

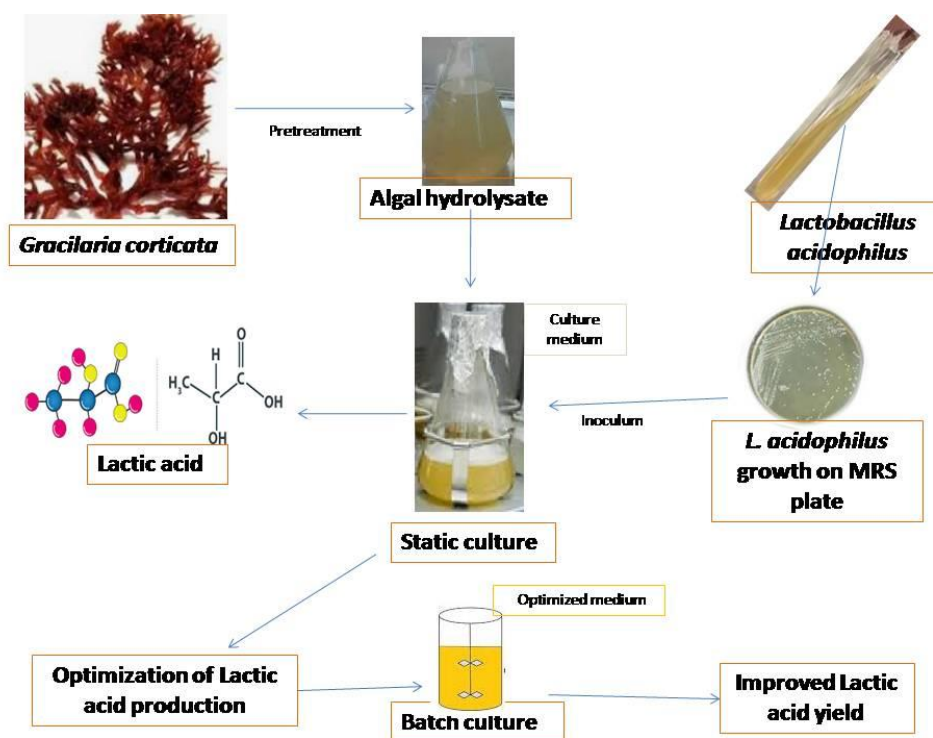
Lactic Acid Production by Fermentation of Hydrolysate of the Macroalga *Gracilaria corticata* by *Lactobacillus acidophilus*

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GRAPHICAL ABSTRACT



Lactic Acid Production by Fermentation of Hydrolysate of the Macroalga *Gracilaria corticata* by *Lactobacillus acidophilus*

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Macroalgae (*Ulva fasciata*, *Gracilaria corticata*, and *Sargassum wightii*) were collected from the marine environment and used as the substrate for lactic acid production. These macroalgae were pretreated with hydrochloric acid (0.2 to 0.4 N) for various times (20 to 60 min). Additionally, the algal hydrolysate was incubated with cellulase for 24 h at 30 ± 1 °C to achieve enzymatic saccharification. Proximate analysis of these macroalgae was performed, and the yield was high in *G. corticata*. The *G. corticata* hydrolysate was composed of $10.01 \pm 0.12\%$ ash content, $1.25 \pm 0.2\%$ total fat, $10.2 \pm 0.1\%$ crude protein, $9.2 \pm 0.2\%$ moisture content, and a higher level of total carbohydrate ($69.33 \pm 1.5\%$) than the other two macroalgae. In *G. corticata*, the enzymatic treatment showed the maximum reducing sugar ($33.5 \pm 2.3\%$) relative to the other macroalgal hydrolysates and was considered for optimization of lactic acid production. *Lactobacillus acidophilus* (MTCC447) utilized pretreated *G. corticata* hydrolysate (enriched with 5% yeast extract), and maximum lactic acid yield was achieved after 72 h, 30 °C incubation temperature, and 6% inoculum (1×10^8 CFU/mL) in static culture condition. Batch fermentation was performed in the 1-L bioreactor at room temperature (30 °C) for 96 h. Lactic acid production was maximum within 72 h and the pH value was depleted. The present finding indicates that *G. corticata* could be used as a substrate for lactic acid production.

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Keywords: Macroalgae; Pretreatment; Algal hydrolysate; Hydrolysable sugar; Lactobacillus; Lactic acid

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INTRODUCTION

In recent years, there has been a growing search for novel biomass sources that could in the future serve as feedstock for an industry and may show an alternative to fossil fuels. Marine macroalgae show high fixation of carbon dioxide emissions, and increased growth rate, and are a promising low-carbon feedstock for various industrial processes. The cultivation of marine macroalgae does not compete with other terrestrial flora and fauna; thus the promoting of marine macroalgae cultivation can improve life of coastal people (Rebours *et al.* 2014). In addition, macroalgae can be cultivated in manmade artificial environments. Macroalgae are widely used in various industrial processes,

including cosmetic, food, plastic, and pharmaceutical industries (Filote *et al.* 2021). According to industrial experts, macroalgae is a major feedstock and highly preferable to other available biomass regarding economic, social, and environmental scenarios. In the EU nations, macroalgal research and development-based renewable energy are considered as an alternative raw material for biorefineries than other alternative biomass from 2010 to 2030 (Fiorese *et al.* 2013).

A number of compounds, including polysaccharides, lectins, pigments, phenolic compounds, and amino acids, have been extracted from seaweed. The main source of agar was derived from the genera *Gelidium*, *Ahnfeltia*, and *Gracilaria*. The amount of agar in macroalgae varies based on the harvesting season, geographical location, and growth conditions (Fleurence 1999). Macroalgae are rich in protein (7 to 31% dry weight), lipids (2 to 13%) and carbohydrates (32 to 60% dry weight) (Michiu *et al.* 2020). Macroalgae are composed of polysaccharides and are used for the development of various industrial products, including butanol and ethanol (Torres *et al.* 2019). Macroalgae are rich in antioxidants, salts, proteins, and minerals that are naturally adsorbed from their growing environment and could be harvested and extracted for industrial processes (Álvarez-Viñas *et al.* 2019; Sadhukhan *et al.* 2019). Macroalgae are rich in lignocellulosic biomass, especially hemicelluloses and cellulose, which break down into xylose and glucose. Macroalgae are considered an alternative to lignocellulosic biomass from the terrestrial environment because of various advantages. Macroalgae has a short generation cycle and can be cultivated in available low-lying areas in the coastal hamlets. It is not considered as a primary crop and does not contain lignin; this makes saccharification easier. The gradual depletion of bioresources from the terrestrial environment has encouraged researchers and industrial entrepreneurs to look closer at macroalgae research. The available macroalgae biomass from the marine environment is higher than lignocellulosic biomass, such as switchgrass and corn stover (Jung *et al.* 2013).

In macroalgae, several polysaccharides, including laminaran and fucoidan (in brown seaweeds), carrageenan and agar (in red seaweeds), glucuronan and ulvan (in green seaweeds), have been commercially exploited for their efficacy for lactic acid production (Sharif Hossain *et al.* 2008). Lactic acid, an odorless and colorless monocarboxylic acid, has received much more attention because of its several applications in the non-food and food industries. There is a growing demand for lactic acid in the production of biodegradable polylactic acid films, which are considered an alternative to petroleum-derived plastics (Mora-Villalobos *et al.* 2020). Lactic acid can be synthesized by either microbial fermentation or the chemical synthesis. Two optical isomers of lactic acid, D(–)-lactic acid, and L(+)-lactic acid are produced during chemical synthesis process. The microbial strains, such as the lactic acid bacteria (Sun *et al.* 2015; Zhang and Vadlani 2015), yeast, including *Pichia stipitis* (Ilmen *et al.* 2007), and fungi, such as *Rhizopus* sp. (Mass *et al.* 2006; Mass *et al.* 2008; Yamane and Tanaka 2013), were utilized for lactic acid production *via* microbial fermentation. The process parameters, including physical factors and medium compositions, were optimized to improve lactic acid production. Optimization of temperature, pH control, and medium supplementation improved lactic acid production (de Lima *et al.* 2010; Abdel-Rahman *et al.* 2013; Nuzzo *et al.* 2019). Macroalgae were used in the production of biofuels and bio-based chemicals (Park *et al.* 2012; Wu *et al.* 2014) and these previous studies revealed the presence of a carbon source in macroalgae. Lactic acid bacteria produce lactic acid *via* fermentation with carbohydrate-rich biomass as a substrate and are considered first-generation feedstocks. In the second generation, lignocellulosic biomass is utilized as feedstock (Han *et al.* 2018). However,

many studies have reported that lactic acid production *via* first- and second-generation feedstocks is associated with both technical and economic issues. First-generation substrates can be used as feed for organisms, whereas second-generation substrates are expensive for product formation (López-Gómez *et al.* 2020). Hence, a sustainable approach is needed to overcome these limitations faced by first- and second-generation feedstocks. The high carbohydrate content and good growth rates of seaweed make it an alternative feedstock for lactic acid production (Chai *et al.* 2021). In this context, macroalgae biomass (*Ulva fasciata*, *Gracilaria corticata*, and *Sargassum wightii*) was utilized as a low-cost culture medium for lactic acid production *via* microbial fermentation.

EXPERIMENTAL

Macroalgae

Macroalgae were collected from the South coast of India (8.088306 N, 77.538452 E) between June 2023 and December 2023. Macroalgae were collected on the rocky shore and transported to the laboratory. The collected macroalgae were identified as *Ulva fasciata*, *Gracilaria corticata*, and *Sargassum wightii*. The characterized macroalgae were dried for two weeks (sun drying) and ground mechanically. The powder was sieved to achieve 1.5 to 2 mm and was stored at -20 °C.

Macroalgae Hydrolysate Preparation

Macroalgae (20 g) was pretreated with hydrochloric acid at three different concentrations (0.2 N, 0.3 N, and 0.4 N) for 20, 40, and 60 min, respectively. The pH of the sample was adjusted to 4.5, and the mixture was treated with commercial cellulase (10 U/mL) (Sigma Aldrich, St. Louis, MO, USA). The algal hydrolysate was incubated for 24 h at 30 ± 1 °C for enzymatic saccharification (Harrigan and McCance 1976).

Proximate Analysis

The crude protein, moisture content, crude fiber, crude lipid, and ash content of macroalgae were determined as described earlier (AOAC 1998).

Reducing Sugar Analysis

The amount of reducing sugar in the macroalgae hydrolysate was determined using the DNS (3,5-Dinitrosalicylic Acid) assay method. Briefly, the hydrolysate (2 mL) was centrifuged at 5000×g for 10 min at room temperature. The supernatant (0.5 mL) was mixed with 0.5 mL DNS reagent and placed in a boiling water bath for 10 min. It was cooled and mixed with 2 mL double distilled water. The absorbance of the mixture was read at 546 nm using a spectrophotometer (ELICO, Bangalore, India). The reducing sugar of macroalgae hydrolysate was calculated (Miller 1959) using the following formula:

$$\text{Reducing sugar yield} = \frac{\text{Reducing sugar content/weight of dried algae powder}}{\text{ratio of carbohydrate}} \times 100\% \quad (1)$$

Microorganisms and Culture Conditions

The Microbial Type Culture Collection *Lactobacillus acidophilus* (MTCC447) was cultured in a Man-Rugosa-Sharpe (MRS) medium containing 2% galactose (Himedia,

Mumbai, India). The selected bacterial strain was cultured for 24 h in MRS broth and was used as an inoculum.

Analysis of Lactic Acid

The amount of lactic acid in the cell-free extract was determined using a high performance liquid chromatography (HPLC) device equipped with a refractive index detector. The samples were filtered using a 0.22- μ m membrane filter and the filtrate was used for analysis. Millipore water was used as the eluent and the flow rate was maintained at 0.5 mL/min (Gardner *et al.* 2001).

Optimization of Lactic Acid Production

To determine the effect of temperature on lactic acid production, the lactic acid bacterial strain was inoculated into macroalgal hydrolysate (*G. corticata*) containing yeast extract (0.5%) and incubated at various temperatures (25 to 40 °C) for 72 h. The cell density of inoculums was 1×10^8 CFU/mL and used for experiments. To optimize the effect of the fermentation period on lactic acid production, the macroalgal hydrolysate medium containing 0.5% yeast extract was incubated for 96 h after inoculation of lactic acid bacteria. To analyze the effect of agitation on lactic acid production, the lactic acid bacteria (2%) was inoculated into macroalgal hydrolysate containing 0.5% yeast extract and incubated at 30 °C for 72 h. To determine shaker speed on lactic acid production, the culture was agitated at various rpm (75 to 150 rpm/min). The static culture was considered as a control. To determine the inoculum concentration on lactic acid production, the inoculum was supplemented at various concentrations (2% to 10%, v/v) and incubated for 72 h. The amount of lactic acid bacteria in the culture medium (CFU/mL), pH of the culture medium, and lactic acid level were assessed.

Production of Lactic acid in Fermentor

Batch fermentation was performed in the 1-L bioreactor at room temperature (30 °C). The culture medium was prepared using macroalgal (*G. corticata*) hydrolysate containing 0.5% yeast extract. The pH of the medium was adjusted to 6.0 using 1 N KOH. The experiment was performed for 72 h and 6% inoculum (LAB) was inoculated initially. After 72 h fermentation, the culture was centrifuged and lactic acid production was analyzed.

Statistical Analysis

Analysis of variance was used to determine statistical significance. The p -value < 0.01 was considered as significant.

RESULTS AND DISCUSSION

Proximate Analysis of Macroalgal Hydrolysate

The proximate compositions of the macroalgae (*U. fasciata*, *G. corticata*, *S. wightii*) are described in Table 1. *Ulva fasciata* was found to be composed of $20.3 \pm 1.1\%$ ash, $1.01 \pm 0.04\%$ fat, $18.1 \pm 0.2\%$ protein, $10.4 \pm 0.1\%$ moisture, and $50.19 \pm 2.2\%$ carbohydrate. *Gracilaria corticata* was composed of $10.01 \pm 0.12\%$ ash content, $1.25 \pm 0.2\%$ total fat, $10.2 \pm 0.1\%$ crude protein, $9.2 \pm 0.2\%$ moisture content, and had a higher level of total carbohydrate ($69.33 \pm 1.5\%$) than the other two macroalgae (Table 1).

Hwang *et al.* (2012) reported ash, crude fat, crude protein, moisture, and carbohydrate content of *Enteromorpha prolifera* to be $13.6 \pm 0.21\%$, $1.2 \pm 0.1\%$, $8.4 \pm 0.2\%$, $12.5 \pm 1.1\%$, and $64.3 \pm 0.2\%$, respectively. In *Enteromorpha prolifera*, the proximate analysis of seaweed hydrolysate was determined. The amount of carbohydrate (51.8%), total protein (30%), ash (17.7%), and fat (0.4%) content was within the range reported previously. The amount of crude protein, carbohydrate, and other nutrient sources varied based on season and time of harvest among *Laminaria hyperborean*, *Laminaria digitata*, and *Ascophyllum nodosum* (Garcia-Vaquero *et al.* 2021). The macroalgae species (*Galaxaura rugosa*, *Sargassum boveanum*, *Caulerpa racemose*, *Caulerpa sertularioides*, and *Bryopsis corticolans*) collected from the Persian Gulf exhibited ranges of biomolecule contents 14.46% to 38.20% crude protein, 1.27% to 9.13% lipid, and 15.50% to 49.14% ash content. These biomasses can be directly used to produce industrial products, including lactic acids (Pirian *et al.* 2020).

Table 1. Proximate Composition of Seaweed Hydrolysates of *Ulva fasciata*, *Gracilaria corticata*, and *Sargassum wightii*

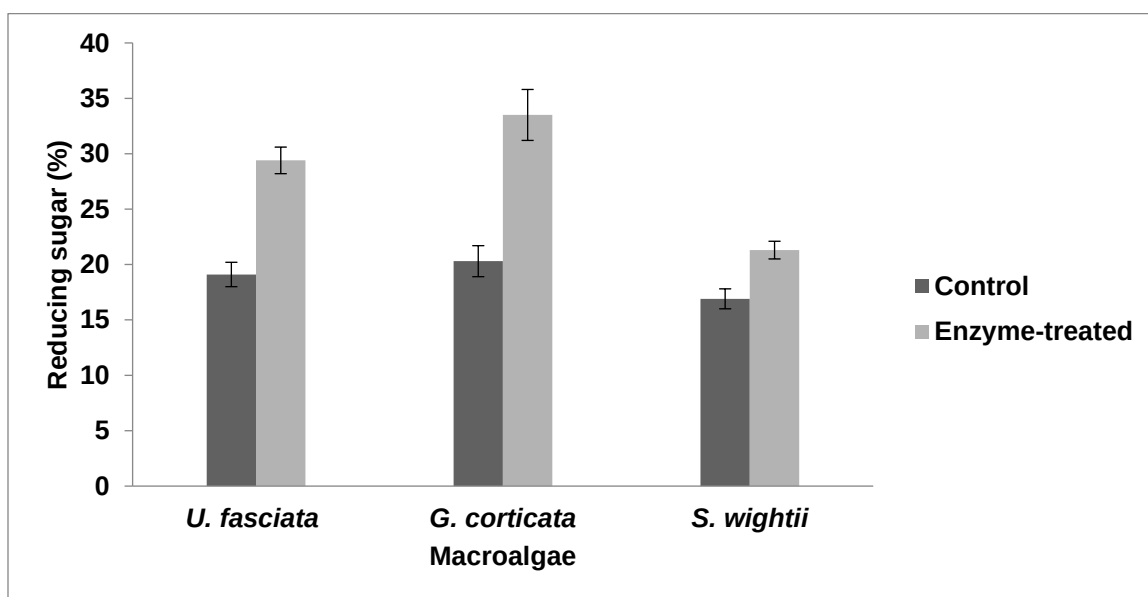
Components	<i>Ulva fasciata</i>	<i>Gracilaria corticata</i>	<i>Sargassum wightii</i>
Ash	20.3 ± 1.1	10.01 ± 0.12	13.6 ± 0.21
Fat	1.01 ± 0.04	1.25 ± 0.2	1.2 ± 0.1
Protein	18.1 ± 0.2	10.2 ± 0.1	8.4 ± 0.2
Moisture	10.4 ± 0.1	9.2 ± 0.2	12.5 ± 1.1
Carbohydrate	50.19 ± 2.2	69.33 ± 1.5	64.3 ± 0.2

Pretreatment of Macroalgae and Determination of Reducing Sugar

The macroalga powder was pretreated with hydrochloric acid at various concentrations, and the resulting % reducing sugar is listed in Table 2. As described in Table 2, increasing concentrations of hydrochloric acid and increased treatment time increased reducing sugar yield ($p < 0.01$). After 40- and 60-min treatment times, reducing sugar yield increased. In *U. fasciata*, the reducing sugar content was $19.2 \pm 0.12\%$ after 60 min incubation with 0.4N hydrochloric acid. In *G. corticata*, the reducing sugar content was $20.1 \pm 0.22\%$ after 60 min incubation in 0.4N hydrochloric acid. In the case of *S. wightii*, the reducing sugar content was $17.5 \pm 0.1\%$ after 60 min treatment. Acid hydrolysis was used for the preparation of reducing sugar recently from *Ulva lactuca* and *Enteromorpha intestinalis*. In *Ulva lactuca*, maximum reducing sugar content was obtained at 0.5 N H_2SO_4 , for 45 min of treatment at 121°C (214.67 mg/g). Moreover, at 0.7 N H_2SO_4 showed less reducing sugar yield (240 mg/g) in *Enteromorpha intestinalis* (Hebbale and Ramachandra 2023) at 120°C after 45 min treatment. Citric acid was used in the pretreatment of macroalgae previously and released significant amount of total reducing sugar from macroalgae, *Gracilaria verrucosa*, and the yield was 50.9% (Kwon *et al.* 2016). In addition to these discussed pretreatment methods, ultrasonication and acid catalyst methods were suggested. These combined methods improved reducing sugar yield and achieved 60.4% reducing sugar at 2% (w/v) treatment with sulphuric acid (100 mM) for 60 min (Park and Jeong 2021).

Table 2. Pretreatment of Macroalgae Powder with Various Concentrations of Hydrochloric Acid at Various Incubation Times

Macroalgae	Treatment Time (min)	HCl (N)	Reducing Sugar (g/L)
<i>Ulva fasciata</i>	20	0.3	5.3 ± 0.12
		0.4	17.1 ± 0.5
	40	0.3	6.01 ± 0.4
		0.4	18.3 ± 0.1
	60	0.3	11.5 ± 0.2
		0.4	19.2 ± 0.12
<i>Gracilaria corticata</i>	20	0.3	5.4 ± 0.42
		0.4	18.5 ± 0.11
	40	0.3	9.5 ± 0.14
		0.4	19.1 ± 0.23
	60	0.3	10.4 ± 0.1
		0.4	20.1 ± 0.22
<i>Sargassum wightii</i>	0	0.3	8.1 ± 0.11
		0.4	14.3 ± 0.09
	40	0.3	8.3 ± 0.2
		0.4	15.4 ± 0.1
	60	0.3	8.6 ± 0.2
		0.4	17.5 ± 0.1

**Fig. 1.** Analysis of reducing sugar level of acid-treated macroalgae hydrolysate with cellulase. The acid-pretreated macroalgae hydrolysate (60 min, 0.4 N HCl) was incubated with cellulases and reducing sugar level was determined. To the control, cellulase was not added.

Effect of Cellulase on Macroalgae Saccharification

The acid pretreated macroalgae hydrolysate from *U. fasciata*, *G. corticata*, and *S. wightii* was treated with cellulase, and the saccharification efficacy was determined. As depicted in Fig. 1, the amount of reducing sugar increased after enzymatic pretreatment, and the reducing sugar level increased. The reduced sugar yield of *U. fasciata* hydrolysate was $29.4 \pm 1.2\%$ after cellulase treatment. The amount of reducing sugar in *S. wightii* hydrolysate was less than other algal sources. In *G. corticata*, the enzymatic treatment showed maximum yield ($33.5 \pm 2.3\%$) than the other macroalgal hydrolysates after cellulose treatment (Fig. 1) ($p < 0.01$). However, the amount of reducing sugar obtained in the current study was lower than citric acid-catalyzed pretreatment, followed by enzymatic hydrolysis (57.8%) (Kwon *et al.* 2016). Park and Jeong (2021) used *G. verrucosa* biomass and pretreated with sulphuric acid, and enzymatic hydrolysis and achieved 76.3% of reducing sugar.

Production of Lactic Acid in Submerged Fermentation

Effect of fermentation period on lactic acid bacterial growth and lactic acid production

The effects of the fermentation period on *L. acidophilus* cell growth and lactic acid production were studied. The growth of bacteria was maximum after 72 h (6.6 ± 0.19 Log CFU/mL), and the corresponding pH of the medium was 4.39 ± 0.13 . The pH of the medium was significantly less after 72 h than 24 h culture, and lactic acid production was high after 72 h (8.01 ± 0.15 g/L) culture (Table 3) ($p < 0.01$). Macroalgal hydrolysate was considered as the third-generation feedstock for lactic acid production. The yield of lactic acid was based on the concentration of fermentable sugar, and types of sugar in the medium (Tong *et al.* 2024). *Lactobacillus plantarum* (MTCC 1407) utilized *U. fasciata* substrate to produce lactic acid and produced maximum amount of lactic acid (0.40 ± 0.07 g, w/w) after 4 days of incubation (Sudhakar and Dharani 2022).

Table 3. Effect of Fermentation Period on Lactic Acid Production Using *Gracilaria corticata* Hydrolysate

Fermentation Period (h)	Bacteria (Log CFU/mL)	pH Value	Lactic Acid (g/L)
24	3.9 ± 0.15	4.86 ± 1.7	7.28 ± 0.05
48	5.2 ± 0.28	4.71 ± 0.04	7.74 ± 0.12
72	6.6 ± 0.19	4.39 ± 0.13	8.01 ± 0.15
96	6.2 ± 0.27	4.61 ± 0.4	7.83 ± 0.09

Effect of incubation temperature on lactic acid bacterial growth and lactic acid production

The optimum temperature for LAB growth and production of lactic acid is known to be species-specific. The effect of optimum temperature for bacterial growth and lactic acid production was performed. The bacteria growth was maximum after 40 °C (6.91 ± 0.41 Log CFU/mL); however, lactic acid production was maximum at 30 °C (10.36 ± 0.38 g/L). The present results revealed that 30 °C was optimum for lactic acid production ($p < 0.01$) (Table 4). The optimum growth of the tested bacterial strain was similar to previous reports with *Lactobacillus plantarum* (Popova-Krumova *et al.* 2024). The lactic acid production was improved from 23 to 42 °C in *Lactobacillus helveticus*, and this strain utilized lactose and the pH depletion in the culture medium indicated lactic acid production

(Tango and Ghaly 1999). The optimum temperature was found to be 37°C for *Lactobacillus* for lactic acid production (Sarkar and Paul 2019).

Table 4. Effect of Temperature on Lactic Acid Production Using *Gracilaria corticata* Hydrolysate

Temperature (C)	Bacteria (Log CFU/mL)	pH Value	Lactic Acid (g/L)
25	6.61 ± 0.12	4.14 ± 0.17	9.41 ± 0.26
30	6.74 ± 0.39	4.06 ± 0.11	10.36 ± 0.38
35	6.84 ± 0.19	4.19 ± 0.29	9.16 ± 0.59
40	6.91 ± 0.41	4.37 ± 0.43	8.19 ± 0.91

Effect of agitation speed on lactic acid bacterial growth and lactic acid production

The effect of agitation speed on lactic acid fermentation was tested at various agitation rates (75 to 150 rpm). The number of bacteria was 6.79 ± 0.47 Log CFU/mL at 75 rpm/min, and the yield was 9.75 ± 0.31 g/L. At higher agitation rates (>100 rpm/min), lactic acid bacteria growth was affected, and the pH value was higher. The increased pH value indicated a decreased level of lactic acid in the medium ($p < 0.01$). In the static conditions, growth was higher and lactic acid production was maximum (10.51 ± 0.04 g/L), which revealed that the anaerobic environmental condition was optimum for lactic acid production (Table 5). The present finding reveals that the tested bacteria utilized an anaerobic environment for maximum lactic acid production because generally, lactobacilli are anaerobic organisms (Salminen 2012). Anaerobic fermentation improved lactic acid yield in *Lactobacillus plantarum*, and the yield was 2.3 times higher than aerobic fermentation (Fu and Mathews 1999). Anaerobic fermentation of apple waste, potato waste, and swine manure was performed for lactic acid production by *Lactobacillus* and *Clostridium*. The supplemented swine manure and apple waste improved lactic acid production than mono digestion (Lian *et al.* 2020).

Table 5. Effect of Agitation on Lactic Acid Production Using *Gracilaria corticata* Hydrolysate

Agitation (rpm/min)	Bacteria (Log CFU/mL)	pH Value	Lactic Acid (g/L)
75	6.79 ± 0.47	4.18 ± 0.07	9.75 ± 0.31
100	6.54 ± 0.21	4.27 ± 0.03	9.05 ± 0.14
125	6.47 ± 0.19	4.31 ± 0.12	8.85 ± 0.11
150	6.01 ± 0.15	4.61 ± 0.29	8.71 ± 0.29
Static culture	7.03 ± 0.17	4.01 ± 0.04	10.51 ± 0.04

Effect of inoculum on lactic acid bacterial growth and lactic acid production

The effect of inoculum (%) on lactic acid production was observed in static culture conditions. Lactic acid production was maximum at 6% inoculum concentration (11.46 ± 0.08 g/L), and the pH of the medium was 4.58 ± 0.04 . At lower and higher concentrations of inoculum, lactic acid production was significantly affected ($p < 0.01$) (Table 6). Djukic-Vukovic *et al.* (2012) analyzed the optimum inoculum on biomass production and achieved maximum inoculums at 5% inoculum concentration. Soybean straw hydrolysate was used

as a low-cost substrate to produce lactic acid and maximum lactic acid production was achieved at 10% inoculum concentration in *Lactobacillus casei* (30 °C and 42 h) (Wang *et al.* 2015). Whey filtrate supplemented with 1% (w/v) beet molasses was used as the culture medium for lactic acid production, and maximum production was achieved at 10% (w/v) inoculum concentration (Chiarini *et al.* 1992).

Table 6. Effect of Inoculum on Lactic Acid Production Using *Gracilaria corticata* Hydrolysate

Inoculum (%)	Bacteria (Log CFU/mL)	pH Value	Lactic Acid (g/L)
2	6.13 ± 0.02	4.86 ± 0.06	10.59 ± 0.05
4	6.28 ± 0.05	4.81 ± 0.05	11.04 ± 0.04
6	6.73 ± 0.08	4.58 ± 0.04	11.46 ± 0.08
8	6.82 ± 0.07	4.84 ± 0.07	11.02 ± 0.02
10	6.94 ± 0.04	4.95 ± 0.05	10.25 ± 0.06

Production of Lactic acid in fermentor in optimized culture medium

The LAB strain hydrolyzed macroalgal hydrolysate was supplemented with 5% yeast extract. At optimized culture conditions, lactic acid production was maximum after 72 h incubation in static culture. The fermentation bioprocess was monitored for 96 h. Figure 2 illustrates that lactic acid production was maximum within 72 h and pH value was depleted.

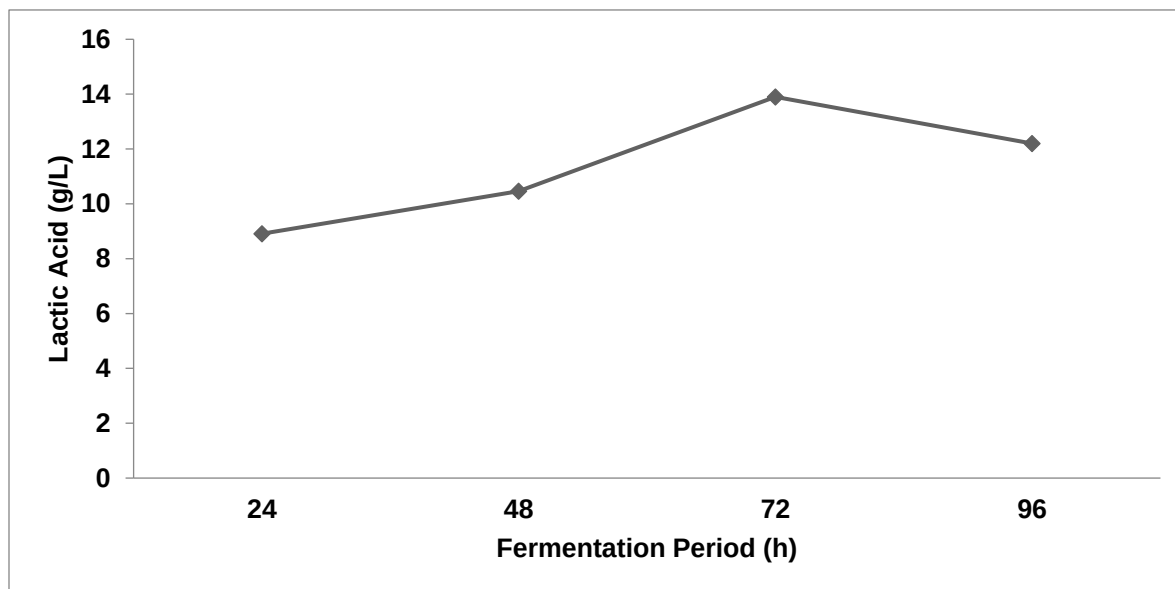


Fig. 2. Lactic acid production in fermentor; The optimized culture medium was subjected to lactic acid production and incubated for 96 h

At 96 h the pH value was increased; then after 72 h of fermentation, the lactic acid production was diminished. The inoculated bacterial strain consumed >91% of reducing sugar and achieved the maximum amount of lactic acid in the medium (13.9 ± 0.4 g/L medium) ($p < 0.01$). This result shows that when the carbon source is depleted in the culture medium, the bacteria begin to consume the lactic acid (de Oliveira *et al.* 2021). During the

fermentation process, the amount of reducing sugar in the fermentor decreases, and the LAB is utilized for lactic acid production. The non-fermentable sugar derived from *Gracilaria corticata* improved the growth of lactic acid bacteria and non-fermentable sugar was not utilized for lactic acid production. Lactic acid was produced in a batch bioreactor by cellobiose fermentation using lactic acid bacteria, and 80 g/L D-lactate was obtained (Abdel-Rahman *et al.* 2011). In batch fermentation, lactic acid production, cell mass, and productivity were high in *Enterococcus mundtii* QU 25 culture (Abdel-Rahman *et al.* 2011). Lactic acid bacteria convert starch to lactic acid directly and this process is preferable for industrial processing.

CONCLUSIONS

1. The macroalga *Gracilaria corticata* hydrolysate exhibited increased amount of fermentable sugar compared to other algae. The algal hydrolysate increased the productivity of lactic acid, and for this reason algal biomass can be considered as a third-generation substrate.
2. Acid treatment and cellulose treatment of algal biomass increased the availability of fermentable sugars. The screened *Lactobacillus acidophilus* utilized sugars for its growth and production of lactic acid.
3. The optimized culture medium improved lactic acid production. The maximum lactic acid yield was achieved after 72 h fermentation, 30°C incubation temperature, and 6% inoculum (1×10^8 CFU/mL) in static culture.

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