Lactic Acid Production from Green Microalgal Biomass Hydrolysates *via* Dilute Acid Pretreatment and Fermentation

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This study explored the feasibility of using green microalgae (*Chlorella vulgaris*) as a renewable feedstock for bio-based lactic acid production. Microalgal biomass was subjected to dilute sulfuric acid hydrolysis under various conditions to optimize fermentable sugar recovery. The optimal hydrolysis condition consisted of 2% sulfuric acid, heating at 121 °C for 20 min, and a 10% solid-to-liquid ratio. This treatment yielded 12.1 g/L glucose and 2.1 g/L xylose. The hydrolysate was then used as the sole carbon source for fermentation by *Lactobacillus casei*, which completely consumed the sugars and produced 10.7 g/L lactic acid within 24 h. The overall sugar-to-lactic acid conversion efficiency reached 98% without any observable inhibition, and the product consisted exclusively of L-lactic acid with no detectable D-isomer. These findings demonstrate the effectiveness of microalgal hydrolysis and confirm the potential for integrating cultivation, pretreatment, and fermentation into a sustainable, carbon-neutral biorefinery process.

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INTRODUCTION

In recent years, the environmental impacts brought about by global climate change have become increasingly severe. Governments and scientific communities worldwide have prioritized carbon reduction as a critical policy goal. According to reports published by the Intergovernmental Panel on Climate Change, in order to achieve the Paris Agreement goal of limiting global temperature rise to within 1.5 °C above pre-industrial levels, the world must reduce carbon dioxide emissions by several billion tons annually (Bell *et al.* 1954; Matthews and Wynes 2022). Confronted with such immense pressure to reduce carbon emissions, traditional mitigation methods are no longer sufficient, thereby accelerating the development of various negative emission technologies. Among these, bioenergy with carbon capture and storage (BECCS) and its extended concept, Bioenergy with Carbon Capture, Utilization, and Storage (BECCUS), which integrates renewable bioenergy with carbon capture, have emerged as core strategies for achieving carbon neutrality and even net-negative emissions (Stavrakas *et al.* 2018; Ganeshan *et al.* 2023; Yeung *et al.* 2024).

Among the many biomass feedstocks available, microalgae have attracted increasing attention in recent years. Microalgae exhibit rapid growth, high photosynthetic efficiency, and can be cultivated on non-arable land (Chen *et al.* 2024; Tahir *et al.* 2024). They do not compete with food crops for resources and are capable of effectively fixing atmospheric CO_2 (Prasad *et al.* 2021). The green algae genus, particularly *Chlorella vulgaris*, is a representative species in this regard. Its biomass contains over 50% carbohydrates by dry weight (Illman *et al.* 2000; Ho *et al.* 2013). With proper pretreatment and hydrolysis technologies, it can release large amounts of fermentable monosaccharides, especially glucose, which can then be used in microbial fermentation to produce high-value bioproducts. Therefore, microalgae are not only an important source for future green biorefineries but also a key resource within the BECCUS framework. Their potential for carbon absorption and reutilization warrants further exploration.

Lactic acid, a versatile organic acid, has steadily growing market demand across the food, pharmaceutical, agricultural, and green materials sectors (Kim *et al.* 2022; Raman *et al.* 2022). With the rising adoption of bioplastics such as polylactic acid (PLA), lactic acid has become a focal point in the development of global bio-based chemical industries. Currently, commercial lactic acid is predominantly produced by fermenting starch-based crops such as corn and potatoes (Forfora *et al.* 2024). However, these sources face issues including volatile pricing, competition with food supply, and inefficient land use. Consequently, identifying alternative non-food-based biological carbon sources has become a pressing challenge.

In contrast, green algae and other microalgal resources offer high carbon fixation efficiency and can be cultivated under harsh environmental conditions, making them particularly promising for sustainable development and resource cycling. Studies have shown that green algae can absorb over 200 tons of CO_2 per hectare per year (Zhao and Su 2020; Onyeaka *et al.* 2021), far surpassing the carbon fixation capacity of traditional crops such as corn and sugarcane. Within the BECCUS framework, cultivating microalgae for lactic acid production—further processed into biodegradable plastics, medical polymers, and other products—enables both short-term carbon storage and delayed carbon release or even permanent sequestration through extended product lifespans. This model constitutes a sustainable, circular, and carbon-negative green process that demonstrates the strategic value of integrating microalgae into biorefinery systems.

Several technical and procedural challenges must be overcome to successfully convert microalgal biomass into lactic acid. First, the rigid cell walls of green algae, composed of cellulose, hemicellulose, and polysaccharides, require effective disruption and hydrolysis to release fermentable sugars. Common treatment methods include physical disruption (*e.g.*, ultrasonication, high-pressure homogenization), chemical hydrolysis (*e.g.*, dilute acid), and enzymatic hydrolysis. Among these, dilute acid hydrolysis is considered the most economically viable and scalable approach that is capable of converting polysaccharides into monosaccharides effectively. Under controlled conditions (concentration, temperature, duration), it can enhance glucose yield (Hebbale and Ramachandra 2021; Wang *et al.* 2022).

The sugar-rich hydrolysate obtained from green algae, after neutralization and preprocessing, can serve as a fermentation substrate for lactic acid bacteria (LAB). LAB, primarily of the genus *Lactobacillus*, are widely used for lactic acid production. These microbes possess high specificity in converting glucose and can maintain robust growth and acid production in acidic environments (Balasubramanian *et al.* 2024). Literature indicates that under optimal conditions, strains such as *Lactobacillus plantarum* and

Lactobacillus casei can achieve yields of up to 0.9 grams of lactic acid per gram of glucose (Haris *et al.* 2023). Recent studies have also engineered genetically modified strains to enhance tolerance to non-conventional carbon sources, thereby expanding the spectrum of substrates usable for lactic acid production (Tu *et al.* 2019).

Furthermore, the fermentation process itself can be considered a form of carbon transformation and sequestration. By calculating the amount of CO_2 absorbed during microalgal growth and the carbon content within the final lactic acid product, along with its end-use (*e.g.*, in PLA products, long-life construction materials, or energy recovery), a complete carbon footprint analysis can be constructed. If carbon loss during this process is minimized and the products are not rapidly decomposed or combusted, the process can fulfill BECCUS objectives of carbon capture and utilization. Industrial application of this strategy can significantly enhance both social and economic value.

In summary, this study focuses on using green algae as a raw material to investigate the efficiency of hydrolysis and glucose release under various conditions. The resulting sugar hydrolysate is then used as a carbon source for lactic acid fermentation. The lactic acid yields of fermentation strains are evaluated, aiming to establish a feasible production process based on microalgal biomass. By integrating microalgae cultivation, hydrolysis techniques, and biological fermentation, this research aspires to develop a sustainable and carbon-negative technological platform, and to provide empirical support for constructing microalgae-based biorefinery chains within the BECCUS framework.

EXPERIMENTAL

Effect of Sulfuric Acid Concentrations on Microalgal Biomass Hydrolysis Efficiency

To evaluate the effect of dilute sulfuric acid concentration on the hydrolysis efficiency of microalgal biomass, a series of batch experiments were conducted in 250 mL Erlenmeyer flasks, with a total working volume of 50 mL per flask. The microalgal biomass used in this study was powdered *Chlorella* obtained from Guang-Bi Co., Ltd. (Yunlin, Taiwan). The moisture content of the biomass was determined using an infrared moisture analyzer. Six concentrations of sulfuric acid were selected for evaluation: 0, 0.1, 0.5, 1, 2, and 4% (v/v), aiming to investigate the influence of acid strength on cell wall disruption and sugar release efficiency.

In all experimental conditions, the solid-to-liquid ratio was maintained at 10%. Specifically, approximately 5.49 g of microalgal powder with a moisture content of 9% was added to each flask, and deionized water was used to adjust the total volume to 50 mL. Before hydrolysis, the flasks were sealed with a sterilizable plug to prevent contamination by condensate. The hydrolysis reactions were carried out in an autoclave at 121 °C for 120 min to ensure effective acid hydrolysis under high-pressure conditions.

Following hydrolysis, the reaction mixtures were immediately cooled to room temperature. A 2 mL aliquot of the hydrolysate was withdrawn from each sample, centrifuged using a benchtop centrifuge, and filtered through a CHROMAFIL® PVDF 0.45 μm syringe filter. The resulting supernatant was then analyzed for sugar concentration using high-performance liquid chromatography (HPLC).

Effect of Heating Time on Microalgal Biomass Hydrolysis Efficiency

To investigate the influence of heating duration on the efficiency of acid hydrolysis, this study conducted a series of experiments under a fixed dilute sulfuric acid concentration of 2% (v/v), using an autoclave for thermal treatment at elevated pressure. Each hydrolysis reaction was carried out with a total volume of 50 mL, maintaining a solid-to-liquid ratio of 10%.

Three heating durations—20, 60, and 120 min—were evaluated at a constant temperature of 121 °C under autoclave conditions. Upon completion of the hydrolysis reaction, samples were immediately cooled to room temperature. A 2 mL aliquot of the hydrolysate was centrifuged using a benchtop centrifuge, and the supernatant was filtered through a CHROMAFIL® PVDF 0.45 μm membrane filter. The filtrate was analyzed for sugar concentration using high-performance liquid chromatography (HPLC).

Effect of Solid-to-Liquid Ratios on Microalgal Biomass Hydrolysis Efficiency

To examine the effect of varying solid-to-liquid ratios on the efficiency of acid hydrolysis, experiments were conducted under fixed conditions of 2% (v/v) dilute sulfuric acid and a temperature of 121 °C using an autoclave for thermal treatment. Three solid-to-liquid ratios were tested: 5%, 10%, and 20% (w/v), with each reaction carried out in a total volume of 50 mL. Based on the specified ratios, approximately 2.74 g, 5.49 g, and 10.98 g of *Chlorella* powder (with a moisture content of 9%) were added, respectively, and deionized water was added to bring the final volume to 50 mL.

All reaction mixtures were subjected to high-pressure hydrothermal treatment at 121 °C for 20 min to induce acid hydrolysis. Following the reaction, samples were rapidly cooled to room temperature. A 2 mL aliquot of the hydrolysate was withdrawn, centrifuged using a benchtop centrifuge, and the supernatant was filtered through a CHROMAFIL® PVDF 0.45 μm membrane filter. The resulting filtrates were analyzed for sugar concentration using high-performance liquid chromatography (HPLC).

Fermentation Using Algal Hydrolysate

For inoculum preparation and scale-up, *Lactobacillus casei* 7BL (Kuo *et al.* 2015) was retrieved from a -80 °C freezer. A 250 μL aliquot of the frozen stock was inoculated into a 15 mL culture tube containing 2.5 mL of sterile medium composed of 1% yeast extract, 2% peptone, and 2% glucose. The culture was incubated at 37 °C and 150 rpm under non-aerated conditions to activate the strain. The activation status was assessed by measuring the optical density (OD) of the culture using a spectrophotometer.

When the OD reached 1.0 (or continued incubating until it did), a 10% (v/v) inoculum was transferred to a 250 mL Erlenmeyer flask containing 25 mL of the same medium and incubated at 37 °C and 150 rpm under similar non-aerated conditions. Upon reaching an OD of 1.0 in this stage, the culture was further scaled up by inoculating 10% (v/v) into a 1 L Erlenmeyer flask containing 250 mL of medium and incubated under the same conditions until an OD of 1.0 was achieved.

The resulting culture was then used to inoculate a 5 L fermenter, with the inoculum comprising 10% of the total working volume. The fermentation medium was prepared using acid-hydrolyzed *Chlorella* hydrolysate supplemented with 1% yeast extract and 2% peptone as nitrogen sources. The hydrolysate was obtained by treating microalgal biomass with 2% (v/v) sulfuric acid at 121 °C for 15 min at a solid-to-liquid ratio of 10%. Before entering the fermentation process, the hydrolysate was centrifuged at 8000 rpm for 10 min to obtain the supernatant for fermentation.

Fermentation was carried out at 37 °C with an agitation speed of 150 rpm. The pH was maintained at 5.8 using 25% ammonium hydroxide. Samples (2 mL) were collected from the fermenter at 0, 2, 4, 6, 8, 24, and 48 h. Each sample was centrifuged using a benchtop centrifuge, and the supernatant was filtered through a CHROMAFIL® PVDF 0.45 μm membrane filter. The filtrates were subsequently analyzed for sugar concentration and lactic acid optical activity to evaluate carbon source utilization and lactic acid production efficiency during the fermentation process.

Analysis Methods

Sugar concentration analysis in this study was performed using a high-performance liquid chromatography (HPLC) system (Agilent 1200 Series, USA). The chromatographic separation was achieved using a column manufactured by Transgenomic (USA), model 87H3, with a length of 30 cm and an internal diameter of 7.8 mm. The stationary phase consisted of sulfonated polystyrene-divinylbenzene (PS/DVB) resin, 8% cross-linked, with a particle size of 9 μm and an ion exchange capacity of 1.7 to 1.9 meq g^{-1}. This cation exchange column is suitable for the analysis of organic acids and carbohydrates.

All sugar standards used were of analytical grade, with purity exceeding 98%, and were purchased from Merck (Germany). The mobile phase consisted of 8 mM sulfuric acid in deionized water, operated at a constant flow rate of 0.5 mL min^{-1}. The system pressure was maintained between 45 and 65 bar, with a maximum allowable pressure of 92 bar.

Column temperature was maintained at 65 °C using a thermostatted column compartment. Each sample was diluted 50-fold prior to injection, with a total chromatographic run time of 20 min per sample. Detection was carried out using a refractive index detector (RID) operated at a sample compartment temperature of 4 °C. All experimental data represent the average of three independent experiments.

The optical characterization of the lactic acid was analyzed by HPLC on SUPELCO Astec CLC-D column (4.6 mm I.D. × 150 mm L, 5 μm) and a mobile phase consisting of CuSO4 (10 mM) with diode array detector at 254 nm. The calibration curves of L-lactic acid and D-lactic acid were linear with regressions being of 0.999 in the concentration range of 10-1000 mg/L.

RESULTS AND DISCUSSION

Optimizing Microalgal Hydrolysis for Fermentable Sugar Release

This study systematically explored the acid hydrolysis of microalgal biomass by examining the influence of three critical operational parameters: dilute sulfuric acid concentration, heating duration, and solid-to-liquid ratio on the efficiency of glucose release. The primary objective was to establish optimized hydrolysis conditions suitable for pretreatment in lactic acid fermentation. Due to their high carbohydrate and protein content, superior carbon fixation efficiency, and non-competition with food crops, microalgae are considered one of the most promising sustainable biomass resources for future biorefineries. By applying effective hydrolysis techniques to release internal carbon sources, microalgae can serve as a renewable carbohydrate feedstock for fermentation, facilitating the production of high-value products such as organic acids, biofuels, and biodegradable materials.

In evaluating the impact of acid concentration on hydrolysis efficiency, the experimental results showed a clear increase in glucose concentration in the hydrolysate as

the sulfuric acid concentration increased from 0% to 4%, yielding 0.5, 0.5, 0.7, 1.9, 11.3, and 12.1 g/L, respectively. When the acid concentration was below 1%, the algal cell walls were not effectively disrupted, resulting in minimal sugar release. However, a substantial increase in glucose yield was observed at 2%, indicating a critical threshold for efficient cell wall breakdown. Additionally, Palmqvist and Hahn-Hägerdal (2000) noted that excessive acid concentrations might cause sugar degradation and formation of inhibitory byproducts, which could negatively impact subsequent fermentation. Therefore, 2% sulfuric acid was identified as the optimal concentration, balancing efficiency and process safety.

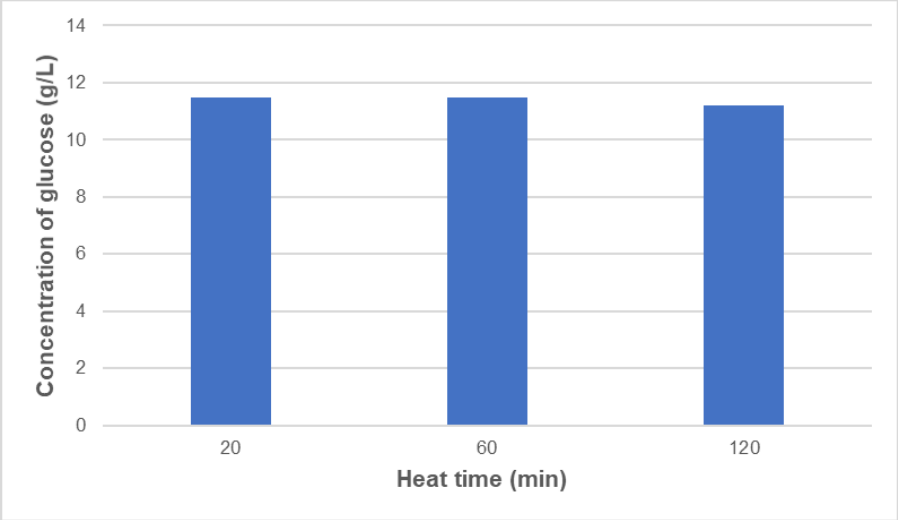

Fig. 1. Hydrolysis profile under different concentrations of dilute sulfuric acid

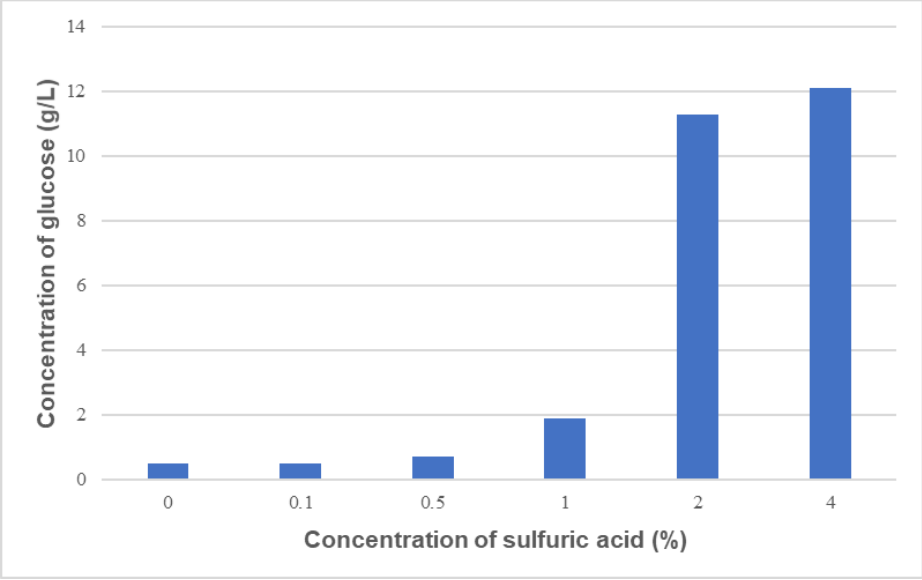

Fig. 2. Hydrolysis profile under different heating times

Regarding the effect of heating time, hydrolysis treatments were performed for 20, 60, and 120 min under 2% sulfuric acid conditions, yielding glucose concentrations of 11.5, 11.5, and 11.2 g/L, respectively. The results indicate that most of the hydrolysis occurred

within the first 20 min, after which the reaction plateaued. This observation aligns with the kinetic analysis by Liu *et al.* (2012), which showed rapid initial hydrolysis followed by a gradual decline in reaction rate as equilibrium was approached. Adopting a short-time, high-efficiency heating strategy not only reduces energy consumption and equipment load but also minimizes thermal degradation and byproduct formation, thereby enhancing process stability and economic viability.

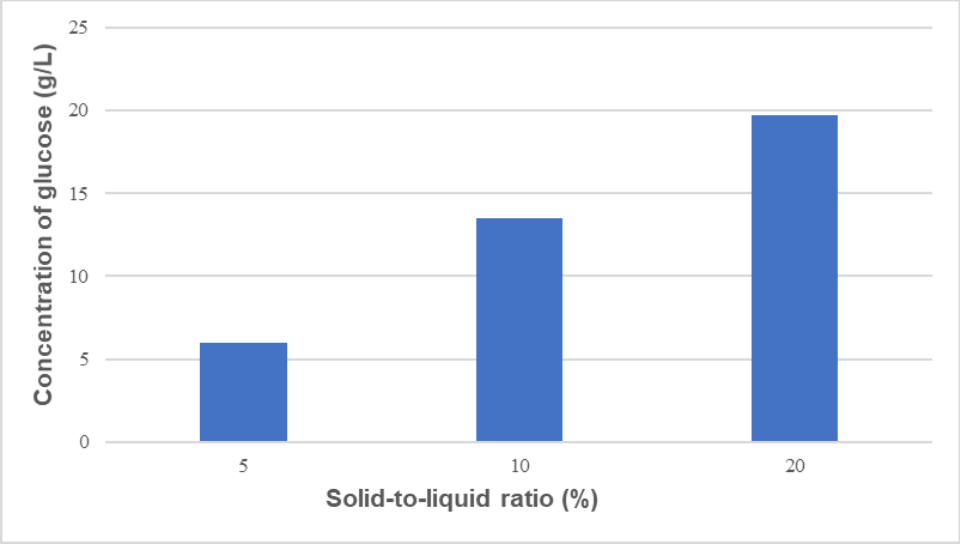

Fig. 3. Hydrolysis profile under different solid-to-liquid ratios

As for the effect of solid-to-liquid ratio, experiments conducted at 5%, 10%, and 20% showed corresponding glucose concentrations of 6, 13.5, and 19.7 g/L. These results demonstrated that increasing the solid content effectively improved substrate concentration and cumulative glucose yield in the hydrolysate. This observation is consistent with the conclusions of Jeya *et al.* (2009), who emphasized the benefits of higher solid-to-liquid ratios in enhancing reaction substrate concentration and conversion efficiency. However, increased solids can also lead to operational challenges, such as reduced mixing uniformity and lower heat transfer efficiency (Modenbach and Nokes 2013; Mishra and Ein-Mozaffari 2019). These issues become more significant in scaled-up systems and must be addressed through optimized reactor design and process control strategies to maintain stable performance and product quality.

Based on the comprehensive evaluation of the three parameters, the optimal hydrolysis condition identified in this study was 2% dilute sulfuric acid, heating at 121 °C for 20 min, and a solid-to-liquid ratio of 10%. Under these conditions, a high concentration of glucose-rich hydrolysate can be obtained, demonstrating excellent potential as a substrate for lactic acid fermentation. Compared to traditional starchy feedstocks, microalgae-derived carbohydrates offer environmental sustainability advantages, avoid food crop competition, and provide a renewable carbon source with carbon fixation benefits. Further research focusing on the impact of hydrolysate composition on microbial growth kinetics and metabolic pathways, along with pilot-scale evaluations, would support the practical and industrial deployment of microalgal biomass in lactic acid and broader bioproduct manufacturing, strengthening their role in green bioprocess development.

Lactic Acid Fermentation from Microalgal Sugars

This study employed hydrolysates derived from green microalgal biomass as the carbon source for lactic acid fermentation. The initial fermentation volume was 2500 mL, which increased to 2700 mL due to microbial metabolism and the addition of pH control agents. Such a volume change is typical of fed-batch or semi-batch processes. As shown in Fig. 4, the concentrations of primary sugars (glucose and xylose) and lactic acid were monitored over time, revealing the metabolic efficiency and sugar utilization capacity of the lactic acid bacteria (LAB) in response to the microalgal hydrolysate.

Regarding glucose, the initial concentration was 8.2 g/L, which decreased to 0 g/L within 24 h, indicating efficient glucose consumption by LAB and a rapid activation of sugar metabolism at the early fermentation stage. Xylose concentration declined more gradually, from 2.1 g/L to 0 g/L over 24 h, which was possibly due to the generally lower capacity of LAB to metabolize pentose sugars. Most wild type *Lactobacillus* strains have limited pentose fermentation capacity unless adapted or genetically modified (Kim *et al.* 2010; Cubas-Cano *et al.* 2019). Nonetheless, the complete xylose depletion observed in this study suggests that the strain used may possess effective xylose assimilation pathways or that certain components within the microalgal hydrolysate enhanced its metabolic activity. This finding warranting further investigation through isolation and genetic analysis.

For lactic acid production, the initial concentration was 1.4 g/L, and it steadily increased within the first 8 h, reaching 10.7 g/L at 24 h. This increase corresponded to the rapid depletion of glucose and xylose, demonstrating strong sugar-to-acid conversion capability and good acid tolerance of the strain. Moreover, the lactic acid yield in this study, calculated from the total initial sugar concentration of approximately 10.3 g/L, approached the theoretical maximum, with a yield of around 1.04 g lactic acid/g sugar. The overall sugar-to-lactic acid conversion efficiency was 98%, and chiral analysis confirmed that the product was exclusively L-lactic acid, with the D-isomer. This result is comparable to those reported by Talukder *et al.* (2012), who achieved a lactic acid yield of 92.8% at sugar concentrations of 3 to 25 g/L using microalgal-derived carbon sources. Notably, no obvious signs of fermentation inhibition were observed in this study, suggesting either a low concentration of inhibitory by-products in the hydrolysate or that the LAB strain exhibits a degree of inhibitor tolerance.

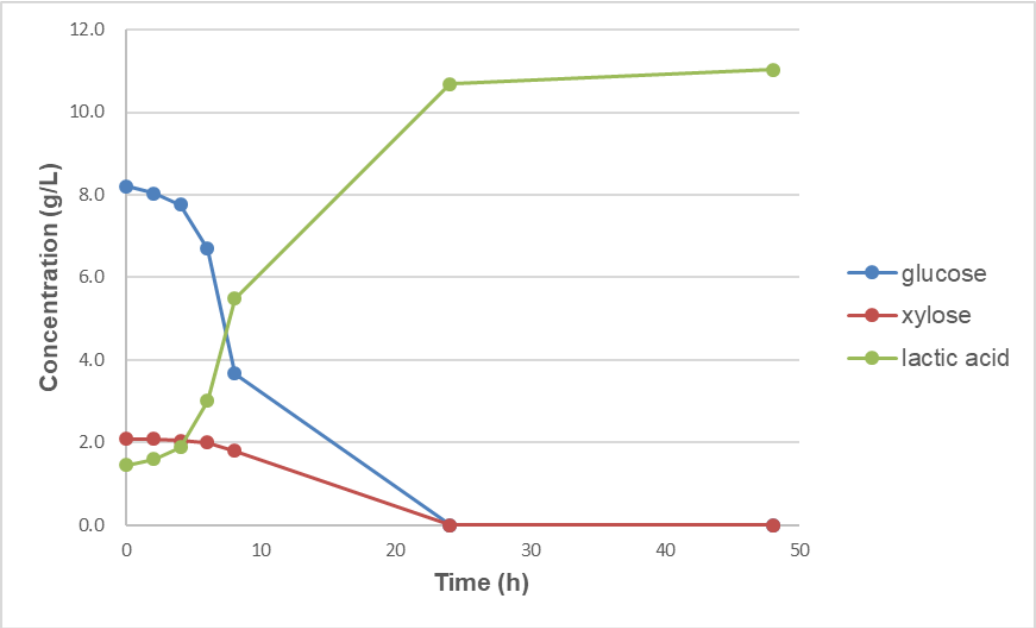

Fig. 4. Concentration profiles of glucose, xylose, and lactic acid during 5L fermentation using green algal hydrolysate

Overall, microalgal hydrolysates were converted into lactic acid, with fermentation performance comparable to or exceeding that of other international studies, especially under complex polysaccharide substrate conditions. These findings not only validate the feasibility of using microalgae as a renewable resource but also provide technical support for the development of non-food-based lactic acid fermentation substrates. Future improvements, such as the use of acid-tolerant or pentose-utilizing engineered strains, pH control systems, optimized feeding strategies, or multi-stage inoculation, could further enhance lactic acid titers and conversion efficiency, broadening the industrial applicability of microalgal biomass in sustainable bioprocesses. From a carbon footprint perspective, integrating microalgae cultivation, hydrolysis, and lactic acid fermentation into a unified platform could potentially achieve carbon-neutral or even carbon-negative biorefinery operations, aligning with global goals for sustainable biotechnology and circular economy development.

CONCLUSIONS

1. Optimized dilute acid hydrolysis of green microalgal biomass (2% H_2SO_4, 121 °C, 20 min, 10% solid-to-liquid ratio) yielded up to 12.1 g/L glucose and 2.1 g/L xylose, confirming the effectiveness of pretreatment in releasing fermentable sugars from microalgal biomass.
2. Subsequent lactic acid fermentation achieved complete sugar depletion within 24 h and a final lactic acid titer of 10.7 g/L, with an overall sugar-to-lactic acid conversion efficiency of approximately 98%, demonstrating strong metabolic performance of the LAB strain.

3. This integrated approach highlights green microalgae as a viable non-food biomass for biorefinery applications, offering both high sugar yields and fermentation efficiency, and supporting its future use in sustainable, carbon-neutral lactic acid production systems.

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