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Studies on Bioethanol Production of Commercial Baker's and Alcohol Yeast under Aerated Culture Using Sugarcane Molasses as The Media

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Abstract

Efforts to optimize bioethanol production were made by modifying culture condition of the yeasts used. Two commercial yeast (New Aule alcohol yeast and New Aule baker's instant dry yeast) were grown in sugarcane molasses under different condition with and without aeration in order to compare the productivity of both yeast. The fermentation processes were carried out in batch condition for 72 hours incubation time. Aeration rate of 0.3 vvm were provided for four hours at the early stage of the aerated cultures. The level of ethanol produced by New Aule Alcohol Yeast was 74.8 g/L with ethanol productivity of 2.078 g/L/h and yield (Yp/s) was at 0.378 g/g. Aeration of 0.3 vvm did not affect the level of ethanol produced, yielded 0.338 gram of ethanol per gram of substrate used. Interestingly, the result showed that the New Aule baker's instant dry yeast produced higher ethanol compare to that of its alcohol yeast. Without aeration, New Aule baker's instant dry yeast produced 102.854 g/L. Meanwhile, the aerated culture of this yeast increase the ethanol production to the level of 120.917 g/L with productivity 3.359 g/L/h and ethanol yield 0.669 g/g, indicating the differences in oxygen sensitivity of both commercial yeast.

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Keywords: batch culture, bioethanol, molasses, aeration, alcohol and baker's yeast

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1. Introduction

Generally, it's a common thing to know that fossil fuels are non-renewable, finite resources and could harm the environment because of its gas emission. Recently, research and development in finding and improving the production of alternative energy to fossil fuels has been increasing rapidly, one of the most known product is bioethanol. The main advantage of using bioethanol as fuel is because its high octane number, can reduce the CO_2 emission, lower the emission of hydrocarbon, carbon monoxide and particles, so does because of its environmental friendliness, indicating bioethanol as a clean and efficient alternative energy (Pokhrel et al., 2008). Bioethanol can be produced using agricultural waste as a substrate through fermentation by microorganisms. Molasses as one of agricultural waste is widely used as sugar resources for bioethanol production. It was used because of its inexpensive price, availability, and sugars content. Hidayat et al. (2006), stated that molasses has high content of sugars, mainly sucrose (32%), fructose (16%), and glucose (14%).

Meanwhile, the most widely used microorganisms for bioethanol production is *Saccharomyces cerevisae,* which can produce ethanol to concentration as high as 18% of the fermentation broth (Lin and Tanaka, 2006). Nowadays, yeasts which are sold in market can be divided into some groups, whether by types (baker's, brewer's, wine, bioethanol, etc), by applications (bakery, alcoholic beverages, non-alcoholic beverages, etc) and by forms (instant, dry, natural, etc). Every of it would give different result once it's applied in bioethanol production because each one has different composition although the main yeast used were the same. Compare to the natural yeast strains, commercial yeast strains has many advantages due to its long shelf life (over one year), high cells viability (up to 4.6 x 10^{10} yeast cells/g), efficiency (reduce the time and trouble of culture work-up), low or no microbial contamination, inexpensive price, and superior characteristics in one package (high thermal, sugar, alcohol, and acid tolerant) (Rose and Harrison, 2012). Although natural yeast strains also has superior characteristics, but it couldn't be compare to the other advantages using commercial yeast strains, moreover in terms of shelf life, yeast management, and population growth, also the ethanol productivity produced by natural yeast strains were tend to be lower than commercial yeast strains (Milkesa, 2009).

Bioethanol production from molasses with different yeast strains and fermentation condition could give different result in ethanol content. Hence, in this present research, in order to extend the knowledge in bioethanol production, the bioethanol production from molasses using commercial yeast strains under different condition is being evaluated. The purpose of this study are 1) to determine the ethanol concentration during bioethanol production of commercial alcohol and baker's yeast under aerated culture using sugarcane molasses as the media 2) to evaluate the effect of aeration 0.3 vvm during bioethanol production by commercial alcohol and baker's yeast.

2. Materials and methods

Molasses was obtained from local sugar factory (PTPN XI Djatiroto, Lumajang, Indonesia). Commercial yeasts used for this research were New Aule Alcohol Yeast and New Aule Baker's Yeast from Xinjiang Shengli Biotechnology Co., Ltd.

2.1 Preparation of fermentation media and starter

Fermentation media used for this research is molasses. Molasses was diluted to 24^{o}Brix and the pH set to 4,3 using citric acid or NaOH 0,4 M. Furthermore, molasses which has already prepared was settled for 24 hours to stabillize the pH and insoluble solids in molasses then removed by filtration. The nutrition for molasses as fermentation media enriched by adding 100 ppm of Diamonium Hidrogen Phospat $(NH_4)_2HPO_4$. The prepared media was sterilized at 121^{o} C for 15 minutes. Starter from commercial yeast was made by weighing the yeast for 1% (w/v) from the total media used in fermentation, then mixed it with glucose solution 2% ± at temperature 42^{o}C and settled for 3 hours in laminar air flow.

2.2 Batch fermentation

Batch fermentation experiments were carried out under different condition (anaerobe and aerobe). Batch fermentation was performed in 1000 ml sampling bottles (under anaerobe condition) and 2L fermenter (aerobe)

((*Continuous Stirred Tank Reactor*) *applikon dependable instrument* 2L (*marine impeller 3 blades*)) with 1000 ml total liquid volume were initiated by transferring 1% starter to the prepared media. Fermentation under aerobe condition, the fermentation media was flushed with air at flow rate of 0,3 vvm and stirring rate at 100 rpm. No change was applied either to the flow rate or to the stirring rate during the fermentation. The experiments were carried out for 72 hours at room temperature. The experiments were monitored by harvesting ±100 ml samples every 12 hours for analysis.

2. 3 Analytical methods

The yeast cell numbers and total soluble solids of the fermentation broth were determined by direct counting method using total plate count (Ristiati, 2000) and hand-held refractometer, respectively. The reducing sugar in terms of total substrates used during fermentation was determined by dinitrosalisilic acid method (Miller, 1959). Ethanol concentration (*P*, g/L) was analyzed by Chamber Conway method and alcohol 96% was used as an internal standard (Kartika et al., 1992). The ethanol yield (*Yp/s*) was calculated by ethanol concentration produced (*P*, g/L) divided by substrates used (*ΔS*, g/L) and expressed as g ethanol per g substrates used (g/g). The ethanol productivity (*Qp*, g/L/h) was calculated by ethanol concentration produced (*P*, g/L) divided by fermentation time. The fermentation efficiency (η) was calculated by using the ethanol produced divided by theoretical ethanol and multiply by 100%. The data obtained were statistically analyzed using real different test (t test).

3. Results **and discussion**

3.1. Profile of bioethanol production

To evaluate the impact of aeration on bioethanol production, two fermentation conditions were tested. A first fermentation was carried out under static condition (non aerated). The second condition was carried out under full aeration, i.e. aeration conditions were set to avoid any oxygen limitation during fermentation. The mass of reducing sugar consumed, ethanol produced, and numbers of yeast population during fermentation are shown in Fig. 1.

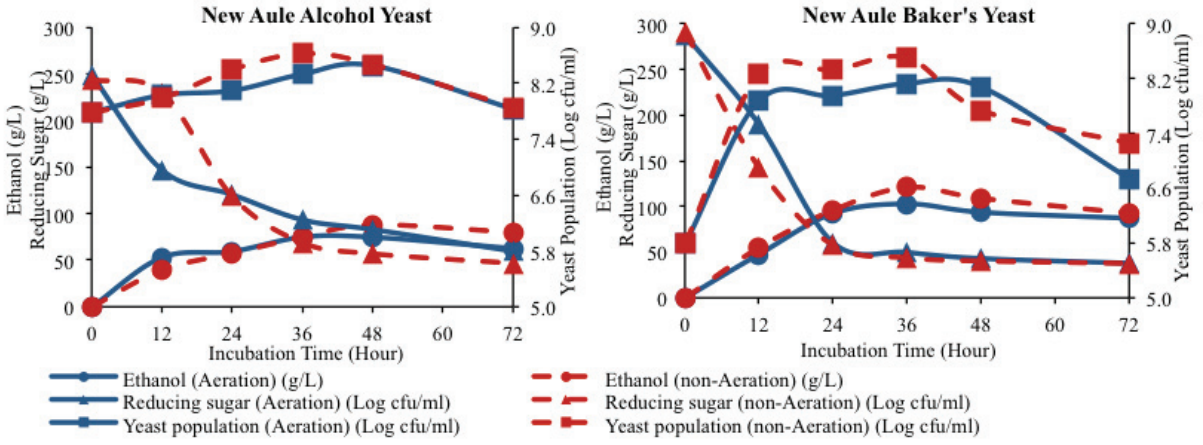

Fig. 1. Changes in measured parameters of yeast population, reducing sugar concentration, and ethanol concentration during bioethanol production by New Aule Alcohol Yeast and New Aule Baker's Yeast under non-aerated and aerated culture.

During fermentation, both yeast under both fermentation condition were well grown and proliferated. The increase of number in yeast population occur until 36 hours of incubation time. The maximum number of yeast population by New Aule Alcohol Yeast And New Aule Baker's Yeast under non-aerated culture were not significantly different throughout fermentation, as respectively the result are 8.33 log cfu/ml and 8.13 log cfu/ml.

Under aerated culture, the maximum number of yeast population by both yeast showed an increase compare to non-aerated culture. The maximum number of yeast population by New Aule Alcohol Yeast And New Aule Baker's Yeast under aerated culture were not significantly different throughout fermentation, as respectively the result are 8.62 log cfu/ml and 8.50 log cfu/ml.

Fig. 1 showed that the increase on population number of yeast strains until 36 hours of incubation time was hypothesized because of the availability of sugars from molasses as the source of energy is high enough for the growth of yeast. The number of yeast population from each strains during the 36 hours until 48 hours of incubation time was tend to be stable. It was hypothesized that in those fermentation time, each yeast strains was going through the stationary phase (in which the phase of yeast growth is slowly decreasing/become limited and the cells stop dividing). The two commercial yeasts were hypothesized going through in an early death phase because of the decreasing number in yeast population during the 48 hours until 72 hours of incubation time. The decreasing of yeast population number was hypothesized because of the sugars content decreased so that it was not possible for the yeast to grow any further.

Based on t-test, the maximum numbers of yeast population under non-aerated culture compare to aerated culture were shown to be significantly different. It means that aeration could affect the number of yeast population during fermentation. Aeration in the culture media gave oxygen supply to yeast because in the earlier stage of incubation time, yeast were undergone aerobic fermentation to optimize its growth, so the result shown the increasement in number of yeast population. Khongsay et al. (2012), reported that aeration in the earlier stage of fermentation is importatnt for yeast due to its needed to synthesize cell membran (sterols and unsaturated fatty acids), which are essential to assure cell membrane integrity as well as to vent out CO_2 that could inhibit yeast growth. Meanwhile, Alfenore et al. (2004), stated that aeration 0.2 vvm in culture under fed-batch fermentation could increase cell viability up to 23%. Hence, the maximum numbers of yeast population under aerated culture were tend to be higher than yeast under non-aerated culture because the oxygen availability in media were well used for yeast growth.

Molasses used as a substrate in fermentation because it has fairly high sugar content, so it could meet the needs of carbon source required by yeast during metabolism. The reducing sugar concentration at 0 hour incubation time by New Aule Alcohol Yeast and New Aule Baker's Yeast under both fermentation condition were approximately 300 g/L. Throughout fermentation, reducing sugar were well used by both yeast indicated by the increase in the number of yeast population and ethanol produced. A sharp drop of reducing sugar concentration during 36 hours incubation time occured and then were slowly going down until 72 hours incubation time for both yeasts. The reducing sugar concentration of both yeast in the end of incubation time decreased more than 50% until 36 hours of incubation time. Buglass (2011) stated that 50% sugar availabilities in substrate were used by yeast during logarithmic phase. Hence, the reducing sugar consumed by both yeast were high during the phase. The reducing sugar consumed by New Aule Alcohol Yeast and New Aule Baker's Yeast under non-aerated culture at 36 hours of incubation time were 196.274 g/L and 237.583 g/L, respectively. Under aerated culture, reducing sugar consumption by both yeast were tend to be higher compare to non-aerated culture, with reducing sugar consumption at 221.880 g/L and 246,649 g/L, indicated that aeration led to increasement in sugar consumption of both yeast. Yan et al. (2009) stated that oxygen availability in the earlier fermentation used by yeast for respiration, so that it could accelerate yeast growth and agitation could homogenize fermentation media, so nutrition and oxygen supply added were well utilized by yeast. Correlated with Rodmui et al. (2008), stated that controlled aeration during lag and log phase could incerase sugar consumption in substrate which could accelerated lag phase and increasement in biomass production.

Saccharomyces cerevisiae produced metabolite such as alcohol dehydrogenase enzyme which can convert sugars to ethanol. During fermentation, sugars content in media used by yeast were not only for growth and proliferation, but also to produce ethanol. Glucose in substrate will be converted to pyruvic acid through glycolysis. The pyruvic acid then converted to acetaldehyde and CO_2, the acetaldehyde further will be converted to ethanol by alcohol dehydrogenase enzyme (Buglass, 2011). Based on Fig.1 showed that the increase in ethanol concentration by both yeast occured until 36 hours of incubation time. The maximum ethanol concentration produced by New Aule Alcohol Yeast and New Aule Baker's Yeast under non-aerated culture were significantly different, with result at 74.80 g/L and 102.85 g/L, respectively. Meanwhile, under aerated culture the ethanol produced by New Aule Alcohol Yeast was not significantly different compare to its non-aerated culture showed that there was no significant effect given aeration in culture media during 36 hours fermentation, but different at 48 hours fermentation led to an

increase on ethanol production until 87.94 g/L. The ethanol produced by New Aule Baker's Yeast under aerated culture were significantly different or tend to be higher compare to its non-aerated culture, with ethanol produced at 120.92 g/L. Based on the explanation above, it showed that aeration affect the ethanol concentration produced by both yeast indicated an increase in ethanol production. Correlated with Alfenore et al. (2004), reported that aeration 0.2 vvm during fed-batch fermentation could increase ethanol production from 128.1 g/L (non-aeration) to 143.8 g g/L.

The different in ethanol concentration produced by both yeast was hypothesized due to its ability by both yeast to adapt under such fermentation condition. The New Aule Alcohol Yeast tend to need longer time (48 hours of incubation time) to produce maximum ethanol concentration under aerated culture, meanwhile New Aule Baker's Yeast only need the exact same incubation time (36 hours) to produce maximum ethanol concentration under aerated culture. It was hypothesized that this condition could happen because of several factors, such as temperature, pH, etc. New Aule Alcohol Yeast is popular for its thermal tolerance (best temperature to be 38-40^{o}C) and acid tolerance (pH 2.5), so due to the uncontrolled temperature and pH during incubation time, this yeast couldn't reach maximum ethanol production, indicating further optimum fermentation condition need to be studied for this yeast.

The decrease in ethanol concentration occurred at the end of incubation time of both yeast. It was hypothesized, both yeast were in a death phase, so that it couldn't convert sugars to ethanol optimally. Throughout fermentation, yeast was not only produce alcohol, but also by products. Mukhtar et al. (2010) stated that during alcoholic fermentation, *Saccharomyces cerevisiae* was not only producing alcohol, but also by product such as organic acids and acetaldehyde which can decrease the alcohol content. The alcohol accumulated in sample interacted with organic acids and formed ester compounds and make the alcohol content of sample decreased.

3.2. Kinetics of bioethanol production

Efficiency of bioethanol production can be evaluated by three parameters, such as yield, productivity, and final product concentrations. Ethanol yield can be referred to either as metabolic yield or process yield, calculated as ethanol produced based on sugars consumed. The maximum metabolic yield for both hexoses and pentoses are 0.51 gram ethanol per gram sugars used (Kent, 2013). Based on Table 1, it showed that both yeast under non-aerated as well as on aerated culture produced ethanol yield up to more than 60% of theoretical ethanol yield, indicating fermentation were doing well. However, ethanol yield produced by New Aule Alcohol Yeast under both fermentation condition were lower than ethanol yield produced by New Aule Baker's Yeast. Lower result in ethanol yield of New Aule Alcohol Yeast was because the ethanol produced by the yeast was not proportional to the sugars consumed.

Table 1. Kinetics parameters of bioethanol production at 36 hours fermentation

Parameters*	New Aule Alcohol Yeast		New Aule Baker's Yeast	
	Non-Aeration	Aeration	Non-Aeration	Aeration
Yp/s (g/g)	0,38	0,34	0,43	0,49
Qp (g/L/ h)	2,08	2,07	2,86	3,36
(η) (%)	74,04	66,08	84,71	95,92

In contrary, higher result in ethanol yield of New Aule Baker's Yeast was because the ethanol produced by the yeast were proportional to the sugars consumed. It means aeration gave better effect to ethanol yield production. Yan et al. (2009), reported that aeration 4-9 mg/L along with 200 rpm agitation could increase ethanol yield production during fermentation. Ethanol yield under static condition only produced ethanol yield 0.249 g/g, in contrary, under aerated culture the ethanol yield produced increase up to 0.471 g/g, indicated higher ethanol produced during fermentation. The commercial strains of *Saccharomyces cerevisiae* usually grow and produce ethanol between 30°C and 32°C, their ethanol theoretical yields are usually among 90-93% (Bai et al., 2008 in Thammasittirong et al., 2013). Therefore, the high ethanol yield at 0,49 g g^{-1} (96%) of New Aule Baker's Yeast from

ethanol fermentation under aerated culture demonstrated the high ethanol production from sugarcane molasses was successful.

Lower ethanol concentration produced by New Aule Alcohol Yeast were also affect the ethanol productivity and fermentation efficiency which shown in Table 1. In general, it showed that aeration mostly did not affect the bioethanol production by New Aule Alcohol Yeast which may be attributed to their low ability under such low temperature (Lin et al., 2012). As already explained above, it was hypothesized due to New Aule Alcohol Yeast ability which has not reached to its optimum point. It was believed that further requirement of proper fermentation conditions need to be explored. Correlated with Mukhtar et al. (2010), reported that bioethanol production by commercial baker's yeast (Saf-Instan) produced ethanol concentration 8.8% (v/v) which was slightly higher than the commercial alcohol yeast (Ethanol red) only produced 8% (v/v). Based on explanation above, aeration improved the bioethanol production by New Aule Baker's Yeast and it has better efficiency in bioethanol production compare to New Aule Alcohol Yeast.

4. Conclusion

The level of ethanol produced by New Aule Alcohol Yeast was 74.8 g/L with ethanol productivity of 2.078 g/L/h and yield (Yp/s) was at 0.378 g/g. Aeration of 0.3 vvm did not affect the level of ethanol produced, yielded 0.338 gram of ethanol per gram of substrate used. Interestingly, the result showed that the New Aule baker's instant dry yeast produced higher ethanol compare to that of its alcohol yeast. Without aeration, New Aule baker's instant dry yeast produced 102.854 g/L. Meanwhile, the aerated culture of this yeast increase the ethanol production to the level of 120.917 g/L with productivity 3.359 g/L/h and ethanol yield 0.669 g/g, indicating the differences in oxygen sensitivity of both commercial yeast.

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