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Short Communications

The Delignification of Plants Residual for Saccharification Growth by the Fungal Consortium: A Practical Approach

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Abstract. The environments have created an abundance of residual plants from all life sectors, which is not optimal for bioethanol. Therefore, this research developed microbial technology that yielded sugar and fermentation testing. The research aimed to discover the delignification process and compare the consuming sugar by *Saccharomyces cerevisiae* between the chemical saccharification and accelerated bio-agent of fungal consortium in the engineered media. The innovation of the bioethanol process was conducted using raw materials from biomass. Based on this study, some preliminary hypotheses were made: (i) arranging fungal substrate which consists of residual sugar, molasses, and enriched residual papaya fruits could provide distinguishable growth of cell mass; (ii) the substrate concentration of 2.5% and 7.5% in the growth medium using enriched residual papaya fruits, respectively, as a medium, could be distinguished using delignification. A benchmark was used to compare the chemical and bio-agent saccharification. The consortium that grew and produced cell mass by times factor in molasses has fulfilled the element needed compared to the natural organic substances from the papaya fruit. The higher concentration of delignification material substrate yielded higher growth-saccharification and the average of 10.45 ± 0.21 % Brix was obtained by the fungal consortium in the broth medium, although the acceleration growth is insignificant. Nonetheless, *Saccharomyces cerevisiae* had successfully fermented saccharification yield sugar from the delignification of plants residual.

Keywords: Cellulose, hemicellulose, hydrolysate, bio-agent

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1. Introduction

Innovation of bioethanol from biomass as raw materials in the second generation has been done over the past few years (Aditya *et al*. 2016). The innovation factors include the types of plants material used, pre-treatment methods, hydrolysis, and fermentation (Robak and Balcerek, 2018). Recently, the fourth bioethanol generation has included algae, a plant-like single-celled organism. Our environments have led to an abundance of residual plants from all life sectors that are not optimal for carbohydrates extract. However, these residual plants are useful in the production of bioethanol. Regardless of the raw material, residual plants are the cheapest for bioethanol production, especially in tropical areas. Ethanol fermentation, using *Saccharomyces cerevisiae* during cell propagation has been researched about the multiple regression models and is ready to apply in the production scale (Syauqi *et al*. 2021).

The pre-treatment of the cellulose solvent- and organic solvent-based lignocellulose fractionation (COLSIF) has a shorter processing time and is expensive compared to the dilute acid (DA) and the enzyme-based method for the determination of cellulose accessibility to cellulases (CAC) (Zhu *et al*. 2009). Usually, the lignocellulose raw material such as corn and rice plants were developed for chemical hydrolyses. The chemical method preparation with glucose (hexose) and xylose (pentose) in the substrate can be fermented to ethanol by microbes; however, the fermentable substrate of xylose is not convincing yet. The study for *Saccharomyces cerevisiae* only on the phenomenon stage was conducted for short-term adaptation (Van Dijk 2021). The glucose molecule is vital in the substrate for the growth of fungi since cell wall formation requires glucans polymer, the chitin that is made up of1-3-ß-glucan, and the 1-6-ß-glucan.

The microbiological method by fungi with xylanase enzyme is preferable due to the cost-effectiveness of the substrate from the lignocellulose raw material (Mondal *et al.* 2019). However, the extraction of enzymes is an expensive application compared to fungal consortium bio-agent for saccharification of biomass raw materials. The raw material from residual plants can be obtained by adding sodium chloride to crack the lignin molecule covering the cellulose and hemicellulose polymer (Richana 2011). The process is used both for chemical and biological saccharification.

A review by Srivastava *et al.* (2019), provides the list for various plants prepared for enzyme activity against substrates. However, optimisation is needed with the immobilised enzyme. Cellulose and hemicellulose are part

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of the fibre in the plant cell wall. They consist of 1-4-ß-glucan and 1-4 ß-xylan polymers. Therefore, this research investigated the preparation and testing for the extracellular enzyme of the fungal consortium and the fermentable yield.

The microbial saccharification method was discovered during species interaction of species. The consortium consists of *Aspergillus niger*, *Trichoderma sp.*, *Hansenula saturnus*, and *Candida sp.* (Syauqi 2008). The individual *Trichoderma viride* showed the capability to hydrolyse carbohydrate polymer (Lailah *et al.* 2017). The *Aspergillus niger* has fifteen xylanases, meanwhile, *Trichoderma harzianum* E58 and *Trichoderma reesei* have xylanase 1 and xylanase 2, respectively, while *Trichoderma viridae* has 13 xylanases (Colin *et al.* 2005). All the xylanases could hydrolyse and break down the cell wall of residual biomass plants that have been prepared for the delignification.

Considering the unique enzymes from the fungal consortium (Syauqi 2007), and adding a member of *Trichoderma* have a potential for saccharification (Syauqi 2008). The fungi consortium optimisation is unknown, and this study aims to apply the broth medium to the delignification product (DP) of residual plants. The fungi consortium is promotional and impacts the application in the industrial field (Mondal 2019). A study on the relationship between the turbidity and *Saccharomyces cerevisiae* population for the propagation of cell mass with potato dextrose broth as a medium displayed faster growth (Syauqi *et al.* 2019).

Therefore, in an attempt to engineer the growth of a fungal consortium while saccharifying substrate from plant residue has linear growth. The outcome of this investigation was the saccharification product that can be fermented by *Saccharomyces cerevisiae*. The fungi consortium interacts with the microscopic fungus that has different functions. The consistency of the consortium is needed since the activity of the bio-agent to hydrolyse fibre is limited, and the effort to study the dynamic population resulted in no linear growth (Adawiyah 2018, Saputri 2018). It is expected that 15 - 50% of xyloglucan might contribute to the lignocellulose optimisation for bioethanol production. *Trichoderma sp.* in the 8% *Reutalis trisperma* exocarp substrate produced xylanase (672.039 U/mL) at pH 5 and 40^{o}C (Mardawati *et al.* 2020). Meanwhile, *Bacillus arseniciselenatis* DSM15340 secreted xylanase activity at pH 8.0 and 50^{o}C (Kamble and Jadhav 2012).

The residual papaya fruit has been studied with hydrochloric acid (HCl) to decrease the pH of substrate and yielded the ethanol fermentation process (Baharuddin, 2014). In the industry, molasses was used from residual sugar production. Molasses application is the leading source of bioethanol production and for the growth of primary culture in a 6% Wang medium that consist of $FeSO_4.7H_2O$, $MgSO_4.7H_2O$, $CaCl_2.2H_2O$ and $CuSO_4$ (Syauqi 2008). The previous enzymatic studies (Collin *et al.* 2005, Syauqi 2007) and synergistic *Aspergillus niger*, *Hansenula saturnus*, and *Candida sp.* had contributed to the apparent saccharification activity. A through follow up of the saccharification by the bio-agent i.e., fungal consortium offers knowledge on the medium and the accelerating the growth towards the DP.

A chemical agent for delignification and saccharification was aimed to explore glucose polymers, cellulose, and hemicellulose. Theoretically, the chemical agent has been applied to liquid sugar production for food raw material. A similar goal in this research is to process a benchmark of saccharification yield by a bio-agent, which is the fungi consortium. The benchmark appeared as a reviewed result for the biomass raw material for biofuels (Srivastava *et al.* 2019). This research also aimed to discover the delignification process and compare the consumed sugar by *Saccharomyces cerevisiae* with the chemical saccharification to the accelerated bio-agent of the fungal consortium. The fungal consortium consisted of *Aspergillus niger*, *Trichoderma viride*, *Hansenula saturnus*, and *Candida sp.* in the engineered media.

2. Materials and Methods

2.1 Materials

This study used HCl (Merck), sodium hypochlorite (NaOCl) 5.25% (Johnson), potato dextrose broth (PDB) (Becton-Dickinson), papaya fruit extract, molasses, dextrose (Sigma-Aldrich), pure water (OTSUKA), iron(II) sulfate heptahydrate ($FeSO_47H_2O$) (Merck), magnesium sulfate heptahydrate ($MgSO_4.7H_2O$) (Merck), calcium chloride dihydrate ($CaCl_2.2H_2O$) (Merck), sulphuric acid (H_2SO_4) (Smart-Lab), and copper(II) sulfate ($CuSO_4$) (Merck). The DP of the residual plants were roots and stems of "kangkung" (water spinach), banana fruit skin, *Citrus sinensis* L. fruit's skin, the lower and upper part of cassava root, lettuce, and the residual plants of household waste. *Saccharomyces cerevisiae* from Sigma-Aldrich and baker's yeast for household activities, the fungi consortium culture of *Trichoderma viride*, *Hansenula saturnus*, *Aspergillus niger*, and *Candida sp.* (Syauqi 2008) were prepared and collected from the laboratory.

2.2 Delignification-saccharification (chemical agent) - fermentation-benchmarking

For the delignification process, this study used a descriptive-explorative-quasi-experiment method at an observation stage on a time series. HCl was used for the saccharification and hydrolysis process, while *Saccharomyces cerevisiae* was involved in the fermentation to obtain the delignification yield. The variables of hydrolysate concentration (%) and yeast (v/v) were: Brix 1: 16 - 4% (G16-R4), Brix 2: 13 - 1% (G13-R1), Brix 3: 11 - 4% (G11-R4), and Brix 4: 10 - 1% (G10-R1+). The temperature was controlled between 26.4 to 35°C at pH 5.38. The hydrolysate analysis replications were conducted in random times as long as fermentation.

Residual plants were peeled and blended with the NaOCl 3 - 3.5% and stored in a plastic container. The paste was added with water until the NaOCl was at 1% before being incubated for five hours (Richana, 2011). The paste was sieved, washed with water, and dehydrated by sun radiation before being baked in an oven at 50 ^{o}C until dry. The hydrolysis process used HCl 18% at 60 ˚C, and the treatments were HCl 1%, 10%, 75 – 90 ˚C (pasteurisation). The hydrolysate was agitated at 138 rpm using a magnetic stirrer and a mixer-hot plate with a regulator voltage to adjust the temperature. Then, the hydrolysate was neutralised by a lime until pH 7.0. The prepared fermentation was adjusted to pH < 7 by adding HCl. The treatments were 10, 11, 13, dan 16% (Brix) with *Saccharomyces cerevisiae* granule added. Next, the 1 L

substrate in the fermenter was incubated. Finally, a hand refractometer was used at random times for the substrate physical analysis.

2.3 Bio-agent growth media

The inoculum was propagated in a Wang medium that consists of 0.278 g $FeSO_4 7H_2O$, 1.5 g $MgSO_4.7H_2O$, 0.128 g $CaCl_2.2H_2O$, 0.25 g $CuSO_4$/L, and dextrose 5% at pH 5.1 (Syauqi 2007). Furthermore, the inoculum was incubated with warm air at 32 – 34˚C and relative humidity (RH) between 50 – 60% with a rotating shaker at 75 rpm for seven days. The standard growth medium was prepared with a PDB, 6% glucose hydrolysate (Adawiyah, 2018), and 0.5% urea (Syauqi *et al.* 2019, Van Dijk 2021) before being sterilised at 121°C for 15 minutes.

Two kinds of dual-energy treatment medium consist of the PDB and enrichment of (I) 1:1 papaya fruit extract and (II) 1:1 molasses at pH 5 with replications was prepared in eleven glass bottles. The bio-agent culture was added for three days (Syauqi 2008, Adawiyah 2018, Saputri 2018, Syauqi *et al.* 2019). The fungi consortium's growth was halted using boiled water steam to the glass bottles for treatment I and II for 10 minutes. Subsequently, the fungus consortium biomass was dried in the oven at 105°C until it reached a constant dry weight.

2.4 Delignification substrate and saccharification bio-agent

The treatments consist of two types of substrate; the DP with 2.5% and 7.5% concentrations in a preliminary Wang nutrition medium at pH 5.1 and a papaya extract at 8% enrichment. There were 11 replications in a glass bottle and incubation with hand agitation every 24 hours for the submerged saccharification process. The three-day incubation period was conducted using warm air at 32 – 34°C, and RH of 50 – 60%, and was terminated when the temperature reached 100°C for 10 minutes by boiling water until it produced steam.

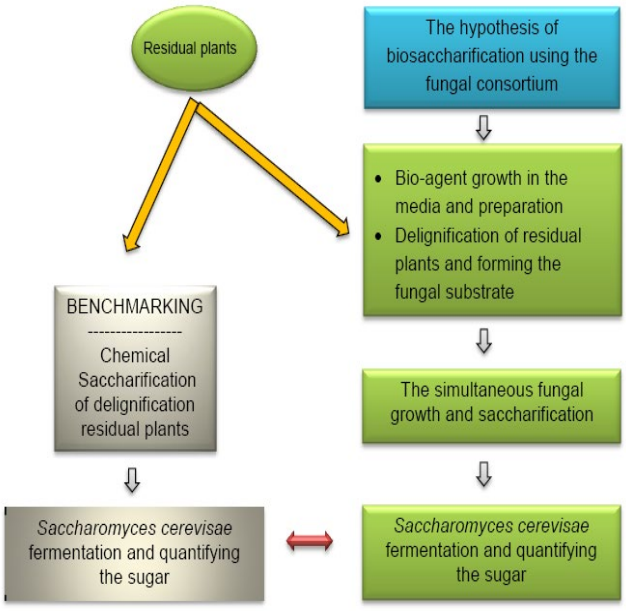

Fig.1. A flow diagram of the comprehensive methods

The hydrolysate analysis used the sulphate-phenol method and was based on the standard curve of the spectrophotometry. Furthermore, the analyte was kept in the refrigerator to prepare for further study. The saccharification at 6, 9, and 12 days incubation at room temperature was performed, and a calibrated hand refractometer measured the hydrolysates.

2.5 Ethanol Fermentation

The hydrolysate was aseptically collected and prepared for 10% and 16% concentration at pH 5.38. Adding two types of *S. cerevisiae* granule to the hydrolysate substrate fulfilled the concentration of 2% and more. The incubation was performed at 30 – 35°C using warmed water-mantel.

2.6 The comprehensive methods

The initiated methods to the end of the research stage are shown in the diagram (Fig. 1). The DP was in powder form, and the substrates were prepared in the liquid state as a fungal media.

2.7 Data Analysis

The experiments consist of an accelerated fungal consortium growth and a media that contained the delignification substrate and papaya extract. Statistics analysed the mean of every treatment at a 95% confidence interval or $p < 0.05$. The ranges of the mean were analysed by t-distribution and the validity value (mean ± standard error). Furthermore, the t-distribution analysis was used to identify the difference of means values. The Excel application was utilised for t-test (equal variance) on Windows 10 operating system (OS). The regression slopes were analysed with analysis of covariance (ANCOVA) approach analysis of variance (ANOVA) computed by DOSbox Megabuild6 of Windows 10 OS.

3. Results and Discussion

3.1 The substrate of plants residue delignification and Bio-agent Saccharification

A decrease in lignin molecules in the organic materials could expose the part of the plant's cell wall and reveal cellulose and hemicellulose amorphous characteristics. Delignification using sodium chloride was adequate for glucose and xylose polymer using 30 - 35% cellulose and 20 - 35% hemicellulose, respectively. The treatment at various concentrations aimed to decrease the application of sodium chloride. The amorphous characteristic of the hydrolysed polymer was emphasised with % Brix instrumentation yield. The % Brix is reputed as an efficient method of preparing chemical digestion for benchmarking.

These treatments had the assumption that the delignification of different plants tissue contains similar lignin of trees and herbaceous plants category. The materials of the root and stem of “kangkung”, banana fruit skin, *Citrus sinensis* L. fruit skin, part of the lower and upper cassava root, and lettuce were delignificated using materials at 33.5% in 1% sodium hypochlorite. The yielded sugar increased using the increased materials and hydrochloric concentration, as shown in Table 1.

The fungal consortium resulted from the spores and yeast on potato dextrose agar (PDA) and dominated green conidia (Fig. 2) from the *Trichoderma viride*. The resulted sporulation showed that the fungus had more conidia with 50% (v/v) *Zea mays* extract with spores number of 2.07 x 10^7 conidia per mL potato medium (Mahruzah 2011). The consortium in the PDA medium had a positive effect on the sporulation of the *Trichoderma viride* (green colour), while *Aspergillus niger* spores (black colour) were absent. However, the black colour appeared in the broth medium. This finding showed that the fungi had no antagonistic or predation interaction. If *Aspergillus niger* was grown first in the substrate followed by the others, it would be dominant in that consortium (Syauqi 2017).

Furthermore, the mycelia grew on Wang medium and appeared like cotton on day eight. The mycelium was later moved to the standard PDB and aerated at 32 - 34°C for 24 hours with 125 rpm agitation. Stirring was performed with a magnet stirrer at DP treatment with 2.5% substrate, and the cell mass appeared on the surface on day four. The solution with 25% substrate was prepared in 1% sodium chloride for delignification before adding the papaya extract.

The consortium growth in the dual-energy medium for the propagation cell produced 157.0 ± 11.66 mg cell mass using papaya extract. In comparison, 300.45 ± 33.66 mg cell mass was formed using the molasses added treatment ($p < 0.05$). The molasses had sulphur in the protein component at the tertiary bond of a protein molecule. The sulphur in molasses is the element in the treatment medium, and the consortium was supported by molasses enrichment. The growth consortium of cell mass was faster with an inorganic component of molasses than the organic element from the papaya extract; (334.0 ± 33.655) mg comparing (157 ± 11.66) mg. Both the substrates enrichment had the monosaccharide for cell energy but yielded different cell masses. *Trichoderma koeningi* with pineapple peel supplement produced xylanase activity of 2,869.8 ± 0.4 IU/g at pH 5.3 and 40 °C (Bandikari *et al.* 2014), which could provide energy for the biomass.

Table 1
Materials of delignification material (%DM), delignification-chemical agent saccharification (HCl:DM), and sugar concentration (% Brix)

% DM *	HCl (%)	Vol. (L)	HCl : DM	Temp. (°C)	Time (hour)	% Brix
10	1	0.2	10 : 1	70 - 75	2	3
		0.2 – 0.6			1.83 - 3	15.4 - 18
	10	1.5	40 : 1		0.5 - 1	15.6– 6.2
33.5		2			1	20

* DM: Delignification Materials

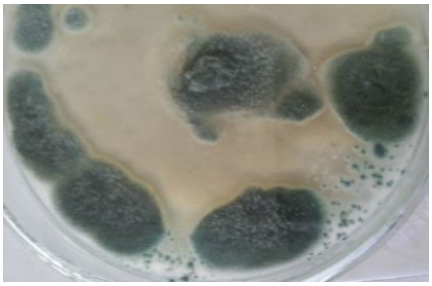

Fig. 2 Green Conidia of *Trichoderma*

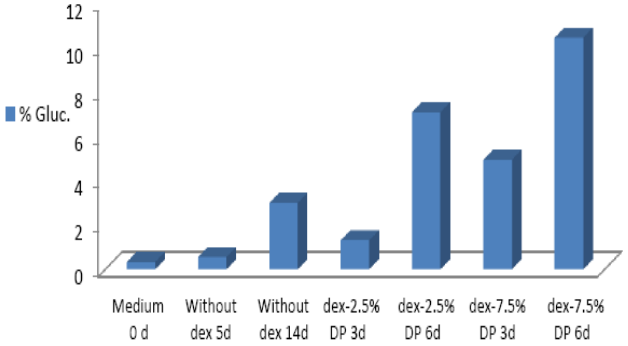

Fig. 3 The comparative of hydrolysate quantity in the medium with or without dextrose and containing the DP

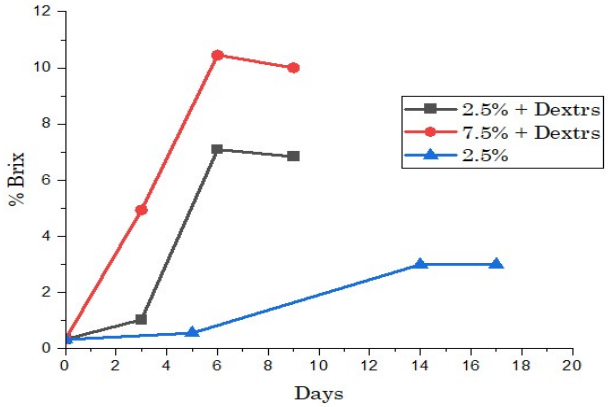

Fig. 4 The glucose concentration growth yielded in the DP medium of 2.5% substrate with or without dextrose and 7.5% substrate for nine days

On the contrary, the *Trichoderma viride* had excreted xylanase and glucans (Tjandra *et al.* 2020). The synergistic interaction of the fungal consortium suggested that the hydrolysate from the delignification substrate could release energy. The hydrolysate of 1-4-ß-glucan was used for *S. cerevisiae* (Van Dijk 2021) and 1-4 ß-xylan polymers for *Candida sp.* (Robak and Balcerek 2018).

The three-day incubation for the saccharification with bio-agent using 8% papaya extract have produced extracellular enzymes. Based on glucose analysis, the hydrolysate is higher with the addition of DP. The delignification of plants for using substrate showed that the extracellular enzyme hydrolysed the cellulose and hemicellulose after the cell mass growth. The glucose quantity was 0.96 - 1.89% (Fig. 3) in the DP with 2.5% substrate, and it had the same pattern as the DP of 7.5% substrate. The trend could be explained by the similar incubation time. Sugar concentration was higher due to the availability of monosaccharide for the growth of cell mass. That cell mass consortium provided the extracellular enzymes for hydrolysing the delignification substrate.

DP with 7.5% substrate incubated for three days produced glucose of 2.71 - 5.07% and was higher than DP with 2.5% substrate (six days incubation period). In the six-day saccharification process, there was a clear liquid at the top of the medium. The growth of consortium cells

Table 2
ANCOVA approach ANOVA on the homogeneity of slopes of Line

Treatment	df	SS	Slope	Correction	df
DextroDP2.5	3	39.715	0.852	7.023	2
DextroDP7.5	3	72.116	0.983	28.592	2
Total	3	111.831		35.616	4

F distribution

Kind of test	Fs	F_{Table}	ANCOVA Conclusion
Linear regression	10.531	6.610	fulfilled
Slope homogeneity	0.043	7.710	fulfilled
Var. X effect to treatment	0.000	5.990	fulfilled

and the liquid changing indicated that the extracellular could be produced and functional.

The glucose quantity for nine days in the DP of 2.5% substrate was (7.09 ± 0.14) %, lower than DP with 7.5% substrate (10.45 ± 0.21) % (Fig. 4). The statistical test for both means of effect results showed a significant difference at $p < 0.01$. The difference indicated that thevariables of delignification substrate and the inoculum quantity of fungus consortium gave a distinguishing effect on the extracellular enzyme's activity. The cells of consortium were agitated by submerging the mycelium for the next 24 hours at room temperature with specific RH; 51 – 81.7%.

The growth periods in six and nine days showed a yellowish liquid in the DP with a 7.5% substrate medium. Meanwhile, green conidia are present in the submerged rhizomorph of consortium growth in DP with a 2.5% substrate medium. A lower concentration of delignification substrate gave a sporulation effect or the use of energy to form the cell's generation device. When comparing the DP with 7.5% substrate in the extracellular enzyme's activity, it appears that only the white rhizomorph had the mycelium without sporulation. The consortium cell activity secreted the enzyme, and the substrate in the medium must be simultaneously hydrolysed to grow the cells. Therefore, the cells mass was submerged every 24 hours by agitation. The process has decreased the sporulation effect of *Aspergillus niger* and *Trichoderma viride*.

The data analysis of the regression coefficient on linear growth is shown in Table 2. The linear growth of glucose concentration in the dextrose-papaya extract enrichment medium was insignificant. The finding indicated that the elements in the papaya extract did not accelerate the growth of cell mass and enzymes activity. Regardless of the slopes regression, DP with 2.5% and 7.5% of substrate treatment in the medium did not significantly affect the saccharification growth. The effect of molasses enrichment in the medium above suggests that higher cell mass provides a higher amount of unique extracellular enzymes in saccharification.

This finding explained that the hydrolysed sugar supported the growth, and unique enzymes have appeared on the engineered medium from *Aspergillus niger*. With ideal significant linear regression, obtaining saccharification yield was expected with the 7.5% DP medium. The concentration of DP as a substrate had induced the formation of the extracellular enzymes from *Trichoderma viride* to yield the sugar. The sugar quantity was higher at a substrate concentration of DP 7.5% than DP 2.5%.

The enzymes activity has no significant difference at Fs 2 ($p < 0.05$; Table 2), which explained that the higher substrate concentration of cellulose and hemicellulose lead to a higher glucose concentration due to the higher units of those enzymes (Collin *et al.* 2005). Solid and liquid media is an essential and reliable medium (Adawiyah, 2018; Saputri, 2018). However, both the solid media are not conducive to accelerated inoculum growth. The growth factor for the fungus consortium in this research needs the elements for cell energy and high cell mass in the broth medium.

3.2 Ethanol fermentation–chemical agent saccharification

The *Saccharomyces cerevisiae* yeast used glucose for fermentation and decomposed the hexose to ethanol and CO_2. This study investigated whether the hydrolysate yield from the substrate could be deteriorated by yeast; at pH 5.38 and 31 – 37°C. At the 10% (Brix) hydrolysate, a CO_2 bubble could be seen. The decomposition activity was slower than the sugar cane substrate. The concentration experiment that initiating hydrolysate sugar with 10% sugar content and 1% yeast granules showed higher activity of ethanol fermentation (Fig. 5). The 16% hydrolysate sugar increased as the yeast hydrolysed the residual cellulose polymer and the chemical agent did. The cell itself could yield the extracellular enzyme and hydrolysed the polymer. However, the hydrolysate sugar at 10% initiation decreased due to the fermentation activity. A *Saccharomyces cerevisiae* strain consumed the xylose (pentose) from the hemicellulose, but this was a short-term adaptation study (Van Dijk 2021).

3.3 Bio-Agent Saccharification and Benchmarking

The hydrolysate sugar was yielded by chemical and bio-agent saccharification, which occurred when 10% of concentration had been reached. The bio-agent resulted in more than 10% of concentration achieved at sixth-day incubation. The finding had raised the question of the accurate measurement of incubation time at which it highest concentration of hydrolysate sugar stopped. In curve analysing, the investigation was performed on the trend line and R^2 value of DP 7.5% for nine and twelve day's incubation.

The nine days incubation has the equation trend line $y = -0.1404x^2 + 2.4141x - 0.008$, $R^2 = 0.966$ and twelve days $y = -0.1248x^2 + 2.2925x + 0.0761$, $R^2 = 0.968$. The optimum for hydrolysate yield is 8.77^{th} days and 18.48 hours and the average (10.45 ± 0.21) % Brix was approximately nine days and 5.52 hours; the optimal incubation time for fungus growth with saccharification was DP 7.5% medium. Applying this hydrolysate sugar for ethanol fermentation of *Saccharomyces cerevisiae* at more than 2% has increased the sugar concentration pattern, and this phenomenon is caused by *Trichoderma viride* hydrolysing the cellulose (Tjandra *et al.* 2020). A trend of using bio-agent (Fig. 6) at 16% hydrolysate is similar to Fig. 5 by a chemical agent.

This phenomenon means a higher cell mass induced the enzymes and secretion to the cellulose and hemicellulose substrates, which hydrolysed those polymers to produce the sugar. *Saccharomyces cerevisiae* at 2% decomposed the hydrolysate and decreased the 10% sugar concentration. The fungal consortium consisted of *Candida sp.*

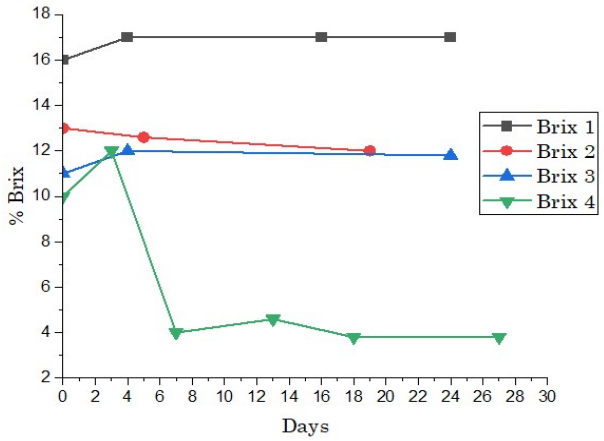

Fig. 5 The consumed hydrolysate sugar from the chemical agent during ethanol fermentation by *Saccharomyces cerevisiae*

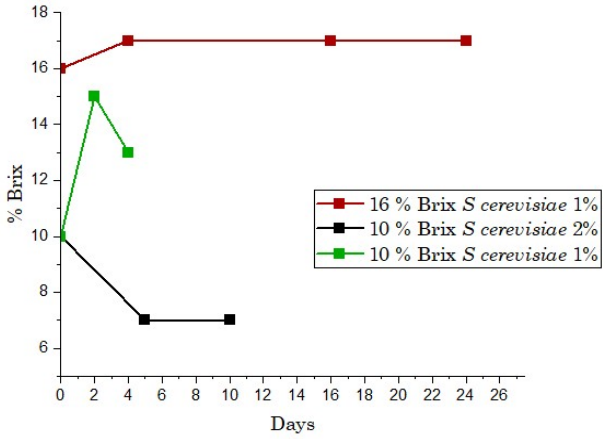

Fig. 6 Applying hydrolysate sugar for ethanol fermentation of *Saccharomyces cerevisiae* during the incubation period (days)

This finding is comparable to a Bruinberg study whereby *Candida utilis* had a pentose-phosphate pathway (Van Dijk 2021), and *Candida Shehatae* decomposed the xylose to ethanol. Meanwhile, *Saccharomyces cerevisiae* is suitable for studying genetic engineering (Robak and Balcerek 2018) or xylose adaptation at the cell propagation stage (Van Dijk 2021).

3.4 Possible future work

A pilot plant programme could apply a similar pattern of consuming saccharification yield in chemical agent and bio-agent by *Saccharomyces cerevisiae*. Therefore, the decreasing sugar concentration at 10% Brix initial with all fermentation process factors needs enhancement for the larger scale. Further research is required to distinguish xylose hydrolysate in the bioethanol process, as Felczak *et al*. (2021) reported the incapability limit of its role in ethanol fermentation.

Additionally, the environmental factor must be considered in the pilot programme, focusing on the residual delignification activity. This delignification process produced waste, and theoretically, the water environment is allowed at 200 ppm, which technically could be met (Rutala *et al.* 2019).

4. Conclusion

At a specified period, *Aspergillus niger*, *Trichoderma viride*, *Hansenula saturnus*, and *Candida sp.* can propagate and grow their cells in a dual-energy medium with the DP as a substrate. Molasses as a source of energy is potentially suitable for cell mass growth than natural organic papaya fruit extract. The DP of 7.5% substrate concentration with enriched papaya fruit extracts produced hydrolysate sugar before fermented by the *Saccharomyces cerevisiae*. The hydrolysate sugar was achieved around 10% Brix at approximately nine days of the incubation period.

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