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Comparing the Efficiency of Various Ethanol Fermentation Methods from *Chaetomorpha* sp. Residue

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ABSTRACT

The *Chaetomorpha* sp. seaweed residue, which is the carbohydrate-rich remainder after protein extraction, has great potential as a raw material for ethanol production. The residue, after protein extraction, was pretreated with H_2SO_4 acid at high temperatures, then converted into ethanol using several methods: separate hydrolysis and fermentation (SHF), simultaneous saccharification and fermentation (SSF), and a combined SHF-SSF method. All fermentation methods used a cellulase enzyme system (including a mixture of cellulase Cellic Ctec2 and β-glucosidase Novozyme 188) and the heat-resistant yeast ThermoSacc® (*Saccharomyces cerevisiae*). The SHF method was performed by converting the raw material into a sugar-containing solution and then fermenting it immediately; the SSF method was carried out by using enzymes for saccharification and yeast for fermentation at the same time; the combined SHF-SSF method was conducted by saccharifying the raw material (using the SHF method), then adding a portion of the seaweed residue and continuing the fermentation process according to the principles of the SSF method. Upon completion, the results show that the fermentation efficiency of the combined SHF-SSF method reached 89.6%, while the SSF and SHF methods achieved efficiencies of 70% and 54%, respectively. The results indicate that the improved method has superior efficiency compared to the two common methods.

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1. Introduction

Ethanol is a fermentation product that has been present since early human history. Currently, ethanol is a raw material widely used across various fields in significant quantities. It plays a crucial role in food technology, chemical technology, biology, medicine, and biofuels, among others. The ethanol production processes that yield high economic efficiency today primarily utilize grains or starchy tubers as raw materials. However, producing ethanol from grains can significantly impact food supplies. This issue becomes even more critical in the context of global climate change, which is causing sea levels to rise and leading to the loss of many agricultural areas. To meet the demand for both ethanol and food, scientists worldwide are actively seeking new raw materials for ethanol production [1]-[3].

The algae *Chaetomorpha* sp. moves with the sea water intrusion and adapts to the brackish water of the Mekong Delta. They are present in large numbers in the brackish water extensive aquaculture ponds in the Southwestern provinces of Vietnam. After the aquaculture harvest, this algae is often wasted and causes environmental pollution. With the advantage of rapid biomass growth and the ability to utilize water surface area for combined cultivation with shrimp, brackish water algae is considered a promising option for future ethanol production [4]. Algae contain many polysaccharides similar to those in plants (e.g., cellulose) but have little to no lignin, and the crystalline structure of the material is not overly stable. Therefore, the ethanol production process from seaweed is simpler and more convenient [5].

The process of producing ethanol from cellulose-containing materials usually goes through steps such as pretreatment to break down the crystalline structure of the material, enzymatic hydrolysis to

convert the material into fermentable sugars, and fermentation of sugars to obtain ethanol. There are two common methods: SHF (Separate Hydrolysis and Fermentation) and SSF (Simultaneous Saccharification and Fermentation). However, due to technical limitations and material characteristics, these methods often do not meet the fermentation efficiency requirements. Therefore, many studies have proposed various solutions to increase ethanol production efficiency [6].

In this study, we conducted and compared two ethanol production methods, SHF and SSF, using *Chaetomorpha* sp. algae residue that had been pretreated to remove protein and ash. In addition, we also experimented and compared with an improved method that combines both SHF and SSF methods.

2. Materials and Methods

2.1. Materials

Chaetomorpha sp. algae: *Chaetomorpha* sp. was collected from shrimp farming ponds in several locations in Bac Lieu province (Vietnam). The algae were washed to remove impurities, dried to a moisture content of 5-10%, and then ground through a 40-mesh sieve. The dried algae were pretreated by soaking in a 1% NaOH solution to separate protein and ash components. Most of the protein and ash in the raw material diffused into the 1% NaOH solution. The entire mixture was filtered, and the residue (containing polysaccharides) was collected and rinsed with clean water to remove excess NaOH. The residue was then dried to a moisture content of 10-14% and stored at room temperature [7].

Enzymes: The cellulase product Cellic Ctech2 (Novozymes, Denmark), with an optimal temperature of 50°C and pH of 5 respectively, had an activity of 150 FPU/ml. The β-glucosidase product Novozyme 188 (Sigma Aldrich, USA), with an optimal temperature of 50°C and pH of 5 respectively, had a total activity of 300 CBU/ml. These enzymes were used to convert the polysaccharides in the algae residue into fermentable sugars.

Yeast: The study used the commercial yeast strain ThermoSac® Dry from LBDS (Lallemand Biofuels & Distilled Spirits, USA). This strain of *Saccharomyces cerevisiae* has the ability to produce ethanol at higher temperatures (37-40 °C) compared to other strains of the same species. The yeast was activated in YPD (Yeast extract Pepton Dextrose) medium with 2% glucose, and after 24 hours, it reached an Abs_{610} density of approximately 5-5.5. The yeast was then used for various fermentation processes (SHF, SSF, and improved methods)

2.2. Pretreatment of Raw Material

The raw material was pretreated using the method described by Nguyen et al. (2013). During the pretreatment process, part of the crystalline structure of the algae residue was disrupted, creating favorable conditions for subsequent enzymatic hydrolysis [8].

2.3. Separate Hydrolysis and Fermentation (SHF) Method

After pretreatment, the algae residue was converted into sugars using two enzymes: Cellic Ctec2 and Novozyme 188. The pretreated algae residue (4 grams) was neutralized with $Ca(OH)_2$ and diluted with distilled water to achieve a solid content of 10% (w/v) and a pH of 5.0. The enzymes were added at a concentration of 30 FPU/g of pretreated material for Cellic Ctec2 and 10 CBU/g of pretreated material for Novozyme 188. The mixture was placed in Schott Duran® 100 mL bottles and subjected to hydrolysis in an Innova 4230 shaking incubator (New Brunswick Scientific, USA) at 50°C for 48 hours. After saccharification, the entire mixture was inoculated with ThermoSac® Dry yeast biomass to achieve an initial yeast density of 7 log CFU/mL. The fermentation process was carried out in the same shaking incubator at 35°C for 48 hours. Environmental parameters were monitored during the fermentation process.

2.4. Simultaneous Saccharification and Fermentation (SSF) Method

Four grams of pretreated algae residue were placed in Schott Duran® bottles and neutralized with $Ca(OH)_2$. Distilled water was added to achieve a pH of 5.0 and a solid content of 9% (w/v). Enzymes (25 FPU/g of pretreated material for Cellic Ctec2 and 4 CBU/g of pretreated material for Novozyme 188) were added to the pretreated material slurry. ThermoSac® Dry yeast biomass was also added to

reach an initial yeast density of 7 log CFU/mL. The SSF process was conducted in an Innova 4230 shaking incubator at 38°C for 60 hours. After fermentation, environmental changes were analyzed.

2.5. Combined SHF-SSF Method

First, the pretreated algae residue was hydrolyzed according to the technical parameters of the SHF method. After completing the hydrolysis step, a portion of the pretreated material (equivalent to 37.5% of the initial material) was added to the hydrolysates. In addition, to enhance the fermentation efficiency, additional enzymes (20 FPU/g of added material for Cellic Ctec2 and 5 CBU/g of added material for Novozyme 188) were included. The subsequent ethanol fermentation process was carried out according to the technical parameters of the SSF method (38 ºC, 48h) with ThermoSac® Dry yeast biomass to achieve a density of 2.5 x 7 log CFU/mL in the fermentation broth. After fermentation, environmental changes were assessed.

2.6. Comparison of Fermentation Efficiency

To compare the ethanol fermentation efficiency of the different methods, we evaluated the following results: remaining solid content (polysaccharides), total reducing sugars (including oligosaccharides and monosaccharides), HMF (hydroxymethyl furfural, an inhibitor of yeast growth), and ethanol production.

2.7. Analytical Methods

Total reducing sugars were determined using the DNS method with glucose as the standard [9]. Glucose content in the fermentation broth was measured using the GOD-PAP colorimetric assay [10]. HMF levels were assessed using UV spectroscopy [11]. Ethanol production was quantified using HPLC [6]. The experimental samples after the end of the process were centrifuged to collect solid residue; then the residue was washed and centrifuged several times; Finally, the residue was dried to a constant weight and weighed to determine the residual polysaccharide content.

The hydrolysis efficiency (Y_H) is calculated by the ratio of the amount of reducing sugars produced during the hydrolysis process (m_S) to the total amount of sugars theoretically generated from the polysaccharides present in the raw material (m_T).

$$Y_H = \frac{m_S}{m_T} \times 100\% \quad (1)$$

where m_T is calculated using formula 2

$$m_T = m_{Pol} \times 1{,}1 \quad (2)$$

Ethanol Fermentation Efficiency (Y_F) is calculated based on the ratio of the ethanol content obtained after the fermentation process (m_E) compared to the theoretical amount of ethanol produced when completely converting the glucose content in the raw material (mg) into ethanol according to formula 3.

$$Y_F = \frac{m_E}{m_g \times 0{,}51} \times 100\% \quad (3)$$

With a conversion factor of 0.51 representing the complete conversion of glucose to ethanol in the theoretical ethanol fermentation from glucose, the estimated amount (m_g) is based on the glucose content present in the total monosaccharides of the raw material (according to preliminary analysis by the research group).

$$m_g = m_{Pol} \times 1.1 \times 0.76 \quad (4)$$

With mpol representing the mass of polysaccharide in the experimental sample, 1.1 as the theoretical conversion factor when completely converting polysaccharide to monosaccharides, and 0.76 as the proportion of glucose within the total monomer content of the polysaccharide present in the raw material.

All experiments were repeated at least three times. The experimental results are presented as the mean value ± *standard deviation* (SD). The Statgraphic Centurion XV software and Multiple Range Test were used to assess statistically significant differences ($p<0.05$) among the experimental results.

3. Results and Discussion

According to preliminary analysis, *Chaetomorpha* sp. algae, after protein separation, primarily consists of polysaccharides (70% dry weight), with the remaining portion being protein (2.6% dry weight) and ash (8.9% dry weight). The monomer composition within the polysaccharide mainly includes glucose (76%), galactose (6.2%), mannose (4.8%), and some other sugars such as rhamnose, xylose, and arabinose. These results indicate that glucose is the predominant monomer, and it is also the primary sugar converted to ethanol by yeast during the fermentation process. Therefore, the analysis in this study focuses on glucose content. These findings align with a similar study by Hoang et al. (2014) that analyzed the polysaccharide composition of *Chaetomorpha* sp. algae [4].

3.1. Raw material pretreatment

The raw material after being pretreated with 1.75% (w/v) H_2SO_4 underwent significant chemical composition changes. A part of the insoluble polysaccharide was decomposed and transformed into insoluble fragments. This is illustrated by the appearance of many reducing sugar radicals in the suspension after preprocessing. Additionally, the ratio of insoluble polysaccharide decreased from 125 g/L to 81 g/L (Table 1). Acid is a strong corrosive agent, so it can hydrolyze the glycosidic bonds in polysaccharides, thereby creating many smaller fragments (reduced sugar residue) and even soluble monosaccharides (glucose) in the solution [6]. However, due to the low acid content, the hydrolysis process was limited, so there was still a large amount of insoluble solids in the mixture of raw materials that have been preprocessed [12]. However, this process partially degraded some of the stable crystal structures of the material and created conditions for subsequent hydrolysis [13], [14].

HMF is an inhibitory compound for microorganisms and can slow down subsequent fermentation processes. It can be observed that the HMF concentration increased to 109 mg/L after the pretreatment process. However, according to published reports, the HMF concentration that can significantly affect microorganisms (yeast) is 250 mg/L. Therefore, the HMF generated during the pretreatment process will not impact the subsequent fermentation process [15].

Table 1. *Pretreatment results of Chaetomorpha sp. with H_2SO_4*

Materials	Insolube polysaccharide (g/L)	Reduced sugar residue (g/L)	Glucose (g/L)	HMF (mg/L)
Before pretreatment	125	0.5 ± 0.02	0.0	0.0
After pretreatment	81 ± 6.3	20.19 ± 0.769	2.2 ± 0.05	109 ± 3.0

3.2. SHF method

SHF is a popular method of fermenting polysaccharide-containing materials. The raw material is hydrolyzed (saccharified) to obtain a fermentable sugar solution; then yeast is used to ferment the sugar solution into ethanol.

3.2.1. Saccharification process

The results of the saccharification process of *Chaetomorpha* sp. residue are presented in Figure 1. The results in the figure show that the hydrolysis process took place quickly in the first 24 hours: the amount of reducing sugar increased 3.0 times (from 19.3 to 57.2 g/L), the amount of glucose increased 11.6 times (from 2.0 to 23 g/L) and the hydrolysis efficiency reached 73.1% at the 24th hour. Polysaccharide hydrolysis begins to occur in the amorphous region of the substrate [16]-[19]. This result shows that the breakdown of the durable structure of the raw material during pretreatment has created favorable conditions for the hydrolysis process.

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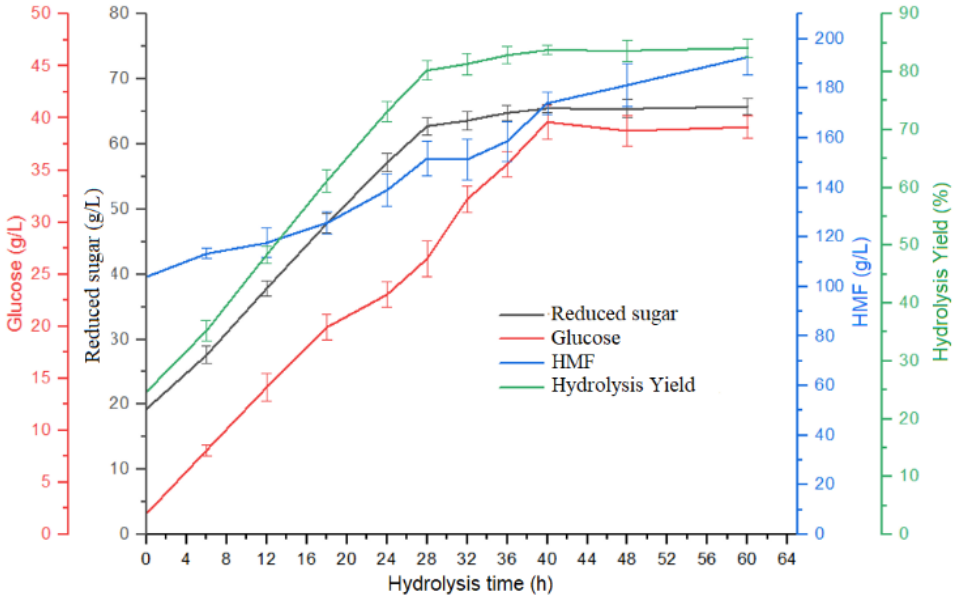

Figure 1. The results of the *Chaetomorpha* sp. residue hydrolysis process in SHF method

During the period from 24th to 36th hour, the total reducing sugar and glucose increased by 13.6% and 72%, respectively, while the saccharification efficiency only rose by an additional 13.4%. From 36th to 60th hour, these values remained relatively stable. We assume that most of the amorphous regions of the material have undergone hydrolysis, leaving a mixture of reactants with crystalline structures. The slow formation of enzyme-substrate complexes is likely to contribute to the gradual pace of the saccharification process. Additionally, the release of a large amount of glucose may lead to reverse inhibition of β-glucosidase enzymes (Feedback Inhibition). Reduced β-glucosidase activity can subsequently diminish the catalytic ability of cellulase enzymes. Similar phenomena was observed during the hydrolysis of green seaweed *Ulva fasciata* Dehli (Trivedi et al., 2013) and *Ulva lactuca* (Poespowati et al., 2018), where the peak reducing sugar content typically occurred between 36th and 40th hour [20], [21].

3.2.2. Fermentation process

After the saccharification process, the entire medium was used to supplement ThermoSac® Dry yeast and carry out the fermentation process. The fermentation results are presented in Figure 2

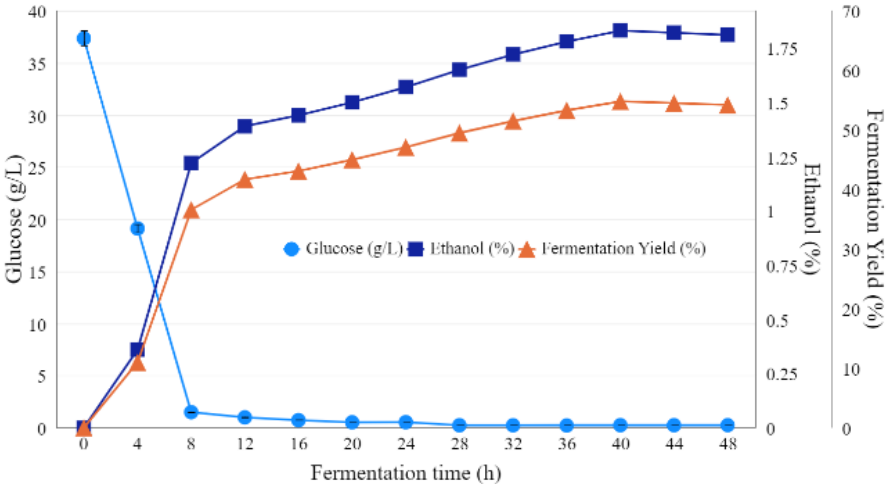

Figure 2. The results of the *Chaetomorpha* sp. residue fermentation in SHF method

The initial 12 hours of fermentation resulted in a decrease in glucose concentration to 0.99 g/L, an increase in ethanol content to 1.39% v/v, and a fermentation efficiency of 41.7%. This indicates that most of the initial sugar in the medium was converted by the yeast. However, the ethanol concentration

continued to rise from 12th to 40th hour, reaching a peak of 1.83%, corresponding to a fermentation efficiency of 54.8%. This phenomenon can be explained by the fact that the cellulase enzyme preparation in the fermentation broth remains active, and its catalytic activity continues during the fermentation process. Once the hydrolysis process is completed, the accumulated amount of glucose becomes quite high, causing inhibition of the enzyme's activity in hydrolyzing cellulose into sugars [22]. Our experimental results show that approximately 60% of the reducing sugars in the saccharification broth were glucose, while the remaining portion consisted of other reducing sugars and oligosaccharides. According to Trivedi *et al*. (2015), as fermentation proceeds, glucose is converted into ethanol by the yeast, leading to a gradual reduction in glucose levels. Consequently, the inhibitory effect of β-glucosidase due to end-product inhibition diminishes. β-glucosidase resumes its activity, hydrolyzing cellooligosaccharide segments into glucose, which the yeast then continues to ferment into ethanol [23].

3.3. SSF method

The SSF method is a fermentation technique that combines both saccharification and fermentation processes simultaneously. The raw material is hydrolyzed by enzymes to produce fermentable sugars, which are immediately converted into ethanol by yeast [6]. Therefore, it can be seen that this method has simplified the process of fermenting ethanol from *Chaetomorpha* sp. residue. The results of the SSF fermentation method are presented in figure 3.

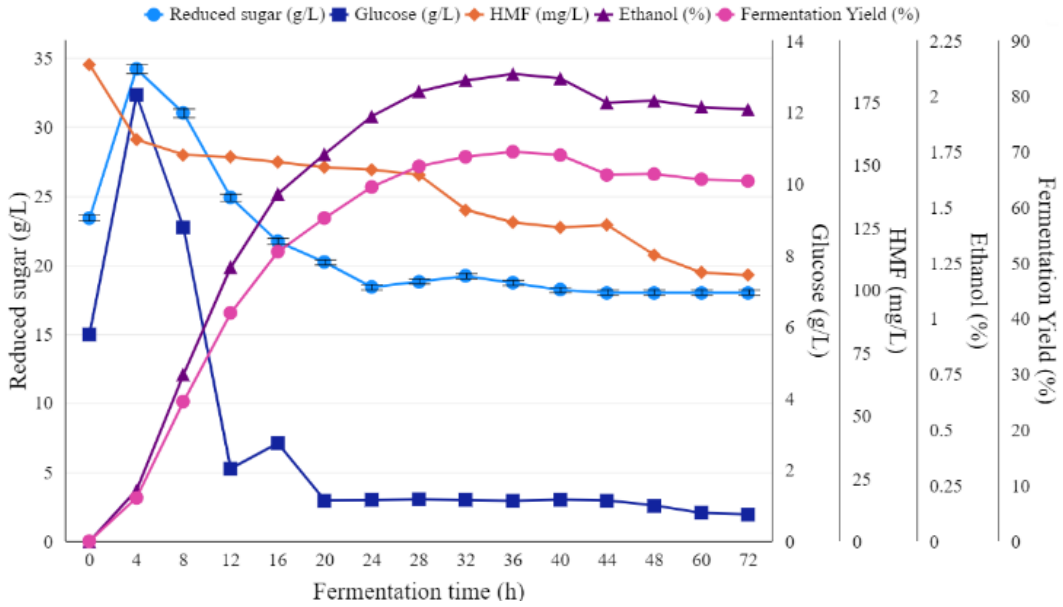

Figure 3. Results of the *Chaetomorpha* sp. residue fermentation by SSF method

During the initial phase from 0 to 4 hours, the ethanol concentration produced was approximately 0.23% v/v (equivalent to 1.84 g/L), while the concentrations of glucose and reducing sugars in the broth increased by 2 times and 1.5 times respectively (from 5.8 to 12.5 g/L and from 23.4 to 34.2 g/L). Thus, in this phase, the hydrolysis process occurred vigorously in the amorphous regions of the polysaccharide, the glucose content in the broth gradually increased to a maximum value, and the conversion of sugar to ethanol was slow. Additionally, the HMF content decreased by 16% in the first 4 hours, indicating that the yeast began to metabolize HMF. Previously, Jung et al. (2015) studied the conversion of rice straw to ethanol and observed a reduction in HMF content from 20 to 65% within the first 5 hours of fermentation [24].

From 4th to 12th hour, the concentrations of glucose and reducing sugars decreased by 6.0 times and 1.4 times respectively, while the ethanol content increased by 5.3 times. At the 12th hour, the concentrations of glucose and ethanol in the broth were 2.0 g/L and 1.23% v/v, respectively. This indicates that the yeast began to adapt to the environment during this phase, enabling the conversion of glucose to ethanol, which explains the rapid decrease in glucose content and the corresponding increase in ethanol content.

From 12th hour to 16th hour, the glucose concentration in the broth increased by an additional 35% (reaching 2.7 g/L), and the ethanol content also increased by 27%. We believe that the decrease in glucose concentration during the 4-12 hour phase reactivated some β-glucosidase enzyme molecules that were inhibited by glucose; these enzyme molecules catalyzed the hydrolysis of cellooligosaccharides to produce glucose, and a portion of the glucose produced was then converted by the yeast into ethanol. Therefore, during the phase from 12th to 16th hour, the concentrations of glucose and ethanol both increased.

From 16th hour to 20th hour, the glucose content decreased and then stabilized from 20th hour until the end of the fermentation process; meanwhile, the reducing sugar content decreased by 15% from 16th hour to 24th hour and the reducing sugars stabilized from 24th hour to 72th hour; conversely, the ethanol content gradually increased and reached its peak at 36th hour (achieving 2.1% v/v corresponding to a fermentation efficiency of 70.0%). These results indicate that the hydrolysis and fermentation processes took place from 16th to 36th hour, with most of the glucose molecules produced being converted into ethanol, hence the glucose content remained almost unchanged in the broth. The results also show that the hydrolytic enzymes continued to operate during this phase to produce glucose for the yeast to convert into ethanol.

After 36 hours, the content of ethanol, reducing sugars, and glucose remained almost unchanged, indicating that both the fermentation and hydrolysis processes were very slow. We assume that the amorphous polysaccharide structure was almost depleted, the amount of easily fermentable sugars like glucose was very low, and the broth contained harder-to-ferment sugars such as rhamnose, mannose, xylose, etc. Additionally, after a period of catalysis, the enzyme activity decreased. Chen and Wang (2016) suggested that some enzyme molecules were trapped within the remaining porous structure of cellulose, thus limiting the hydrolysis reaction [25].

The HMF content tended to decrease throughout the saccharification and simultaneous fermentation period. This indicates that the reduction in HMF content was associated with the ethanol fermentation activity of *Saccharomyces cerevisiae* [24], [26]. Previously, Kuglarz et al. (2018) used the SSF method to convert oilseed rape straw into ethanol with *Saccharomyces cerevisiae* and also observed a gradual decrease in HMF content to zero during the saccharification and simultaneous fermentation period [27].

3.4. Combined SHF-SSF method

Experimental results shown in Figure 4 indicate that, within the first four hours, the levels of reducing sugars and glucose decreased rapidly; the ethanol content reached 0.87% v/v at the 4th hour and was 2 to 3 times higher compared to the SHF and SSF methods (Figures 2 and 3). The reason is that the initial fermentation medium already contained glucose (35.5 g/L), and the hydrolysis of the added raw materials continued to produce new glucose during the first 4 hours.

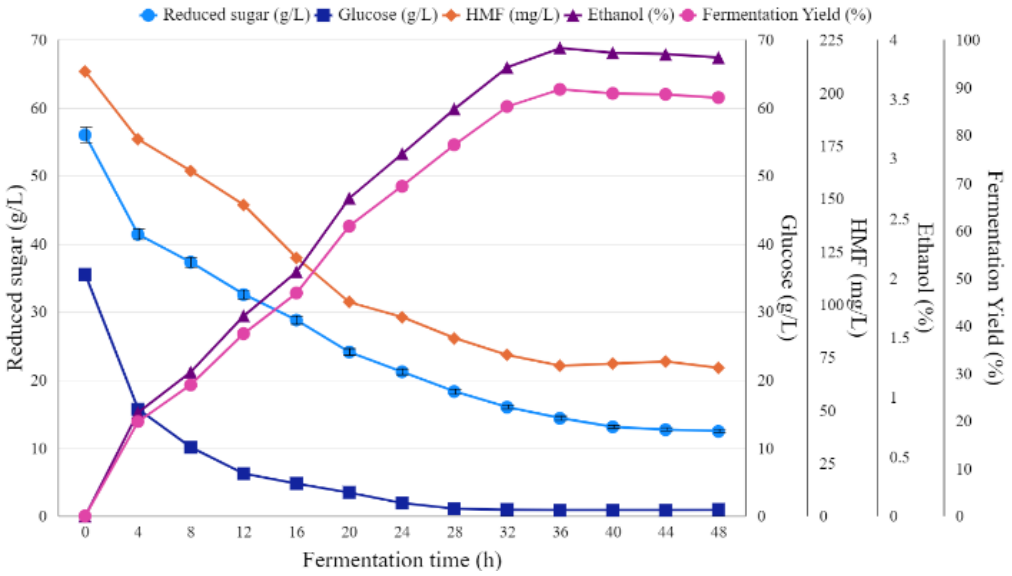

Figure 4. Results of the *Chaetomorpha* sp. residue fermentation using the combined SHF-SSF method

From the 4th to the 24th hour, the levels of reducing sugars, glucose, and HMF continued to decrease, while the ethanol content rapidly increased, reaching 3.04%, corresponding to a fermentation efficiency of 69.3% at the 24th hour. This indicates that fermentation was very strong during this period, with a large amount of raw material being converted into ethanol. The vigorous fermentation also led to a significant reduction in the HMF content in the broth.

The reducing sugar content decreased to 14.4 g/L at the 36th hour, a 32.2% reduction from the 24th hour. Similarly, the glucose content also decreased by 43.5% from the 24th hour to the 28th hour (remaining at 1.1 g/L) and then stabilized until the end of the fermentation process. However, the ethanol content continued to increase from the 24th hour onwards, peaking at the 36th hour with a concentration of 3.93% v/v, a 29.3% increase from the 24th hour, achieving a fermentation efficiency of 89.6%. From the 36th hour to the 48th hour, the levels of reducing sugars and ethanol remained nearly unchanged, due to the hydrolysis of most amorphous polysaccharide molecules in the added raw materials. The remaining crystalline structures were slowly hydrolyzed due to the poor adsorption of cellulase enzymes onto the crystalline regions of the substrate [16].

Additionally, the HMF content also continuously decreased over the fermentation time. From the start until the 24th hour of fermentation, the HMF content decreased by 55.2%. This period corresponds to the strong ethanol fermentation phase of the yeast, with an ethanol content of 3.04% v/v corresponding to a fermentation efficiency of 69.2%. From the 24th hour to the 36th hour, the HMF content decreased more slowly, with an additional reduction of 24.4%; the yeast fermentation process also slowed down during this period, with only a 22.1% increase in ethanol content. From the 36th hour, the HMF content remained almost unchanged, and the ethanol content did not change during this stage. These results once again demonstrate that when yeast is active, it will metabolize and reduce the HMF content [24], [28], [29].

3.5. Comparison of fermentation methods

The effectiveness of the methods for converting seaweed into ethanol is presented in Table 2.

For both SHF and SSF methods, the initial amount of raw material in the fermentation medium was almost the same (10% and 9% w/v, respectively). Due to the relatively low input of raw material, a high ethanol content could not be obtained at the end of the fermentation process. The combined SHF-SSF method helped increase the total input of raw material to 13% (w/v), which is 30% and 44% higher than the SHF and SSF methods, respectively; therefore, the ethanol content obtained after fermentation was higher.

Table 2. *Comparison of some technical parameters in SHF, SSF and the combined SHF-SSF method*

Method		SHF	SSF	Combined SHF-SSF
Raw material (% w/v)	Initial	10	9	10
	Additional	0	0	37,5% Initial material
Cellulase activity (FPU/g material)	Initial	30	25	30
	Additional	0	0	20 FPU/g Additional material
β-glucosidase activity (CBU/g material)	Initial	10	4	10
	Additional	0	0	5 CBU/g Additional material
Ethanol concentraion (% v/v)		1,83[a]	2,10[b]	3,93[c]
Fermentation Yield (%)		54,8[a]	70,0[b]	89,6[c]
Total process time (h)		80	36	76
	Hydrolysis time (h)	40	0	40
	Fermentation time (h)	40	36	36

* *Different characters (horizontally) represent statistically significant differences (p<0.05).*

In terms of time, the SHF method included two separate hydrolysis and fermentation stages, resulting in a total duration of up to 80 hours, while the SSF method carried out both hydrolysis and fermentation process simultaneously, reducing the total time to 36 hours, which was 2.2 times shorter than the SHF method. The combined SHF-SSF method had a longer duration, 111% longer than the SSF method, but 5% shorter than the SHF method.

Regarding fermentation efficiency, the combined SHF-SSF method reached the highest efficiency (89.6%), which was 28.0% higher than the SSF method (70.0%) and 63.5% higher than the SHF method (54.8%), respectively. Previously, Cho et al. (2013) also studied the SHF method on *Enteromorpha intestinalis* seaweed and achieved a fermentation efficiency of only 30.5% [30]; similarly, Park et al. (2012) also achieved a fermentation efficiency of 38% when studying on *Gelidium amansii* red seaweed [31]. For the SSF method, other studies on *Saccharina japonica* seaweed yielded similar results to ours, achieving a fermentation efficiency of approximately 70% [32], [33].

In terms of ethanol content obtained after fermentation, the combined SHF-SSF method achieved ethanol levels that were 115% and 87% higher than those of the SHF and SSF methods, respectively. Compared to many similar studies on ethanol fermentation from seaweed, the combined method also produced superior ethanol yields. Wu et al. (2014) conducted an SHF fermentation process (using acid and enzymatic hydrolysis), achieving an ethanol yield of 0.6% with the red seaweed *Gracilaria* [34]. In contrast, ethanol yields of 1.62% and 1.17% were obtained with the brown seaweed *Undaria pinnatifida* [35] and the green seaweed *Ulva fasciata* [20], respectively.

Regarding enzyme usage, the combined SHF-SSF method required the highest amount of enzymes, while the SSF method used the least. This is a disadvantage of the combined SHF-SSF method as it increases the cost of enzyme usage.

The above analyses show that the combined SHF-SSF method has superior advantages in terms of efficiency in converting substrates into products and the ethanol content obtained after the fermentation process.

4. Conclusions

The research results indicate that the combined SHF-SSF method achieved higher ethanol recovery efficiency compared to the two conventional methods, SHF and SSF. However, the combined SHF-SSF method still has several aspects that need improvement. In terms of processing time, the combined method had a total duration time comparable to the SHF method and was twice as long as the SSF method. The total amount of enzymes used was also higher than that of the SHF and SSF methods. Although the fermentation efficiency was significantly improved, further research is needed to enhance the economic feasibility of the combined SHF-SSF method.

Conflict of Interest

The authors declare no conflict of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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