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Evaluation of Growth Kinetics and Biomass Yield Efficiency of Industrial Yeast Strains

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ABSTRACT

The aim of the study was to compare the growth and biomass yield efficiency of baker's, wine and brewing yeast strains on cane molasses using different nitrogen sources. Biochemical composition of cane molasses was assayed before it was used for the growth of the yeast strains. The result indicated that, biochemical composition of cane molasses was in the acceptable value. Following biochemical analysis, the three yeast strains were cultured in YPD medium and in different cane molasses concentrations, 5% (w/v), (10% w/v) and (15% w/v), using different nitrogen sources to compare biomass production and growth rate efficiency of these strains. The result revealed that biomass production and growth kinetics was higher in 10 % (w/v) and 15% (w/v) sugar concentration of baker's yeast than the others. However, in 5% (w/v) substrate sugar concentration, wine yeast showed higher biomass production and growth kinetics than the rest of the other strains. In addition, results of the study showed that, biomass yield and growth kinetics of the three strains were significantly affected by the type of nitrogen source used. High yield of biomass and growth rate was achieved in fermentation medium containing 2 % (w/v) ammonium sulphate ($(NH_4)_2SO_4$). However, potassium nitrate (KNO_3) had suppressive effect. From the result of this study, it is concluded that yeast biomass production can be industrialized using baker's yeast strain, 10 % to 15% (w/v) cane molasses sugar concentration as a substrate and ammonium sulphate as a nitrogen source.

Keywords: Biomass yield, growth kinetics, molasses, *saccharomyces cerevisiae*, single cell protein, yeast strains.

INTRODUCTION

Yeasts can be used in many industrial processes, such as the production of alcoholic beverages, biomass (baker's and fodder) and various metabolic products. Among these categories, biomass in general and baker's yeast specifically has developed from sugarcane molasses by using different strains and substrate concentration [1]

Now a day, the scientific knowledge and technology allow the isolation, construction and industrial production of yeast strains with specific properties to satisfy the demands of the baking and fermentation industry (beer, wine, baking) [2]. Yeasts are included in starter cultures, for the production of specific types of fermented foods like bread, sourdoughs, fermented meat and vegetable products, etc. The significance of yeasts in food technology as well as in human nutrition, as an alternative source of protein to cover the demands in a world of low agricultural production and rapidly increasing population makes the production of food grade yeasts extremely important [3].

A large part of the earth's population is malnourished, due to poverty and inadequate food production. Scientists are concerned whether the food supply can keep up with the world's increase in population. Therefore, the production of microbial biomass for food consumption is a main concern for the industry and the scientific community in minimizing the above mentioned problem. The impressive advantages of microorganisms for single cell protein (SCP) production compared with conventional sources of protein (soybeans or meat) are well known.

Microorganisms have high protein content and short growth times, leading to rapid biomass production, which can be continuous and is independent from the environmental conditions [4]. In addition, these cells require only cheap and widely available substrate in the environment. The most widespread and commonly used substrates for SCP production have been those where the carbon and energy source is derived from carbohydrates, molasses, whey, methanol and cellulose. This is because their building blocks (mono and disaccharides) are natural microbial substrates, and that the raw materials are a renewable resource, which are widely distributed.

The principal raw materials used in developing baker's yeast were the pure yeast culture and molasses. Cane and beet molasses were used as the principal carbon sources to promote yeast growth and supplies all the sugar that yeast needs for growth and energy source along with part of the needed nitrogen [5].

The rationale of this work was to investigate the efficiency of cane molasses in supporting the industrial production of baker's (*Saccharomyces cerevisiae*), wine and brewery (*Saccharomyces carlsbergensis*) yeast and evaluating the growth kinetics and biomass yield of these three strains at different substrate concentration and different nitrogen sources.

MATERIALS AND METHODS

Materials

Cane molasses, were collected from Wonji Shoa Sugar Factory and analyzed for its biochemical composition before it was used for fermentation. Baker's yeast (*Saccharomyces cerevisiae*), brewery yeast strain (*Saccharomyces carlsbergensis*) and wine yeast strain were collected from commercial market, Meta Abo Brewery Factory, and from the laboratory, respectively. All strains were labeled and kept in the refrigerator to avoid contamination and activity loss.

Methods

Biochemical compositions analysis of cane molasses

The biochemical composition of cane molasses was analyzed by using the method described by Ethiopian standard [6].

Determination of total solids: About Ten gram cane molasses was weighed, well mixed and diluted with 50gm of distilled water to get 60gm of total weight. The resulting solution was stirred with glass rod until completely dissolved. Then, it was fluted through whatman no 41 filter paper covered on the funnel with a watch glass to minimize evaporation. The first 20ml was ejected and sufficient filtrate were collected in a 150ml beaker for determination of refractometric brix and reading from refractometer was taken after adjusting the molasses solution at 20 °C using water bath [6].

Determination of sulphated ash: A 1:1 w/w solution, 20g of water with 20g of cane molasses, was prepared. After the solution was mixed, 10g of distilled water was added and mixed. Then, 2 ml of sulphuric acid was added and heated in a fume cupboard until the solution was completely carbonized. The solution was placed in a muffle furnace at 550 °C for 2 hrs. Two milliliters of H₂SO₄ acid solution was added after it was cooled and then it was allowed to evaporate on Bunsen burner in a fume cupboard. Finally, it was incinerated at 650 °C for 30 minutes and cooled in the desiccators. The final weight of the samples was taken and used to calculated sulphated ash of the cane molasses in percent [6].

Determination of total reducing sugars: This method rely upon the reducing of Fehling's solution by reducing sugars present in the molasses under standard conditions. 50 g/l hydrolyzed cane molasses solution was prepared and 25ml of the cane molasses solution was transferred in to a 250ml volumetric flask. Five ml of the 6.34 N HCl acids was added to the flask and mixed gently. The flask was immersed in the water bath at 60 °C and swirled gently for 3 minutes to raise the temp of the sample. It was left in the water bath for further 12 minutes. The flask was cooled and diluted with water to approximately to a volume of 125ml with water and then a few drops of phenolphthalein solution were added in to it. About 4.4 ml of 2N NaOH solution was added to impart a reddish color to the solution. During the addition of the alkaline, the solution was gently agitated. The red color of the solution was discharged by adding a few drops of 0.5N HCl, 4.0ml of the EDTA solution. Twenty ml Fehling's solution was pipetted in the boiling flask which had 20 ml of the hydrolyzed cane molasses solution with 2 - 4 drops of paraffin. The flask which had Fehling's solution and standard invert solution was boiled on Bunsen burner and 4 drops of the methylene blue solution was added. Titration was preceded by initial addition of 2ml molasses solution and progressively reducing the additions down to 0.2ml and attempting to obtain the end point in about two minutes from the time the solution commencing boiling. The end point was denoted by the disappearance of the blue color.

Further testing was carried out on the next sample by adding 20ml of Fehling's solutions to the boiling flask and adding sufficient water to give volume of 75 ml. After 2 minutes of boiling four drops of methylene blue solution were added. Titration was made by adding the hydrolyzed molasses solution drop wise and the total reducing sugar concentration was calculated according to modified method of John [5, 6].

Determination of nitrogen: Molasses sample was weighed and transferred into a tector tube. Five ml of NH_4Cl solution was added in to each tector tubes and 6 ml mixed acid (5 parts of conc. Orthophosphoric acid with 100 parts of conc. Sulphuric acid) and the solution was mixed carefully. Hydrogen peroxide (3.5 ml) was added step-by-step. Then, 3 grams of the catalyst mixture were added for 30 min before digestion. Distillation was carried out on a 250 ml conical flask containing 25 ml of the boric acid, indicator solution under the condenser of the distiller with its tip immersed into the solution. The digested and diluted solution was transferred in to the sample compartment and 25 ml of the 40 % sodium hydroxide solution was added into the compartment, it was rinsed down with a small amount of water, and the steam was switched on. It was distilled until it becomes 100 ml then continued till it becomes a total volume of a few ml of water before the receiver was removed. Then it was titrated with 0.1N sulphuric acid to a reddish color using the radio meter pH stat. The percentage of nitrogen in a sample was calculated [6].

Determination of calcium: Wet Ashing method was used to test calcium content of the cane molasses using 60ml tri-acid solutions to 4g molasses sample in beaker or conical flask cover with a watch glass and allowed to stand for 8hours. Initially, it was digested at low heat on a hot plate but after a while temperature was raised to 200 °C and digested until oxidation is completed. Three ml concentrated HNO_3 was added and reheated. Temperature was increased until more fumes were evolved. The solution was cooled and 20ml 2N HCl was added. The resulting solution was boiled for 2-4 hrs, transferred to 100ml volumetric flask and filtered through whatman no 541. One ppm, 2ppm and 3ppm standard solution were prepared for calibration and reference reading of sample testing in the atomic absorption. Atomic absorption reading were taken and used to calculate calcium content of the sample cane molasses [6].

Production of yeast

Preparation of culture media from sugar cane molasses

The amount of sugar in the substrate cane molasses was estimated by Dinitrosalicylic acid method. The absorbance readings taken at 540nm wave length were converted in to the corresponding sugar amount from standard curve. Based on this result, culture media from sugar cane molasses substrate was prepared in 5 % (w/v), 10 % (w/v) and 15 % (w/v) sugar concentration. Finally, the pH was adjusted within the range of 5-5.6 and sterilized at 120 °C for 20 minutes in the autoclave [7].

Preliminary yeast growth experiment

The experiment was carried out by taking 5 g baker's, wine and brewery yeast strains into YPD media, and 5% (w/v) molasses and 5 % (w/v) molasses having 1.8 % NH_4NO_3 . Yeast growth was measured every two hrs for 14 hrs at 540 nm wave length. Based on the data the microbial growth curves were plotted for each strain.

Biomass yield of the three strains in different substrate concentration

Five gram pure strain of baker's, wine and brewery yeast were inoculated in 100 ml of YPD medium and shaken with 120rpm for more than 12 hrs. Ten milliliters of cultured yeast was transferred in to a test tube and centrifuged with 4500 rpm for 20 minutes. Supernatant was removed and the pellet was collected and inoculated into 5 % (w/v), 10 % (w/v) and 15 % (w/v) cane molasses samples which was enriched with 2 % (w/v) NH_4NO_3 , $(\text{NH}_4)_2\text{SO}_4$ or KNO_3 .

Analytical method used for measuring and analyzing yeast growth

Yeast growth kinetics

Five ml of cultured medium sample was taken in every 12 hours time interval until the 36th hour. The resulting solution optical density was measured at 540 nm wave length. A blank solution was used for reference reading. The results were recorded and used in plotting growth curve of the yeast cells in different sugar concentration of cane molasses [8].

Biomass estimation

At the end of the yeast growth, the biomass was harvested centrifuging the culture medium at 4500 rpm for 20 minutes. During centrifuging the supernatant and pellet was separated. The liquid part of the culture, supernatant, was utilized for the determination of the residual sugar estimation. The remaining pellet was diluted with 5 ml distilled water and its optical density was measured at 540 nm [8, 9].

Residual sugar estimation by Dinitrosalicylic acid (DNS) method

The glucose concentration in different substrate concentration was determined by the 3, 5-dinitrosalicylic acid (DNS) method [7]. Standard glucose solutions were prepared in concentrations of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9, and 1.0 g ml⁻¹. One ml of standard glucose solution was pipetted into a test tube and 3 ml of DNS reagent was added to it. The mixture was placed in boiling water for 5 minutes to develop color. After 5 minutes, the sample was cooled at room temperature. About 2 ml of the sample was placed in a cuvette and the absorbance was measured at 540nm. The readings were used in plotting the graph.

Total protein concentration estimation

Estimation of protein concentration in baker's, wine & brewery yeast in different substrate concentration of sample was determined following standard procedures [10]. Sample to be analyzed was centrifuged at 4500rpm for 20 minutes after testing the yeast cell growth. Supernatant was removed; the remaining pellet was diluted with 5 ml distilled water and added 1 ml of 0.85 % (w/v) NaCl solution. Pellet solution, 0.1 ml, was dissolved in 2.9 ml of distilled water in different test tubes which is followed by the addition of 1ml alkaline copper sulphate reagent. The resulting solution was mixed and incubated at room temperature for 10 minutes. Then, 0.5 ml of the solution was added to each tube, vortexed and incubated for 30 min in dark area. Spectrophotometer readings were recorded at 720 nm. Protein concentration (g/l) was calibrated using appropriate standard graph BSA (Bovine Serum Albumin).

Statistical analysis of the experiments

Completely randomized design (CRD) for the experimental analysis was used and data were analyzed using SPSS/14.0 software. Analysis of variance (ANOVA) was conducted to test least significance differences (LSD). Significance was accepted at 0.05 level of probability ($p < 0.05$).

RESULTS AND DISCUSSION**Biochemical composition of cane molasses**

Molasses composition shows wide variation. Its composition is influenced by factor such as soil type, ambient temperature, moisture, season of production, variety and technology of sugar mills which all these factors can control the amount of sucrose extracted [11]. Because of this the sugar content of molasses produced in different countries varies according to the production technology employed. According to Curtin [12], changes in the design of centrifuges used to separate sugar and syrup constitute one of the major advancements in the cane sugar industry. Continuous centrifugation results in more sugar extraction with a corresponding decrease in the amount of sugar left in molasses. Molasses biochemical composition analysis result of this study was acceptable to the standard set by Ethiopian standards [6] and was in agreement with the study of John [5] and Curtin [12] as it is presented in Table 1.

Table 1 Summary of laboratory results and comparing the result with other publications

Parameter	Ethiopian standards(w/v)	John (w/v)	Curtin (w/v)	Laboratory Results(w/v)	Remarks
Total solids	85%	79%	79.5%	80%	✓
Total reducing sugar	50%	45-55%	46%	47.7%	✓
Reducing sugar	14%	10-15%	-----	11.16%	✓
Nitrogen	---	0.15-.8%	-----	0.88%	✓
calcium	---	0.08-.5%	0.8%	0.42%	✓
Ash	14%	8.1%	8.1%	8.3%	✓

[5, 6, 12]= Acceptable or within the standard range

Preliminary growth kinetics analysis of the yeast strains in molasses

Many studies reported that sugar cane molasses can be used as a substrate for the production of yeast biomass [13, 14, and 15]. The preliminary study conducted to test the potential of the cane molasses in supporting the growth of yeast cells indicated that, the yeast strains were significantly able to grow by assimilating the sugar present in molasses regardless of the initial amount of the sugar concentration (Fig 1, Fig 2, Fig 3 and Table 2). Higher efficiency of sugar utilization was observed by baker's yeast and wine yeast strains.

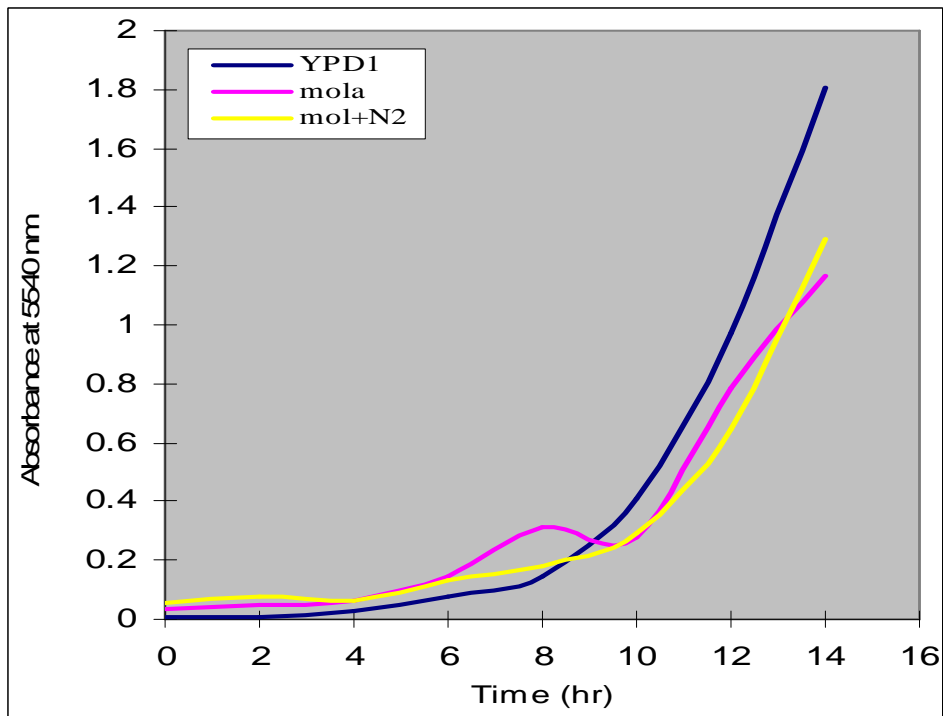


Fig 1. Baker's yeast growth on YPD, molasses and molasses with NH_4NO_3

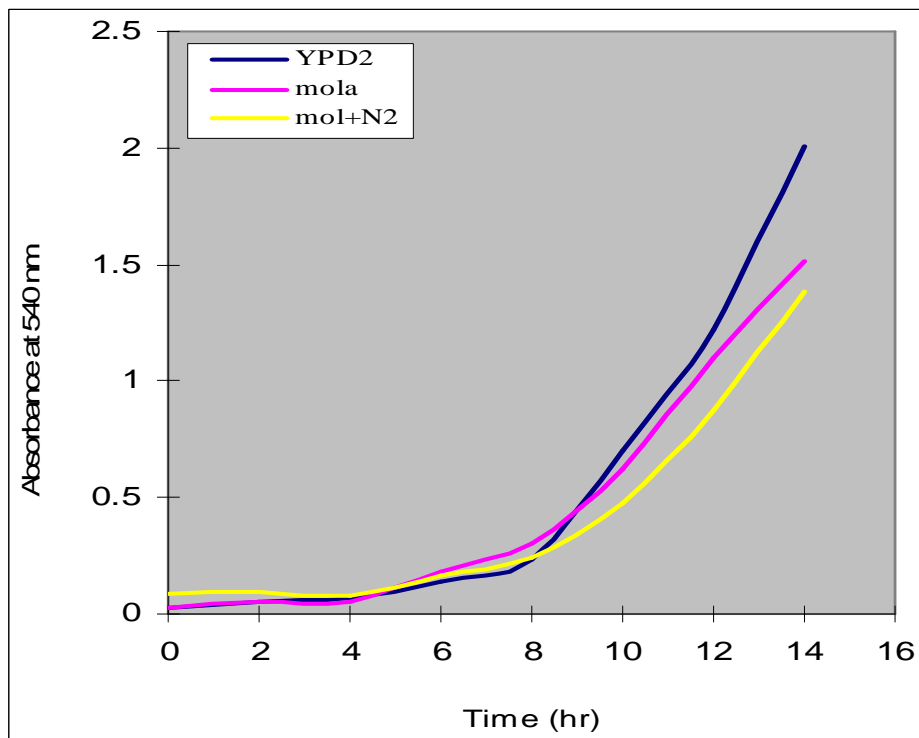


Fig 2. Wine yeast growth on YPD, molasses and molasses with NH_4NO_3

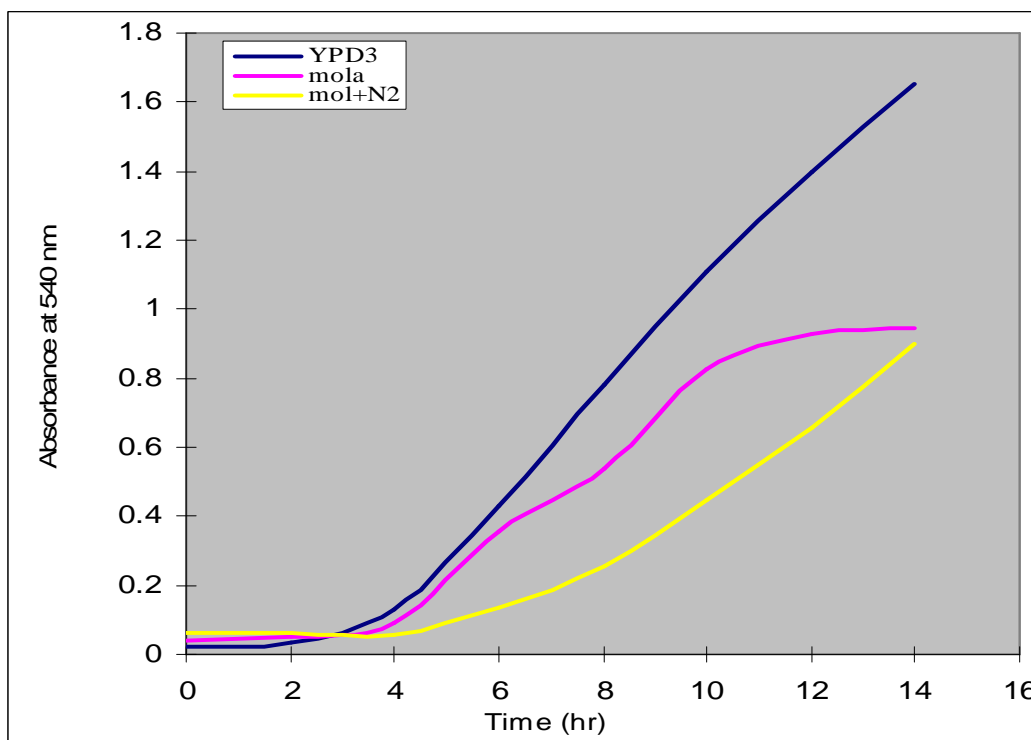


Fig 3. Brewery yeast growth on YPD, molasses and molasses with NH₄NO₃

Table 2 Percentage of sugar assimilation efficiency on baker’s, wine and brewery yeast strain in YPD, molasses & molasses with 1.8 % NH₄NO₃

Types	Baker’s yeast			Wine yeast			Brewery yeast		
	YPD	Mol.	Mol. + NH ₄ NO ₃	YPD	Mol.	Mol. + NH ₄ NO ₃	YPD	Mol.	Mol. + NH ₄ NO ₃
Sugar assimilation (%)	92.7	88.6	79	93	71	30.8	24	49	5.5

YPD= Yeast extract, peptone and dextrose medium, Mol. = Cane molasses

Growth Kinetics and biomass yield of the strains on different substrate sugar concentration

Elevated sugar consumption by yeasts causes a high substrate conversion to ethanol and other bi-products. This will severely affect cell physiology, bioprocess performance, and biomass yield [16]. As a result, it is empirical to determine the optimum substrate sugar concentration suitable for production of yeast biomass. In this study after growing for 36 hrs, baker’s, wine and brewery yeast strains showed the least residual sugar concentration in 5%, 15% and 10% (w/v) substrate sugar concentration, respectively (Table 3). Wine yeast used more than 60% of the available sugar and also produced high protein yield at 5% (w/v) substrate sugar concentration than the other strains during their 36 hrs of fermentation (Table 3, Fig 4, 5 and 6).

Table 3 Residual sugar concentration at 5%, 10% & 15% molasses sugar concentrations of the yeast strains

Time(hr)	Baker’s yeast			Wine yeast			Brewery yeast		
	5 % (w/v)	10 % (w/v)	15 % (w/v)	5 % (w/v)	10 % (w/v)	15 % (w/v)	5 % (w/v)	10 % (w/v)	15 % (w/v)
0	63.5	103	161.5	71.5	145	162	82.5	102	165
12	43	89	92	31	29.5	130.5	78	72	62
24	40	69.5	79	24.5	60	64.5	73	72.6	40.3
36	40	54.2	86	24	58	61	64.5	24.5	36.5

Even though brewery yeast showed higher sugar utilization potential (around 75 %) than the other yeast strains in 10% and 15 % (w/v) sugar concentration of the culture media, the strains were unable to produce significant amount of protein as it is presented on Table 3, Fig 4, 5 and 6. This might be due to the conversion of the available sugar in to alcohol rather than to assimilate it to their biomass [17]. On the other hand, baker’s yeast used only less than 50% of the available sugar in the media during 36 hours of fermentation period. However, they produced high protein yield than the other yeast strains in 10% and 15% of sugar concentration (Table3, Fig 4, 5 and 6). The study of Highina [18] also showed that around 17% molasses sugar concentration were optimal for the growth of baker’s

yeast. Similarly, Ortiz-Muñiz *et al* [20] showed that, some strains of baker's yeast were osmotolerant and showed high growth rate and biomass yield between 10 % (w/v) to 20% (w/v) of molasses sugar concentration.

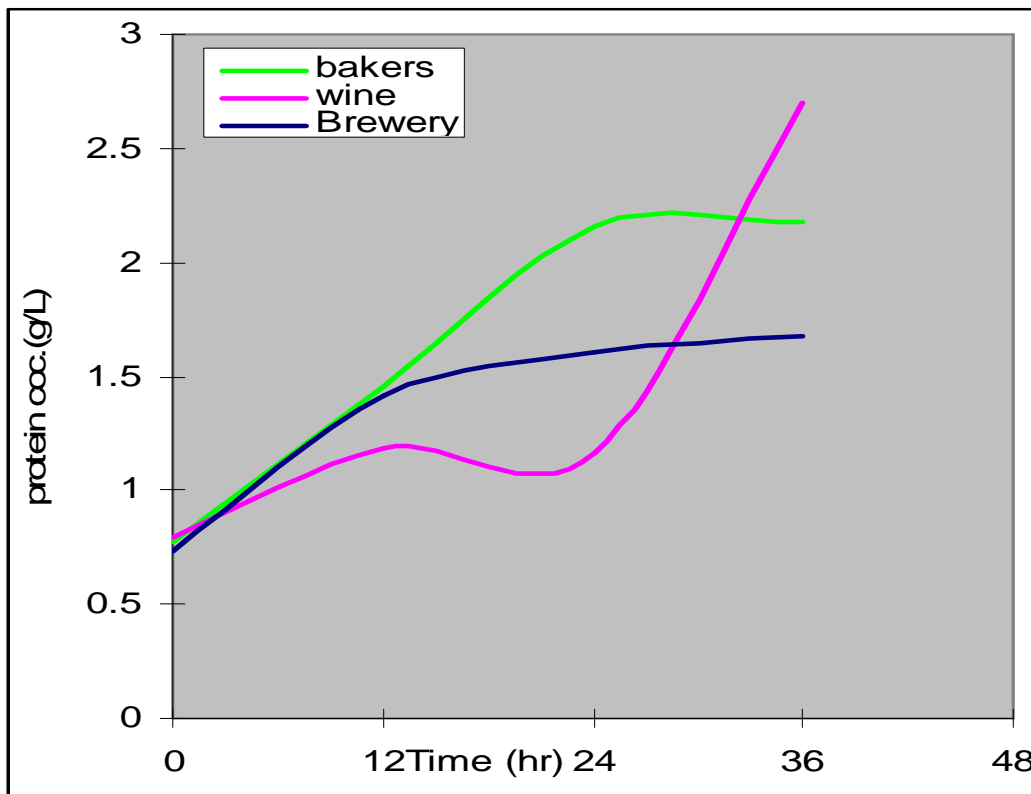


Fig 4. Protein conc. on baker's, wine and brewery yeast on 5% sugar conc.

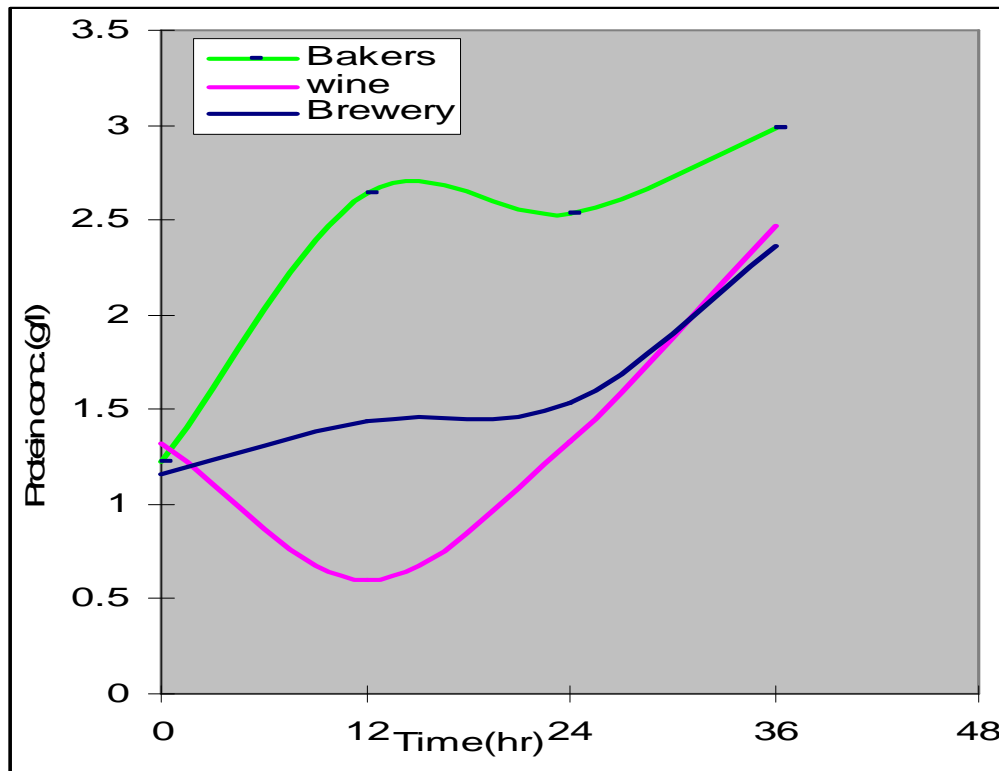


Fig 5. Protein yield of baker's, wine and brewery yeast strains on 10 % sugar conc.

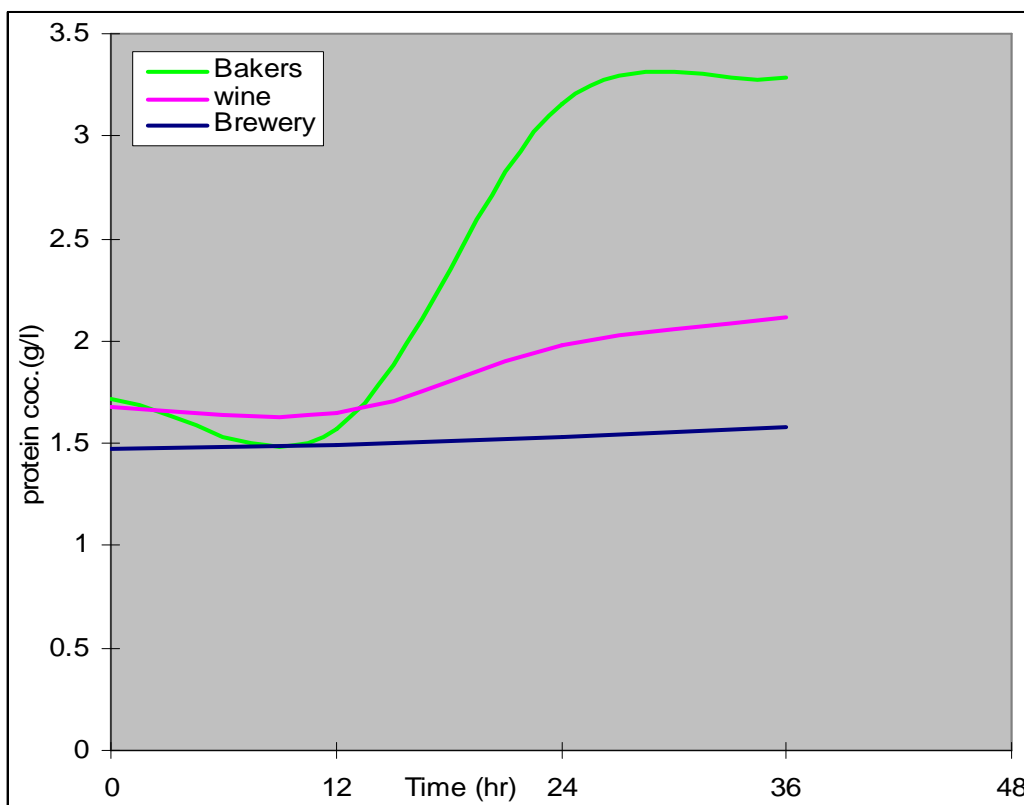


Fig 6. Protein yield of baker's, wine and brewery yeast strain on 15 % sugar conc.

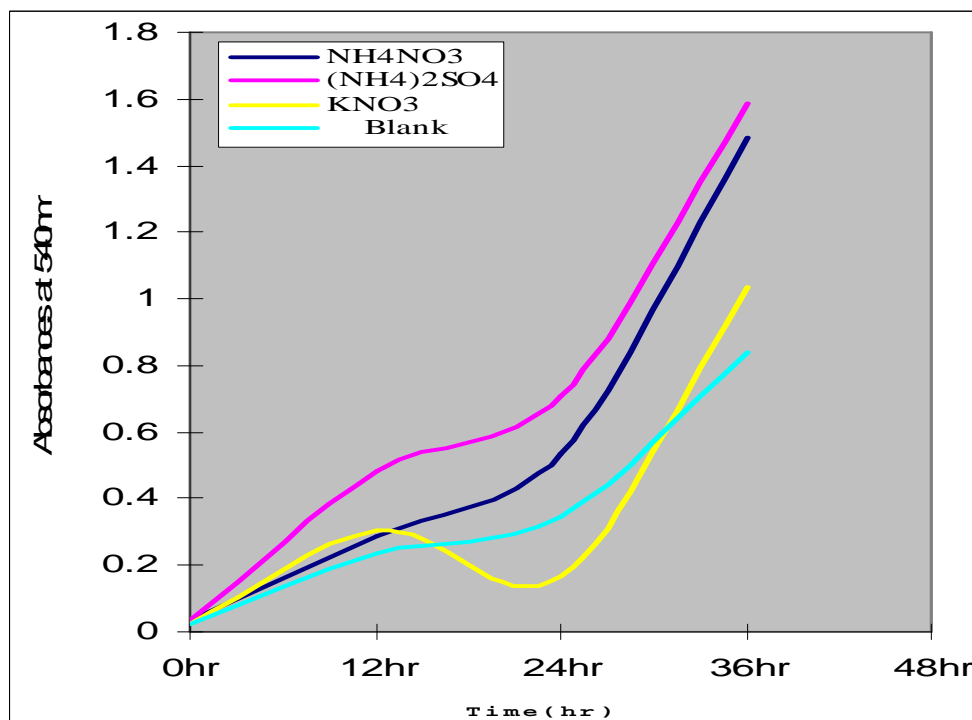


Fig 7. Growth of 10 % sugar conc. on baker's yeast with NH₄NO₃, (NH₄)₂SO₄ and KNO₃

Effect of NH₄NO₃, (NH₄)₂SO₄ and KNO₃ on the kinetic and biomass yield of the three yeast strains in 10 % molasses

According to Curtin [12], cane and beet molasses are not rich in basic elements like nitrogen, phosphors, calcium and magnesium which are vital for the growth of yeast strains. Nitrogen is the basic nutrient in enhancing yeast growth. According to Gutierrez *et al* [20] nitrogen deficiencies are one of the main causes of stuck or sluggish Fermentations. Thus, addition of nitrogen sources is needed; generally in the form of ammonium salts, aqueous

ammonia, soluble proteins or urea in addition to the amino acids present in molasses [21]. In this experiment, nitrogen was supplied as 2 % (w/v) NH_4NO_3 , $(\text{NH}_4)_2\text{SO}_4$ and KNO_3 salts in investigating which of these nitrogen sources are effective for high biomass yield of the three strains.

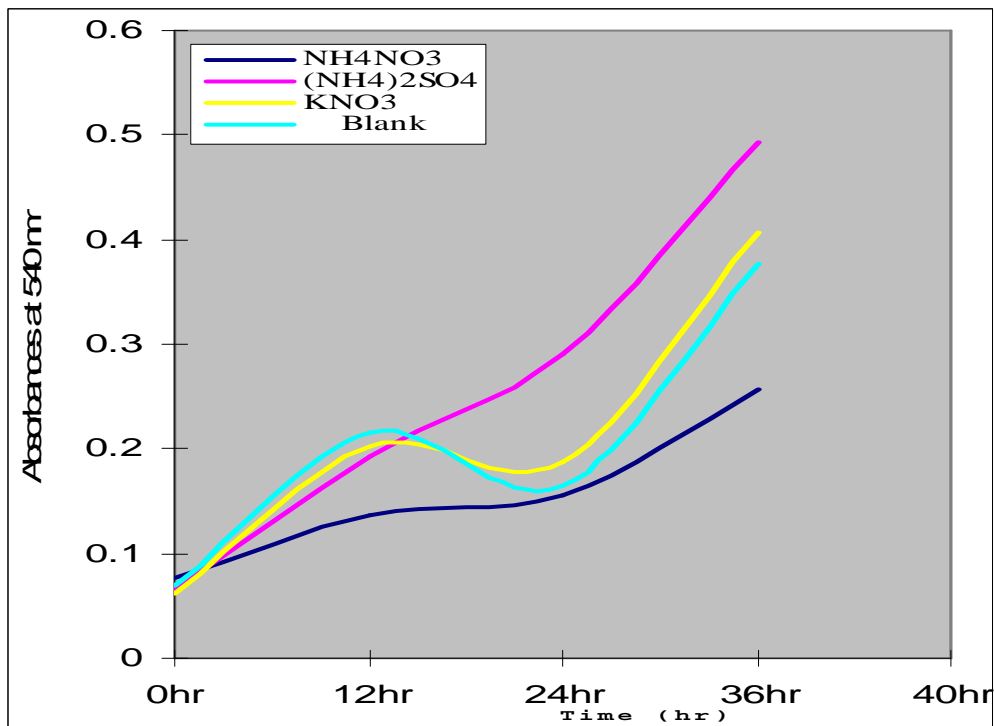


Fig 8. Growth of 10 % sugar conc. on wine yeast with NH_4NO_3 , $(\text{NH}_4)_2\text{SO}_4$ and KNO_3

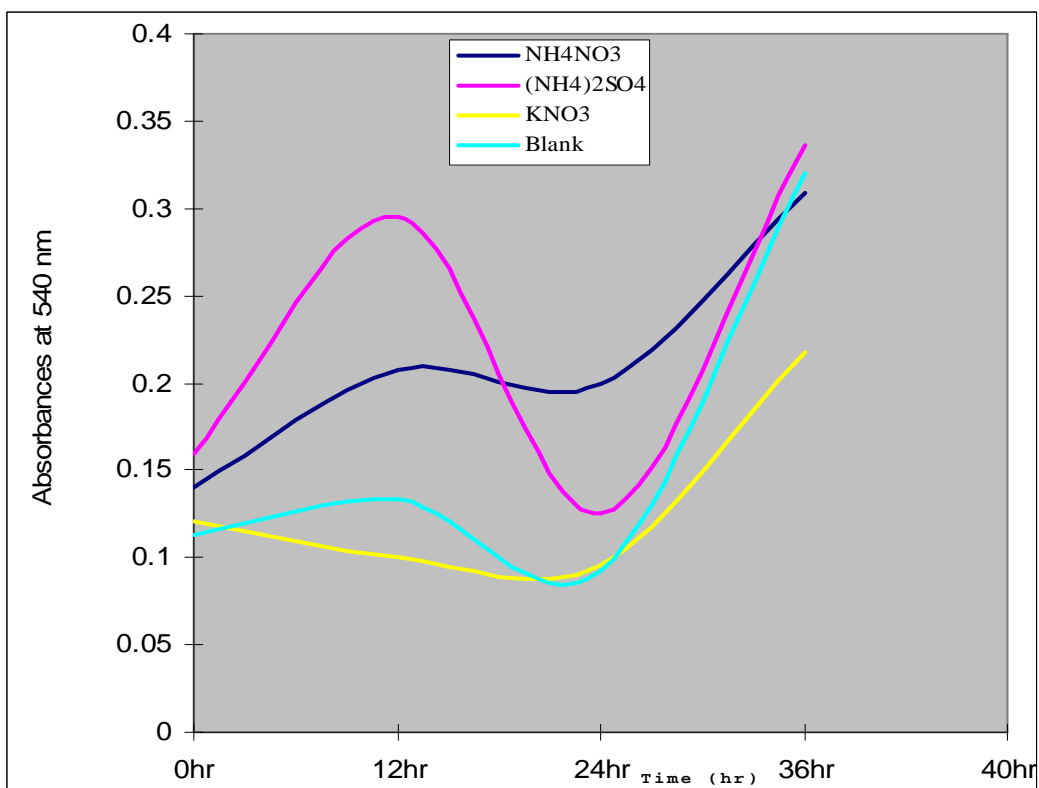


Fig 9. Growth of 10 % sugar concentration of brewery yeast with NH_4NO_3 , $(\text{NH}_4)_2\text{SO}_4$ and KNO_3

Generally, there was growth in all yeast strains in all nitrogen sources at different level. Yields of biomass varied considerably and were found to be dependent on the types of the yeast strain and the type of nitrogen source used.

From the growth curve indicated on Fig 7, Fig 8 and Fig 9, substrate with 2 % (w/v) ammonium sulfate had maximum growth rate and biomass production in all yeast strains than the other nitrogen sources tested in this study. The study of Arrizon and Gschaedler [22] also clearly showed that ammonium sulfate was efficient nitrogen sources of different yeast strains.

Preson [23] demonstrated that, if sugars are fermented in the presence of adequate amount of nitrogen, less alcohol is formed because the environment is more favorable for the growth of the yeast. Moreover, the additions of ammonia on the molasses will hydrolysis some of the sucrose to a reducing sugar. As the data indicated in Table 4, baker's yeast had high protein yield than the other strains. However, wine and brewery yeast strains biomass yield were insignificant during 36 hrs of fermentation period. Gellissen [24] reported that very few species have ability to utilize nitrate as nitrogen source.

Table 4 Protein concentration of 10 % (w/v) substrate concentration in all three strains at every 12 hrs in (g/L)

Time(hr)	Baker's yeast strain				Wine yeast strain				Brewery yeast strain			
	a	b	c	d	a	b	c	d	a	b	c	d
0	1.13	0.82	1.2	1.46	0.9	1.3	1.0	1.08	1.1	1.0	0.8	1.2
12	1.40	1.87	1.4	2.00	1.5	2.3	1.1	1.3	0.5	0.9	1.1	1.1
24	2.17	3.53	1.7	3.3	1.1	1.8	1.2	1.27	1.1	1.0	1.1	1.0
36	4.52	5.07	2.7	3.11	1.5	1.2	1.3	2.1	1.0	1.0	1.0	1.1

NH_4NO_3 , $b = (NH_4)_2SO_4$, $c = KNO_3$, $d = blank$

CONCLUSION

From the result of this study it is concluded that the best strain for the production of biomass at 10 % (w/v) and 15% (w/v) molasses sugar concentration was baker's yeast. In addition they also higher growth kinetics at this sugar concentration. Similarly, 5% (w/v) concentration was suitable for wine yeast biomass yield and to achieve better growth kinetics. On the other hand, brewery yeast was unable to produce significant amount of biomass even though the strain had the highest sugar assimilation efficiency than the other strains. Among the nitrogen sources, ammonium nitrate was the best for all yeast strains in the production of biomass.

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