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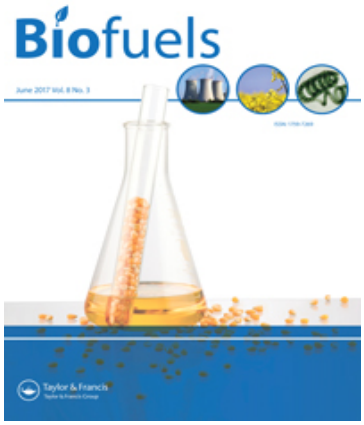

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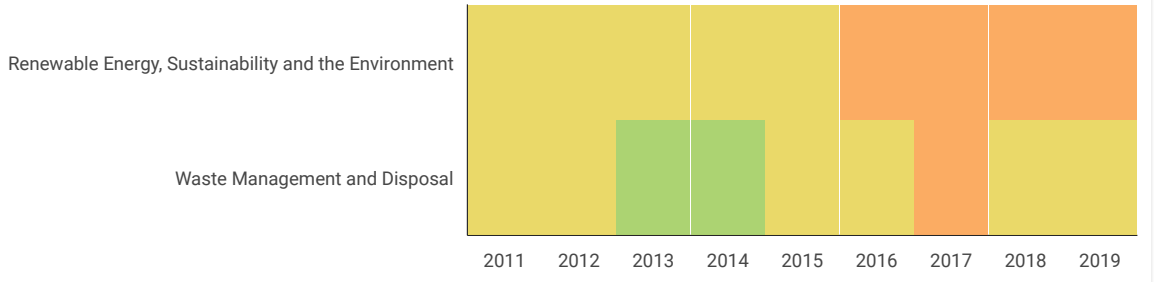

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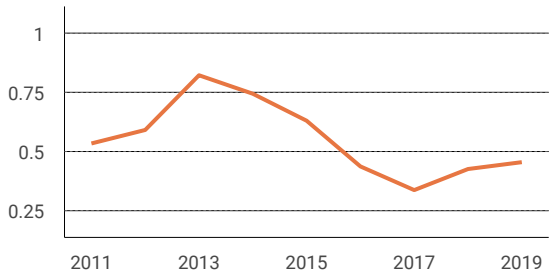

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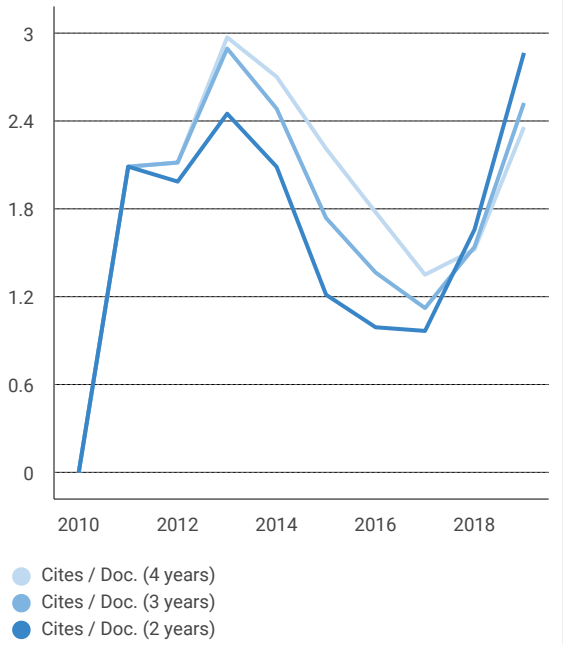

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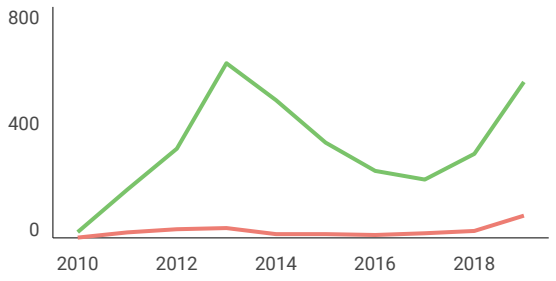

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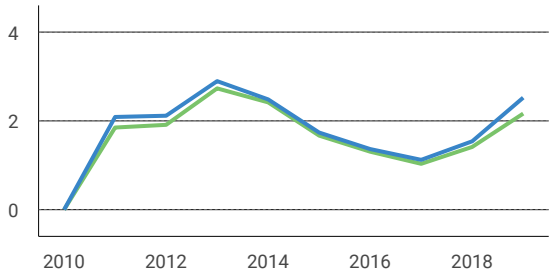

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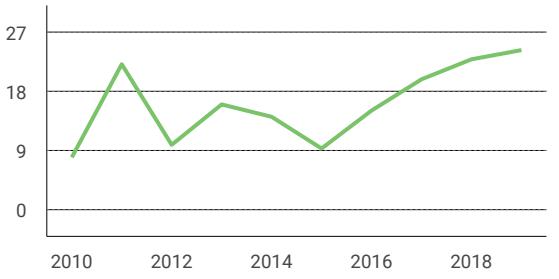

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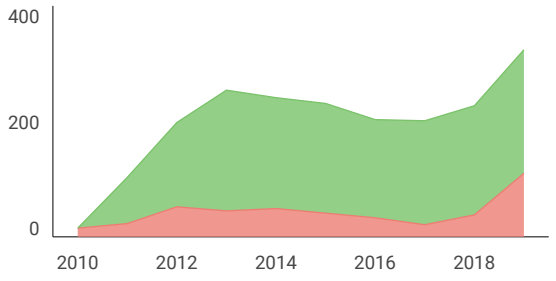

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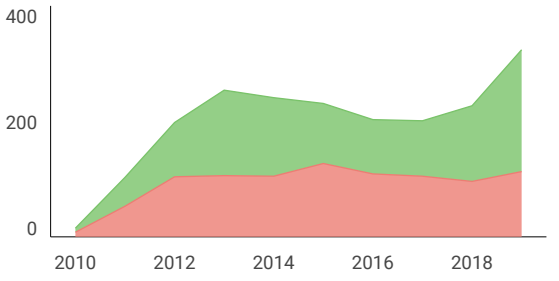

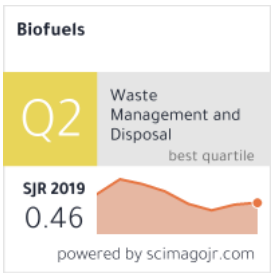

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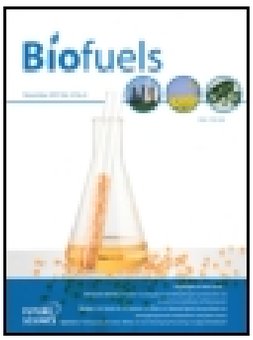

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Studies on The Hungate technique for ethanol fermentation of algae Spirogyra hyalina using Saccharomyces cerevisiae

Sulfahri, Ni'matuzahroh & Sri Wulan Manuhara

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Studies on The Hungate technique for ethanol fermentation of algae *Spirogyra hyalina* using *Saccharomyces cerevisiae*

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ABSTRACT

In this study, the feasibility of the Hungate technique was studied using the alga *Spirogyra hyalina*. This study aimed to determine the effect of different types of gas, culture pH and the duration of fermentation on cell biomass, total reducing sugars, and total ethanol produced from the fermentation of *Spirogyra hyalina* hydrolysates using *Saccharomyces cerevisiae*. The experimental trials were carried out in batches with two types of gas (nitrogen and hydrogen), three pH levels (pH 4, 5, 6), and four fermentation times (0, 24, 48 and 72 hours). The fermentation process was carried out under anaerobic conditions. Anaerobic conditions were ensured using the Hungate technique, namely by injecting nitrogen gas or hydrogen gas into the fermentor. The results show that the average levels of ethanol produced tended to be higher in the presence of hydrogen gas when compared to the presence of nitrogen gas. The highest level of ethanol was achieved in the presence of hydrogen gas at pH 4 after 72 hours of fermentation.

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Bioethanol; reducing sugar; anaerobic; pH; nitrogen; hydrogen

Introduction

Fuel needs are increasing with the increase in the human population [1–3]. Globally, most fuel oil is derived from fossil fuels, which are non-renewable [4,5]. The amount of fossil fuels is thus very limited, since the formation process requires millions of years. Conversion and diversification of fuel oil must be done intensively due to the limited oil resources derived from fossil fuels, as fuel consumption is increasing [6,7]. Bioethanol fuel is a potential substitute for fossil fuels. Unlike fossil fuels, bioethanol is more environmentally friendly because it is a renewable energy source, and it is considered one of the most promising replacements for gasoline as a transport fuel [4,8]. The utilization of algae as a raw material for bioethanol production is a realistic solution to reduce the use of fossil fuels [8–10]. The use of Spirogyra algae as a feedstock for bioethanol production has been successfully demonstrated using a fermentation process [11,12].

The fermentation of Spirogyra algae is most effectively done using the Hungate technique with the addition of nitrogen gas [13]. The Hungate technique is a method for generating anaerobic conditions by the addition of gas into the fermentor space. Studies on the addition of nitrogen gas using the Hungate technique have shown this gas to be less effective, because nitrogen cannot act as a reducing agent. Unlike nitrogen gas, the addition of hydrogen gas plays a role in anaerobic conditioning as it functions as a reducing agent for the formation of NADH. This is due to the reduction potential of hydrogen is -0.4 V vs. a normal hydrogen electrode (NHE), while the reduction potential of NADH is -0.32 V vs. NHE [13,14]. The difference in reduction potential allows hydrogen to act as a reducing agent for the formation of NADH. In the metabolism of *Saccharomyces cerevisiae* cells, NADH functions in the formation of pyruvic acid and ethanol [15,16]. Therefore, the addition of hydrogen gas into the fermentor space is important to increase the level of NADH by converting NAD^+ to NADH. The availability of NADH in the fermentation medium will increase the production of ethanol.

An increase in NADH in the fermentation medium due to the addition of hydrogen with the Hungate technique will certainly affect the pH of the culture. Culture pH is an important factor in ethanol fermentation, because microbial fermentation will only occur at a given pH. Therefore, determining the initial pH of the fermentation medium is essential to optimize growth during microbial fermentation. Besides the pH, another factor that may affect the ethanol fermentation process due to the addition of NADH is the duration of fermentation. Fermentation time is an important factor in ethanol fermentation because it affects production costs. Therefore, the effect of the use of the Hungate technique through the addition of nitrogen or hydrogen gas is important. This study aimed to determine the effect of different types of gas (nitrogen and

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hydrogen), the initial pH, and the duration of fermentation on cell biomass, pH, the amount of reducing sugars, and the level of ethanol produced from the fermentation of *Spirogyra hyalina* hydrolysates using *Saccharomyces cerevisiae.*

Material and method

Pretreatment and hydrolysis process of Spirogyra hyalina

Spirogyra hyalina was collected from a pond located in Surabaya, Indonesia, with the coordinates 07 °28′60.44″S and 112 °79′65.69″W. *Spirogyra hyalina* was collected and identified, then dried in an oven at 80 °C for 24 hours. Following this, *Spirogyra hyalina* was dried, then crushed and sieved through a 40 mesh size sieve. The dried *Spirogyra hyalina* weighed 62.5 g and was mixed with 1 L of distilled water; this was heated for two hours at 100 °C, and then cooled to the optimal temperature of the α-amylase enzyme, i.e. 45 °C [11,17]. The hydrolysis process was initiated by the addition of 8.1 kilo Novo units (KNU) of α-amylase (Liquozyme Supra, Novozymes, Denmark), and incubated for 80 minutes [11,13]. The hydrolyzed *Spirogyra hyalina* was then centrifuged at 9000 rpm for 15 minutes. The reducing sugars were then measured in the supernatant using the Luff Scholar method.

Starter preparation of Saccharomyces cerevisiae

Saccharomyces cerevisiae was inoculated into 50 mL Erlenmeyer flasks containing 5 mL of sterile *S. hyaline* hydrolysate that had been set to pH 4 by adding sodium citrate buffer, then incubated in a rotary shaker with an agitation speed of 15 rpm at 30 °C for 24 hours (activation I). A total of 1 mL of activation I was inoculated again into 50 mL Erlenmeyer flasks containing 9 mL of *S. hyalina* hydrolysate, which was then incubated in a rotary shaker with an agitation speed of 15 rpm at a temperature of 30 °C for 24 hours (activation II). A total of 5 mL of activation II was inoculated again into 100 mL Erlenmeyer flasks containing 50 mL of *S. hyalina* hydrolysate, then incubated in a rotary shaker with an agitation speed of 15 rpm at a temperature of 30 °C until the time point at which log phase of *S. cerevisiae* occurred (in accordance with the growth curve) (activation III) [17,18].

Fermentation process

The starter inoculum was added at a concentration of 10% ($OD_{600nm} = 0.5$) to a 100 mL bottle fermentor containing 50 mL of the substrate (*S. hyalina* hydrolysate), then incubated by varying the fermentation duration (0, 24, 48, and 72 h) at room temperature (30 °C). The fermentation process was carried out under anaerobic conditions. Anaerobic conditions were ensured using the Hungate technique, namely by injecting nitrogen gas or hydrogen gas into the fermentor. The fermentor was closed with a rubber stopper and then the gas (nitrogen or hydrogen) was supplied for 2 minutes. After that, the fermentation incubation was carried out without agitation, for a duration according to the study design. After the period of incubation (0, 24, 48, or 72 h), the amount of *S. cerevisiae* biomass, the pH, the reducing sugar concentration, and the ethanol concentration were measured [13,19].

Measurement of Saccharomyces cerevisiae *biomass*

Saccharomyces cerevisiae cell biomass measurements were performed using the dry cell weight (DCW) method. DCW was calculated by first centrifuging 50 mL of fermentation medium at a speed of 9000 rpm for 10 minutes. After centrifugation, a supernatant and a cell pellet were obtained. The supernatant was removed by gentle aspiration using a pipette. The pellet was washed by adding 0.1 M phosphate buffer to the cell pellet, which was centrifuged again at 9000 rpm for five minutes. Pellets were suspended in distilled water and then aspirated using a pipette and transferred to filter paper with a pore size of 0.47 μm, which had previously been heated in an oven until a constant weight was obtained (W1). Filter papers that contained pellets were then dried in an oven at a temperature of 80 °C for 24 hours and weighed (W2). DCW was calculated as the difference between the final weight of the filter paper and the initial filter paper weight (W2–W1), which was expressed as the DCW with units of grams/liter (g/L).

Measurement of total reducing sugars

Total reducing sugar measurements were performed using the Luff-Schoorl method. Reducing sugars were determined by the reaction of the water-soluble portion of the sample with an excess of standard copper sulfate in alkaline tartrate (Fehling's solution) under controlled conditions of time, temperature, reagent concentration, and composition. The reducing sugar concentration was estimated by the iodometric determination of the unreduced copper remaining after the reaction [20].

Measurement of ethanol concentration

Measurement of the ethanol concentration was performed using the specific gravity method and gas chromatography method. The fermentation culture was distilled before its specific gravity was measured using a glass pycnometer at room temperature (30 °C). The specific gravity method is used to measure the ethanol content based on the ratio of the density of a

substance (distilled fermentation culture) to the density of a reference substance (distilled water). Specific gravity is then determined using a specific gravity table from the Association of Official Analytical Chemists and the ethanol percentage is calculated [12,13,20].

Results and discussion

Saccharomyces cerevisiae *biomass*

The success of ethanol fermentation can be determined by assessing microbial growth in the fermentation medium. The biomass of *S. cerevisiae* (g/L) in *Spirogyra hyalina* hydrolysate-containing medium is presented in Table 1.

Based on Table 1, it was found that *S. cerevisiae* biomass increased with an increased duration of fermentation. Significant increases occurred from 0 to 24 h. After 24 h, the biomass tended to stagnate. The highest amount of biomass occurred with an initial pH 5 for nitrogen gas and at an initial pH 4 for hydrogen gas. According to the ANOVA results, the type of gas and the duration of fermentation significantly affected the biomass of *S. cerevisiae*. The pH did not significantly affect the biomass of *S. cerevisiae*.

pH change during the fermentation process

During the fermentation process, acids are produced, such as pyruvic acid and acetaldehyde, which will affect the pH of the fermentation medium. The pH of the fermentation medium is important for microbial growth, because certain enzymes will break down the substrate only at a specific pH [21–23]. In this study, measurements of pH were carried out at 0, 24, 48, and 72 h. This was done to assess the changes in pH during fermentation. The results regarding the pH value of the culture medium during fermentation in the presence of nitrogen gas and hydrogen gas are presented in Figure 1. Based on the initial pH of the medium, the Tukey test at the 95% confidence level indicates that there was no significant difference between the initial pH 4, 5 and 6, in the presence of both nitrogen gas and hydrogen gas. In Figure 1, it can be seen that the pH decreased during fermentation. The decrease in pH was caused by the formation of ethanol metabolites and other products such as organic acids. Organic acid production could lead to a lower pH [12,21,24].

Table 1. Biomass of *S. cerevisiae* (g/L) in *Spirogyra hyalina* hydrolysates medium.

Gas	pH medium	Fermentation duration			
		0 hour	24 hours	48 hours	72 hours
N_2	4	0.302 ± 0.10^{b}	2.167 ± 0.24^{a}	2.181 ± 0.14^{a}	2.141 ± 0.10^{a}
	5	0.308 ± 0.11^{b}	2.197 ± 0.18^{a}	2.219 ± 0.15^{a}	2.186 ± 0.10^{a}
	6	0.303 ± 0.09^{b}	2.158 ± 0.10^{a}	2.179 ± 0.30^{a}	2.053 ± 0.22^{a}
H_2	4	0.345 ± 0.14^{b}	2.037 ± 0.34^{a}	2.140 ± 0.23^{a}	2.153 ± 0.43^{a}
	5	0.311 ± 0.15^{b}	1.792 ± 0.21^{a}	2.133 ± 0.19^{a}	2.140 ± 0.46^{a}
	6	0.347 ± 0.22^{b}	1.660 ± 0.31^{a}	2.127 ± 0.33^{a}	2.131 ± 0.34^{a}

Information: same latter in each column and row are not significantly different based on Tukey test at the 95% confidence interval.

Reducing sugar concentration

The growth of microorganisms in fermentation medium will surely consume the substrate as the carbon source. Reducing sugars are a source of carbon found in *S. hyalina* hydrolysates. The amount of reducing sugars during the fermentation process was monitored to assess reducing sugar utilization by *S. cerevisiae*. The amount of reducing sugars consumed by *S. cerevisiae* in the presence of nitrogen gas and hydrogen gas are presented in Figure 2. Based on Figure 2, reducing sugars continued to decrease throughout fermentation. The amount of reducing sugars at the initial fermentation time point (0 h) was 9.12%, which decreased during fermentation. Reducing sugars were not depleted at the end of the fermentation period (72 hours), as the reducing sugar content ranged from 2.27 to 3.82%. Based on the regression calculation, reducing sugars were expected to be depleted between 96 and 120 h of fermentation. *S. cerevisiae* converts reducing sugars into ethanol [11–13]. Higher ethanol levels led to a decrease in the reducing sugar content of the fermentation substrate [11,22,23].

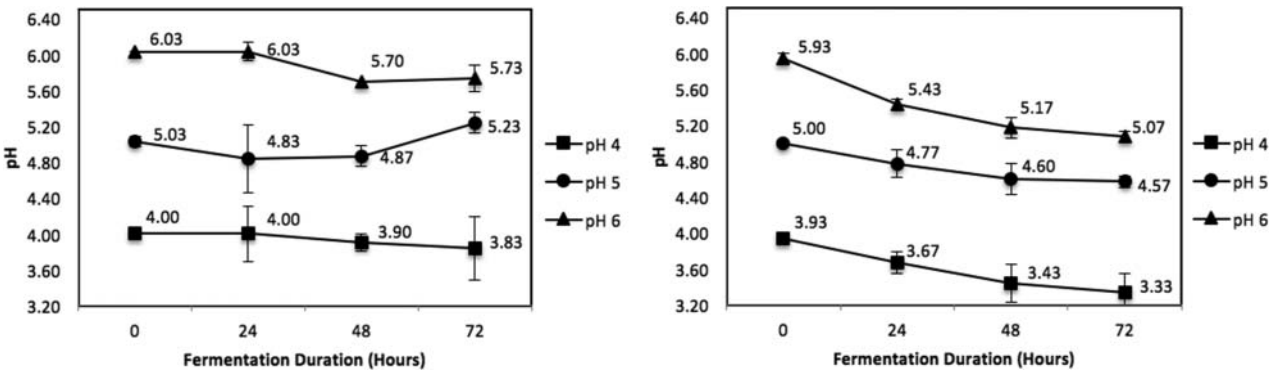

Figure 1. Graph of the culture pH value of *Saccharomyces cerevisiae* in fermentation medium with the presence of nitrogen gas (left) and hydrogen gas (right).

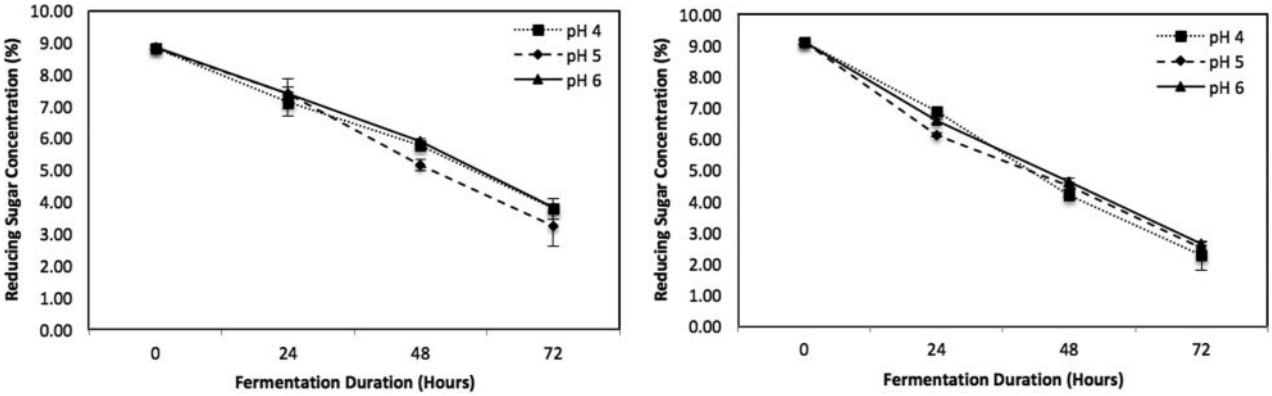

Figure 2. Graph of reducing sugar concentration that was generated by *S. cerevisiae* in the presence of nitrogen gas (left) and hydrogen gas (right).

Ethanol concentration

Measurement of the ethanol concentration was conducted using the specific gravity method. The amount of ethanol that was produced by *S. cerevisiae* in the presence of nitrogen gas and hydrogen gas is presented in Figure 3. Based on Figure 3, the ethanol content continued to increase throughout fermentation. Ethanol levels were highest at 72 hours, with the ethanol content ranging from 4.80 to 6.76%. Based on ANOVA followed by the Tukey test at the 95% confidence level, it can be seen that the duration of fermentation affected the level of ethanol. The highest ethanol content was found at an initial pH of 5 in the presence of nitrogen gas and at an initial pH of 4 for hydrogen gas. Therefore, the initial pH of the medium influenced the amount of ethanol produced.

The results show that the average levels of ethanol produced in the presence of hydrogen gas tended to be higher when compared to fermentation in the presence of nitrogen gas. The result of the Tukey test at the 95% confidence level indicates that the average levels of ethanol in the presence of hydrogen gas were significantly different to the average levels of ethanol in the presence of nitrogen gas. The highest level of ethanol in the presence of hydrogen gas was 6.76% with the use of reducing sugar as much as 75.05%; the highest level of ethanol in the presence of nitrogen gas was only 5.92% with the use of reducing sugar as much as 63.15%. This is consistent with the results of other studies showing that the presence of hydrogen gas increases the level of ethanol, compared to using nitrogen gas [11].

The results show that the amount of reducing sugars decreased with an increasing duration of fermentation (Figure 4). The more reducing sugars that were used by the microbial cells, the higher the levels of ethanol produced [24,25]. The use of more reducing sugars will produce higher ethanol levels [22,23]. Similar results have been reported in other studies, i.e. that higher ethanol levels lead to a decrease in reducing sugars in the fermentation substrate [13]. The decrease in reducing sugar levels accompanied by increased ethanol levels during fermentation under optimum conditions is shown in Figure 4.

Higher ethanol yields were found with the use of reducing sugars in the presence of hydrogen gas compared to nitrogen gas, showing that hydrogen gas likely acts as a reducing agent for the formation of NADH. NADH has functions in cell metabolism and in the formation of pyruvic acid and ethanol [15,26,27].

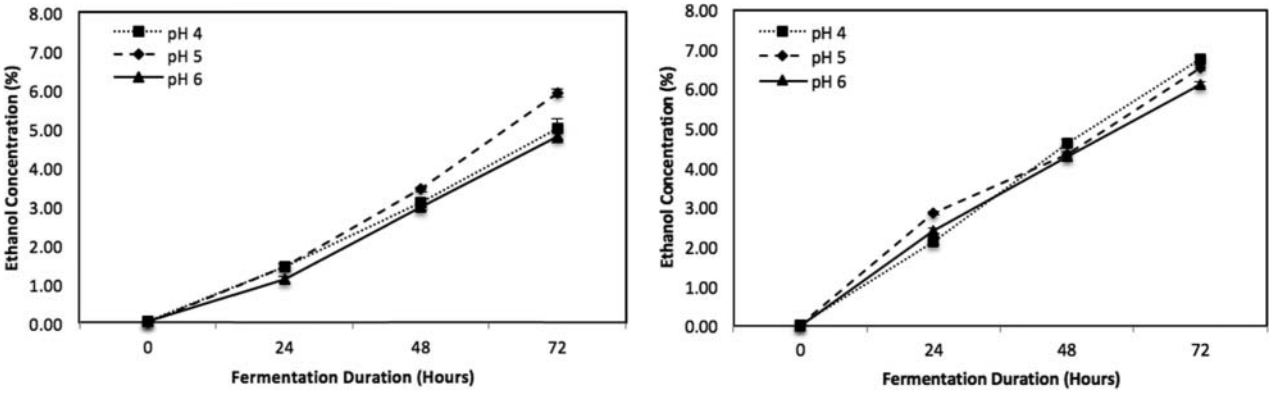

Figure 3. Graph of ethanol concentration that was produced by *S. cerevisiae* in the presence of nitrogen gas (left) and hydrogen gas (right).

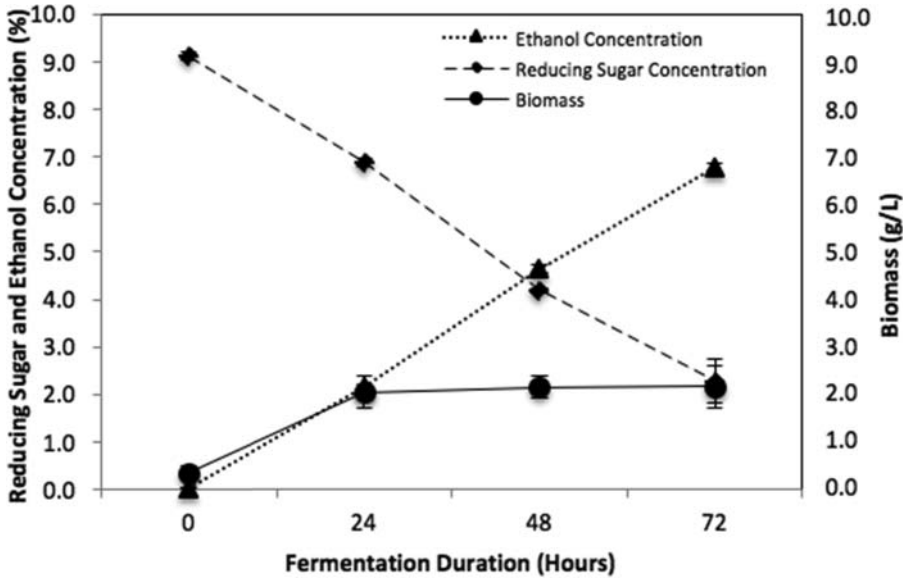

Figure 4. Graph of biomass production, reducing sugar concentration and ethanol concentration during fermentation process in optimum condition.

Therefore, when hydrogen acts as a reducing agent for the formation of NADH, ethanol levels increase, while nitrogen does not act as a reducing agent and cannot increase the ethanol content. The level of ethanol production was higher in the presence of hydrogen gas than nitrogen gas. This premise was supported by the pH, which did not decrease significantly during 72 hours with the addition of nitrogen gas. However, pH decreased significantly over 72 hours with the addition of hydrogen gas. A higher amount of ethanol produced is associated with more pyruvic acid production. Pyruvic acid is an acidic substance that can decrease the pH; thus, the presence of hydrogen gas will decrease the pH more rapidly than the presence of nitrogen gas.

The results show that the *S. cerevisiae* takes 96–120 h to convert reducing sugars from *S. hyalina* hydrolysates into ethanol; this is longer than *Zymomonas mobilis*, which takes only 72 hours [11]. This is because *Z. mobilis* and *S. cerevisiae* have different metabolic pathways; *Z. mobilis* has the Entner-Doudoroff metabolic pathway, while *S. cerevisiae* has the Embden-Meyerhof metabolic pathway. *S. cerevisiae* uses carbon sources through the Embden-Meyerhof pathway, in which 2 moles of ATP are produced per mole of glucose [28,29]. Meanwhile, *Z. mobilis* can use glucose, fructose or sucrose as the carbon source via the Entner-Doudoroff metabolic pathway [11,30,31], which produces only 1 mole of ATP per mole of glucose or fructose, so *Z. mobilis* uses sugar at a higher rate in order to produce enough energy for growth. Because only 1 mole of ATP is produced per mole of sugar, *Z. mobilis* transforms glucose quickly to provide ATP. This causes *Z. mobilis* to produce ethanol faster when compared with *S. cerevisiae*.

Ethanol fermentation using the Hungate technique with hydrogen gas produced 14.19% more ethanol when compared to nitrogen gas. This shows that ethanol fermentation using *Spirogyra hyalina* should be done by replacing the gas contained in the fermentor space with hydrogen gas. This is supported by other studies showing that the Hungate technique using hydrogen gas increases ethanol production and speeds up the fermentation process [13]. Using the Hungate technique with hydrogen is effective for ethanol formation by *Saccharomyces cerevisiae*. Subsequent studies are suggested to evaluate the Hungate technique with the addition of nutrients to the fermentation medium. This study also supports the finding that hydrogen serves as a reducing agent for the formation of NADH, which increases the ethanol content.

Conclusion

The highest levels of ethanol and biomass were achieved by *Saccharomyces cerevisiae* in the presence of hydrogen gas. This shows that hydrogen gas injected into the fermentor space can act as a reducing agent for the formation of NADH, which in the metabolism of *S. cerevisiae* plays an important role in the formation of ethanol. The highest ethanol levels were achieved by *S. cerevisiae* in the presence of hydrogen gas at pH 4 after 72 hours of fermentation, i.e. about 6.76% (v/v) ethanol produced with the use of 75.05% reducing sugars.

Disclosure statement

No potential conflict of interest was reported by the authors.

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References

[1] Speirs J, McGlade C, Slade R. Uncertainty in the availability of natural resources: fossil fuels, critical metals and biomass. Energ Policy. 2015;87:654–664.
[2] Tvinnereim E, Ivarsflaten E. Fossil fuels, employment, and support for climate policies. Energ Policy. 2016;96:364–371.
[3] Atilgan B, Azapagic A. Life cycle environmental impacts of electricity from fossil fuels in Turkey. J Clean Prod. 2015;106:555–564.
[4] Li K, Liu S, Liu X. An overview of algae bioethanol production. Int J Energ Res. 2014;38(8):965–977.
[5] Jang J, Cho Y, Jeong G, et al. Optimization of saccharification and ethanol production by simultaneous saccharification and fermentation (SSF) from seaweed, *Saccharina japonica*. Bioprocess Biosystems Eng. 2012;35(1):11–18.
[6] Soha RA, Khalil AA, Abdelhafez EA, et al. Evaluation of bioethanol production from juice and bagasse of some sweet sorghum varieties. Ann Agr Sci. 2015;60(2):317–324.
[7] Faraco V. Lignocellulose conversion: enzymatic and microbial tools for bioethanol production. Berlin, Heidelberg: Springer-Verlag; 2013.
[8] Vassilev SV, Vassileva CG. Composition, properties and challenges of algae biomass for biofuel application: an overview. Fuel. 2016;181(1):1–33.
[9] Sarkar D, Shimizu K. An overview on biofuel and biochemical production by photosynthetic microorganisms with understanding of the metabolism and by metabolic engineering together with efficient cultivation and downstream processing. Bioresource Bioprocess. 2015;2(17):1–19.
[10] Jones CS, Mayfield SP. Algae biofuels: versatility for the future of bioenergy. Curr Opin Biotech. 2012;23(3):346–351.
[11] Sulfahri, Ni'matuzahroh, Manuhara YSW. Optimization of the bioconversion of *spirogyra hyalina* hydrolysates to become ethanol using *Zymomonas mobilis*. J Appl Environ Biol Sci. 2012;2(8):374–379.
[12] Sulfahri, Amin M, Sumitro SB, et al. Bioethanol production from algae *Spirogyra hyalina* using *Zymomonas mobilis*. Biofuels. 2016.
[13] Sulfahri, Nurhidayati T, Nurhatika S. Aerobic and anaerobic processes of Spirogyra extract using different doses of *Zymomonas mobilis*. J Appl Environ Biol Sci. 2011;1(10):420–425.
[14] Jeon YB, Hwang TS, Park DH. Electrochemical and biochemical analysis of ethanol fermentation of *Zymomonas mobilis* KCCM11336. J Microbiol Biotechn. 2009;19(7):664–674.
[15] Hou J, Scalcinati G, Oldiges M, et al. Metabolic Impact of Increased NADH availability in *Saccharomyces cerevisiae*. Appl Environ Microb. 2009;76(3):851–859.
[16] Knudsen JD, Carlquist M, Grauslund MG. NADH-dependent biosensor in *Saccharomyces cerevisiae*: principle and validation at the single cell level. AMB Express. 2014;4:81.
[17] Zhang K, Feng H. Fermentation potentials of *Zymomonas mobilis* and its application in ethanol production from low-cost raw sweet potato. Afr J Biotechnol. 20109(37):6122–6128.
[18] Cazetta ML, Celligoi MAPC, Buzato JB, et al. Fermentation of molasses by *Zymomonas mobilis*: effect of temperature and sugar concentration on ethanol production. J Bioresource Technol. 2007;98:2824–2828.
[19] Briyant MP. Commentary on the hungate of anaerobic bacteria. Am J Clin Nutr. 1972;25:1324–1328.
[20] Horwitz W, Alan S, Helen R, et al. Official methods of analysis of the AOAC, 12th edition. AOAC, 1975, Washington DC.
[21] Chaudhary, Naureen, Qazi JI. Microbiological saccharification and ethanol production from sugarcane bagasse. J Biotechnol. 2006;5(4):517–521.
[22] Limtong S, Sumpradit T, Kitpreechavanich V. Effect of acetic acid on growth and ethanol fermentation of xylose fermenting yeast and *Saccharomyces cerevisiae*. Nat Sci. 2000;34:64–73.
[23] Arroyo-López FN, Orlić S, Querol A, et al. Effects of temperature, pH and sugar concentration on the growth parameters of *Saccharomyces cerevisiae*, *S. kudriavzevii* and their interspecific hybrid. Int J Food Microbiol. 2009;131(2–3):120–127.
[24] Itelima J, Ogbonna A, Pandukur S. Simultaneous saccharification and fermentation of corn cobs to bioethanol by co-culture of *Aspergillus niger* and *Saccharomyces cerevisiae*. Int J Environ Sci Dev. 2013;4(2):239–242.
[25] Wiratno EN, Ardyati T, Wardani AK. Effect of reducing sugar and total nitrogen to ethanol production from molasses by *Saccharomyces cerevisiae*. J Exp Life Sci. 2014;4(2):50–55.
[26] Wecker MSA, Zall RR. Production of acetaldehyde by *Zymomonas mobilis*. J Appl Environ Microbiol. 1987;53(12):2815–2820.
[27] Geermen JM, Van Dijken JP, Pronk JT. Engineering NADH metabolism in *Saccharomyces cerevisiae*: formate as an electron donor for glycerol production by anaerobic, glucose-limited chemostat cultures. FEMS Yeast Res. 2006;6(2006):1193–1203.
[28] Tsantili IC, Nazmul K, Maria IK. Quantifying the metabolic capabilities of engineered *Zymomonas mobilis* using linear programming analysis. J Microb Cell Fact. 2007;6(8):1–23.
[29] Reddy GK, Lindner SN, Wendisch VF. Metabolic engineering of an ATP-neutral Embden-Meyerhof-Parnas pathway in *Corynebacterium glutamicum*: growthrestoration by an adaptive point mutation in NADH dehydrogenase. Appl Environ Microbiol. 2015;81(6):1996–2005.
[30] Nowak J. Ethanol yield and productivity of *Zymomonas mobilis* in various fermentation methods. Electronic Journal of Polish Agricultural Universities. 2000;3(2):4–10.
[31] Rutkis R, Kalnenieks U, Stalidzans E, et al. Kinetic modelling of the *Zymomonas mobilis* Entner–Doudoroff pathway: insights into control and functionality. Microbiology. 2015;159:2674–2689.