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Research Article

The Selection and the Growth Condition Optimization of Ethanol-Producing Microbes Isolated from *Ragi* Tapai

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ABSTRACT

Ethanol is a biofuel produced from renewable resources, which potentially plays an important role in solving future fuel problems. This study aimed to select the highest ethanol-producing isolate from candidates obtained from previously isolated candidates from *ragi* and cassava tapai. The selection process was conducted in 2 stages, namely: 1) Selection of the highest ethanol-producing isolate from seven isolate candidates using PYG media containing peptone, yeast extract, and glucose at 0.75%, 0.75%, and 15%, respectively and was followed by: 2) Optimization of the growth conditions of the highest ethanol-producing isolate, which was conducted at various temperatures of 27, 30, 33, 35, 37, and 40°C with the combination of various pH of 3.5, 4.5, 5.5, and 6.5. The experimental results showed that the R5I3 isolate was the highest ethanol-producing performance isolate, which yielded approximately 4.69±0.25% (*v/v*). Following the temperature and pH optimization of the fermentation processes, the optimum growth conditions were at 35°C and pH 5.5, where the ethanol produced was increased to 8.63 ± 0.04% (*v/v*). With these results, this new strain has the potential to be used in bioethanol production processes and other industrial applications.

Keywords: Cassava tapai, Ethanol-producing microbe, Optimization condition, Ragi tapai

Introduction

The demand for petroleum fuel is increasing rapidly in developed and developing countries [1]. One solution to overcome the use of non-renewable fossil energy, which results in global warming and environmental pollution, is using bioethanol as fuel [1–4]. Bioethanol is one of the most critical fuels sourced from renewable energy, which will play an essential role in effectively solving future problems [5].

Renewable energy is very important in reducing the use of fossil energy [6]. Renewable energy development involves microbial activity to convert biomass into biofuels such as bioethanol, biogas, and biodiesel [7, 8]. Production of ethanol as an eco-friendly renewable fuel is one of the solutions constantly being developed [6]. Bioethanol is a renewable energy source obtained from fermentation of sugar and plant starch components [7]. The production from sugar-containing substrates requires potential microbes to convert sugar into ethanol [7].

Potential microbes in producing ethanol can be obtained by isolating microbes from several sources such as fruit, yeast, soil, and fermentation products. These ethanol-producing microbes can also easily be found in the Indonesian marketplace in yeast form. *Ragi* tapai is a dry starter culture made from rice flour, spices, and water or sugarcane juice/extract [9, 10]. Various amylolytic and fermentative microbes have been isolated from

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various brands of *ragi* tapai acquired from various places and markets, such as *Amylomyces rouxii, Rhizopus oryzae, Endomycopsis burtonii, Mucor* sp., *Candida utilis, Saccharomycopsis fibuligera, Saccharomyces cerevisiae* and some lactic acid bacteria *Pediococcus pentosaceus, Lactobacillus plantarum,* and *L. fermentum* [10–12]. Studies from the Philippines, Malaysia, Thailand, and Vietnam found similar microbial species in the inoculum of tapai [10].

Isolation of ethanol-producing microbes from natural resources to produce higher ethanol as a replacement for fossil fuels is essential in energy trends [1, 4]. Gunam *et al.* [10] previously isolated microbes from *ragi* (tapai fermentation starter) and cassava tapai; about seven isolates capable of producing amylase enzymes were obtained. The isolates were tested for their ability to produce ethanol using Peptone Yeast Glucose (PYG) media. Some amylolytic microbes can ferment starch into sugar and convert into ethanol [13]. The isolates may have different optimal growth conditions that need to be researched to maximize the yield of sugar bioconversion into ethanol.

According to Sonwani *et al.* [14], yeast has optimum activity at a temperature of 30–35ºC and a pH of 4.5–5.5. Hashem *et al.* [15] reported the optimal temperature of ethanol fermentation at 30ºC and pH 5. Meanwhile, Hashem *et al.* [16] research obtained the optimal temperature at 35ºC and a pH of 5.5. Thus, this research aimed to select the highest ethanol-producing isolate from previously obtained candidates and optimize fermentation temperature and pH to produce maximum ethanol yield [14], in which optimization results would provide convenience in larger-scale ethanol production using this isolate in the future.

Material and Methods

Microbe selections

A total of seven isolate candidates from *ragi* and cassava tapai were obtained from previous work [10] (R2I5.1, R5I3, R5I4, T1I4, T2I2.1, T2I2.2, and T2I6.1), which were stored in the glycerol. The stocks were rejuvenated and cells were multiplied using PYG media (Peptone 0.45 g/L, yeast 0.75 g/L, and glucose 5 g/L in 100 mL of distilled water). The rejuvenated isolate candidates were incubated at room temperature for 24 hours, and further propagation was carried out for 48 hours using a rotator shaker at 125 rpm. Following cell propagation, each of the cells were washed twice with sterile 0.85% NaCl solution, vortexed, and centrifuged for 3 minutes at 10,000 rpm at 4ºC. The precipitated cultures were obtained at the bottom of the centrifugation tube after the cell washing stage [1, 17, with modifications] for further use.

Fermentation test for selection from the seven candidates and later optimization of the highest ethanol-producing isolate were conducted using PYG media with the concentration of peptone 0.45% (*w/v*), yeast 0.75% (*w/v*), and glucose 15% (*w/v*). Each isolate candidate was used as a fermentation agent with 500 mL PYG as media, where 4% (*w/v*) culture isolate OD_{660} ±5 was added into the fermentation jar, followed by fermentation for 96 hours at room temperature. The alcohol distillation of each fermentation liquid was carried out according to the procedure used by Khan [18], and the ethanol results from the distillation of the candidates and the highest-ethanol-producing isolate were measured using an alcohol meter and then gas chromatography, respectively [19, 20, with modifications].

Media pH and fermentation temperature optimization of the highest ethanol-producing isolate

Optimization of the ethanol yield from the fermentation process of the highest ethanol-producing isolate was carried out by testing factors of fermentation temperature and pH of media. Mohseni *et al.* [5] researched by optimizing fermentation conditions at temperatures 25, 30, 35, and 40°C and at pH 2, 4, 6, and 8. Sonwani *et al.* [14] also conducted optimization of fermentation at temperatures of 25, 30, 35, 40, and 45°C, with pH 5, 6, 7, 8, and 9. Thus in this experiment, the first factor was the fermentation temperature to be conducted at 27, 30, 33, 35, 37, and 40ºC, while the second factor is the initial pH of media which decided at 3.5, 4.5, 5.5, and 6.5 so 24 combinations were obtained.

After obtaining the highest ethanol-producing isolate producing the highest ethanol yield from the seven candidates, the fermentation temperature and media pH were optimized using previously determined temperature and pH variables. The fermentation media was PYG, with the initial pH adjusted according to the determined treatment before the addition of the isolate culture, where fermentation was conducted at predetermined

temperatures. All experimental treatments were repeated twice to obtain 48 experimental units.

Observed parameters

The pH was measured using a standardized pH meter with buffer 4 and distilled water to select the highest ethanol-producing isolate from the seven candidates. The pH meter was immersed in the fermentation media before and after fermentation, which is presumed to contain ethanol, and the value is shown [21, 22]. The total dissolved solids measurements was carried out using a hand-refractometer, which prior of using were rinsed with distilled water and wiped with clean soft cloth. The sample was dripped onto the refractometer prism, and the degree of Brix was measured [22, 23]. The total reducing sugar test using 3,5-dinitrosalicylic acid (DNS) reagent and absorbance were measured at a maximum wavelength of 540 nm using a UV spectrophotometer [1, 24]. The ethanol content was determined using an alcohol meter, calibrated at concentrations of 5, 10, 15, 30, 50, and 70% (*w/v*). After calibration, the alcohol meter was dipped into each sample distillate, and the results were read to measure the concentration of ethanol produced. Meanwhile, for fermentation for the highest ethanol-producing isolate, the resulting alcohol content was accurately measured using Gas Chromatography (GC) variant 3300. The ethanol content in the sample was calculated by the following equation [1]:

$$\text{Ethanol}=\left(\frac{Sampling\ area}{Standard\ area}\right)\text{x Standard concentration}$$

Data analysis

The data obtained were analyzed using variance (ANOVA), and when there was a significant effect of treatment, it was continued with the Tukey test.

Results and Discussion

Microbial selection

Based on total dissolved solids (TDS), pH, and the resultant ethanol concentration, seven microbial isolates were chosen as the most promising for future ethanol production [10] .

The starting total dissolved solids content of the media used in this microbial selection varied from 14.10±0.14 %brix to 15.00±0.00 %brix. At the end of the fermentation, the total dissolved solids content for all samples were reduced which

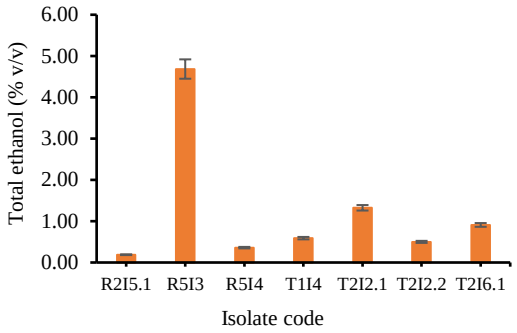

Figure 1. Total ethanol of each candidate at the ethanol-producing isolate selection stage

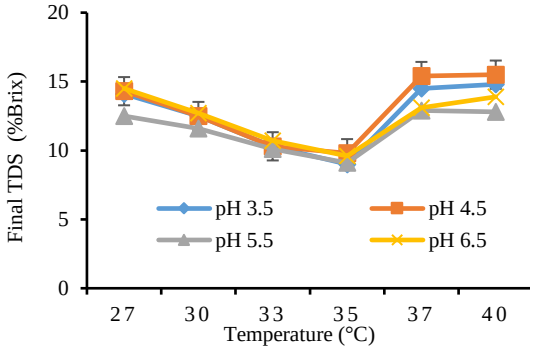

Figure 2. Optimization of the fermentation temperature and pH

ranged from 9.20±0.28 to 13.90% Brix. Based on data from Table 1, only 3 isolates were able to reduce total dissolved solids, namely isolates R5I3, T2I2.1, and T2I6.1. Isolate R5I3 reduced the highest total dissolved solids with a reduction of 5.60 %brix. Total dissolved solids represent the content of sugar, minerals, metals, and salts [25]. According to Mellicha *et al.* [1], the decrease in total dissolved solids occurs because microbes break down glucose to decrease the glucose content, which results in the total value of dissolved solids. Furthermore, [1] decreased total dissolved solids were reported due to glucose convertion into ethanol by microbes. Substrate and nutrients contained in the media were decreased during the fermentation process with the total dissolved solids [1]. Napitupulu *et al.* [22] reported that after fermentation for 92 hours, there was a decrease in the value of total dissolved solids using *S. cerevisiae*.

Changes in the TDS value also affect the final pH value produced during fermentation. The decrement in the TDS value indicates during the fermentation process the microbes actively convert glucose into ethanol, and also produce by-products which result in more acidic pH of media. The initial pH value is an essential factor affecting the

Table 1. Changes in the value of total dissolved solids at the start and the end of fermentation

Isolate code	Initial total dissolved solids (%brix)	Final total dissolved solids (%brix)	Reduction in total dissolved solids
R2I5.1	14.30 ± 0.14 b	13.90 ± 0.14 a	0.40
R5I3	14.80 ± 0.28 a	9.20 ± 0.28 b	5.60
R5I4	14.10 ± 0.14 b	13.70 ± 0.14 a	0.40
T1I4	14.10 ± 0.14 b	13.80 ± 0.00 a	0.30
T2I2.1	15.00 ± 0.00 a	13.90 ± 0.42 a	1.10
T2I2.2	14.80 ± 0.28 a	13.90 ± 0.14 a	0.90
T2I6.1	14.90 ± 0.14 a	13.90 ± 0.14 a	1.00

Notes: Fermentation was carried out at room temperature with initial pH of 6–7 for 96 hours. Different letters behind the mean value indicate significant differences at $p \leq 0.05$ (Tukey's HSD test).

Table 2. The value of pH changes during fermentation

Isolate code	Initial pH	Final pH	Reduction pH
R2I5.1	6.80 ± 0.00 ab	4.35 ± 0.49 a	2.45
R5I3	6.85 ± 0.07 a	4.00 ± 0.00 a	2.85
R5I4	6.75 ± 0.07 abc	4.40 ± 0.00 a	2.35
T1I4	6.80 ± 0.00 ab	4.25 ± 0.07 a	2.55
T2I2.1	6.60 ± 0.00 bcd	4.20 ± 0.00 a	2.40
T2I2.2	6.50 ± 0.00 d	4.35 ± 0.07 a	2.15
T2I6.1	6.55 ± 0.07 cd	4.50 ± 0.00 a	2.05

Note: Fermentation was carried out at room temperature with initial pH of 6–7 for 96 hours. Different letters behind the mean value indicate significant differences at $p \leq 0.05$ (Tukey's HSD test).

fermentation process. Table 2 with the decrement of media initial pH shows all seven isolate candidates were producing organic acids after fermentation for 96 hours. However, each isolate type had different decrement of pH values. According to Napitupulu *et al.* [22] and Gunam *et al.* [4], CO_2 and other organic acids were formed, include acetic, pyruvic, and lactic, which can lower the pH value. In contrast, butyric and fatty acids have little effect in decreasing the pH of the substrate [1]. In this study, isolate R5I3 had higher pH reduction of pH value from an initial pH of 6.85±0.07 to 4.00±0.00, with delta of 2.85. Thus, it can be concluded that isolate R5I3 can produce higher organic acids which may indicate higher ethanol yield.

At the selection stage, each isolate candidate showed different ability to convert existing glucose. The changes in the final value of TDS and pH might greatly affect the total ethanol produced during fermentation. Figure 1, it can be seen that each candidate can convert glucose into ethanol althouh with varying yields. The isolate with the highest and lowest ability to produce ethanol was R5I3 and R2I5.1, repectively. After fermentation for 96 hours, isolate R5I3 was able to produce ethanol of 4.69±0.25% v/v. The research of Choi *et al.* [26] similarly produced 4.04±0.14% (*w/v*) ethanol after 68 hours of fermentation using CHFY0201 isolate yeast. Mohseni *et al.* [5] found that yeast Zym6 isolate produced ethanol of 6.28 g/L. At this stage, isolate R5I3 was found to produced the highest total ethanol among other candidates, thus selected as highest ethanol-producing isolate for further optimization.

Optimization of fermentation temperature and pH

The previous research stage determined the most potential isolate which found to be R5I3 producing highest ethanol yield. Further experiments were then conducted to determine the optimum conditions of the fermentation process using PYG as media and 96 hours fermentation time, at several temperatures and pH's according to the experimental design.

The total dissolved solids (TDS) were measured at the end of the fermentation, where the initial TDS have been equalized by 15–16% Brix. The final total soluble solids produced after fermentation for 96 hours with isolate R5I3 are presented in Figure 2. The temperature and pH of fermentation affected the total dissolved solids produced. From 27–35°C, a decrease of total dissolved solids were observed. However, when the fermentation was carried out above 35°C, the final total dissolved solids did not decrease further. Similarly, the pH measurement (Figure 3) showed different results, and Figure 2 shows that the fermentation treatment of 35°C and pH 5.5 resulted in the smallest final total dissolved solids value. The fermentation temperature of 35°C and pH of 5.5 might result in optimal conditions that can reduce the total dissolved solids. The significant decrease in total soluble solids may be due to the isolate optimally metabolizing the fermentation medium and glucose into ethanol and CO_2 [22]. Total dissolved solids tend to be constant at low pH because sugar consumption is in the stationary phase, or the acidity of the media can kill or inactivate microbes in converting glucose into alcohol [1, 4, 22].

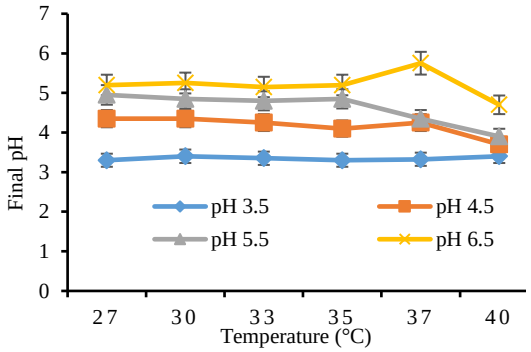

Figure 3. Changes in final pH value after fermentation

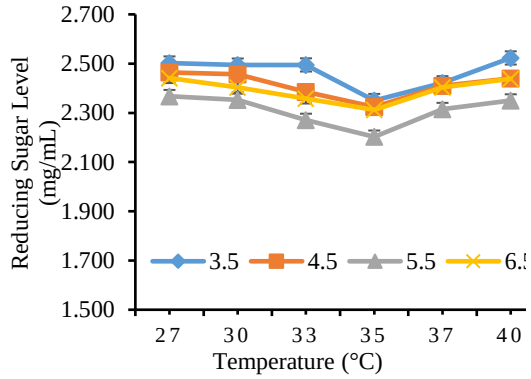

Figure 4. Changes in the remaining reducing sugar content upon fermentation

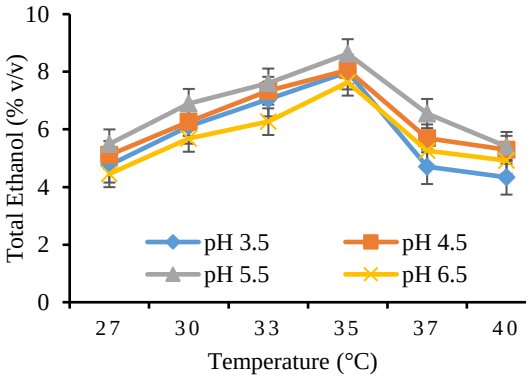

Figure 5. The average value of total ethanol at different initial temperatures and pH treat-

This study adjusted the initial pH before fermentation to pH 3.5, 4.5, 5.5, and 6.5 using a citric acid buffer. Figure 3 shows the resulting final pH did not considerably vary regardless of the starting pH value. In addition to the initial pH of the fermented medium, the temperature may also affects the final pH produced. The higher the fermentation temperature, the lower the resulting pH was observed. Microbial and yeast growth occurs in the pH range of 2–9 [21] and 4–6 [21, 27]. Mohseni *et al.* [5] stated that the initial pH of the medium below 4 could not convert glucose into ethanol. This is due to the medium's acidic nature which prevents microbes' growth. Similarly, Sonwani *et al.* [14] stated that the pH significantly affects the microbes' performance.

The reducing sugar content test was carried out to determine the optimal condition of the R5I3 isolate. Figure 4 shows the lower the initial pH of the fermentation medium, the higher the remaining reducing sugar content. Decrease in the value of reducing sugar from 27°*C* to 35°*C* and increased at 37°*C* and 40°*C* was observed. The lowest value of reducing sugar content was found at a temperature of 35°*C* and a pH of 5.5. Melicha *et al.* [1] stated that the more reduced sugar consumed by microbes, the higher the ethanol produced.

The fermentation process was carried out using isolate R5I3 for 96 hours, and distillation was conducted at a temperature of 78–80°C. The average value of total ethanol (*% v/v*) is presented in Figure 5. Figure 5 shows each pH treatment experienced an increase in total ethanol from a fermentation temperature of 27 to 35°*C*. Figure 4 provides a clue of the optimal fermentation temperature and pH for R5I3 isolate upon producing ethanol are at 35°*C* and with pH of 5.5 with a total ethanol value of 8.63±0.04% (*v/v*). Furthermore, Choi *et al.* [26] reported that the maximum growth temperature of isolate CHFY0201 was 35°*C*. In comparison, Ogbonda *et al.* [21] stated that the optimal temperature and pH of *Blastomyces* sp. from the palm weevil intestines were at 30°*C* and 5.5, producing 6% ethanol. Esmaeili and Keikhosro [28] used Mucor hiemalis to produce ethanol of 5.5% (*v/v*) with optimal temperature and pH at 30°*C* and at pH 5.5. Mohseni *et al.* [5] stated that the optimum conditions for ethanol production were media with pH ranging from 5–6 with a growth temperature of 35°*C*. From these several types of research, the R513 as the highest ethanol-producing microbe isolate, was found to have slightly different optimum conditions for ethanol production. However, the results still coincide with the optimal pH for ethanol productions, which were between 5 and 6, with the optimal fermentation temperature of 30–35°*C*.

Conclusion

In conclusion, the highest ethanol-producing isolate in ethanol production was R5I3 which yielded 4.69 ± 0.25% (*v/v*) ethanol. After optimization of temperature and pH of growth conditions, the optimum conditions were obtained at 35°*C* with pH 5.5, which would yielded total ethanol of 8.63 ± 0.04% (*v/v*), where fermentation temperature above 35°*C* was found to reduce the ethanol yield. These results indicates R5I3 isolate has the potential to produce high ethanol yield upon use on a larger scale in the future.

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References

1. Mellicha SV, Gunam IBW, Antara NS, Arnata IW (2021) Production of bioethanol from wild cassava crude starch (*Manihot glaziovii* Muell. Arg) using different microbial types and fermentation times. IOP Conf. Series: Earth and Environmental Science 913(2021)012032 doi:10.1088/1755 -1315/913/1/012032.
2. Arnata IW, Gunam IBW, Anggreni AAMD, Wijaya IMM, Sartika D (2021) Utilization of solid tapioca waste for bioethanol production by co-fermentation of baker's and tapai yeast. IOP Conf. Series: Earth and Environmental Science724(2021)012058 doi:10.1088/1755-1315/724/1/012058
3. Gunam IBW, Sitepu A, Antara NS, Triania IGAL, Arnata IW, Setiyo Y (2021) bacterial desulfurization of dibenzothiophene by *Pseudomonas* sp. strain KWN5 immobilized in alginate beads. Jurnal Teknologi 83(2): 107–115doi: 10.11113/jurnalteknologi.v83. 15080.
4. Gunam IBW, Dewi IAPK, Antara NS, Anggreni AAMD, Setiyo Y (2021) Bioethanol production from sugarcane bagasse by *Saccharomyces cerevisiae* ATCC 9763 immobilized in Na-alginate. IOP Conf. Series: Earth and Environmental Science 824(2021)012054doi:10.1088/1755-1315/82 4/1/012054.
5. Mohseni M, Ebrahimi H, Chaichi MJ (2013) Isolation and optimization of ethanol producing bacteria from natural environments of Mazandaran province in Iran. Journal of Genetic Resources 1(1): 35–44 doi: 10.22080/jgr.2015.1120.
6. Pranata WS, Suryadarma P, Mangunwidjadja D (2018) Glycerol utilization as substrate for ethanol production in *Escherichia coli* recombinant under an aerobic condition. The Journal of Tropical Life Science (8(1): 81 – 86 doi: 10.11594/jtls.08.01.14
7. Soetarto ES, RN Putri (2016) Bioethanol fermentation from sugarcane bagasse using ragi tape. AIP Conference Proceedings 1744, 020020 doi:10.1063/1.4953494
8. Gunam IBW, Antara NS, Anggreni AAMD, Setiyo Y, Wiguna IPE, Wijaya IMM, Putra IWWP (2019) Chemical pretreatment of lignocellulosic wastes for cellulase production by *Aspergillus niger* FNU 6018. AIP Conference Proceedings 2155,020040 doi:10.1063/1.5125544
9. Azmi AS, Ngoh GC, Mel M, Hasan M (2010) Ragi tapai and *Saccharomyces cerevisiae* as potential coculture in viscous fermentation medium for ethanol production. African Journal of Biotechnology 9(42): 7122-7127 doi: 10.5897/AJB10.933.
10. Gunam IBW, Sujana IGA, Wijaya IMM, Setiyo Y, Putra IWWP, Suriati L (2021). Isolation and selection of amylase-producing microbes isolated from ragi tape and cassava tape available on the markets. IOP Conf. Series: Earth and Environmental Science 913(2021)012041 doi:10.1088/1755-1315/913/1/012041
11. Sujaya IN, Mikumo D, Orikasa Y, Urashima T, Oda Y (2011) Baking properties of *Saccharomyces cerevisiae* strains derived from *brem*, a traditional rice wine from Bali. Food Science and Technology Research, 17 (4): 369–373 doi: 10.3136/fstr.17.369.
12. Buraimoh OM, Ogunyemi AK, Oyegbite IE, Amund OO, Ilori MO (2020) Potentials of hydrocarbon utilization by wood-digesting bacteria isolated from the Lagos Lagoon, Nigeria. The Journal of Tropical Life Science 10 (1): 1–10. doi: 10.11594/jtls.10.01.01
13. Azmi AS, Hasan M, Mel M, Ngoh GC (2009) Single step bioconversion of starch to bioethanol by the coculture of ragi tapai and *Saccharomyces cerevisiae*. Chemical engineering transactions 18: 557–562. doi:10.3303/CET0918090
14. Sonwani RK, Pandey S, Yadav SK, Giri BS, Katiyar V, Singh RS, Rai BN (2021) Construction of integrated system for the treatment of Acid orange 7 dye from wastewater: Optimization and growth kinetic study. Bioresource Technology 337(2021) 125478:1–7 doi:10.1016/j.biortech.2021.125478.
15. Hashem M, Alamri SA, Asseri TAY, Mostafa YS, Lyberatos G, Ntaikou I (2021) On the optimization of fermentation conditions for enhanced bioethanol yields from starchy biowaste via yeast co-cultures. Sustainability (13)1890: 1–13 doi:10.3390/ su13041890.
16. Hashem M, Asseri TYA, Alamri SA (2019) Feasibility and sustainability of bioethanol production from starchy restaurants' bio-wastes by new yeast strains. Waste Biomass Valorization 10**:**1617–1626 doi:10.1007/s12649-017-0184-7.
17. Ma'As MF, Ghazali HM, Chieng S (2020) Bioethanol production from Brewer's rice by *Saccharomyces cerevisiae* and *Zymomonas mobilis*: evaluation of process kinetics and performance. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects 1–14 doi:10.1080/15567036.2020.-1815901.
18. Khan RA, Nawaz A, Ahmed M, Khan MR, Azam FD, Ullah S, Sadullah F, Ahmad A, Shah MS, Khan N (2012) Production of bioethanol through enzymatic hydrolysis of potato. African Journal of Biotechnology 11(25): 6739–6743 doi:10.5897/AJB11. 2791.

19. Lai YT, Cheng KC, Lai CN, Lai YJ (2019) Isolation and identification of aroma producing strain with esterification capacity from yellow water. PLoS ONE 14(2) doi:10.1371/journal.pone.0211356.
20. Chang YH, Chang KS, Chen CY, Hsu CL, Chang TC, Jang HD (2018) Enhancement of the efficiency of bioethanol production by *Saccharomyces cerevisiae* via gradually batch-wise and fed-batch increasing the glucose concentration. Fermentation 2018(4) doi:10.3390/fermentation4020045.
21. Ogbonda KH, Kiin-Kabari DB (2013) Effect of temperature and pH on ethanol production by a *Blastomyces* species isolated from the intestine of oil palm weevil (*Rhynchophorus palmarum, coleoptera*). African Journal of Biotechnology 12(6): 588-591, doi: 10.5897/AJB10.147.
22. Napitupulu AMM, Suhendra L, Gunam IBW (2021) Effect of *Saccharomyces cerevisiae* ATCC 9763 concentration and fermentation time on bioethanol content from corn stover crude cellulose substrate. IOP Conf. Series: Earth and Environmental Science 913(2021)012026 doi:10.1088/1755-1315/913/1/012026.
23. Sarungallo RS, Mellolo A, Melawaty L, Djonny M (2020) The effect of boiler size of distillation to the concentration of bioethanol and yield from fermentation of sweet sorghum juice. IOP Conference Series: Materials Science and Engineering 885(1): 12–14 doi:10.1088/1757-899X/885/1/ 012014.
24. Efeovbokhan, V. E., L. Egwari, E. E. Alagbe, J. T. Adeyemi and O.S. Taiwo. 2019. Production of bioethanol from hybrid cassava pulp and peel using microbial and acid hydrolysis. BioResources. 14(2):2596–2609 doi:10.15376/biores.14.2.2596.2609
25. Rusydi AF (2018) Correlation between conductivity and total dissolved solid in various type of water: A review. IOP Conf. Series: Earth and Environmental Science 118(2018)012019 doi:10.1088/1755-1315/118/1/012019.
26. Choi, Gi-Wook, Um HJ, Kim M, Kim Y, Kang HW, Chung BW, and Kim YH (2010) Isolation and characterization of ethanol-producing *Schizosaccharomyces pombe* CHFY0201. Journal of Microbiology and Biotechnology 20(4): 828–834 doi:10.4014/ jmb.0910.10003
27. Narendranath NV, Thomas KC, Ingledew WM (2001) Effects of acetic acid and lactic acid on the growth of *Saccharomyces cerevisiae* in a minimal medium. *Journal of Industrial Microbiology and Biotechnology* 26(3): 171–177. doi:10.1038/sj.jim.7000090.
28. Esmaeili H Keikhosro K (2018) Optimization of fermentation conditions for efficient ethanol production by *Mucor hiemalis*. Turkish Journal of Biochemistry 43(6): 587-594. doi:10.1515/tjb-2017-0290.

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