Preliminary Analysis of Sugar Supplementation on Alcoholic Fermentation by *Meyerozyma guilliermondii*

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Abstract: Non Saccharomyces yeast strains consume a diverse range of sugars, capable of producing ethanol at different quantities and concentrations. The ability of such wild type indigenous strains to do so and compete with industrial strains of *Saccharomyces cerevisae* is not common in Nigeria. This study aimed at comparing the ability of *Meyerozyma guilliermondii* with a strain of *Saccharomyces cerevisiae*, to consume sugars (fructose, galactose, glucose, lactose, sucrose and molasses) and to convert them into ethanol during fermentation. Yeast extract (6g/L), peptone (10g/L), malt extract (6g/L) broth was supplemented with different concentrations (5g/L, 10g/L, 20g/L, 30g/L) of fructose, galactose, glucose, lactose and sucrose respectively. Sugar utilization post incubation for 96 hours at 120 rpm, 30°C was measured using a refractometer. The alcoholic yield using molasses for *Meyerozyma guilliermondii* 9.2±0.45 (mg/ml) was significantly higher than that of *Saccharomyces cerevisiae* strain T (4.8±1.15 mg/ml) at 96 hours. Ethanol production from the consumption of fructose as the sole carbon source was more favourable for *M. guilliermondii* 2.1, 3.0, 8.11 and 9.06 (mg/ml) compared to 1.08, 3.12, 8.06 and 6.0 (mg/ml) for *S. cerevisiae*. Both strains displayed similar adaptation to galactose metabolism at all tested concentrations. With glucose, *M. guilliermondii* yielded more than its *S. cerevisiae* counterpart at 1.0% (4.15, 3.18 mg/ml) and 2.0% glucose (4.25, 3.3 mg/ml). At 3.0% glucose broth content, 8.15 and 9.08 mg/ml ethanol was obtained for *M. guilliermondii* and *S. cerevisiae* respectively. Sucrose utilization resulted in a 10.18 mg/ml yield of ethanol compared to a 7.06 mg/ml yield for *M. guilliermondi* and *S. cerevisiae* respectively at 3.0% sugar supplement. *Meyerozyma guilliermondii* displayed its ability as a highly adaptable non Saccharomyces yeast specie capable of producing ethanol from a variety of sugars indicative of local feedstock as a suitable alternative.

Keywords: Ethanol, *Meyerozyma guilliermondii,* Fructose, Glucose, Galactose, Lactose, Sucrose

1. Introduction

Amongst the rich diversity of microorganisms in the world, yeasts species represent one of the most studied and documented species [1]. Several studies geared towards their industrial applications, response to different stress conditions and genetic modification attempts via knowledge of its complete genome sequence, makes *Saccharomyces cerevisiae* the most prominent specie known to man [2], [3] [4]. *S. cerevisiae* has been widely employed in the brewing sector due to its ease of converting sugar-rich substrates into ethanol coupled with its unique tolerance to the end-product

and other inhibitory metabolic intermediates [5], [6]. However, studies also suggest that in light of the growing demand for bioethanol due to global energy consumption, several governments are sourcing for sustainable energy solutions whilst attempting to balance the long-term, and in some cases, the short-term needs of the environment [7]. In Nigeria and many developing countries, there is a lot of untapped feedstock material that are rich in a range of sugar types (arabinose, galactose, glucose, mannose, galactose, xylose, xylitol). Preliminary studies on the production of alcohol from these alternative carbon sources would aid in the supply chain, emulating countries like Brazil and America who generate bioethanol from their excess sugar cane and corn resources [7]. For that reason, research into the consumption and fermentation of pentose sugars would be a logical step in the production of ethanol from alternative organic stock [8]. As research is turning to the exploration of unconventional organic substrates for ethanol production, known limits of *S. cerevisiae* in alcoholic fermentation are being stretched [9], [10]. Different organic raw materials rich in lignocellulose is the foremost choice as these are selected based on the fact that its use does not hamper for production nor deplete agricultural resources materials [11]. The ability for non-*Saccharomyces* yeast strains to consume intricate nutrient types whilst showcasing industrially robust traits to fermentation process inhibitors (such as weak acids, furaldehydes, phenolics, etc) makes them ideal starter cultures that can compete effectively with *S. cerevisiae* [12], [13]. Reports from different studies reveal that such non *Saccharomyces* yeast species exist in nature and are commonly obtained from a range of contaminated foods as the main spoilage organism [14], [15]. Some of these unique non *Saccharomyces* yeasts include *Zygosaccharomyces rouxii* [14], [16], [17], [18]. *Kluyveromyces marxianus* [19], [20], *Pichia kudriavzevii* [21], [22], *Dekkera bruxellensis* [23], *Zygosaccharomyces bailii* [24]. *Meyerozyma guilliermondii*, a telemorph of *Candida guilliermondii* or wine yeast is one of such promising non *Saccharomyces* yeasts obtained from environmental samples with unique biotechnological applications and biological control potential [25], [26], [27], [28]. This yeast specie is known for its ease of consumption of xylose, naturally converting it to xylitol [29], [30]. In a previous study, *M. guilliermondii* was obtained as the main yeast strain obtained from soil samples within the environs of a local distillery in Bayelsa state, Nigeria [31].

In a bid to find a durable solution to the high ethanol demand, this study was conducted to tested the ability of *M. guilliermondii* to consume selected simple sugars coupled with its ethanol production potential in comparison to a known strain of *Saccharomyces cerevisiae*.

2. Materials and Methods

Meyerozyma guilliermondii strain of yeast used in this study was obtained and identified from previous studies at the Biotechnology Advanced Research Centre of SHESTCO whereas the control sample (Fali) was obtained from the Scotch whiskey Research Institute, Aberdeen, Scotland. Both yeast isolates were kept at a temperature of 4°C in Potato Dextrose Agar (PDA) and sub-cultured every three weeks. Analytical grade media; fructose, galactose, glucose, lactose, sucrose, Malt extract and agar agar were all purchased from Sigma Aldrich while yeast extract and peptone from Difco.

2.1. Ethanol Production Using Molasses

A loop of each isolate obtained from previous experiments was used to inoculate 100ml of autoclaved yeast extract, peptone, dextrose (YPD) broth in 250ml Erlenmeyer flasks. The flasks were incubated at 30°C, 120rpm for 24 hours. 10ml of each yeast cell suspension was transferred into 125ml broth composed of 6g/L yeast extract; 10g/L peptone; 6g/L malt extract; 2g/L glucose media. The pH of each medium was adjusted to 5.5. 25mls of autoclaved molasses was then introduced into each flask under the laminar flow hood. The flasks were then incubated for 96 hours at 30°C, 120rpm. At 24 hour intervals, samples were collected to measure sugar utilization and ethanol production. Ultra Violet visible (UV-Vis) spectrophotometry was used to measure yeast growth. All experiments were performed in triplicate and the data reported is the average of the three replications.

2.2. Sugar Uptake

24 hour cultures (10ml in YPD broth) were used to inoculate flasks containing yeast extract (6g/L), peptone (10g/L) and malt extract (6g/L) supplemented with different concentrations of fructose, galactose, glucose, lactose and sucrose (5g/L, 10g/L, 20g/L, 30g/L) respectively. The growth kinetics was characterized via absorbance measurements (OD_{580}) after 4 days to ensure a reasonable degree of fermentation. Sugar utilization was measured using a refractometer.

2.3. Statistical Analysis

All the experiments were conducted in triplicate and analysed using one way ANOVA.

3. Results

Meyerozyma guilliermondii was grown and used in the fermentation of molasses against the control *S. cerevisiae* strain T for an incubation period of 96 hours at 28°C. Consumption of simple pentose sugar tests revealed that at the different concentrations (0.5%, 1%, 2% and 3%) of fructose, *M. guilliermondi* did considerably better than its *S. cerevisiae* counterpart (figure 2A). The residual fructose concentrations in the broth was 1.7 ± 0.55, 1.9 ± 0.35, 2.1 ± 0.5 and 2.5 ± 0.25 g/L respectively for *M. guilliermondi*.
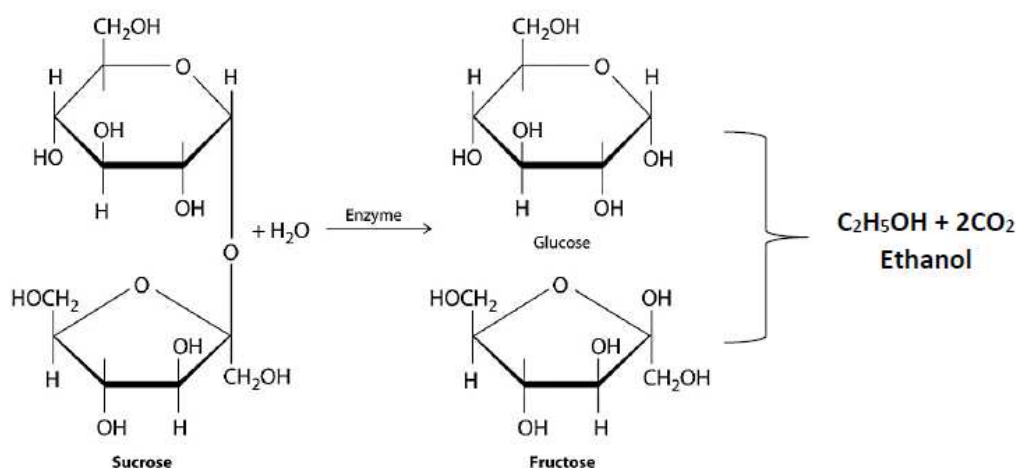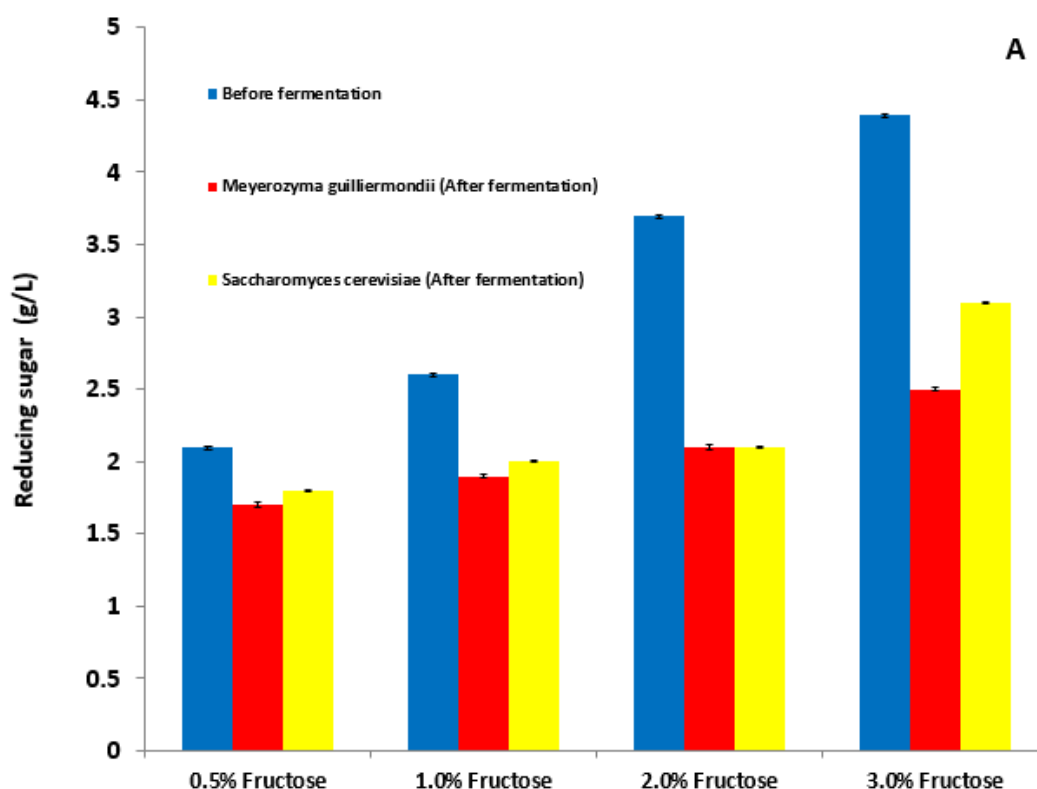
Figure 1. Sucrose hydrolysis and ethanol formation.

Concentrations of 1.8 ± 0.15, 2.0 ± 0.45, 2.1 ± 0.75 and 3.1 ± 0.35 (g/L) for the control sample was obtained. The corresponding values for ethanol obtained in the fructose supplemented YMP broth were 2.1 ± 0.25, 3.0 ± 0.55, 8.11 ± 0.95 and 9.06 ± 1.05 (mg/ml) in the *M. guilliermondi* samples while *S. cerevisiae* resulted in 1.08 ± 0.10, 3.12 ± 0.30, 8.06 ± 0.35 and 6.0 ± 0.75 (mg/ml) as depicted in figure 2B. Galactose Adaptation to galactose test (figure 4A) revealed that at either concentration of the simple sugar, both strains of yeast consumed fractional quantities for 0.5%, 2.0% and 3.0%. Broth samples containing 1.0% galactose was the most utilised of this sugar, which resulted in the highest measured concentration of ethanol produced (figure 4B). In the glucose consumption test, the best ethanol producing concentration was 3.0% glucose which produced 8.15 ± 0.20 and 9.08 ± 0.45 mg/ml of ethanol (figure 3A-3B). The negative control; lactose, employed in this study was not converted into ethanol (figure 4). The consumption of this sugar did not give any substantial value. Sucrose utilization (figure 5A, 5B) revealed that *M. guilliermondi* samples produced more ethanol (10.18 ± 1.25mg/ml) than that of *S. cerevisiae* (7.06 ± 0.90mg/ml) at 3.0% sugar supplement. Daily alcoholic fermentation data using molasses is captured in figure 6. The results showcase a steady increase in ethanol production of 0.9 ± 0.15ml, 2.0 ± 0.35ml, 3.4 ± 0.85ml and 4.8 ± 1.15ml for *S. cerevisiae* strain T and 1.6 ± 0.10ml, 3.9 ± 1.20ml, 6.5 ± 0.95ml and 9.2 ± 0.45ml for *M. guilliermondii* respectively (figure 6).
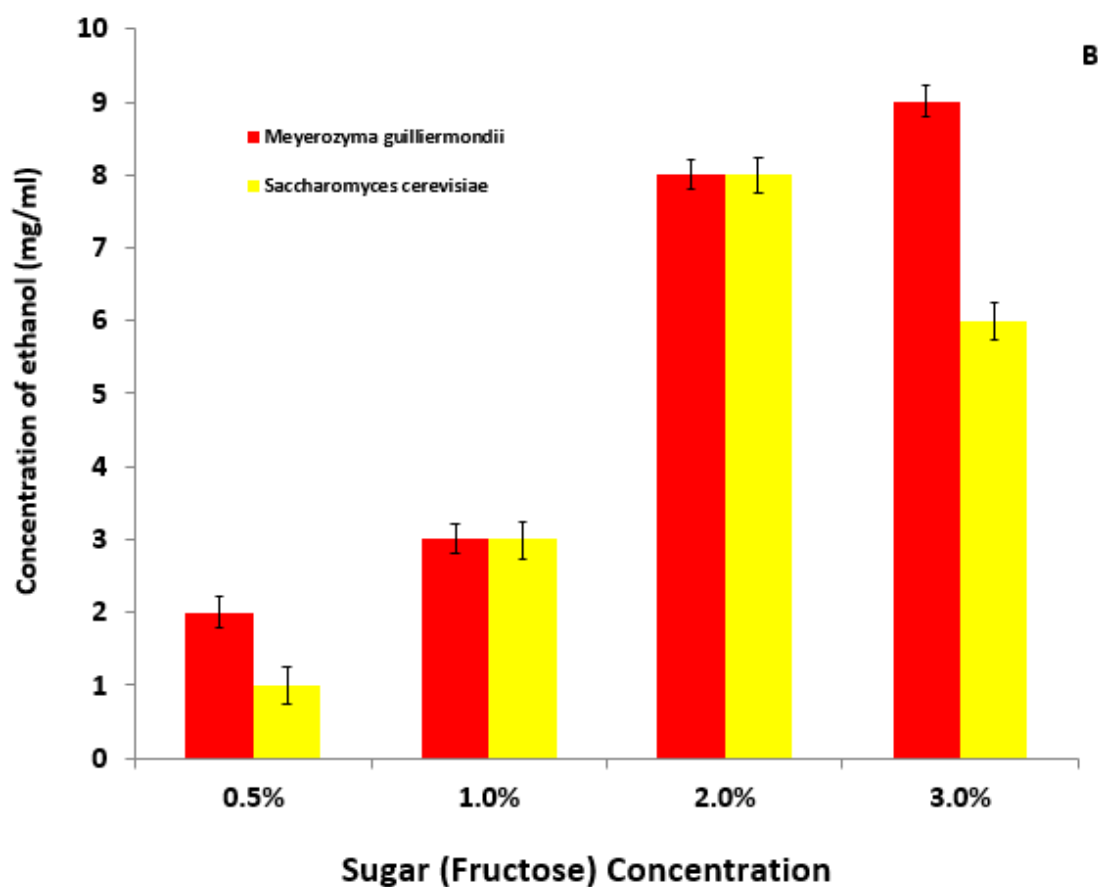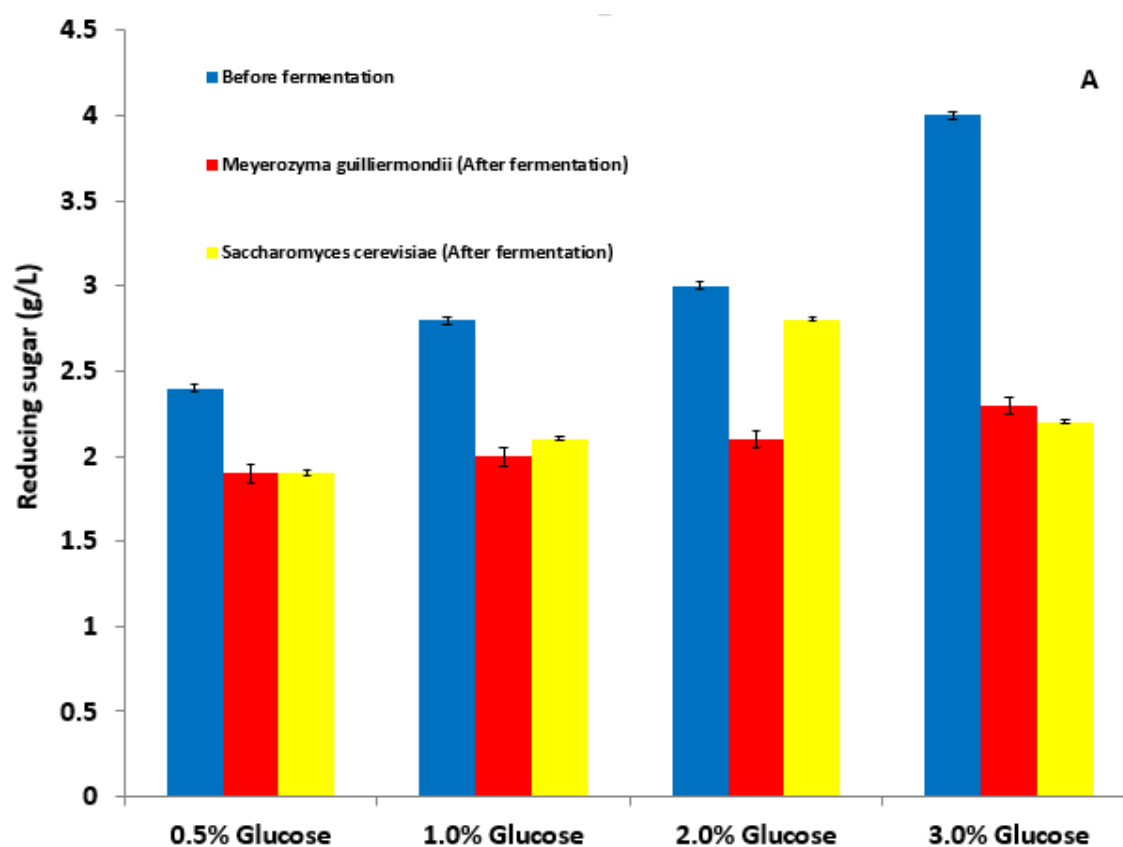
Figure 2. *Graphical representation of sugar consumption rates (A) pre and post fermentation for variable concentrations of fructose in YPM broth. B represents rate of ethanol production using fructose following 96 hours of incubation.*

Figure 3. *Graphical representation of sugar consumption rates (A) pre and post fermentation for variable concentrations of glucose in YPM broth. B represents rate of ethanol production using glucose following 96 hours of incubation.*
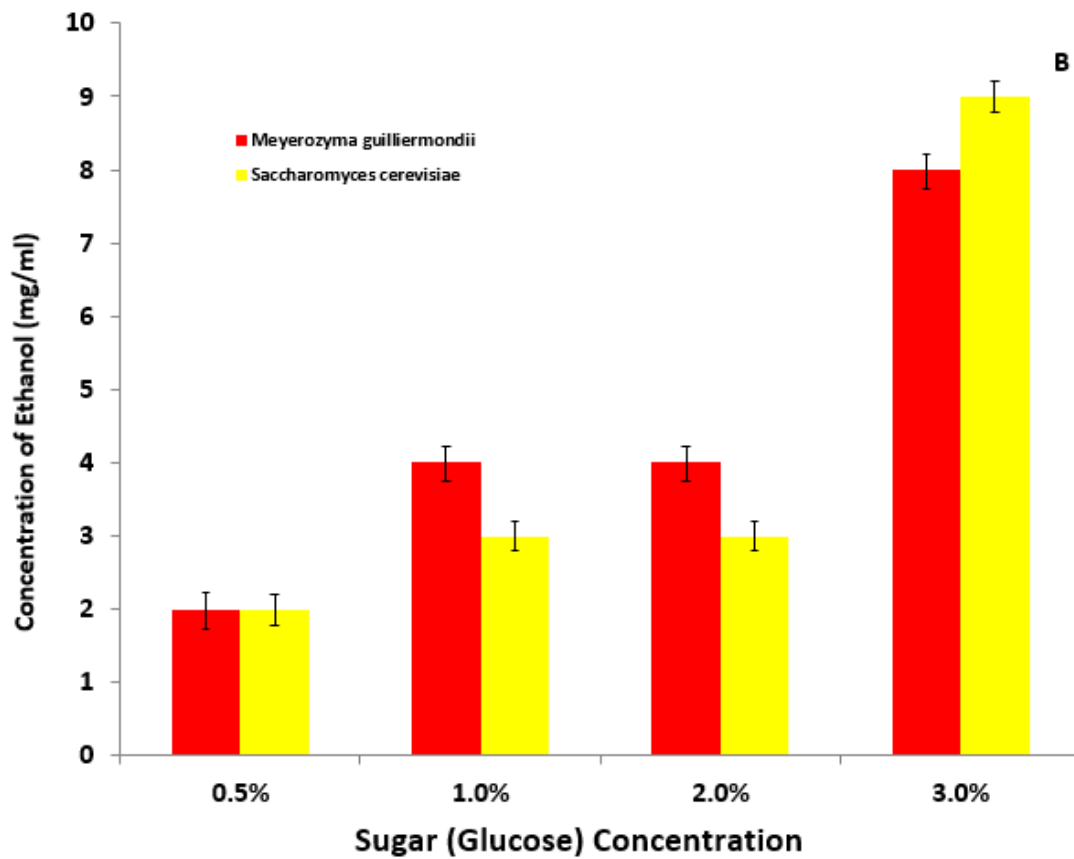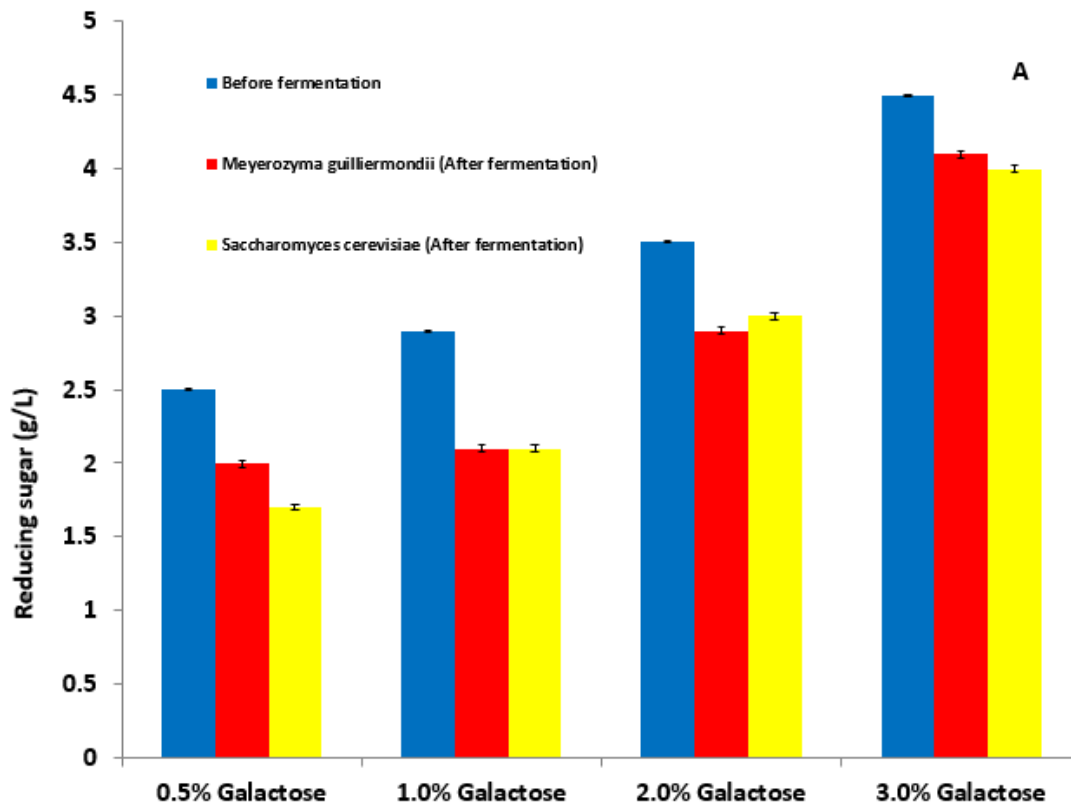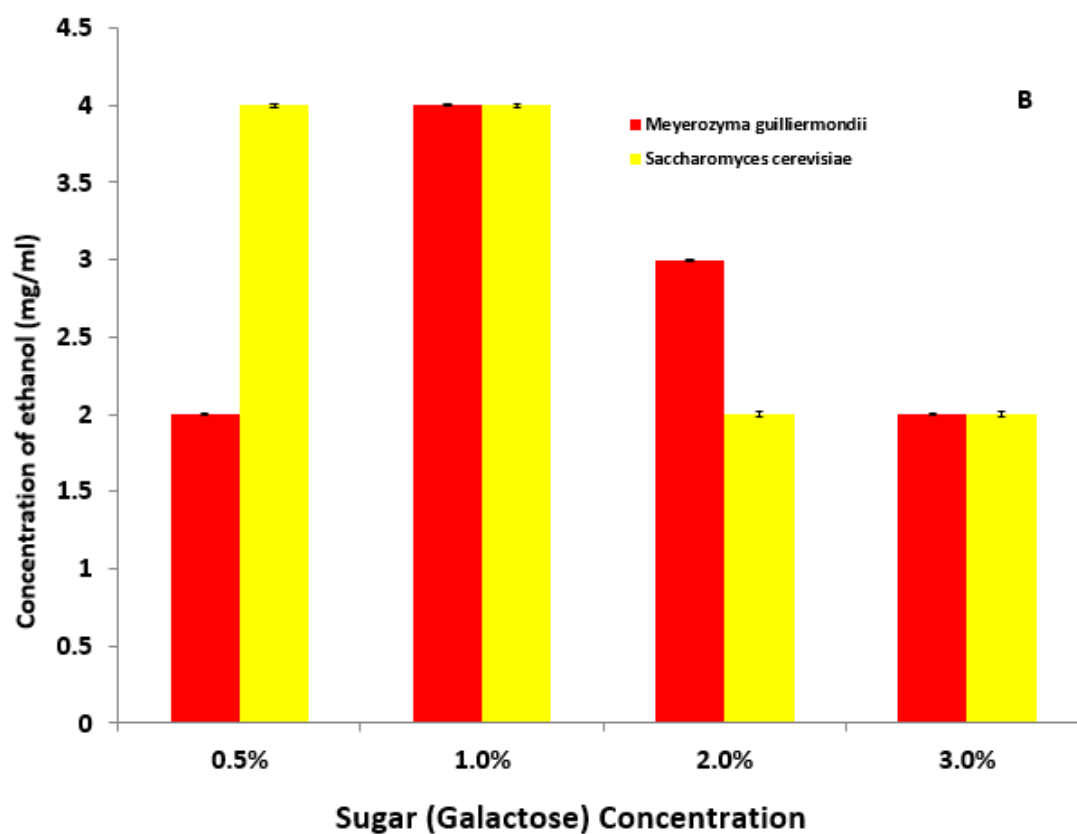
Figure 4. Graphical representation of sugar consumption rates (A) pre and post fermentation for variable concentrations of galactose in YPM broth. B represents rate of ethanol production using galactose following 96 hours of incubation.
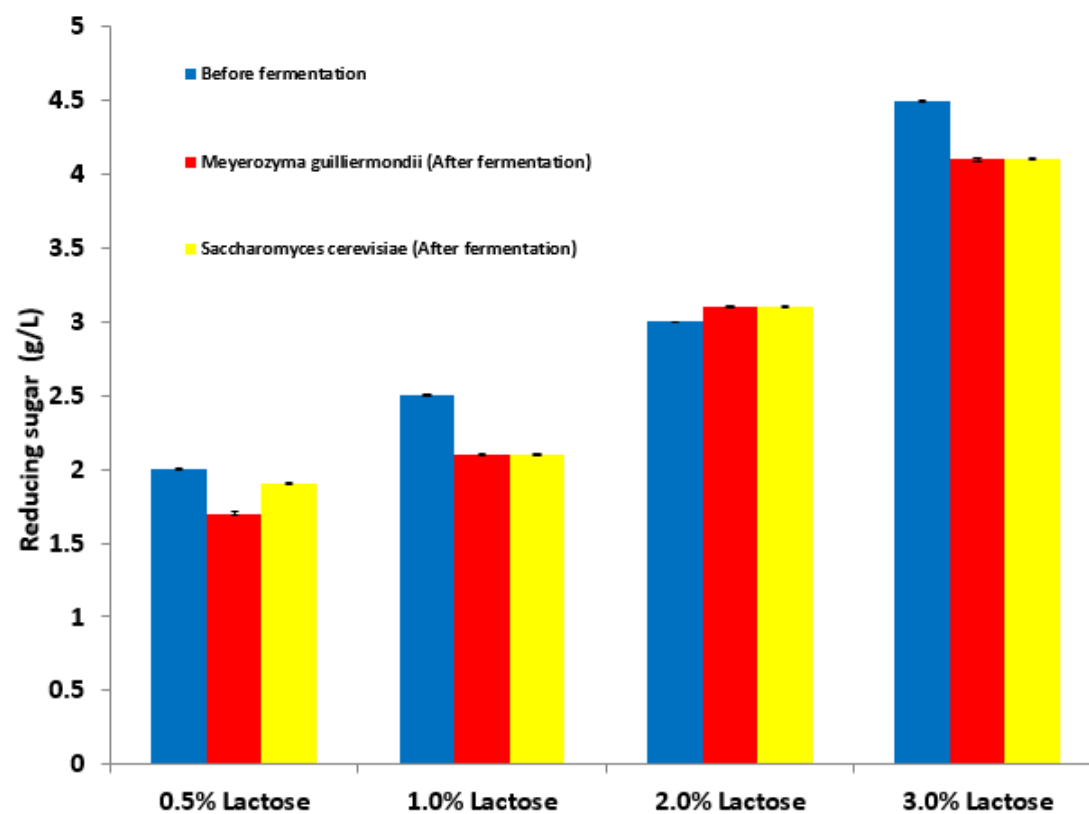

Figure 5. Graphical representation of sugar consumption rates (lactose) in YPM broth following 96 hours of incubation.
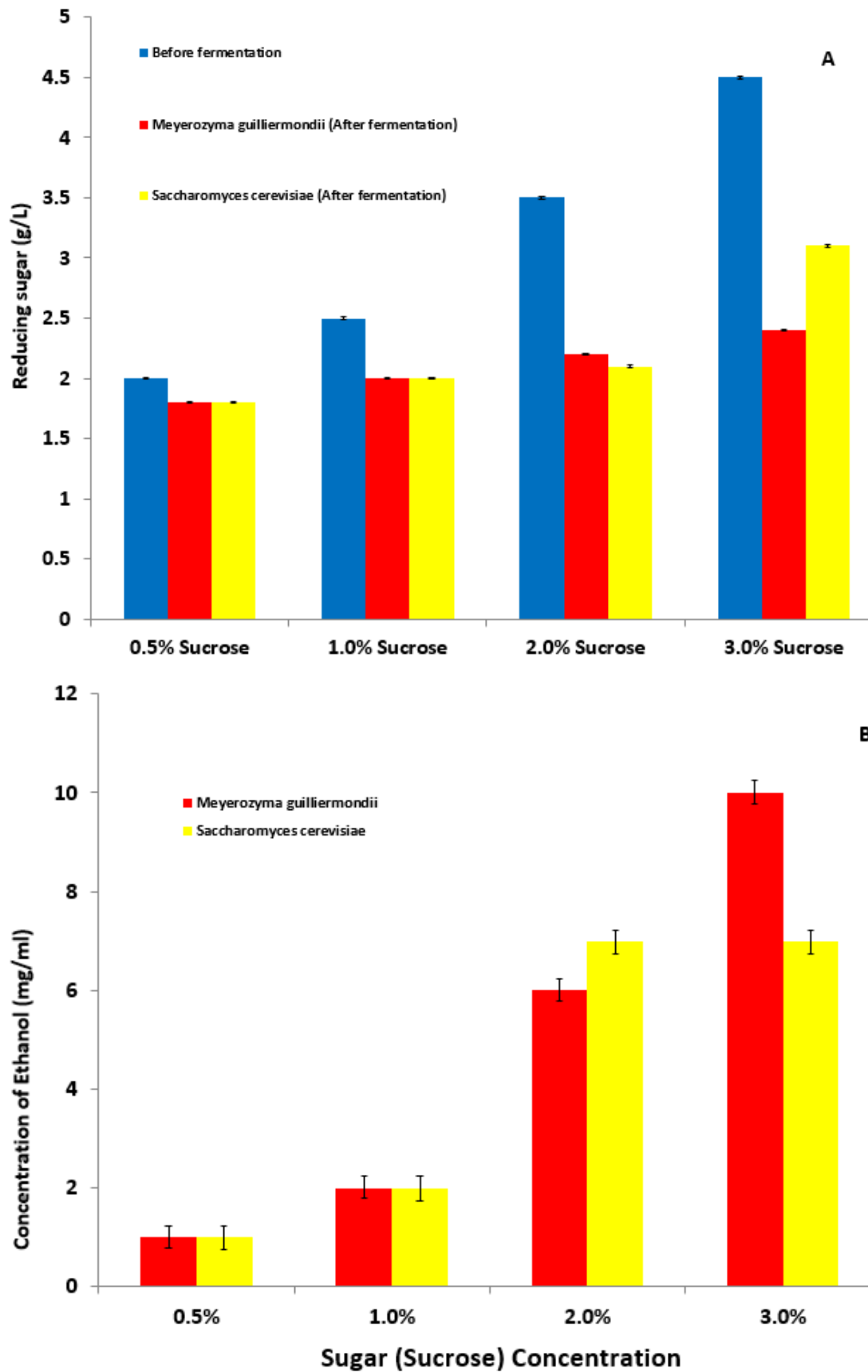
Figure 6. *Graphical representation of sugar consumption rates (A) pre and post fermentation for variable concentrations of sucrose in YPM broth. B represents rate of ethanol production using sucrose following 96 hours of incubation.*
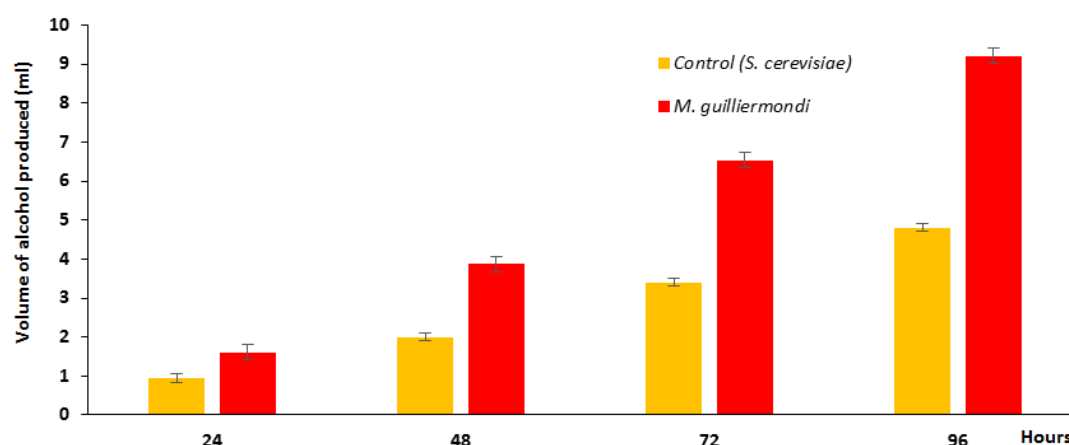
Figure 7. *Graphical comparison of the ethanol production rate from molasses by Saccharomyces cerevisiae and Meyerozyma guilliermondi.*

4. Discussion

Sluggish fermentation reported in the wine-making industry is associated with the incomplete conversion of pentose sugars into ethanol [32]. Studies suggest that most yeast species are more inclined towards high ethanol production from the conversion of pentose sugars like glucose than most other simple organic carbohydrate materials [32], [33], [34]. Research into the use of sugars like fructose for ethanol production revealed that only a small quantity of the carbon fluxes into ethanol while a large percentage lingers as unconverted fructose [35]. Yeast species with that exhibit good consumption of fructose is expected to be excellent in the conversion of glucose and sucrose, the former due to the ease or lateral conversion of its ring structure (figure 1) whereas the latter is via simple hydrolytic reactions. The correlation between sugar consumption and ethanol production was accessed (figure 2) with the hope of deducing alternative feedstock material with high concentrations of simple sugars like fructose, galactose, etc for indigenous yeast isolates. The data indicated that fructose consumption alongside ethanol production using *M. guilliermondii* increased concomitantly to increased sugar concentration (figure 2A, 2B). This was also observed in broth samples inoculated with *S. cerevisiae* (figure 2A, 2B). Assessment of strain performance revealed that the strain of *M. guilliermondii* used in this study was a better consumer of fructose at the different tested concentrations than that of *S. cerevisiae*, producing higher concentrations of ethanol particularly at 0.5 and 3.0%. This is indicative that in feedstock materials with high concentrations of fructose, this non-Saccharomyces yeast strain would be a more suitable candidate for ethanol production than the conventional *Saccharomyces* yeast. Due to the unique metabolic relationship between fructose and glucose, it would be expected that the tested yeast strains would demonstrate good consumption as well as high production of ethanol using glucose as the fermenting material. This study showed that glucose consumption was significantly higher at the highest concentration in direct proportion to the rate of ethanol production by both the test and control yeast strains (figure

3A, 3B). Unlike in the broth samples containing fructose as the sole carbon source, the indigenous strain of *M. guilliermondii* produced ethanol at a slightly lower concentration than the foreign *S. cerevisiae*. This could be as a direct result of the up-regulation of genes involved in respiration, gluconeogenesis, in the uptake and absorption of fructose which inherently favours the forward reaction of fructose better than that of glucose [34], [36], [37]. It is conceivable that intermediate compounds and the availability of enzyme cascades via the activation of certain regulatory genes creates competition for the available carbon whereby some are diverted to the formation of intermediates rather than partake fully in the catalytic events by glucose transporters to produce ethanol. This concept would help in explaining the possibility of glucose repression whereby yeast strains demonstrate adaptation to alternative carbohydrates like galactose or lactose. Although acclimatization to galactose is uncommon, it is still worth investigating phenotypic traits as this to rule out the possibility to employing evolutionary distinct yeast species to fermentation of unique carbohydrate sources. In this study, both *M. guilliermondii* and *S. cerevisiae* exhibited a low degree of flexibility towards the conversion of galactose to ethanol (figure 4B). The weak assimilation of galactose tallies with reports that suggest most yeast species are not fully adaptable towards galactose metabolism [21], [38]. This observation could be attributed to several factors including but not limited to its proton transport assembly and slow substrate affinities of its metabolic enzymes which could eventually cause feedback inhibition, thus decreasing the output. A lower concentration of galactose in the media of 0.5-1.0% mixed with glucose, fructose or sucrose may enhance ethanol production by these isolates.

Like most crab positive yeast species, *M. guilliermondi* and *S. cerevisiae* are not able to utilize lactose (figure 5). Using varying concentrations of lactose, this study revealed that both yeast strains were not capable of carrying out fermentation. This study also found that the rate of consumption increased parallel to increased sucrose concentrations, yielding increased amounts of ethanol. Also, *S. cerevisiae* appeared to have a sucrose optima of 2.0% as its

level of ethanol production dipped at 3.0% (figure 6A, 6B). In contrast, consumption and production of ethanol with *M. guilliermondi* improved at higher concentrations of sucrose. Overall, the data obtained validates results from other studies that propose a partial bioconversion of sugar into ethanol, the outcome of which would be the low yield of ethanol in comparison with the quantity obtained using molasses [34], [35], [39]. Total yield of ethanol from *M. guilliermondii* and *S. cerevisiae* using molasses medium (initial reducing sugar concentration of 18.3 w/v%) revealed that *M. guilliermondii* produced higher volumes (10 ± 0.2 mg/ml) than that of *S. cerevisiae* (9 ± 0.2 mg/ml) at 96 hours (figure 7). The demand curve for assortment of low, medium to high alcohol content wines has informed the decision to study various tactics towards the production of low-medium [27]. Data from this study suggests that the non-*Saccharomyces* yeast; *Meyerozyma guilliermondi*, can be applied towards the production of various categories of wine. Since grapes are the most predominantly used material in wine making, this results (figure 2A, 2B) suggests that *M. guilliermondi* can be applied in wine making. Overall, the unique results obtained from *M. guilliermondi* with regards to its rate of consumption of the different sugar types tested, aligned with its high yield of ethanol and in comparison with the *Saccharomyces cerevisiae* control strain tested suggests a robust genetic and physiological make-up. The sugar consumption and ethanol production pattern spanning the entire duration of incubation suggested that for the purpose of commercial production of ethanol *Meyerozyma guilliermondi* is an effective alternative to *Saccharomyces cerevisiae* for use.

5. Conclusion

This study indicated that indigenous strains of *Meyerozyma guilliermondi* are capable of growing and producing ethanol at room temperature using molasses. Ineffective sugar consumption is correlated to a reduction in ethanol production capacity. The use of local feedstock which are high in fructose content would be best utilised as an alternate material should a shortage in supply of conventional raw materials occur. The dynamics of the fructose transport carriers tempers with glucose metabolism thereby enhancing the rate of ethanol production in *M. guilliermondii*. Lactose adaptation was not apparent for both yeast species utilised in this study, whereas both displayed a tangible degree of adaptation to only small concentrations of galactose. A lower concentration of galactose in the media of 0.5-1.0% mixed with glucose, fructose or sucrose may enhance ethanol production by *M. guilliermondii*.

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