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Evaluation of Generic Fertiliser as an Alternative Inorganic Nitrogen Source for Ethanolic Glucose Fermentation by *Saccharomyces cerevisiae*

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ABSTRACT

In the studies and production of bioethanol, the preferred fermenting yeast (*Saccharomyces cerevisiae*) is usually cultured in liquid broth that contains yeast extract and peptone. However, the use of these laboratory and scientific grade chemicals is costly, making them impractical for mass bioethanol production. Therefore, this study was conducted to evaluate the feasibility of glucose ethanolic fermentation by *S. cerevisiae* using generic fertiliser formulations to provide inorganic nitrogen, phosphorus, potassium and trace elements. Fermentation media of different generic fertiliser strength at 0.5X, 1.0X and 2.0X Fertiliser Nitrogen Equivalents (FNE), as compared to the conventional Yeast Extract-Peptone (YEP) medium as control, was used as fermentation broth during the ethanolic fermentation of glucose. Based on the results, *S. cerevisiae* cultured in YEP broth produced the highest cell concentration for both wet (21.93 g/L) and dry cells (3.87 g/L), with rapid increment observed in the first 72 h of fermentation. By the end of the fermentation period, lactic acid (3.14 g/L) and acetic acid (0.96 g/L) levels were recorded to be the lowest in YEP medium while their concentration (lactic acid, 8.08 g/L) and (acetic acid, 2.67 g/L) were highest in 2.0X FNE fertiliser medium. Results indicated that the best theoretical ethanol yield (TEY) among the fertiliser media was achieved when fermentation was performed in the 0.5X FNE fertiliser medium, with a TEY of 86.18%. TEY yields were 78.68% and 51.54% in broth with 1.0X and 2.0X FNE, respectively. In general, all three fertiliser media supported ethanolic fermentation of glucose, with the 0.5X FNE fertiliser broth showing a yield that is significantly close to the conventional YEP medium, as seen in the statistical analysis. Similarities in other fermentation profiles such as acetic acid, lactic acid, and biomass production, as well as glucose utilisation, between the results from the YEP samples and samples from the fertiliser broths (at 0.5X and 1.0X FNE) have also shown that generic fertiliser has the potential to be used as an alternative medium to replace the conventional YEP to produce ethanol at a lower cost.

Keywords: Bioethanol, ethanolic fermentation, Fertiliser Nitrogen Equivalents (FNE), generic fertiliser, *Saccharomyces cerevisiae*

INTRODUCTION

Global energy demands, along with instability in fuel prices, have prompted intensive explorations for sustainable energy sources to satisfy current and future energy consumption across the world (Hung *et al.*, 2018; Wong & Vincent, 2019). These problems are further intensified by environmental pollution and global warming following the indiscriminate use of fossil fuels as the main energy sources. Therefore, environmentally friendly, renewable and sustainable energy alternatives such as bioethanol and biodiesel are currently mass produced worldwide (Azhar *et al.*, 2017; Vincent *et al.*, 2018).

Bioethanol, also known as ethyl alcohol, is a clear and colourless liquid biofuel with the chemical formula of CH_3CH_2OH (Azhar *et al.*, 2017). It is a clear and colourless liquid biofuel that is volatile and flammable at room temperature (Susmozas *et al.*, 2020). Bioethanol consists of 35% oxygen that contributes to the complete combustion of fuel. This property minimises dangerous tailpipe emissions, as well as particulate emissions that pose health hazards, making bioethanol a promising alternative to reduce vehicular pollution (Khan & Dwivedi, 2013). Bioethanol is an environmentally friendly fuel as it is biodegradable and water soluble (Vincent *et al.*, 2018). When used as fuel, bioethanol burns completely to produce only carbon dioxide and water, making it a

significantly cleaner fuel in comparison to conventional fossil fuel in any application. The quantity of carbon dioxide produced by bioethanol combustion is equal to the amount of carbon dioxide absorbed by the plant for photosynthesis which renders its status as being carbon neutral (Hu *et al.*, 2008; Sanchez & Cardona, 2008).

Bioethanol can be produced from various renewable bioresources rich in carbohydrates, usually via ethanolic fermentation using the yeast *Saccharomyces cerevisiae* (Saini *et al.*, 2015; Hung *et al.*, 2018). Although many other microorganisms such as non-*Saccharomyces* yeast species and bacteria have been widely used during fermentation, *S. cerevisiae* is still the preferred microorganism used for bioethanol production because of its high ethanol yields (Naghshbandi *et al.,* 2019). In a typical fermentation process, the fermentation medium provides the yeast cells with the macronutrients and micronutrients that are needed through the addition of yeast extract and peptone (Sanchez & Cardona, 2008). However, these laboratory grade chemicals are costly. To make ethanol production economically viable, it is important to use more cost-effective materials, such as generic fertilisers, to provide the yeast cells with the necessary nutrition. Fertilisers are materials that contain one or more nutrient elements, such as nitrogen, phosphorus, and potassium (NPK), in the form of either organic or inorganic chemical compounds (Masarirambi *et al*., 2013; Bhatt *et al*., 2019).

To date, no study has been done on using a generic fertiliser to replace the conventional yeast extract and peptone (YEP) powder that is frequently used in bioethanol studies and production. Therefore, this study was carried out to investigate the feasibility of ethanolic fermentation of glucose in the presence of inorganic nitrogen, potassium, phosphorus, and trace element (NPK-TE) from selected generic fertiliser. To gauge the effects of fertiliser, different nitrogen equivalence (in comparison to the standard YEP formulation) were tested. This nitrogen equivalence is termed as Fertiliser Nitrogen Equivalence (FNE), with the values chosen as 0.5X, 1.0X and 2.0X FNE. The effects of different FNE on the yeast cell accumulation and ethanolic fermentation performances of *S. cerevisiae* were then compared between the conventional YEP medium and the three different fertiliser broths.

MATERIALS AND METHODS

Preparation of Glycerol Stock and Working Culture of *Saccharomyces cerevisiae*

Saccharomyces cerevisiae ATCC 24859 was obtained from the Microbiology Laboratory 2 (Faculty of Resource Science and Technology, UNIMAS). *Saccharomyces cerevisiae* was cultured in Yeast Malt Broth (YMB) at ambient temperature on a shaker (NB-101MT Multi Shaker, N-Biotel, Korea) 150 rpm for two days. Separately, 50 mL of YMB was mixed with 25 mL of glycerol (R&M Marketing, UK) and 1.2 mL of the mixture was transferred to 2 mL centrifuge tubes. After autoclaving, 0.8 mL of the *S. cerevisiae* culture was transferred into the centrifuge tubes containing the YMB-glycerol solution and stored in the freezer (SJC203, Sharp, Malaysia) -20 ºC until further use. For the working *S. cerevisiae* inoculum preparation, the glycerol stock containing the yeast cells was cultured overnight in YMB at ambient temperature and with a constant agitation speed of 150 rpm. After 24 h, the *S. cerevisiae* cells were harvested via centrifugation (BK-1032J Low Speed Centrifuge, Biobase, China) at 4,500 rpm for six min. The harvested cell pellet was then transferred to the respective fermentation medium.

Fermentation Broth Preparation

Prior to the fermentation broth preparation, yeast extract, peptone and fertiliser samples were sent for nitrogen content analysis (i-Testchem Laboratory Services, Malaysia). The nitrogen content was recorded as 10.9 g/100 g for the yeast extract powder, 14.1 g/100 g for the peptone powder and 25.0 g/100 g for the fertiliser powder. All fermentation broths were prepared in batch cultures of 500 mL using 1 L Schott bottles with the pH maintained between 4.8 and 5.4 using 50 mM citrate buffer. The control fermentation (YEP) medium was prepared by mixing yeast extract (YE) (Bacto, USA), citrate buffer (CB, pH 4.76) and peptone (P) (Bendosen Laboratory Chemicals, Malaysia). To tally the total of nitrogen in YEP (which is 3.90 g in 1 L of 0.05 mM citrate buffer) (Table 1) with equal strength in the fertiliser (1.0X FNE), a total amount 15.60 g of fertiliser

powder (with nitrogen content of 25 g per/100 g fertilizer) was added to 1 L of citrate buffer to contain 3.90 g of nitrogen in the fermentation medium. Table 2 shows the amount of fertiliser powder used to formulate the fermentation media based on the different Fertiliser Nitrogen Equivalents (FNE) of 0.5X, 1.0X and 2.0X FNE against conventional YEP. Next, all broths were supplemented with 5% glucose (R&M Chemical, United Kingdom). Finally, the broths were autoclaved for sterilisation purposes.

Table 1. The composition of nitrogen in the YEP medium

	In 1 g per 100 g powder	Sources in 1 L (g)	Nitrogen in 1 L (g)
Yeast extract	11	10	1.10
Peptone	14	20	2.80
Total	25	30	3.90

Table 2. The formulation of the fermentation media according to the respective Fertiliser Nitrogen Equivalent (FNE) strength (0.5X, 1.0X, and 2.0X FNE)

Exp No.	Fertiliser Nitrogen Equivalent (FNE)	NPK-TE Fertiliser (g)	Glucose (g)	Citrate Buffer (mL)
1	0.5X	7.80	50.0	1000
2	1.0X	15.60	50.0	1000
3	2.0X	31.20	50.0	1000

Ethanolic Fermentation of Glucose

Saccharomyces cerevisiae culture (10^6 - 10^8 cells/mL) were harvested and added into all the sterilised fermentation broth containing glucose. Fermentation process was conducted in triplicates (n = 3) for five days and shaken at 150 rpm, at ambient temperature. Samples were taken at 0, 6, 12, 24, 36, 48, 72, 96 and 120 h under strict aseptic conditions. Firstly, 1 mL of samples were transferred into 2 mL centrifuge tubes. For the Phenol-Sulphuric Acid (PSA) assay and High Performance Liquid Chromatography (HPLC) analyses, the samples were initially centrifuged at 13,500 rpm for 3 min. The supernatant collected was then filtered through a 0.45 µm nylon syringe filter (Whatman, USA) into a fresh tube to eliminate any solid residues. The samples were stored at -20 ºC until further analyses were done.

Cell Biomass Profiles

Once fermentation has started, the wet and dry biomass of the yeast cells were recorded at 24, 48, 72, 96 and 120 h (Wong & Vincent, 2019). Initially, 50 mL of the fermentation broth was transferred into a clean falcon tube. The cells were harvested by centrifugation at 4,500 rpm for 6 min after discarding the supernatant. Then, the wet cell biomass was weighed. After that, the falcon tube containing the wet yeast cells was dried in an oven (Shel Lab, USA) at 70 ºC for 24 h. The dry cell biomass was weighed after 24 h of drying.

High Performance Liquid Chromatography (HPLC) Analyses

The fermentation samples from the ethanolic fermentation process were analysed using High-Performance Liquid Chromatography (HPLC) (Waters 2695 Separations Module, Alliance HPLC System, USA) to detect and quantify ethanol, residual glucose, lactic acid, and acetic acid, based on the retention times and curves of HPLC-grade standards. The HPLC system used was equipped with a column heater, refractive index detector and a computer controller. The separation and analyses of the fermentation constituents were done on a Bio-Rad Aminex HPX-8711 column (150 × 7.8 mm; Bio-Rad, USA) using 5 mM H_2SO_4 as the mobile phase, operating at a flow rate of 0.8 mL/min and temperature of 65 °C (Vincent *et al*., 2015; Vincent *et al*., 2018).

Phenol-Sulphuric Acid (PSA) Total Carbohydrate Assay

Phenol-Sulphuric Acid (PSA) total carbohydrate assay was done to determine the total carbohydrate content in the fermentation samples according to Crawford and Pometto

(1988). Approximately 0.2 mL of sample from the fermentation medium was transferred into a test tube. Then, 0.2 mL of 5% phenol and 1 mL of H_2SO_4 (HmbG Chemicals, Germany) were added into the test tube. Next, 5.6 mL of distilled water was added to make a final volume of 6 mL in the test tube. The mixture was then thoroughly mixed via vortex, followed by analysis using a spectrophotometer (SP-880 Metertech, Taiwan) at the wavelength of 490 nm. The amount of total carbohydrate present in the sample was calculated based on a standard curve that was plotted prior to the assay.

Statistical Analysis

Statistical analysis was performed using SPSS Statistics Software version 21 via Tukey's post hoc tests to analyse the significant differences in theoretical ethanol yields from different fermentation broth.

RESULTS

Growth Profiles of *Saccharomyces cerevisiae*

The cell biomass of *S. cerevisiae* was recorded gravimetrically throughout the study period of 120 h to examine the yeast growth profiles during fermentation in YEP broth and different types of fertiliser broth of 0.5X, 1.0X and 2.0X FNE. The wet cell biomass profiles of *S. cerevisiae* are shown in Figure 1(a), while Figure 1(b) shows the patterns of dry cell accumulation over time (120 h). In general, based on the results as shown in Figure 1, the *S. cerevisiae* cells were observed to be multiplying in all the broths tested, peaking at 72 h. After 72 h point, the cell concentration gradually decreased until the end of the fermentation period. In the YEP-glucose broth, cell concentration was recorded at 22.46 g/L, followed by 15.93, 18.93 and 17.80 g/L of cell weight in broths containing 0.5X, 1.0X and 2.0X FNE, respectively (at 72 h) (Figure 1(a)). The dry cell concentration of *S. cerevisiae* in all types of broths containing glucose is represented in Figure 1(b). For all conditions, dry cell concentration was observed to increase significantly during the first 24 h. The highest cell concentration was recorded at 72 h for all types of broths. The dry cell concentration for YEP, 0.5X, 1.0X and 2.0X FNE were 4.60, 2.20, 3.26 and 3.00 g/L, respectively at 72 h. After that, the dry cell concentration was on a slow decline until the end of the fermentation period.

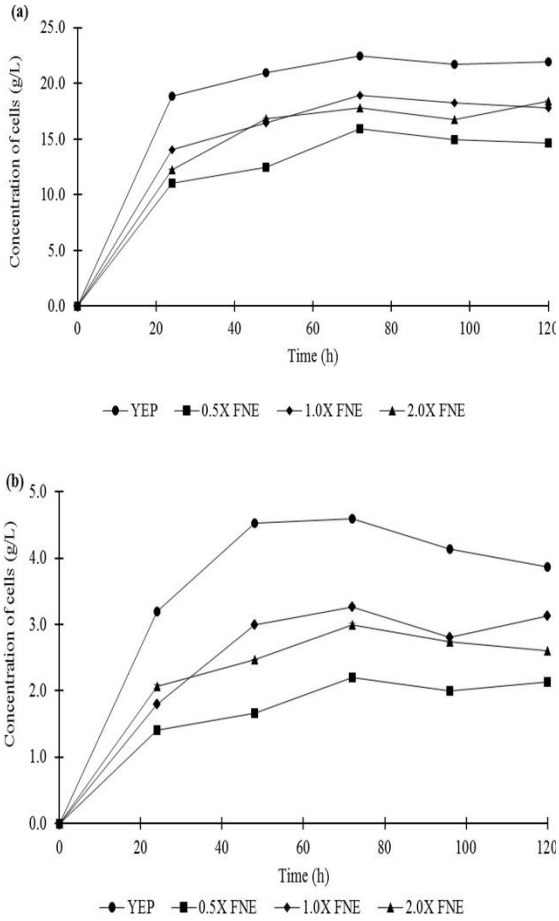

Figure 1. Time course of (a) wet cell, (b) dry cell concentration in fermentation broth using different fermentation media (YEP, 0.5X, 1.0X and 2.0X FNE fertiliser media). The data points represent the average data of three independent experiments (n = 3)

Ethanolic Fermentation of Glucose

The concentration of ethanol (g/L) produced during the fermentations using different broth, as determined via HPLC analysis, is shown in Figure 2. Although the ethanol concentration produced by every fermentation varied, the highest concentration was all achieved at 120 h with values ranging from 13.14 (in 2.0X FNE fertiliser medium) to 22.27 g/L (YEP medium). From the results, ethanol was first produced at 12 h only in fermentation using YEP, while fermentations using other broth started to produce ethanol only after 24 h. The ethanol concentration in all fermentations continued to increase until the end of the fermentation period.

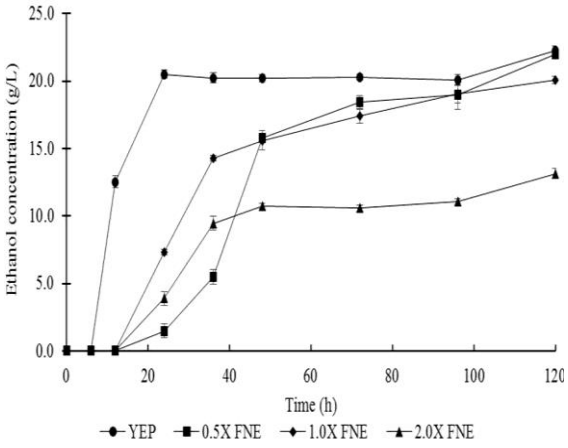

Figure 2. Time course of ethanol concentration (g/L) produced in fermentation using different fermentation media (YEP, 0.5X, 1.0X and 2.0X FNE fertiliser media). The data points represent the average data of three independent experiments (n = 3)

Figure 3 depicts the glucose concentration profiles during the ethanolic fermentation. The initial concentration of glucose was 44.16, 46.73, 45.58 and 43.51 g/L for YEP, 0.5X, 1.0X and 2.0X FNE fertiliser broth, respectively. In YEP broth, the glucose concentration decreased from 44.16 to 7.30 g/L within the first 12 h. After 24 h, no glucose was detected until the last sampling point of 120 h, which indicated that the glucose concentration was 100% consumed from 24 h onwards. In contrast to YEP broth, all the broths using different strengths of fertiliser showed different patterns in glucose concentration profiles. For samples taken from the 0.5X FNE medium, glucose concentration declined from 46.73 to 6.71 g/L at 48 h. After 72 h, no glucose was further detected. As for the glucose 1.0X FNE medium broth, the concentration decreased from 45.58 to 0.06 g/L at 36 h and after that, no glucose was observed from 48 h to 120 h of sampling. Therefore, when using 0.5X and 1.0X FNE as fermentation broth, the glucose consumption was 100% from 72 h and 48 h, respectively. The glucose concentration in 2.0X FNE broth decreased from 43.51 to 7.53 g/L at 36 h. In contrast to the other broth, glucose concentration remained constant until the end of the sampling hours thus, indicating that the glucose consumption was not 100% (120 h).

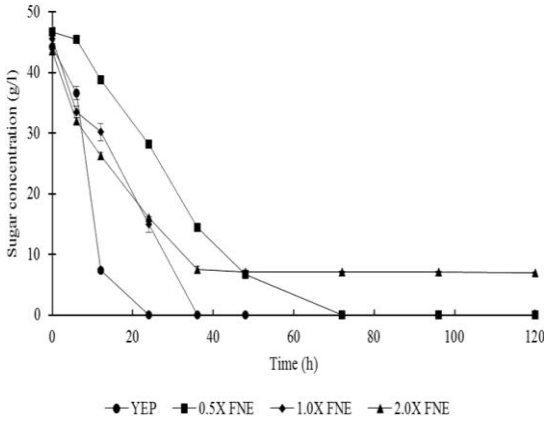

Figure 3. Time course of residual glucose concentration (g/L) produced in fermentation using different fermentation media (YEP, 0.5X, 1.0X and 2.0X FNE fertiliser media). The data points represent the average data of three independent experiments (n = 3)

In general, lactic acid produced in fermentations using FNE broth was higher than that produced in fermentation using YEP medium (Figure 4). The maximum lactic acid concentration in fermentations using 0.5X, 1.0X and 2.0X FNE was 6.98, 6.54 and 8.08 g/L, respectively. Meanwhile, the maximum concentration of LA produced in YEP culture was 3.69 g/L.

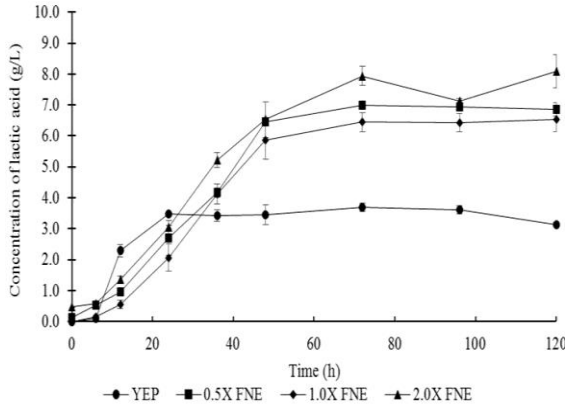

Figure 4. Time course of lactic acid concentration (g/L) produced in fermentation using different fermentation media (YEP, 0.5X, 1.0X and 2.0X FNE fertiliser media). The data points represent the average data of three independent experiments (n = 3)

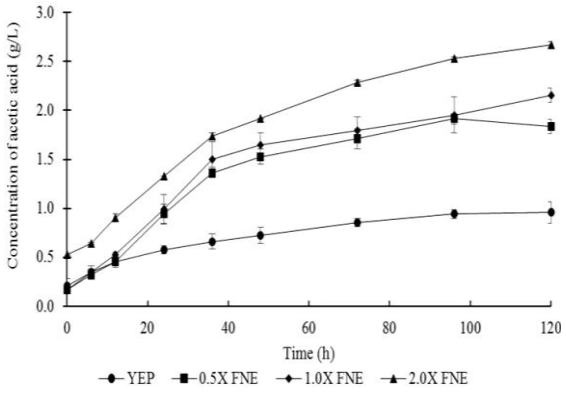

Figure 5. Time course of acetic acid concentration (g/L) produced in fermentations using different fermentation media (YEP, 0.5X, 1.0X and 2.0X FNE fertiliser media). The data points represent the average data of three independent experiments (n = 3)

The maximum ethanol production and theoretical ethanol yield (TEY) are summarised in Table 3. Ethanol produced in YEP medium was recorded at 22.27 g/L, corresponding to a TEY of 87.33%. Ethanol produced in 0.5X FNE fertiliser broth was slightly lower at 21.97 g/L, which represents 86.18% of TEY. Results showed that ethanol productions decrease in relation to higher FNE values. For samples analysed from the 1.0X FNE broth, ethanol concentration was 20.06 g/L (TEY of 78.68%), followed by 13.14 g/L (51.54% TEY) for samples from 2.0X FNE broth. Statistical analyses indicated no significant differences in the ethanol yields between samples taken from YEP medium and 0.5X FNE broth.

Table 3. Maximum ethanol production (g/L) and theoretical yield (%) of glucose ethanolic fermentation in Yeast Extract-Peptone (YEP) broth and fertiliser media at different Fertiliser Nitrogen Equivalents (FNE) concentrations

Broth	Maximum production of ethanol (g/L)	Theoretical ethanol yield (TEY, %)
YEP	22.27 ± 0.29	87.33 ± 1.13[a]
0.5X FNE	21.97 ± 0.36	86.18 ± 1.43[a]
1.0X FNE	20.06 ± 0.30	78.68 ± 1.17[b]
2.0X FNE	13.14 ± 0.37	51.54 ± 1.47[c]

*Data are mean of triplicates ± S.D (Different superscript letters in the same column show significant differences at $p<0.05$).

DISCUSSION

This study was performed to investigate *Saccharomyces cerevisiae* growths and ethanolic fermentation of glucose when grown in YEP broth against a generic fertiliser medium with three different nitrogen equivalent values of 0.5X, 1.0X and 2.0X FNE. To provide an overview of the effect of nitrogen concentrations (in relation to the nitrogen value in YEP) on cell propagation, the wet and dry cell biomass of *S. cerevisiae* in different broth were recorded gravimetrically until the end of the fermentation period of 120 h (Figure 1). Based on the results obtained, the growth profiles of *S. cerevisiae* follow the first three growth phases of the classical sigmoid curve that highlights the lag, exponential and stationary phases. During the lag phase which was from 0 h to 24 h, little growth was observed as the cells try to adapt to the new environment physiologically. The lag phase was followed by the exponential growth phase, which involved active cell propagation. In this phase, both wet and dry biomass accumulation of *S. cerevisiae* cultures were relatively higher in the YEP medium compared with those of the fertiliser broth (0.5X, 1.0X and 2.0X FNE) for all carbon sources tested. For example, the results shown in Figure 1(a) revealed the wet biomass was in the range of 15.00 to 19.00 g/L as compared to the wet biomass in the YEP medium which was recorded at 22.46 g/L. The same patterns were also observed for the dry cell biomass. *Saccharomyces cerevisiae* grown in the YEP medium showed better yields at 4.60 g/L when compared to the highest value of the fertiliser medium which was 3.26 g/L for yeast cultured in 1.0X FNE fertiliser medium.

One possible explanation for these results is a nutritional imbalance, especially excess nutrients in nitrogen, phosphorus, and potassium (NPK) from the fertiliser that may likely cause lethal effects to yeast. According to Tesniere *et al.* (2013), the presence of high nitrogenous compounds in liquid culture inhibits essential

lipid production which then leads to rapid loss of yeast growth and eventually cells death will follow. Other important factors also include high hydrostatic and osmotic pressure (Englezos *et al.*, 2018) due to the high concentration of fertiliser in the culture broth. Yeast cells are prone to death when they are unable to maintain protection responses caused by physical stress (Bai *et al.*, 2008; Walker & White, 2017; Eardley & Timson, 2020). In addition, unlike the inorganic form of nitrogen in the fertiliser used, organic-based macro and micronutrients in YEP are well documented for supporting the optimal growth of *S. cerevisiae*, as reported by previous studies (Ishmayana *et al.*, 2011; Djektif *et al.*, 2016). Hence, this may explain why the cell biomass when using YEP broth is the highest among all the broth tested.

Regardless of the culture broth used for fermentation, the yeast cell growth started to decline slightly after 72 h as shown in Figure 1(a). These observations correspond to the same period when ethanol production peaked. According to Eardley and Timson (2020), the high concentration of ethanol severely affects the yeast cells, resulting in reduced yeast viability and vigor, as well as lower ethanol yield thereafter. Although the overall results suggest that the conventional YEP medium performed better for *S. cerevisiae* growth (wet and dry cell biomass) as compared to using the fertiliser broth, it is worth noting that *S. cerevisiae* were also able to grow rapidly in the fermentation broth containing fertiliser, especially at 0.5X and 1.0X FNE. Judging from the results of the three different concentrations of fertiliser media tested, it is suggested that low fertiliser concentration of 0.5X and 1.0X FNE is recommended for the cultivation of *S. cerevisiae* during ethanolic fermentation of carbohydrates. To date, this study is the first to report this finding.

The results of ethanol yield from different broth to gauge ethanol production is shown in Table 3. In general, results indicated noticeable differences in fermentation performances as seen in ethanol production and sugar consumption profiles of *S. cerevisiae* using glucose. Also, fermentation using YEP medium produced a slightly higher ethanol yield of 87.33 TEY than the other broth used. According to Ishmayana *et al.* (2011), a conventional growth medium such as YEP allows the yeast cells to utilise sugar completely and rapidly which results in an improved glucose consumption rate. Due to its rich content of variety amino acids, water-soluble vitamins, peptides, growth factors, trace elements and carbohydrates, YEP is commonly used as a crucial medium component for growing yeast (Zhang *et al.*, 2003). Early ethanol detection could be due to high yeast concentration as a result of the addition of yeast extract and peptone during the fermentation process may result in a higher yield of ethanol (Sankh *et al.*, 2011; Duhan *et al.*, 2013; Jacob *et al.*, 2019). Other studies have also concluded that yeast extract and peptone can increase sugar concentration up to 90% with the fermentation efficiency of about 78% (Sharma *et al.*, 2018).

Ethanol was also produced in fermentations employing fertiliser broth although the yields were generally lower compared to that in the YEP medium. When glucose was used as the sole carbon source, *S. cerevisiae* in the 0.5X FNE medium produced the highest ethanol at 86.18% TEY, followed by 78.68% TEY (1.0X FNE), while the lowest was 51.54% TEY in 2.0X FNE medium. The results suggested that cultivating *S. cerevisiae* in fermentation using 0.5X FNE fertiliser medium produced the highest ethanol concentration. When the amount of nitrogen increases, the quantity of ethanol produced substantially decreases. Therefore, fermentation using 2.0X FNE broth is not recommended as the high fertiliser concentration in the 2.0X FNE medium may be detrimental towards the overall fermentation process. Meanwhile, the ethanol production by *S. cerevisiae* in 0.5X FNE was significantly different ($p<0.05$) when compared to yields obtained using 1.0X FNE medium (Figure 2).

Theoretically, sugar consumption correlates to nitrogen concentration in the fermentation medium (Barahona *et al.*, 2019). In order to produce the desired amount of ethanol, the initial sugar concentration plays a key factor as high starting sugar concentrations often result in higher ethanol yields in batch fermentation (Azhar *et al.*, 2017). *Saccharomyces cerevisiae* cultivated in the YEP medium consumed glucose at the fastest rate compared to all the fertiliser broth, where at 24 h glucose was completely utilised (Figure 3). Among all the fertiliser media, the fastest glucose consumption was observed when using 1.0X FNE medium as glucose was fully consumed at 48 h. Glucose

was only completely utilised at 72 h in the 0.5X FNE medium, while in the 2.0X FNE medium, glucose was still detected (at 7.0 g/L) even after 120 h of fermentation.

In addition to ethanol, *S. cerevisiae* fermentation may also produce organic acids such as lactic acid and acetic acid as reported by Vincent *et al.* (2018) and Hung *et al.* (2018). This observation is usually observed during a long incubation period that could lead to increased organic acid concentration. The results from this study have also detected the presence of lactic acid and acetic acid (Figure 4 and 5). The lowest acetic acid was detected in fermentation using YEP medium. Based on the results, fermentation in the 2.0X FNE fertiliser medium generated the highest lactic acid and acetic acid concentrations, compared to that using 0.5X and 1.0X FNE broths. According to several previous studies, fermentation by-products such as lactic acid and other hazardous metabolites, can halt the fermentation process resulting in low production of ethanol (Reddy & Reddy, 2006; Joshi & Kumar, 2017; Zabed *et al.*, 2017). In short, lactic acid and acetic acid production during fermentation are undesirable because these organic acids could inhibit yeast development and reduce ethanol yield (Vincent *et al.*, 2015; Zabed *et al.*, 2017). Acetic acids act by dissolving plasma membrane proton gradients and distressing cell pH when they separate into ions in the yeast cytoplasm and lead to the decline of intracellular pH, hence causing the antizymotic action (Walker & White, 2017). Beyond the limit, the formation of by-products, such as acetic acid may have consumed some of the substrates and reduced the efficiency of ethanol fermentation (Lin *et al.*, 2012; Joshi & Kumar, 2017). Furthermore, excessive acidic conditions in the fermentation broth can cause cellular stress to *S. cerevisiae* cells as they become exhausted while attempting to maintain pH in the plasma membrane (Walker & White, 2017; Eardley & Timson, 2020).

Saccharomyces cerevisiae cannot fix atmospheric nitrogen, and therefore it is essential to provide readily assimilable organic nitrogen (such as, amino acids) or inorganic nitrogen (such as, ammonium salts) for growth and fermentative metabolism (Walker & Stewart, 2016). Because of its function in control of growth and fermentation, the availability of nitrogen has been highlighted as a crucial parameter. The results of the study demonstrated a clear preference for lower fertiliser concentrations at 0.5X FNE compared to 1.0X and 2.0X FNE (Figure 6). Among all the media used, YEP broth and 0.5X FNE medium produced the highest ethanol in broth containing glucose as substrate. A total of 22.27 g/L of ethanol was produced in YEP broth, corresponding to a TEY of 87.33%. Ethanol produced in the 0.5X FNE fertiliser medium was slightly lower at 21.97 g/L, which represents 86.18% of the TEY. Results for 1.0X FNE medium recorded ethanol concentration of 20.06 g/L (78.68% TEY), while ethanol yield for the 2.0X FNE medium was the lowest at 13.14 g/L, corresponding to a TEY of 51.54%. Further statistical analysis was conducted using the Tukey *post hoc* test. Figure 6 indicates the statistically significant experimental theoretical ethanol yield among the different types of broth, with $p<0.05$. SPSS analysis was conducted to compare the ethanol yield of each broth tested. Figure 6 showed that there are no significant differences with ($p<0.05$) in the TEY produced in fermentation using YEP and 0.5X FNE.

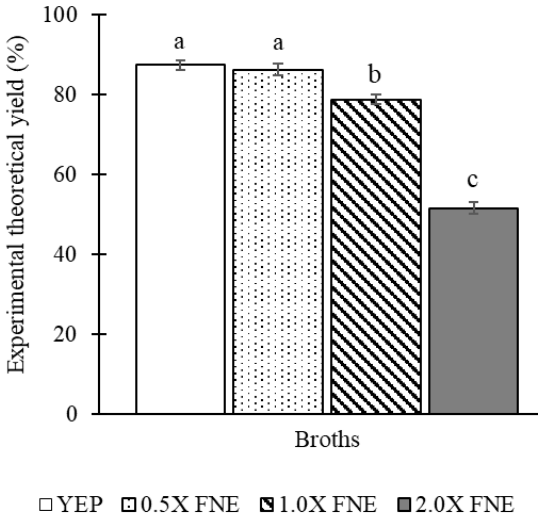

Figure 6. Experimental theoretical ethanol yield (TEY, %) produced using different fermentation media (YEP, 0.5X, 1.0X and 2.0X FNE fertiliser media). Different letters on top of the column indicate significant differences at $p<0.05$

CONCLUSION

In general, all three fertiliser broth formulations supported ethanolic fermentation of glucose. Based on glucose utilisation, as well as the other fermentation products, samples from the fertiliser media at 0.5X and 1.0X FNE also suggested that generic fertiliser has the potential to be used as an alternative medium to replace the conventional YEP for the production of ethanol at lower cost. Among the different fertiliser media, the 0.5X FNE fertiliser broth exhibited a notable ethanol yield (21.97 g/L, 86.18% TEY) that is not significantly different to the conventional YEP medium, as seen in the statistical analyses. In conclusion, the 0.5X FNE fertiliser medium has the potential to be used as an alternative medium to replace conventional YEP to produce ethanol at a lower cost.

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