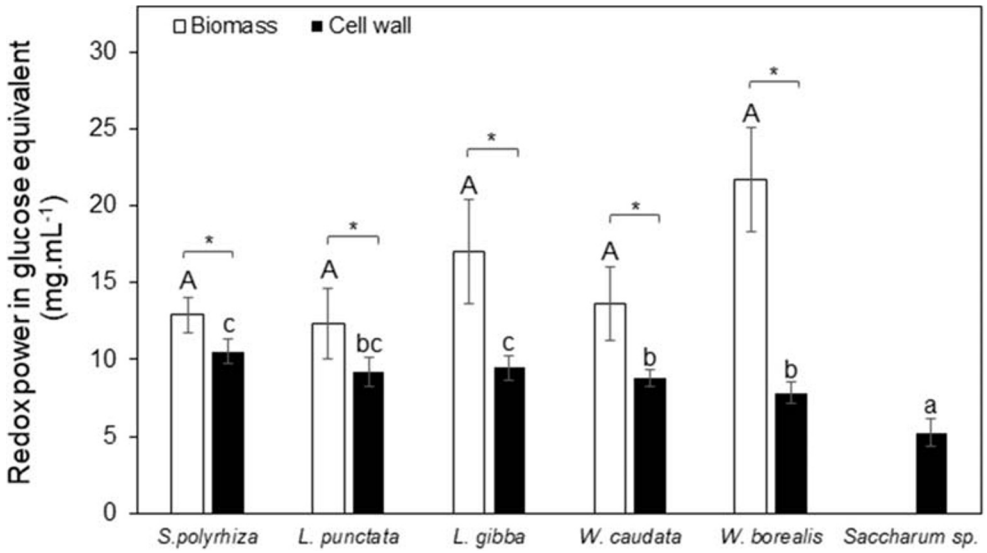

Fig. 3 Comparison of saccharification of duckweed biomass and cell wall. Data are represented by glucose equivalent ± standard error ($n = 5$). Capital letters on top of white bars are the significant differences by Tukey's test ($p < 0.05$) for biomass saccharification. Lowercase letters on top of dark bars mean significant differences regarding cell wall saccharification. Asterisks indicate significant differences by t test ($p < 0.05$) for biomass and cell wall saccharification

of duckweed (~ 5%) corroborate the ones described in the literature (3–12.2%). Because the starting material used for cell wall extraction was not delignified, the remaining phenolic compounds along with polysaccharides (pectins, xyloglucan, and arabinan) found within the cellulose fraction (residue) might be due to lignin interaction, which could be responsible to some degree for recalcitrance (Table 1, Fig. 2).

One of the classes of polysaccharides found in duckweeds is pectins. The majority of the polymers are found as apiogalacturonans and xylogalacturonans, which help distinguish between the subfamilies *Lemnoideae* and *Wolffioideae* [14]. Apiogalacturonans cannot be hydrolyzed by pectinases [46]. This poses a problem for the use of enzyme cocktails in hydrolysis.

Furthermore, apiose and xylose (Table 1), two of the leading hydrolysis products of apio- and xylogalacturonans (besides galacturonic acid), are expected to impact ethanol production negatively. Apiose and xylose are both pentoses and, therefore, difficult to be metabolized by yeasts during fermentation [37]. Although xylogalacturonan's enzymatic hydrolysis has not been reported in the literature, one promising result was obtained by Chen et al. [43]. The mentioned study found an improvement of 142% in ethanol production from *L. punctata* (which accumulates apiogalacturonan) using pectin transaminase. These findings highlight the fact that apio- and xylogalacturonans demand further studies regarding hydrolysis.

According to the fractionation process adopted in this work, the hemicellulose content was 35.2% (Fig. 1) in which the dominant neutral polymers appear to be arabinan, xylan, and xyloglucan (see contents of xylose, arabinose, glucose, and galactose in Table 1). As previously mentioned, hemicellulose content and its interactions with the other cell wall polysaccharide and lignin confer recalcitrance to biomass by limiting the access of hydrolases to cellulose [7, 41]. Also, the high content of arabinose (20.65%) (Suppl. Table 2), as mentioned by Li et al. [8], can affect the lignocellulosic crystallinity and, consequently, the production of 2G ethanol.

The cellulose content in duckweeds is reported as low (~ 10% dry mass) when compared to terrestrial plants (~ 40% dry mass) [17, 23, 47]. The results confirmed the ones previously found in duckweeds, which represented 14.6% of the biomass. This low proportion of cellulose makes polysaccharides in duckweeds walls possibly more susceptible to hydrolysis, and consequently, the cost for ethanol production could be lower [44].

Among the monosaccharides present in duckweeds' cell walls, glucose, galactose, and mannose would be promptly fermentable since they are easily transported and consumed by yeasts during the fermentation process to alcohol production [48]. Therefore, biomass with a high content of hexoses and less recalcitrance is advantageous for this purpose. Considering the fermentable sugars plus xylose, which can be fermented as well, but more slowly than the others [48, 49], the species evaluated in this study contain approximately 50% of the cell wall useful for bioethanol production.

In general, a high proportion of pentoses is not desirable for ethanol production. The higher pentose: hexose ratio on destarched AIR showed that the duckweed biomass might impose problems for 2G bioethanol production due to the difficulty in the fermentation of pentoses (Fig. 2B) since the latter is usually less efficient to be metabolized by yeast. However, new advances in the genetic engineering of fermenting organisms, such as *Saccharomyces cerevisiae* and *Zymomonas mobilis*, make it possible to ferment some pentoses, such as xylose, therefore increasing ethanol yield from biomass [50, 51]. It is essential to remember that apiose is also a pentose and that studies on its fermentation by microorganisms are needed to improve 2G processes that use duckweeds as bioenergy feedstocks.

Another issue regarding recalcitrance is the assembly and interactions of the cell wall polysaccharides [52]. Thus, the architecture of the duckweed cell wall (a porous pectic wrap with hemicelluloses containing a limited number of linkages to phenolic compounds and low cellulose levels) makes it a promising feedstock for biofuels. Indeed, even with a high pentose proportion, the duckweed cell wall was more prone to saccharification than sugarcane, one of the most important bioenergy feedstocks under industrial use to date [53] (Fig. 3). Concerning non-treated biomass saccharification (light bars in Fig. 3), the different features of plants' cell walls from the subfamilies *Lemnoideae* and *Wolffioideae* did not influence the saccharification with the Cellic and Ctec2 (Novozymes®) cocktail. The nonstructural carbohydrates' presence might have increased up to 2.5 times the *Wolffia borealis* biomass' saccharification due to the higher starch accumulation [15], as mentioned above.

Conclusion

The duckweed biomass seems to be suitable for bioenergy production purposes since they display relatively low recalcitrance. They contain relatively large proportions of nonstructural carbohydrates that can be readily metabolized, therefore adequate for 1G bioethanol production. Furthermore, duckweeds also display approximately 50% of fermentable cell walls with low lignin levels and thus easily applicable in 2G bioethanol production. Duckweeds do not need land for production as they are aquatic plants. A further advantage is that these plants can also clean up the water, providing a useful environmental service for urban areas. Besides that, the duckweed cell wall displays a considerable saccharification potential that surpasses sugarcane. All these features highlight that duckweeds as bioenergy feedstocks would bring even higher sustainability to biofuel applications as a measure to mitigate the effects of global warming.

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Authors' Contribution MB, DP, AG, and EL planned the work; DP performed the experiments; DP and AG analyzed the data; DP, AG, and MB wrote the manuscript.

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Data Availability Not applicable.

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no competing interests

Ethics Approval Not applicable.

Consent to Participate All authors from this work consent the participation in this work.

Consent for Publication All authors consent the research article for publication.

Abbreviations 4M NaOH, sodium hydroxide four molar; AIR, alcohol insoluble residue; AmnOx, ammonium oxalate; H, hour; HPAEC-PAD, high-performance anion-exchange chromatography with pulsed amperometric detection; *L. gibba*, *Lemna gibba* stream DWC128; *L. punctata*, *Landoltia punctata* stream 7624; MBTH, 3-methyl-2-benzothiazolinone hydrazone; Min, minutes; RDSC, Rutgers Duckweed Stock Corporation; *S. polyrhiza*, *Spirodela polyrhiza* stream 9509; TFA, trifluoroacetic acid; *W. borealis*, *Wolffia borealis* stream 9144; *W. caudata*, *Wolffiella caudata* stream 9139

References

1. Soccol CR, Vandenberghe LP de S, Medeiros ABP, et al. (2010) Bioethanol from lignocelluloses: status and perspectives in Brazil. Bioresour Technol 101:4820–4825. https://doi.org/10.1016/j.biortech.2009.11.067
2. Hood EE (2016) Plant-based biofuels. F1000Research 5:1–9. https://doi.org/10.12688/f1000research.7418.1
3. de Souza AP, Grandis A, Leite DCC, Buckeridge MS (2014) Sugarcane as a bioenergy source: history, performance, and perspectives for second-generation bioethanol. Bioenergy Res. 7:24–35
4. Conab (2020) Acompanhamento da safra brasileira: cana-de-açúcar. Safra 2019/20. v.7, n.1, Brasilia, Brazil. pp 1–62
5. Phitsuwan P, Sakka K, Ratanakhanokchai K (2013) Improvement of lignocellulosic biomass in planta: a review of feedstocks, biomass recalcitrance, and strategic manipulation of ideal plants designed for ethanol production and processability. Biomass and Bioenergy 58:390–405. https://doi.org/10.1016/j.biombioe.2013.08.027
6. Cui W, Cheng JJ (2015) Growing duckweed for biofuel production: a review. Plant Biol 17(Suppl 1):16–23. https://doi.org/10.1111/plb.12216
7. Xu N, Zhang W, Ren S, Liu F, Zhao C, Liao H, Xu Z, Huang J, Li Q, Tu Y, Yu B, Wang Y, Jiang J, Qin J, Peng L (2012) Hemicelluloses negatively affect lignocellulose crystallinity for high biomass digestibility under NaOH and H 2SO 4 pretreatments in Miscanthus. Biotechnol Biofuels 5:58. https://doi.org/10.1186/1754-6834-5-58
8. Li F, Zhang M, Guo K, Hu Z, Zhang R, Feng Y, Yi X, Zou W, Wang L, Wu C, Tian J, Lu T, Xie G, Peng L (2015) High-level hemicellulosic arabinose predominately affects lignocellulose crystallinity for genetically enhancing both plant lodging resistance and biomass enzymatic digestibility in rice mutants. Plant Biotechnol J 13:514–525. https://doi.org/10.1111/pbi.12276
9. Wang YY, Huang J, Li Y, Xiong K, Wang Y, Li F, Liu M, Wu Z, Tu Y, Peng L (2015) Ammonium oxalate-extractable uronic acids positively affect biomass enzymatic digestibility by reducing lignocellulose crystallinity in Miscanthus. Bioresour Technol 196:391–398. https://doi.org/10.1016/j.biortech.2015.07.099

10. Carpita NC, Gibeaut DM (1993) Structural models of primary cell walls in flowering plants: consistency of molecular structure with the physical properties of the walls during growth. Plant J 3:1–30. https://doi.org/10.1111/j.1365-313X.1993.tb00007.x
11. Buckeridge MS (2017) The evolution of the Glycomic Codes of extracellular matrices. BioSystems 164:112–120. https://doi.org/10.1016/j.biosystems.2017.10.003
12. Ivakov AA, Flis A, Apelt F et al (2017) Cellulose synthesis and cell expansion are regulated by different mechanisms in growing Arabidopsis hypocotyls. Plant Cell:tpc.00782.2016. https://doi.org/10.1105/tpc.16.00782
13. De Souza AP, Kamei CLA, Torres AF et al (2015) How cell wall complexity influences saccharification efficiency in Miscanthus sinensis. J Exp Bot 66:4351–4365. https://doi.org/10.1093/jxb/erv183
14. Avci U, Peña MJ, O'Neill MA (2018) Changes in the abundance of cell wall apiogalacturonan and xylogalacturonan and conservation of rhamnogalacturonan II structure during the diversification of the Lemnoideae. Planta 247:953–971. https://doi.org/10.1007/s00425-017-2837-y
15. Pagliuso D, Grandis A, Igarashi ESES et al (2018) Correlation of apiose levels and growth rates in duckweeds. Front Chem 6:1–10. https://doi.org/10.3389/fchem.2018.00291
16. Cao HX, Fourounjian P, Wang W (2018) The importance and potential of duckweeds as a model and crop plant for biomass-based applications and beyond. In: Hussain C. (eds). Handbook of Environmental Materials Management. Springer International Publishing, pp 1–16. https://doi.org/10.1007/978-3-319-58538-3-67-1
17. Soda S, Ohchi T, Piradee J, Takai Y, Ike M (2015) Duckweed biomass as a renewable biorefinery feedstock: ethanol and succinate production from Wolffia globosa. Biomass and Bioenergy 81:364–368. https://doi.org/10.1016/j.biombioe.2015.07.020
18. Zhao X, Moates GKK, Wellner N et al (2014) Chemical characterisation and analysis of the cell wall polysaccharides of duckweed (Lemna minor). Carbohydr Polym 111:410–418. https://doi.org/10.1016/j.carbpol.2014.04.079
19. Landolt E (1992) Lemnaceae duckweed family. J Arizona-Nevada Acad Sci 26:10–14
20. Les DHD, Crawford DJD, Landolt E et al (2002) Phylogeny and systematics of Lemnaceae, the duckweed family. Syst Bot 27:221–240. https://doi.org/10.1043/0363-6445-27.2.221
21. Verma R, Suthar S (2015) Utility of duckweeds as source of biomass energy: a review. Bioenergy Res 8:1589–1597. https://doi.org/10.1007/s12155-015-9639-5
22. Xu J, Zhao H, Stomp AM, Cheng JJ (2012) The production of duckweed as a source of biofuels. Biofuels 3:589–601. https://doi.org/10.4155/bfs.12.31
23. Ge X, Zhang N, Phillips GC, Xu J (2012) Growing Lemna minor in agricultural wastewater and converting the duckweed biomass to ethanol. Bioresour Technol 124:485–488. https://doi.org/10.1016/j.biortech.2012.08.050
24. Sowinski EE, Gilbert S, Lam E, Carpita NC (2019) Linkage structure of cell-wall polysaccharides from three duckweed species. Carbohydr Polym 223:115119. https://doi.org/10.1016/j.carbpol.2019.115119
25. Xu Y, Ma S, Huang M, Peng M, Bog M, Sree KS, Appenroth KJ, Zhang J (2015) Species distribution, genetic diversity and barcoding in the duckweed family (Lemnaceae). Hydrobiologia 743:75–87. https://doi.org/10.1007/s10750-014-2014-2
26. Perniel M, Ruan R, Martinez B (1998) Nutrient removal from a stormwater detection pond using duckweed. Appl Eng Agric 14:605–609. https://doi.org/10.13031/2013.19429
27. Xiu SN, Shahbazi A, Croonenberghs J, Wang LJ (2010) Oil production from duckweed by thermochemical liquefaction. Energy Sources Part A 32:1293–1300. https://doi.org/10.1080/15567030903060408
28. Duan P, Zhang C, Wang F, Fu J, Lü X, Xu Y, Shi X (2016) Activated carbons for the hydrothermal upgrading of crude duckweed bio-oil. Catal Today 274:73–81
29. Arenque BC, Grandis A, Pocius O, de Souza AP, Buckeridge MS (2014) Responses of senna reticulata, a legume tree from the amazonian floodplains, to elevated atmospheric co2 concentration and waterlogging. Trees - Struct Funct 28:1021–1034. https://doi.org/10.1007/s00468-014-1015-0
30. de Souza AP, Leite DCCC, Pattathil S et al (2013) Composition and structure of sugarcane cell wall polysaccharides: implications for second-generation bioethanol production. Bioenergy Res 6:564–579. https://doi.org/10.1007/s12155-012-9268-1
31. Filisetti-Cozzi TMCC, Carpita NC (1991) Measurement of uronic acids without interference from neutral sugars. Anal Biochem 197:157–162. https://doi.org/10.1016/0003-2697(91)90372-Z
32. Van Acker R, Vanholme R, Storme V et al (2013) Lignin biosynthesis perturbations affect secondary cell wall composition and saccharification yield in Arabidopsis thaliana. Biotechnol Biofuels 6:46. https://doi.org/10.1186/1754-6834-6-46
33. Fukushima RS, Kerley MS (2011) Use of lignin extracted from different plant sources as standards in the spectrophotometric acetyl bromide lignin method. J Agric Food Chem 59:3505–3509. https://doi.org/10.1021/jf104826n
34. Xue FC, Chandra R, Berleth T, Beatson RP (2008) Rapid, microscale, acetyl bromide-based method for high-throughput determination of lignin content in Arabidopsis thaliana. J Agric Food Chem 56:6825–6834. https://doi.org/10.1021/jf800775f
35. Gomez LD, Whitehead C, Barakate A, Halpin C, McQueen-Mason SJ (2010) Automated saccharification assay for determination of digestibility in plant materials. Biotechnol Biofuels:3. https://doi.org/10.1186/1754-6834-3-23
36. Somerville C, Youngs H, Taylor C et al (2010) Feedstocks for lignocellulosic biofuels. Science (80-.) 329:790–792
37. Amorim HV, Lopes ML, De Castro Oliveira JV et al (2011) Scientific challenges of bioethanol production in Brazil. Appl Microbiol Biotechnol 91:1267–1275
38. Hart DA, Kindel PK (1970) Isolation and partial characterization of apiogalacturonans from the cell wall of Lemna minor. Biochem J 116:569–579. https://doi.org/10.1042/bj1160569
39. Duff RB (1965) The occurrence of apiose in Lemna (duckweed) and other angiosperms. Biochem J 94:768–772. https://doi.org/10.1042/bj0940768
40. Yin Y, Yu C, Yu L, Zhao J, Sun C, Ma Y, Zhou G (2015) The influence of light intensity and photoperiod on duckweed biomass and starch accumulation for bioethanol production. Bioresour Technol 187:84–90. https://doi.org/10.1016/j.biortech.2015.03.097
41. DeMartini JD, Pattathil S, Miller JS et al (2013) Investigating plant cell wall components that affect biomass recalcitrance in poplar and switchgrass. Energy Environ Sci 6:898. https://doi.org/10.1039/c3ee23801f
42. Zhao X, Moates GK, Wilson DR, Ghogare RJ, Coleman MJ, Waldron KW (2015) Steam explosion pretreatment and enzymatic saccharification of duckweed (Lemna minor) biomass. Biomass and Bioenergy 72:206–215. https://doi.org/10.1016/j.biombioe.2014.11.003
43. Chen Q, Jin Y, Zhang G, Fang Y, Xiao Y, Zhao H (2012) Improving production of bioethanol from duckweed (Landoltia punctata) by pectinase pretreatment. Energies 5:3019–3032. https://doi.org/10.3390/en5083019
44. Yadav D, Barbora L, Bora D, Mitra S, Rangan L, Mahanta P (2017) An assessment of duckweed as a potential lignocellulosic feedstock for biogas production. Int Biodeterior Biodegrad 119:253–259. https://doi.org/10.1016/j.ibiod.2016.09.007

45. Li Q, Song J, Peng S, Wang JP, Qu GZ, Sederoff RR, Chiang VL (2014) Plant biotechnology for lignocellulosic biofuel production. Plant Biotechnol J 12:1174–1192. https://doi.org/10.1111/pbi.12273
46. Hartt DA, Kindell PK (1969) A novel reaction involved in the degradation of apiogalacturonans from Lemna minor and the isolation of apibiose as a product. Biochemistry 9:2190–2196
47. Su H, Zhao Y, Jiang J, Lu Q, Li Q, Luo Y, Zhao H, Wang M (2014) Use of duckweed (*Landoltia punctata*) as a fermentation substrate for the production of higher alcohols as biofuels. Energy Fuels 28:3206–3216. https://doi.org/10.1021/ef500335h
48. van Maris AJAA, Abbott DA, Bellissimi E et al (2006) Alcoholic fermentation of carbon sources in biomass hydrolysates by *Saccharomyces cerevisiae*: current status. Anton Leeuw Int J Gen Mol Microbiol 90:391–418. https://doi.org/10.1007/s10482-006-9085-7
49. Cunha JT, Soares PO, Romaní A, Thevelein JM, Domingues L (2019) Xylose fermentation efficiency of industrial Saccharomyces cerevisiae yeast with separate or combined xylose reductase/xylitol dehydrogenase and xylose isomerase pathways. Biotechnol Biofuels 12:20. https://doi.org/10.1186/s13068-019-1360-8
50. Kobayashi Y, Sahara T, Ohgiya S, Kamagata Y, Fujimori KE (2018) Systematic optimization of gene expression of pentose phosphate pathway enhances ethanol production from a glucose/xylose mixed medium in a recombinant Saccharomyces cerevisiae. AMB Express 8:1–11. https://doi.org/10.1186/s13568-018-0670-8
51. Liu CG, Xiao Y, Xia XX, Zhao XQ, Peng L, Srinophakun P, Bai FW (2019) Cellulosic ethanol production: progress, challenges and strategies for solutions. Biotechnol Adv 37:491–504
52. Yu HT, Chen BY, Li BY, Tseng MC, Han CC, Shyu SG (2018) Efficient pretreatment of lignocellulosic biomass with high recovery of solid lignin and fermentable sugars using Fenton reaction in a mixed solvent. Biotechnol Biofuels 11:287. https://doi.org/10.1186/s13068-018-1288-4
53. Jaiswal D, De Souza AP, Larsen S et al (2017) Brazilian sugarcane ethanol as an expandable green alternative to crude oil use. Nat Clim Chang 7:788–792. https://doi.org/10.1038/nclimate3410