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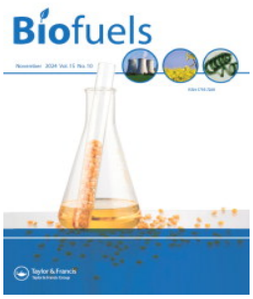

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Optimized bioethanol production from *Lemna minuta* biomass harvested from polluted water via acid and enzymatic hydrolysis

Elis Regina Dana Schutz[a], Daiane Salete Broch Mignoni[b], William Michelon[c] and Estela de Oliveira Nunes[d]

[a]Universidade do Oeste de Santa Catarina, Videira-SC, Brazil; [b]Universidade Estadual de Campinas, Faculdade de Ciências Aplicadas Laboratório de Biotecnologia, BraPhyto. Limeira-SP, Brazil; [c]Universidade do Contstado, Concordia-SC, Brazil; [d]Brazilian Agricultural Research Corporation, Embrapa Swine and Poultry, Environment department, Concordia-SC, Brazil

ABSTRACT

The contamination of water bodies through domestic, agricultural, and industrial discharges remains a critical environmental challenge, leading to eutrophication and harmful impacts on aquatic ecosystems and public health. In response, phytoremediation, which utilizes aquatic plants for pollutant removal, have gained attention. This study investigates the potential of *Lemna minuta* biomass, harvested from a polluted pond, for bioethanol production. The research evaluates carbohydrate content and explores the efficiency of acid and enzymatic hydrolysis in converting the biomass into fermentable sugars. The study's findings reveal that *Lemna minuta* exhibits a carbohydrate content of 36.46 ± 1.69%. Acid hydrolysis demonstrated a high conversion efficiency, with optimal conditions achieving up to 99.20% efficiency and 18.09 g L^{-1} total reducing sugars. Enzymatic hydrolysis, while effective, yielded lower efficiencies, indicating the need for further optimization. Fermentation tests using *Saccharomyces cerevisiae chardonnay* resulted in ethanol production of 1.5 g L^{-1}, highlighting the potential of *Lemna minuta* as a sustainable bioethanol feedstock. These findings highlight the potential of *Lemna minuta* as a sustainable feedstock for bioethanol production while contributing to environmental remediation, reinforcing its dual role in renewable energy and ecosystem restoration.

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Acid hydrolysis; aquatic biomass; biofuel; duckweeds; enzymatic hydrolysis; phytoremediation

Introduction

Water pollution is a persistent environmental challenge driven by domestic, agricultural, and industrial activities [1,2]. Untreated wastewater from these sources introduces excessive nutrients, particularly nitrogen and phosphorus, into aquatic ecosystems, leading to eutrophication and posing significant threats to both biodiversity and human health [3,4]. As the need for sustainable water management intensifies, nature-based solutions are emerging as effective strategies [5]. Among these, phytoremediation—a process that utilizes aquatic plants to remove pollutants—offers an environmentally friendly and cost-effective method for restoring water quality [6].

Phytoremediation not only aids in environmental clean-up but also enhances the biomass yield of the plants involved, thereby increasing their potential as feedstocks for bioethanol production [7]. The absorption of nutrients, such as nitrogen and phosphorus, by duckweeds in polluted waters accelerates their growth, resulting in substantial biomass accumulation [8,9]. This increase in biomass responds to a challenge in the production of biofuels, the availability of sufficient and sustainable feedstock [10,11].

Concurrently, the global shift away from fossil fuels towards renewable energy sources [12,13] has reinforced the interest in bioethanol as a sustainable alternative [14]. Traditionally derived from first-generation feedstocks like sugarcane and corn, bioethanol production has increasingly focused on second-generation feedstocks, such as lignocellulosic materials, to alleviate concerns over food security and land use [15,16]. Among these potential feedstocks, aquatic plants, particularly duckweeds, are promising due to their rapid growth, high carbohydrate content, and ability to absorb nutrients from wastewater, effectively serving dual roles in bioethanol production and water remediation [17]. For example, *Pistia stratiotes* biomass produced 31.0 g L^{-1} of reducing sugars after enzymatic hydrolysis using a commercial cellulase enzyme [18]. Similarly, wild duckweed (comprising *Landoltia* ≥ 90%, *Spirodela* 3–5%, *Lemna* 2–4%, and *Wolffia* ≤ 1%) harvested directly from ponds and paddies demonstrated efficient bioethanol production through separate hydrolysis and fermentation, achieving a mean ethanol yield of 4.98 g [19].

Despite extensive research on the phytoremediation capabilities of various duckweed species, including *Lemna minuta*, the potential of their biomass post-phytoremediation remains underexplored. The environmental stress experienced during phytoremediation, such as exposure to high nutrient loads, can induce the accumulation of carbohydrates, especially starch, which are key substrates for ethanol fermentation [17]. Thus, biomass derived from phytoremediation not only offers quantity but also enhanced quality, potentially improving the yield of fermentable sugars during hydrolysis [20]. Although species like *Lemna* sp. [21,22], *Spirodela* sp. [23,24] and *Wolffia* sp. [25,26] have been extensively studied, the potential of *Lemna minuta* in polluted environments has not been fully realized. For instance, Ceschin et al. [27] demonstrated that *Lemna minuta* exhibits significant phytoremediation potential, with a nearly tenfold increase in biomass and doubling of mat

CONTACT William Michelon eng.williammichelon@gmail.com Victor Sopelsa, 3000, Concordia 89711-330, Brazil.
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thickness during synthetic wastewater treatment. The species also showed high nutrient uptake, with phosphate and nitrate levels increasing by 165% and 10%, respectively, establishing *Lemna minuta* as a hyperaccumulator of these nutrients. However, the potential of this biomass as a bioethanol feedstock post-phytoremediation was not assessed.

In this regard, this study focuses on evaluating the carbohydrate content of *Lemna minuta* biomass collected from a polluted pond, investigating the efficiency of acid and enzymatic hydrolysis for fermentable sugar conversion, and optimizing the conditions for maximizing ethanol yield, thereby advancing its potential for integrated environmental and energy solutions.

Methods

General design

The biomass for this study was sourced from a natural water pond located at 26°56'13.90" S and 51°15'45.07" W in a rural area near Videira City, Santa Catarina State, Brazil. *Lemna minuta* samples were accurately identified, and specimens were deposited in the Campo Grande Herbarium of Mato Grosso do Sul (CGMS) under voucher number CGMS-52914. Samples were collected across all four seasons (spring, summer, autumn, and winter) as described by Rodríguez and Preston [28]. An initial 25 kg of fresh biomass was harvested, sun-dried, and sieved. The dried samples were then stored in low-density polyethylene (LDPE) vacuum-sealed packages to preserve them until further testing. Four subsamples were combined to create a total of 8 kg of dry biomass, which was homogenized and ground in a porcelain crucible. This material was then divided into three portions for carbohydrate content analysis, hydrolysis, and fermentation experiments. Figure 1 illustrates the summary of the methodology used in this study to produce bioethanol from *Lemna minuta* biomass.

Carbohydrates content

Carbohydrate concentration was determined using the Fehling method, as outlined by Instituto Adolfo Lutz [29]. Two grams of each biomass sample were placed in flat-bottomed flasks containing 200 mL of distilled water and 5 mL of HCl. Thermal digestion was carried out under reflux for 3 h, using Digester Block (model TE-008/50-04, Piracicaba, SP, Brazil). After cooling to room temperature for 30 min, the digested samples were neutralized with a 40% NaOH solution until the pH was approximately 7.0. The hydrolysate was then filtered, and the liquid fraction was titrated with Fehling solution until the endpoint was reached.

Saccharification

Acid hydrolysis

Acid hydrolysis pretreatment tests of *Lemna minuta* biomass were used an initial biomass concentration of 60 g L^{-1} [30]. Three grams of the biomass sample were mixed with 50 mL of distilled water, and acid (HCl or H_2SO_4) was added at concentrations of 5, 10, and 15% (v v^{-1}). The mixtures were incubated varying temperature at 100, 130, and 150 °C and time at 20, 40, and 60 min. The experimental design, detailed in Table 1, follows a 2^3 factorial design to systematically investigate the effects of acid concentration, temperature, and time. Post-incubation, the hydrolyzed solutions were cooled in an ice bath for 15 min, and the pH was adjusted to 6.0 using a 2 N NaOH solution. The liquid fraction was separated from the biomass by centrifugation at 10,800 rpm for 20 min.

Enzymatic hydrolysis

Enzymatic hydrolysis was performed using Rohalase® Barley enzyme, comprising cellulases, xylanases, and pectinases. The goal was to optimize the hydrolysis process by testing a range of enzyme concentrations under different pH levels and temperatures. The experimental design is detailed in Table 2. Hydrolysis tests were conducted in 250 mL Erlenmeyer flasks containing 50 mL of distilled water. The hydrolysis tests were carried out in 250 mL Erlenmeyer flasks containing 50 mL of distilled water. Buffered solutions of Na_2PO_4 and citric acid were prepared at pH levels 4.0, 5.0, 6.0, and 7.0. Three grams of *Lemna minuta* were added to each flask, homogenized, and the initial sugar content was determined. Following this, 1 mL of the enzyme solution was added, and the flasks were incubated at 40, 55, and 70 °C with constant agitation. The hydrolysis was performed over three different time intervals—60, 120, and 240 min.

Reducing sugars quantification

The supernatant containing residual sugars was collected and its pH adjusted to 5.5 using 1 M NaOH. Residual sugar concentration was measured using the DNS (dinitrosalicylic acid) method, with glucose serving as the standard for the calibration curve [31]. To quantify sugars, 0.75 mL of glucose solution was mixed with 0.5 mL of DNS reagent and heated at 100 °C for 5 min. After cooling to room temperature, 3 mL of water was added. Sugar concentrations were determined spectrophotometrically at 540 nm using a spectrophotometer (model DM-ESPEC1, Digimed, SP, Brazil).

Fermentation tests

Alcoholic fermentation tests were conducted in triplicate using the commercial yeast strain *Saccharomyces cerevisiae chardonnay* (Proenol®). The yeast was pre-inoculated in a 100 mL Schott® borosilicate glass bottle containing 50 mL of sterilized distilled water. One gram of *Saccharomyces cerevisiae chardonnay* and 1 g of nutrient were added and diluted in water to 30 °C for 1 h to activate the yeast, forming the yeast suspension solution. The hydrolyzed biomass was filtered under vacuum, and the filtrate pH was adjusted to 4.5 with a 2 N NaOH solution. Erlenmeyer flasks were autoclaved for 15 min, cooled to room temperature, and 5 mL of yeast suspension was aseptically added to each flask. Fermentation occurred at 30 °C for 48 h. Microbial kinetics were monitored by measuring the absorbance of the yeast suspension at 600 nm. Yeast concentration (cells mL^{-1}) was calculated using viable cell counting with a Neubauer chamber (Improved Bright-line 0.025 mm^2), where 0.05 mL of yeast suspension (diluted to 0.1 g L^{-1}) and one drop of 1% methylene blue dye were used to identify viable cells. Visualization was done with a

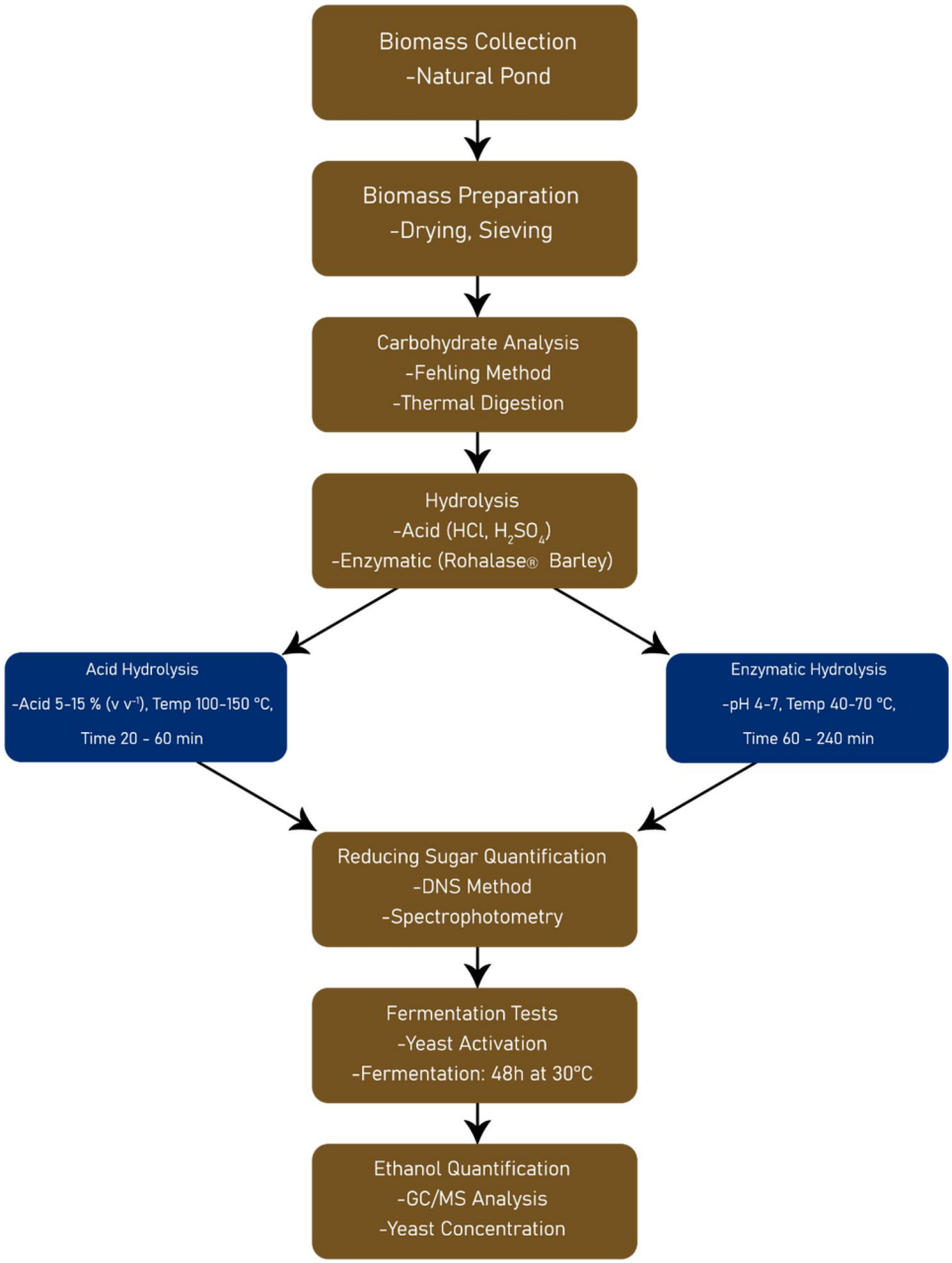

Figure 1. Overview of the study's methodology, including biomass collection and preparation, carbohydrate analysis, hydrolysis (acid and enzymatic), reducing sugar quantification, fermentation, and ethanol quantification.

Table 1. Experimental design for acid hydrolysis: 2^3 factorial.

				Acid (%)		
Treatments	Actual levels			HCl or H_2SO_4	Temperature (°C)	Time (min)
1	1	−1	−1	5	100	20
2	−1	−1	1	5	100	60
3	−1	1	−1	5	150	20
4	−1	1	1	5	150	60
5	1	−1	−1	15	100	20
6	1	−1	1	15	100	60
7	1	1	−1	15	150	20
8	1	1	1	15	150	60
9	0	0	0	10	125	40
10	0	0	0	10	125	40
11	0	0	0	10	125	40

Nikon Eclipse E100 microscope at 40× magnification, showing an initial viable cell concentration of 42,400 cells mL^{-1}.

Ethanol quantification

Ethanol quantification was performed at the Beverage Technology Laboratory of the National Service for Industrial Learning in Pinheiro Preto City, Santa Catarina. Ethanol levels were measured using a GC/MS method with an Agilent gas chromatograph (Model 7890 A, Santa Clara, CA, USA) coupled to a single quadrupole mass detector (Model 5975, Santa Clara, CA, USA).

Statistical analysis

The estimated effects of variables and regression coefficients model for the responses found were submitted to analysis of variance (ANOVA), considering a 95% confidence level ($p \leq 0.05$). The statistical processing was performed using the software STATISTICA 8.0 (StatSoft trial version).

Results and discussion

Carbohydrate content

Table 3 provides a comparative analysis of carbohydrate content across various aquatic plants, highlighting the

Table 2. Enzymatic hydrolysis experimental conditions.

Treatments	Temperatures (°C)	Times (min)	pH
1	40	60	4
2	40	60	5
3	40	60	6
4	40	60	7
5	40	120	4
6	40	120	5
7	40	120	6
8	40	120	7
9	40	240	4
10	40	240	5
11	40	240	6
12	40	240	7
13	55	60	4
14	55	60	5
15	55	60	6
16	55	60	7
17	55	120	4
18	55	120	5
19	55	120	6
20	55	120	7
21	55	240	4
22	55	240	5
23	55	240	6
24	55	240	7
25	70	60	4
26	70	60	5
27	70	60	6
28	70	60	7
29	70	120	4
30	70	120	5
31	70	120	6
32	70	120	7
33	70	240	4
34	70	240	5
35	70	240	6
36	70	240	7

Table 3. Carbohydrate composition of duckweed harvested from phytoremediation of polluted water or wastewater.

Species	Carbohydrate (%)	References
Wolffia globosa	22–28 (starch)	[50]
Spirodela polyrhiza	38–41	[51]
Landoltia punctata	24.5 (starch)	[41]
Lemna sp.	22–43.3	[52]
Spirodela sp.	18–33	[52]
Lemna sp.	18.2–35	[53]
Lemna minor	4 (starch)	[54]
Lemna minor	11–12.5 (starch)	[32]
Lemna minuta	36.46 ± 1.69	This study

potential of *Lemna minuta* as a feedstock for carbohydrate production. *Lemna minuta* exhibited a carbohydrate content of 36.46 ± 1.69%, which is comparable to *Spirodela polyrhiza*, known for its carbohydrate range of 38–41%. This similarity suggests that *Lemna minuta* could be equally effective as *Spirodela polyrhiza* in bioethanol production due to its high availability of fermentable sugars. Additionally, the data illustrates variability among species within the Lemnoideae family, with *Lemna minuta* demonstrating competitive carbohydrate levels. These results highlight the potential of *Lemna minuta* as a bioenergy feedstock, as further evidenced by its carbohydrate concentration being higher than that of other reported species (Table 3).

Duckweed biomass, including *Lemna minuta*, typically contains lower cellulose (around 10% dry weight) compared to terrestrial plants (approximately 40% dry weight). This low cellulose content, coupled with an absence of lignin and low hemicellulose levels, makes duckweed biomass less resistant to saccharification, simplifying its conversion to ethanol [32]. Carbohydrates in *Lemna* sp. species primarily accumulate in response to environmental stressors, such as nutrient limitations (e.g. nitrogen or phosphorus deficiency). Under these conditions, the plant alters its metabolic priorities, increasing the synthesis of storage compounds like starch. This adaptation is driven by the need to store energy in a readily accessible form, ensuring survival during unfavorable conditions [33]. Various studies, including Yu et al. [33] and Guo et al. [34] have shown that nutrient deficiencies, particularly nitrogen, can significantly increase starch content in duckweed species.

Saccharification

Acid hydrolysis

Table 4 showed the Total Reducing Sugar (TRS), hydrolysate yield, and dry mass for the acid hydrolysis treatments of *Lemna minuta* using hydrochloric and sulfuric acids. For hydrochloric acid treatments, treatment 4 resulted in the highest TRS at 18.09 g L^{-1} with a dry mass of 0.36 g g^{-1}, aligning with the optimal conditions predicted by Response Surface Methodology (RSM). Treatments 1 and 3 also produced notable TRS values, 16.26 g L^{-1} and 16.44 g L^{-1}, respectively, with corresponding dry masses. Lower TRS values were observed in treatments such as 5 (13.08 g L^{-1}) and 10 (9.09 g L^{-1}), reflecting the impact of less favorable hydrolysis conditions. Similarly, in sulfuric acid hydrolysis, treatment 4 achieved the highest TRS of 17.55 g L^{-1} with a dry mass of 0.35 g g^{-1}. Other treatments, such as 3 and 1, also showed significant TRS levels at 16.86 g L^{-1} and 15.48 g L^{-1}, respectively. The lower TRS values in treatments 8 (12.21 g L^{-1}) and 9 (15.87 g L^{-1}) further emphasize the variability in hydrolysis outcomes based on the conditions applied. Acid hydrolysis, used in this study with acid (HCl or H_2SO_4), breaks down polysaccharides into fermentable sugars by protonating and cleaving glycosidic bonds. This method converts the carbohydrates in biomass into reducing sugars such as glucose [35].

Su et al. [36] investigated ethanol and isopentanol production from duckweed (*Landoltia punctata*) *via* fermentation, achieving an ethanol production range of 7 to 15 g L^{-1} using selected mutants. Remarkably, the yields of ethanol and isopentanol from acid hydrolyzed duckweed were 15 times higher than those from yeast fermentation. Unlike lignocellulosic energy crops, which produce microbial growth inhibitors like furfural during acid hydrolysis, duckweed generates minimal toxic by-products, enhancing the efficiency of microbial fermentation [36]. The chemical pretreatment of *Lemna minor L.* biomass was assessed using H_2SO_4 and NaOH at concentrations of 5, 10, and 20% at 80 °C. The results demonstrated that dried duckweed biomass yielded a higher concentration of sugars compared to fresh biomass. Notably, the treatment with 5% H_2SO_4 on dried biomass produced the highest reducing sugar content, reaching 796 mg L^{-1}. In contrast, the lowest yield of reducing sugars, 200 mg L^{-1}, was obtained from the treatment of fresh biomass with 20% NaOH [37].

Statistical analysis and RSM modelling

The response surface analysis (Figure 2(A)) for hydrochloric acid hydrolysis indicates that efficiency increases with higher temperatures and moderate HCl

Table 4. Sugar content of *Lemna minuta* from hydrolysis with hydrochloric and sulfuric acid.

		Total Reducing Sugar (TRS)			Total Reducing Sugar (TRS)	
Treatment	Hydrolysis	Hydrolysate (g L-1)	Dry Mass (g g-1)	Hydrolysis	Hydrolysate (g L-1)	Dry Mass (g g-1)
1	Hydrochloric acid	16.26 ± 0.08	0.32 ± 0.01	Sulfuric acid	15.48 ± 0.03	0.31 ± 0.001
2		16.05 ± 0.04	0.32 ± 0.001		15.51 ± 0.04	0.31 ± 0.001
3		16.44 ± 0.01	0.33 ± 0.001		16.86 ± 0.13	0.34 ± 0.01
4		18.09 ± 0.01	0.36 ± 0.001		17.55 ± 0.25	0.35 ± 0.02
5		13.08 ± 0.06	0.26 ± 0.001		13.92 ± 0.15	0.28 ± 0.01
6		11.67 ± 0.08	0.23 ± 0.01		15.45 ± 0.02	0.31 ± 0.001
7		15.78 ± 0.03	0.31 ± 0.001		15.30 ± 0.01	0.31 ± 0.001
8		12.03 ± 0.18	0.24 ± 0.01		12.21 ± 0.17	0.24 ± 0.01
9		10.89 ± 0.08	0.22 ± 0.01		15.87 ± 0.06	0.32 ± 0.001
10		9.09 ± 0.05	0.24 ± 0.001		16.35 ± 0.02	0.33 ± 0.001
11		10.29 ± 0.26	0.21 ± 0.02		15.51 ± 0.05	0.31 ± 0.001

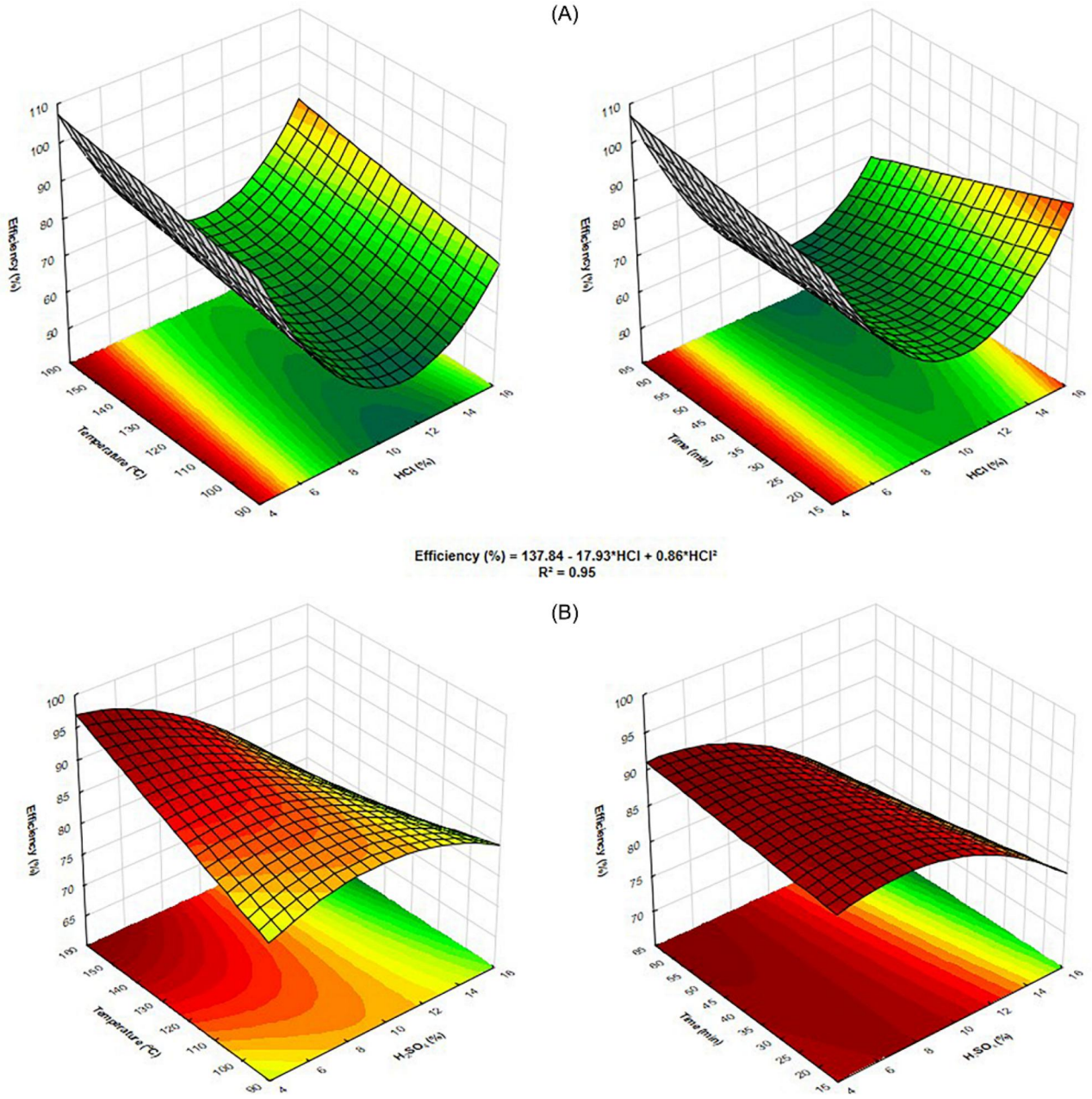

Figure 2. (A) Response surface showing the effects of hydrochloric acid concentration (HCl, %), temperature (°C), and reaction time (min) on hydrolysis efficiency (%). (B) Response surface showing the effects of sulfuric acid concentration (H_2SO_4, %), temperature (°C), and reaction time (min) on hydrolysis efficiency (%).

concentrations, peaking at 150 °C and 4–6% HCl. The quadratic nature of the response is supported by the R^2 value of 0.95, suggesting a strong model fit. Notably, only HCl concentration was a significant factor in hydrolysis efficiency. For sulfuric acid hydrolysis (Figure 2(B)), the response surface indicates that changes in temperature and acid concentration have minimal impact on efficiency within the tested ranges, as confirmed by ANOVA results. The relatively flat response surface suggests that sulfuric acid hydrolysis may require further refinement to achieve efficiencies comparable to hydrochloric acid hydrolysis.

Table 5. Sugar content of *Lemna minuta* from enzymatic hydrolysis and efficiency.

				Total Reducing Sugar (TRS)		
Treatments	Temperatures (°C)	Times (min)	pH	Hydrolysate (g L^{-1})	Dry Mass (g g^{-1})	Efficiency (%)
1	40	60	4	0.285 ± 0.105	0.017 ± 0.008	4.693 ± 1.733
2	40	60	5	0.543 ± 0.059	0.034 ± 0.003	8.943 ± 0.972
3	40	60	6	0.520 ± 0.024	0.031 ± 0.002	8.558 ± 0.388
4	40	60	7	0.533 ± 0.025	0.032 ± 0.002	8.764 ± 0.414
5	40	120	4	0.374 ± 0.128	0.022 ± 0.009	6.151 ± 2.111
6	40	120	5	0.460 ± 0.008	0.031 ± 0.009	7.567 ± 0.135
7	40	120	6	0.514 ± 0.014	0.033 ± 0.007	8.461 ± 0.376
8	40	120	7	0.457 ± 0.033	0.027 ± 0.002	7.512 ± 0.548
9	40	240	4	0.287 ± 0.070	0.021 ± 0.008	4.721 ± 0.178
10	40	240	5	0.170 ± 0.009	0.013 ± 0.009	2.795 ± 0.140
11	40	240	6	0.320 ± 0.103	0.030 ± 0.010	5.271 ± 1.703
12	40	240	7	0.507 ± 0.169	0.030 ± 0.012	8.351 ± 2.77
13	55	60	4	0.500 ± 0.057	0.030 ± 0.004	8.228 ± 0.939
14	55	60	5	0.240 ± 0.010	0.014 ± 0.001	3.351 ± 0.166
15	55	60	6	0.206 ± 0.061	0.012 ± 0.004	3.387 ± 1.005
16	55	60	7	0.307 ± 0.019	0.018 ± 0.001	5.051 ± 0.309
17	55	120	4	0.103 ± 0.040	0.006 ± 0.003	1.695 ± 0.665
18	55	120	5	0.196 ± 0.010	0.012 ± 0.001	3.222 ± 0.159
19	55	120	6	0.174 ± 0.004	0.011 ± 0.000	2.864 ± 0.658
20	55	120	7	0.319 ± 0.178	0.019 ± 0.013	5.243 ± 2,936
21	55	240	4	0.260 ± 0.031	0.016 ± 0.002	4.281 ± 0.505
22	55	240	5	0.242 ± 0.012	0.015 ± 0.001	3.978 ± 0.202
23	55	240	6	0.411 ± 0.291	0.025 ± 0.021	6.756 ± 4.781
24	55	240	7	0.376 ± 0.113	0.023 ± 0.008	6.192 ± 1.80
25	70	60	4	0.140 ± 0.027	0.008 ± 0.001	2.300 ± 0.440
26	70	60	5	0.098 ± 0.011	0.005 ± 0.001	1.613 ± 0.186
27	70	60	6	0.098 ± 0.010	0.005 ± 0.001	1.613 ± 0.159
28	70	60	7	0.272 ± 0.039	0.016 ± 0.002	4.473 ± 0.642
29	70	120	4	0.124 ± 0.018	0.005 ± 0.001	2.039 ± 0.292
30	70	120	5	0.357 ± 0.043	0.021 ± 0.003	5.876 ± 0.707

Enzymatic hydrolysis

The results of enzymatic hydrolysis are shown in Table 5. The highest yield was observed at 40 °C, 60 min, and pH 5, with a hydrolysate concentration of 0.543 g L^{-1} and an efficiency of 8.943%. In general, enzymatic hydrolysis yielded lower efficiencies (1.613–8.943%) compared to acid hydrolysis, indicating a need for further optimization. These findings suggest that while enzymatic hydrolysis is effective under certain conditions, it currently underperforms relative to acid hydrolysis in terms of sugar yield and efficiency.

The enzymatic hydrolysis of duckweed biomass was performed using commercial enzymes α-amylase, pullulanase, and amyloglucosidase, following a protocol like that used for the saccharification of corn starch [38]. This process yielded a hydrolysate with 0.5 g $_{sugar}$ g $_{dry\ biomass}^{-1}$ [38]. Subsequent fermentation using yeast produced an ethanol yield of 258 mg per gram of dry biomass. These results suggest that duckweed biomass can generate significant amounts of starch, which can be efficiently fermented into ethanol [38]. Xu et al. [39] employed a 14-L continuous stirred tank reactor to perform enzymatic hydrolysis followed by yeast fermentation on high-starch *Spirodela polyrrhiza* (31% starch). They applied a similar enzymatic hydrolysis technique to duckweed biomass using α-amylase, pullulanase, and amyloglucosidase for saccharification. The process resulted in a recovery of reducing sugars amounting to 96.8% of the theoretical maximum.

Zhao et al. [40] enhanced the yield of fermentable sugars by introducing cellulase enzymes, demonstrating the process's effectiveness despite the cost of the enzymes. Similarly, Chen et al. [41] found that pectinase treatment also improved sugar yield. Focusing on the cell wall composition of five duckweed species across all five genera, Pagliuso et al. [42] used the enzyme cocktail Cellic Ctec2 (Novozymes) and observed low recalcitrance to hydrolysis, likely due to the low lignin and cellulose content in duckweed. While enzymatic hydrolysis is effective, acid hydrolysis offers a viable alternative for breaking down starch into sugars. For instance, Rana et al. [43] achieved a 99.4% conversion of starch to glucose by treating starch-rich *Spirodela polyrhiza* with 0.1% sulfuric acid at 121 °C for 1h.

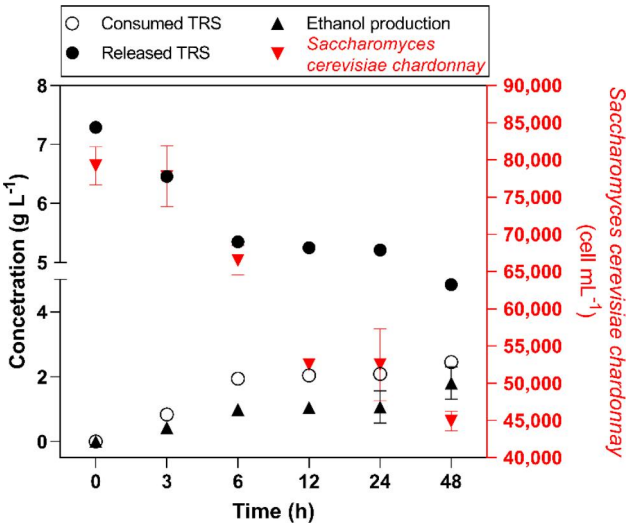

Figure 3. Time-course of TRS release and consumption, ethanol production, and *Saccharomyces cerevisiae chardonnay* cell concentration during fermentation.

Ethanol production

Figure 3 illustrates the time-course of ethanol production during the fermentation of hydrolyzed *Lemna minuta* biomass. The optimized hydrolysate, derived under conditions of 5% HCl at 150 °C for 60 min, was subjected to fermentation. Ethanol production peaked at 1.5 g L^{-1} after 48 h, aligning with a decrease in available TRS and an increase

Table 6. Comparison of saccharification and ethanol production from various duckweed species using different hydrolysis processes.

Specie	Initial Dry Mass (g)	Saccharification	Hydrolasate (g L^{-1})	Ethanol Production	Reference
Spirodela polyrrhiza	–	Enzymatic	0.51 g g^{-1}	0.258 g g^{-1}	[38]
Lemna minor	20	Enzymatic	2.5	18.8 g g^{-1}	[55]
Landoltia punctata, Lemna aequinoctialis, Spirodela polyrrhiza, and Wolffia arrhiza	5	Enzymatic	10.6–13.2	0.16–0.19 g g^{-1}	[48]
Lemna minor	46.3	Enzymatic	10	4.5 g L^{-1}	[32]
Lemna aequinoctialis	20	Enzymatic	7.8	3.38 g L^{-1}	[56]
Landoltia punctata	10	Enzimatic modified	80	24.6 g L^{-1}	[36]
Landoltia punctata	10	Enzimatic modified	60	1.15 g L^{-1}	[57]
Pool of duckweeds (*Landoltia* $\geq$ 90%, *Spirodela* 3–5%, *Lemna* 2–4%, $\leq$ *Wolfa*)	19	Enzymatic	100	0.262 g g^{-1}	[19]
Wolffia globosa	20	$NaOH + H_2O_2$	5.5	2 g L^{-1}	[50]
Lemna minuta	3	HCl	18	1.5 g L^{-1}	This study

in TRS consumption, reflecting efficient sugar conversion by *Saccharomyces cerevisiae chardonnay*.

Saccharomyces cerevisiae ferments sugars, primarily glucose, into ethanol and carbon dioxide. Through glycolysis, glucose is metabolized into pyruvate, producing ATP and NADH. Pyruvate is then decarboxylated to acetaldehyde and reduced to ethanol by alcohol dehydrogenase, regenerating NAD^+ for glycolysis to proceed [44,45]. Notably, even after a significant reduction in yeast concentration at 12 h, ethanol production continued to increase, suggesting sustained metabolic activity in the remaining cells. This observation highlights the potential for optimizing fermentation processes by enhancing yeast viability and metabolic efficiency, which could reduce operational costs in industrial bioethanol production.

Similar results were reported by Masami et al. [46], who achieved a maximum ethanol production of 0.9–1.5 g L^{-1} from the saccharified solution of water hyacinth powder. Additionally, Takagi et al. [47] obtained a maximum ethanol yield of 2.33 g L^{-1} from water hyacinth using a saccharified solution treated with 3% (v v^{-1}) sulfuric acid at 121 °C for 1 h. However, the ethanol yield of 0.5 g $_{ethanol}$ g $_{dry\ biomass}{}^{-1}$ observed in this study for *Lemna minuta* surpasses those reported for other duckweed species (Table 6), highlighting its efficiency as a bioethanol feedstock. In instance, *Landoltia punctata* yielded 0.1 g $_{ethanol}$ g $_{dry\ biomass}{}^{-1}$ For instance, *Landoltia punctata* yielded 0.1 g $_{ethanol}$ g $_{dry\ biomass}{}^{-1}$, *Lemna aequinoctialis* 0.17 g, *Spirodela polyrrhiza* 0.19 g, and *Wolffia arrhiza* 0.16 g $_{ethanol}$ g $_{dry\ biomass}{}^{-1}$ [48].

The potential ethanol production from *Lemna minuta* biomass is approximately 634.85 L ton^{-1}, which is significantly higher compared to traditional feedstocks used for bioethanol production. For instance, the bioethanol production potential of other feedstocks as sugar cane (70 L ton^{-1}), sugar beet (110 L ton^{-1}), sweet potato (125 L ton^{-1}), potato (110 L ton^{-1}), cassava (180 L ton^{-1}), maize (360 L ton^{-1}), rice (430 L ton^{-1}), barley (250 L ton^{-1}), wheat (340 L ton^{-1}n), sweet sorghum (60 L ton^{-1}), and bagasse and other cellulose biomass (280 L ton^{-1}) [49]. Although other studies have reported higher ethanol yields using different species of aquatic plants (Table 6), it is important to emphasize that one of the main objectives of this work is to utilize biomass harvested from polluted environments. This approach not only provides a sustainable method for disposing of this biomass but also adds value by transforming it into bioethanol. *Lemna minuta*, when harvested from contaminated environments, plays a key role in environmental remediation by removing nutrients and pollutants from water while simultaneously serving as a renewable source for bioethanol production. This dual approach—combining environmental remediation with renewable energy production—highlights the importance of integrating sustainable practices that promote environmental protection and economic value generation from low-cost waste and biomass.

Conclusions

This study identifies *Lemna minuta* biomass, harvested from a polluted pond, as a promising feedstock for bioethanol production, with a carbohydrate content of 36.46%. Acid hydrolysis, particularly using 5% HCl at 150 °C for 60 min, proved to be the most effective method, achieving up to 99.20% efficiency in sugar conversion. Ethanol production peaked at 1.5 g L^{-1} after 48 h of fermentation, demonstrating the feasibility of using *Lemna minuta* for bioethanol production.

The findings of this study pave the way for future research to explore large-scale applications of *Lemna minuta* biomass in bioethanol production, with a focus on enhancing enzymatic hydrolysis efficiency and reducing process costs. Further studies could investigate integrating *Lemna minuta* in circular economy models, utilizing residual biomass for co-products such as animal feed or biofertilizers. From a societal perspective, this study contributes to sustainable energy solutions by demonstrating a renewable feedstock that mitigates dependence on fossil fuels. Additionally, enjoying *Lemna minuta* for environmental remediation, this dual-purpose approach supports water quality improvement and pollutant removal. These benefits collectively promote energy security, environmental sustainability, and a circular bioeconomy, aligning with global efforts to combat climate change and resource depletion.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Data availability statement

The data will be available from the authors upon request.

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