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BIOETHANOL PRODUCTION FROM SEAWEED PROCESSING WASTE BY SIMULTANEOUS SACCHARIFICATION AND FERMENTATION (SSF)

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ABSTRACT
Seaweed processing has been used for bioethanol production through simultaneous saccharification and fermentation (SSF). SSF is commonly used for bioethanol production to shorten the process and increase the yield of ethanol produced using *Trichoderma reseei* and *Saccharomyces cerevisiae.* The aim of the research was to obtain the best concentration of T. reseei and S. cerevisiae to produce bioethanol by SSF. The various concentration of *T. reseei* dan *S. cerevisiae* used was 0 (contro)l, 5, 10, 15 and 20 % (v/w), then in SSF process using shaking incubator at 35 °C and rotation of 150 rpm for 3 days. The seaweed processing waste used for the study had moisture content of 12.94±0.08% and 15.38±0.19%, ash content of 16.72±0.08% and 18.39±0.19%, lignin content of 15.38±0.11% and 12.74±0.38%, and cellulose content of 26.92±0.57% and 34.57±0.81% for untreated and hot water treatment, respectively. Result of SSF process of seaweed waste showed that different concentration of *T. reseei* and *S. cerevisiae* (control, 5, 10, 15 and 20 %) yielded significant effect ($p<0.05$) of total reducing sugars and ethanol produced. DMRT test showed that the treatment 10% of *T. reseei* and *S. cerevisiae* concentration was the best treatment producing highest yield of ethanol.

Keyword: bioethanol, treatment, seaweed processing waste, SSF

1. Introduction

Seaweed is one of aquatic commodities which abundantly available and useful material for feedstock, food industry, cosmetic and pharmacy as well as in medicine. Based on the Food and Agricultural Organization of the United Nations (2014) data the volume of seaweed and water plants of the world reached 23.7 million tons in 2012, and Indonesia ranks the second with a volume of 6.5 million tons. Indonesia seaweed production in 2013 reached 9.3 million tons and increased in 2014 by 36.86% (Ministry of Marine Affairs and Fisheries of Indonesia 2014). Seaweed is a potential carbohydrate source with a content of 70-72 % (Nahak *et al.* 2011). Carbohydrate contents of seaweed are varied which is composed of hydrocolloid and hemicellulose, cellulose and lignin with different content depending on the type. Nowdays, there are agar industries in Indonesia, producing on solid waste. Bioethanol is one of prospective product from seaweed processing waste utilization of from industrial agar.

Lignocellulose from seaweed has a low lignin, making it easier for bioethanol production (Wei et al. 2013). Conversion of lignocellulose biomass into bioethanol is conducted through several stages, namely the initial processing of lignocellulosic biomass (pretreatment), the saccharification process using enzymes, and the fermentation process (Borines et al. 2013). Pretreatment is performed to decrease lignin levels that can inhibit the process of saccharification. El-Naggar et al. (2014) reported that the optimum condition for bioethanol production from lignocellulose is strongly influenced by the raw materials, the type of degradation microorganism and the fermentation condition. The processing waste of agar industry containing 13.87% hemicellulose, 30.18% cellulose and 5.76% lignin (Andhikawati 2014). Martosuyono et al. (2013) reported that 4-5% NaOH pretreated seaweed solid waste contained 5.30% and 3.86% lignin; and 26.31% and 24.85% of cellulose.

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The production of bioethanol from seaweed has been reported using *Sargassum* sp. (Borines et al. 2013), *Gelidium* sp. (Kim et al. 2015) and *Gracilaria* sp. (Adini et al. 2015). Uju et al. (2015) reported enzymatic saccharification from carrageenan industrial waste with peracetic acid (PPA) and ionic liquid (IL) which were able to produce cellulose conversion of 77%. Production of bioethanol from *Gelidium amansii* with SSF using β-glucosidase and *S. cerevisiae* at 37 ° C, pH 4.8 for 48 hours yielding 84.9% bioethanol (Kim et al., 2015). Simultaneous saccharification and fermentation (SSF) is a method that combines two stages into one phase which aims to shorten the process time and increase the yield of bioethanol produced. The process is more efficient because two reactions occur simultaneously, the conversion of cellulose into sugars and the conversion of sugar into bioethanol through the fermentation process by using cellulose enzyme and yeast (El-Naggar et al. 2014).

This study aims to convert seaweed processing waste into bioethanol production using SSF method through application of *T. reseei* and *S. cerevisiae.*

2. Materials and Methods

2.1 Characterization of Seaweed Processing Waste

The seaweed solid waste was taken from smallscale agar processing unit in Pameungpeuk, District of Garut, West Java. The fresh processing waste material was sun dried and grounded to size 60 meshes prior to the pretreatment. The hot water treatment of seaweed processing waste were carried out at 121 °C for 30 minutes. The ratio of seaweed waste : aquades was 1:5 (1 g of sample added by 5 ml of aquades) (Pasanda et al. 2016). The sample of seaweed processing waste was analyzed for the cellulose and lignin content by Chesson method (Datta 1981), water content with SNI 03-1971-1990, salt content with SNI 01-2359-1991, and characteristic of seaweed processing waste analyzed by Scanning Electrone Microscope (SEM) in Agricultural Postharvest Laboratory, Ministry of Agriculture and Fourier-Transform Infrared Spectroscopy (FTIR) in Instrument Laboratoty, Research and Development Center for Marine and Fisheries Product Competitiveness and Biotechnology.

2.1 Saccharification and fermentation

The SSF process followed Tan and Lee methods (2014), converting carbohydrate in the waste into ethanol using *T. reesei* and *S. cerevisiae* with different concentrations. Preparation of *T. reesei* and *S. cerevisiae* cultures was performed using PDA (Potato Dextrose Agar) medium at 35 °C at 120 rpm for 48 hours using shaking incubator.

Inoculum starter was added on to 0 (control), 5, 10, 15, and 20% of substrate volume and 50 mM citrate buffer pH 8. The SSF was performed with shaking incubator at 35 °C, 150 rpm for 120 hours. To determine the best concentration of substrate, sampling at 0, 24, 48 and 120 hours was conducted for the analysis of reducing sugar and ethanol content. The sample was centrifugated at 10,000 x g for 15 minutes before conducting the analysis. All experiments were conducted in triplicate otherwise specifically stated.

Sugar concentration was measured by reacting the saccharification product with 3,5-dinitrosalycilic acid (DNS) and read the absorbance at 575 nm (Miller 1959). Determination of pH value using pH meter (SNI 06-6989.11-2004), The ethanol yield from saccharification and fermentation process was measured using Gas Chromatography (GC). Calculation of ethanol content (%) was based on standard ethanol curve by measuring the area and then used to calculate the ethanol content of the sample.

The data were analyzed using Factorial Complete Random Design (RAF) with two factor experiments. This treatment was analyzed using Analysis of Variance (ANOVA). Treatment at the determination stage of culture concentration that gives a real effect ($p<0.05$) on ethanol content will be tested further with Duncan Multiple Range Test (DMRT) using SPSS 17.0 software.

3. Results and Discussion

3.1 Characteristics of Seaweed Processing Waste

The sample used in this research was seaweed processing waste from smallscale agar processing unit after the production process. Seaweed processing waste was sun dried and grounded to size 60 meshes mesh particle size to expand the surface.. The chemical composition of seaweed processing waste is shown in Table 1

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Lignin, hemicellulose and cellulose content of untreated seaweed processing waste (SWU) were 15.38%, 16.11% and 26.92%, and hot water treatment (SWT) were 12.74%, 13.99% and 34.57% respectively. The high content of lignin in seaweed processing waste was affected by various types of seaweed that are used as the raw materials in the small scale agar industry in Pamengpeuk, Garut, West Java. Mixture of seaweed such as Gracillaria and Gelidium were usually used in the processing of agar. However, the content of lignin and hemicellulose was decreased after treatment with hot water at high temperature.

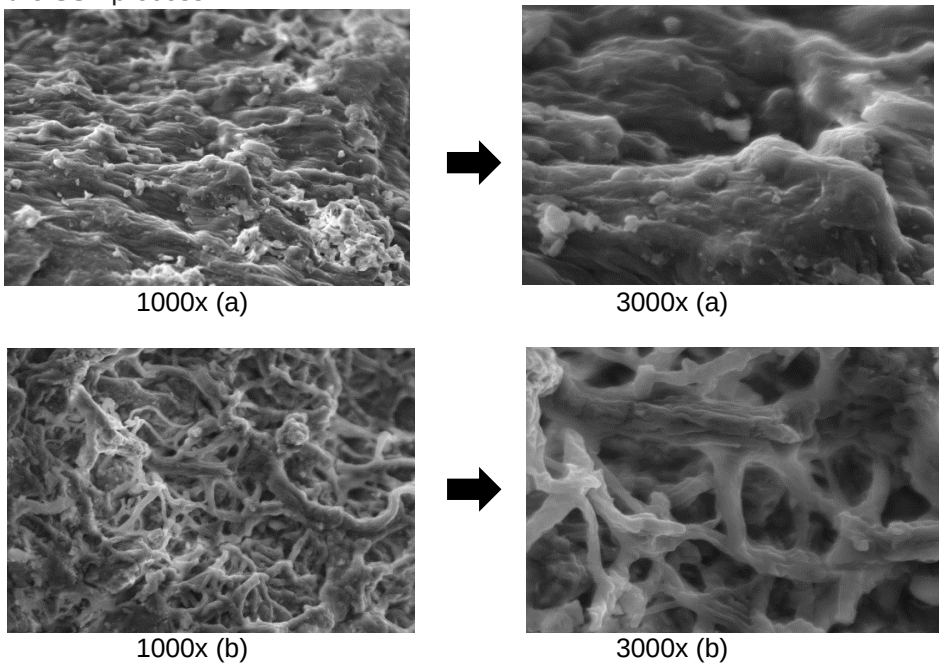
Cellulose content of seaweed processing waste increased from 26.92% to 34.57% after hot water pretreatment at 121°C. Cellulose content of seaweed processing waste used in this study was higher than that of reported by Sari et al. (2013), i.e 20.17±0.03% (*Gracilaria* sp.). Pasanda et al. (2016) reported that seaweed waste hot water treatments was decreased the lignin content from 4.24% to 0.64% and was increased the cellulose content from 18.98% to 21.35%. Changes in the composition was due to partly dissolved lignin along with hemicellulose. Based on the degree of crystallinity, classified into crystalline cellulose and amorphous was resulted cellulose into soluble.

Table 1. Characteristics of seaweed processing waste

Seaweed processing waste	Concentrations (%)					
	cellulose	Hemicellulose	Lignin	Ash	Moisture	Salt
SWU	26,92±0,07	16,11±0,75	15,38±0,12	16,72±0,16	12,94±0,08	3,77±0,07
SWT	34,57±0,32	13,99±0,815	12,74±0,38	18,39±0,28	15,38±1.85	1,41±0,11

SWU : Untreated seaweed processing waste
SWT : Hot water treatment seaweed processing waste

Cellulose and hemicellulose contents that can be converted into sugars for bioethanol production, were increased to more than 30%. The moisture, ash and salt content of untreated seaweed processing waste were 12.94 ± 0.08%, 16.72 ± 0.16% and 3.770 ± 0.07% respectively, while after the pretreatment using hot water were 15.38 ± 0.18%, 18.39 ± 0.28% and 1.41 ± 0.11%., respectively. Hot water treatment causing moisture and ash content increased. In contrast, the salt content decreases due to the addition of aquades and high temperatures in the treatment so that the salt dissolves, where salt content greatly affects microbial activity and growth in raw materials (waste), which means good when bioethanol production in the SSF process.



1000x (a) 3000x (a)

1000x (b) 3000x (b)

Figure 1. Scanning Electrone Microscopy of untreated (a) and hot water treatment (b) seaweed processing waste

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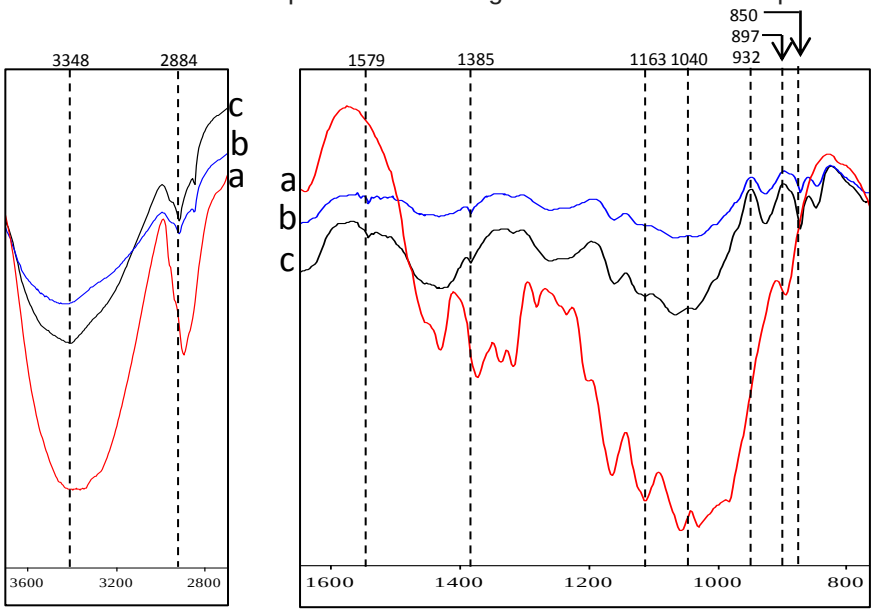
Scanning Electrone Microscope (SEM) was used to observe microstructures of cellulose and lignin in the waste before and after treatment. As shown in Figure 1 the difference in cellulose structure of untreated seaweed processing waste and hot water treatment. The structure of cellulose (cellulose bonds with lignin) after hot water treatment became more rooged and loosed. The solid structure became destroyed and loose with many small fragments after the treatment process. The hot water treatment was caused swelling, breaks the bond between lignin and carbohydrate, increases cellulose surface and decreases polymerization and crystallinity levels, which triggers impaired lignin structures. All of these effects make cellulose more accessible for enzymatic fermentation, and ultimately can improve the efficiency of bioethanol production (Zhang et al. 2013). Uju et al. (2015) reported that paracetic acid (PPA) and ionic liquid (IL) treatments used for carrageenan industrial waste showed more profound effect loosening of lignin and cellulose bonds to form cavities in cellulose fragments. The research results of Martosuyono et al. (2015) shown that the structure of the seaweed waste processing may change after treatment with acid and base, and may be able to increase the surface area that was easily accessible for enzymatic hydrolysis. It confirmed the disintegration of samples of cellulose structures due to treatments on solid seaweed waste that would produce more sugars than untreated samples.



Gambar 2. Fourier-Transform Infrared Spectroscopy of cellulose standard (a) untreated (b) and hot water treatment (c) seaweed processing waste

The characteristics of seaweed processing waste was analysed also with Fourier-Transform Infrared Spectroscopy (FTIR) using infrared spectrum that affected the molecular changes to produce the absorbant peak. According to Stevulova et al. (2014), peak at wavelength 2800-3400 cm^{-1} indicates polysaccharides, 1300-1400 cm^{-1} cellulose, 1028 and 1733 cm^{-1} shows hemicellulose groups, whereas wavelength 1500-1600 cm^{-1} was a lignin group. Figure 2 shows that the standard FTIR spectrum of cellulose and the waste looks similar. The FTIR spectrum of cellulose standard and seaweed processing waste show the presence of crystalline cellulose groups at wavelengths of 1385 cm^{-1}. While the lignin group was seen in the untreated and hot water treatment seaweed processing waste at wavelength 1579 cm^{-1}. Uju et al. (2015) reported that the high cellulose crystallinity characteristics in the untreated and PAA-pretreated of seaweed waste biomass from the carrageenan industry were indicated by a strong and sharp peak appearance at 1425 cm^{-1} but a weak appearance at 897 cm^{-1}. The results suggest that treatment caused the pretreated seaweed waste was changed to more amorphous fractions. Sharp large peaks are visible from 3000 to 3600 cm^{-1}, which indicates OH stretching, these peaks were centered at 3348 cm^{-1}. Its a weakening of the hydrogen bonds occurred in the cellulose structure. Nurhayati and Kusumawati (2014) reported that the cellulose spectrum from the seaweed processing waste was appeared in the absorption areas of 1430 and 1638 cm^{-1}.

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3.2 Biotehanol Production By SSF

a. Total reducing sugars

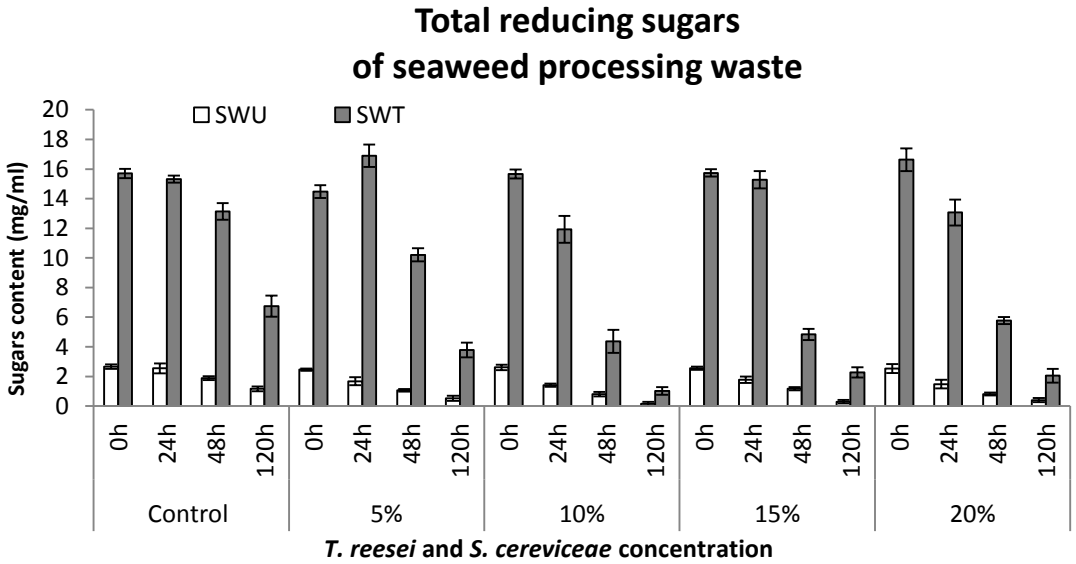

Figure 3. Total reducing sugars of seaweed waste processing

The total reducing sugars of simultaneous saccharification and fermentation of seaweed processing waste used *T. reesei* and *S. cerevisiae* (Figure 3). The reducing sugars were the result of cellulose hydrolysis in the saccharification process with *T. reesei*. The reducing sugar produced will be used *S. cerevisiae* as fermentation substrate in bioethanol production. The total reducing sugars produced was 2-16 mg/ml hot water treatment seaweed processing waste (SWP), while the untreated seaweed processing waste (SWU) ranged from 0.3-2.7 mg/ml. The total reducing sugar produced from seaweed waste with hot water pretreatment (SWT) was higher than untreated (SWU). The decreased of lignin content and high cellulose content in the seaweed processing waste with hot water treatment was affected the total reducing sugar produced.

Different concentrations of *T. reesei* and *S. cerevisiae* (control, 5, 10, 15 and 20%) had a significantly different effect on total reducing sugars produced ($p<0.05$). The total reducing sugars were higher than that of Sari et al. (2013) which value was ranging from 4.65-10.77 mg/ml using raw materials of industrial seaweed waste (*Gracilaria* sp.). The total reducing sugars decreased with the length of time of simultaneous saccharification and fermentation. The sugar produced directly converted into ethanol. The reducing sugar became an important element as a carbon source for *S. cerevisiae* growth in bioethanol production (Adini et al 2015).

b. Ethanol Contents

Seaweed processing waste became one of the potential feedstock in bioethanol production because it has high cellulose content that can be converted into sugar to ethanol. As shown in Figure 4 the ethanol content from the SSF process of seaweed processing waste using *T. reesei* and *S. cerevisiae*. The production of bioethanol from seaweed processing waste through SSF using different concentrations of *T. reseei* and *S. cerevisiae* (controls, 5, 10, 15 and 20%) gave a significantly different effect on ethanol content per time ($p<0.05$).

Ethanol contents increased with the time of saccharification and fermentation. Increasing levels of ethanol are affected by total reducing sugars produced during the process of SSF The highest ethanol content was generated from seaweed processing waste with hot water treatment at the 120 hour or on the 3rd days of SSF with 10% *T. reesei* and *S. cerevisiae* concentrations, resulting ethanol of 1.068±0.082%. While the ethanol content in untreated seaweed processing waste was 0.615 ± 0.061% with the concentration of *T. reesei* and *S. cerevisiae 20%*.

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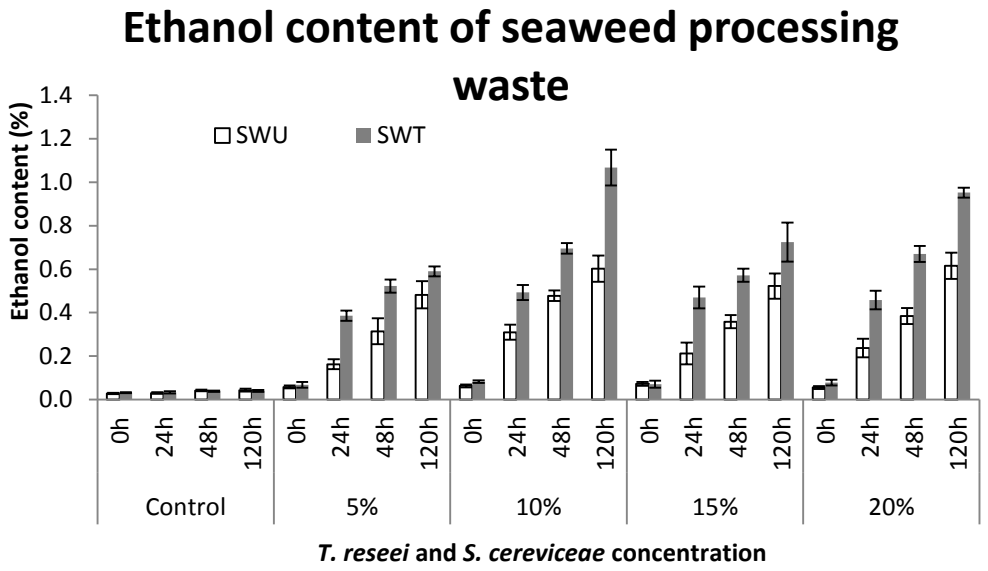

Figure 4. Ethanol contents of seaweed waste processing

The hot water treatment and untreated seaweed processing waste showed a significant effect on the resulting ethanol content ($p<0.05$). The ethanol content of seaweed processing waste with hot water treatment (SWT) was higher than untreated seaweed processing waste (SWU). The high contents of cellulose and total reducing sugars produced on saccharification was thus affecting the ethanol yield. Sari et al. (2013) reported that the ethanol content of industrial seaweed waste for fermentation (*Gracilaria* sp.) using *T. viride* and *S. cerevisiae* was 0.47±0.08%. Based on DMRT test, the 10% of *T. reseei* and *S. cerevisiae* concentration was the best concentration based on the ethanol content produced on the seaweed processing waste with the pretreatment of hot water. Wahono et al. (2015) reported that the ethanol production from sugarcan bagasse was resulted 0.748 5 % after 5 days incubation time by SSFmethod using *S. cerevisiae* and cellulose enzymes of *T. reesei.*

Conclusion

The seaweed processing waste used for the study had moisture content of 12.94±0.08% and 15.38±0.19%, ash content of 16.72±0.08% and 18.39±0.19%, lignin content of 15.38±0.11% and 12.74±0.38%, and cellulose content of 26.92±0.57% and 34.57±0.81% for untreated and hot water treatment, respectively. The simultaneous saccharification and fermentation process using *T. reesei* and *S. cerevisiae* for 120 hours resulted in total reducing sugar content of 16 mg/ml and 1.08% ethanol content. Based on DMRT test showed that the 10% treatment of *T. reseei* and *S. cerevisiae* concentration was the best concentration to obtain the highest yield of ethanol produced.

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