Kinetics of Bio-ethanol production on the molasses-based medium by Saccharomyces cerevisiae

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Abstract:

Bioethanol is a renewable and environmentally friendly biofuel because it is produced from renewable sources such as sugarcane molasses. One strategy for lowering production costs and making ethanol fuel economically competitive with fossil fuels could be to use overland yeast with somnolence and ethanol resistance. and low nutritional requirements This work focuses on the kinetics of ethanol production by *Saccharomyces cerevisiae* on untreated or treated molasses-based medium with the development of a mathematical model considering the effect of substrate concentration, inoculum size, and pretreatment of molasses on the growth rate, substrate consumption, and product concentration. Experiments were carried out in batch mode, with substrate concentration varying from 100 to 250 g L^{-1} and inoculum size from 1 to 4 gL^{-1}.. It was discovered that there were significant effects on cell growth, substrate utilization, and ethanol production rates. The kinetic parameters were calculated using linear and non-linear regression methods. A Monod model was applied to obtain a more accurate fitting of kinetic parameters. The parameters such as maximum specific growth rate (μmax), saturation constant (Ks), substate to biomass (Yx/s), the substrate to product (Yp/s), cell to product factor ($Y_{P/X}$)and the important parameter of fermentation efficiency (FE) (Y_X) was shown to be dependent on substrate concentration. The best concentration of molasses-based medium was given the highest bioethanol concentrations and fermentation efficiency was 150 and 250 gL^{-1} on untreated and treated based-molasses medium, respectively.

Keywords: Bioethanol; Saccharomyces cerevisiae; Kinetics; Molasse; Fermentation Efficiency

1 Introduction

At the present, fossil fuels are the most common fuel source, but this energy cannot be replenished and will soon be depleted. The depletion of fossil fuel reserves will result in volatile gasoline prices as well as increased environmental and political pressures **(Alair A.etal., 2021)** The rising demand for fossil fuels will almost certainly lead to a decrease in global fuel reserves, resulting in a lack of supply of this fossil fuel and a dramatic increase in price **(Thomas C. etal., 2016)**. The use of fossil fuels as primary energy resources has caused global environmental issues **(James P. etal., 2006)**. One of the most significant potential contributors is the emission of carbon dioxide (CO2) from automobiles and other industries. Nowadays, many researchers are attempting to discover alternative energy sources derived from biomass as renewable sources to substitute the use of fossil fuels. Bioethanol production consists primarily of four important components that play a significant role in production efficiency: I fermentable sugars, (ii) an efficient microbial strain, (iii) nutrients, and (iv) optimized cultural conditions for best fermentation. Almost 80% of the world's ethanol supply is managed to meet by fermentation of either sugar/starch-containing crops or agricultural and industrial byproducts.

Molasses can be produced from a variety of sources, including sugarcane, sugar beets, and fruits. Molasses is a thick substance obtained after the sugar crystallizes and is separated from the mother liquor. Molasses contains various sugars such as sucrose, glucose, and fructose, resulting in a total sugar concentration of 45 to 60 % (w/v). The most common types of molasses are black strap, refinery and invert, and high-test molasses. Sugarcane molasses contains less sucrose but more invert sugars, such as fructose and glucose. Furthermore, it

contains very little nitrogen, which is required for various metabolic activities and amino acid generation in fermenting organisms, as well as a low raffinose content. Molasses has a dark brown or black color and a higher buffer capacity **(W Borzani 2001)**. Pretreated sugar cane molasses is used for ethanol production; however, molasses requires very little pretreatment as compared to other substrates like cereals, grains. **(Yadav et al., 1997)** investigated the effect of pretreatment on sugarcane molasses prior to fermentation). Before yeast inoculation, molasses was treated with sulfuric acid H_2SO_4 and $K_4Fe\ (CN)_6$, and it was discovered that this is an effective method for reducing various inhibitory compounds. This chemical pretreatment reduces inhibitory substances such as iron (Fe), calcium (Ca), and copper (Cu), resulting in increased ethanol production.

Polysaccharides are converted into ethanol by a wide range of microorganisms. There are only a few bacteria capable of alcoholic fermentation. Zymomonas mobilis and Bacillus subtilis are the most used. The yeasts of the genera Saccharomyces and Kluyveromyces are best suited to produce ethanol from fermentable sugars. At the present, the industrial species used are Saccharomyces cerevisiae and Zymomonas mobilis. Because of its ability to ferment a wide range of sugars, Saccharomyces cerevisiae (*S. cerevisiae*) is the preferred choice for ethanol fermentation. In addition to tolerating both the high osmotic pressure of sugar and the toxicity of high ethanol concentration.

Saccharomyces cerevisiae, the typical microbe used in ethanol production due to its capacity to ferment a wide range of sugars, high ethanol tolerance, and high ethanol productivity, may manufacture bioethanol from micro-algal biomass via a fermentation process (**P.S. Nigam, A. Singh 2011**). Because it separates easily from the fermentation medium without

centrifugation, it lowers the cost of cell recovery . On this fermentation, commercial instant dry yeast was chosen because it can be used directly as a starter, simplifying the production process to reduce the risk of bacterial contamination. Bioethanol production mainly contains four important components that have a major role in production efficiency i.e. (i) fermentable sugars (ii) an efficient microbial strain (iii) nutrients and (iv) Optimized cultural conditions for best fermentation. In this line, studies have shown that Saccharomyces cerevisiae may produce bioethanol from molasses (**Periyasamy et al.,2009).**

Aerobic fermentation occurs in the presence of oxygen. It usually happens at the beginning of the fermentation process. Aerobic fermentation is typically a more rapid and intensive process.. The most used strategy for improving oxygen transfer rate ORT is to keep the culture at a high cell density by using a high agitation and/or aeration rate [152–153]. In recent years, biological systems have been using higher air/O2 pressure to improve the OTR or dissolved oxygen DO level in When high sugar concentration is present in the fermentation broth, it increases the ethanol yield (**Thatipamala et al., 1992**) because in the presence of higher concentration the yeast (even under high dissolved oxygen concentration) changes its oxidative metabolism to oxido- reductive or fermentative metabolism (**Lei et al., 2001**). This phenomenon is referred to as the Crabtree effect **(Converti et al. 1985, Lei et al. 2001 and Thatipamala et al. 1992)**. fermenting liquid to create more desirable products (metabolites The overall reaction of ethanol fermentation was expressed by scientist Gay-Lussac that forms the basis of calculating fermentation efficiency.

$C_6H_{12}O_6$	$2C_2H_5OH + 2CO_2$
1 kg	51.1 kg + 48.9 kg

pH, temperature, time, inoculum size, and agitation rate are all parameters that affect the effectiveness of the fermentation process and *S. cerevisiae* development. To get the highest ethanol output, various process factors like as incubation temperature, incubation period, initial pH, initial sugar concentration, pretreatment molasses, and nitrogen sources were investigated . Cultural conditions play an essential impact in microbial development and ethanol production, according to studies by **(S. H. Mohd Azhar etal.,2017)** To achieve more efficient bioethanol production و an appropriate and adequate inoculum size is necessary **(Rorke D etal., 2017)** .However, according to a study by **(Zabet et al.,2014)** inoculum concentration has no significant effect on ultimate ethanol concentration, although it does alter sugar consumption and ethanol generation. In addition, according to **(H. Erten et al 2006).** the amount of the inoculum influences yeast development and the course of fermentation. The researchers also discovered that the amount of yeast inoculum had a substantial impact on the fermentation process. It sped up the fermentation process. With increasing inoculum size, non-Saccharomyces yeasts eliminated soon . optimum of inoculum size in yeast is in a range of 3 to 10% v/v.

Sugar Concentration's Effects: In Preliminary research found that increasing the molasses concentration in the fermentation media improved ethanol production efficiency and *S. cerevisiae* cell viability. As a result, several studies were carried out to assess the behavior of fermentation when scaling up to reactors in a batch operation. The fundamental impediment to achieving increased ethanol production is yeast's resistance to high sugar concentrations and, as a result, to the ultimate product, ethanol. When sugar concentrations are too high, yeast metabolic cycles are inhibited. As a result, determining the amount of sugar that will produce the most ethanol while using the least amount of

substrate is critical **(Peña-Serna et al., 2011)**. According to **(Xin et al. 2003)**, utilizing 35% (w/v) glucose concentration resulted in a maximum of 16.5% (w/v) ethanol production. Due to strong osmotic pressure, bacterial growth was completely inhibited when this concentration exceeded 45%. Batch fermentation was used by **(Sree et al. 2000)** to determine ethanol production using varied sugar (glucose) concentrations of 150, 200, and 250 (g/L) at 30°C. This procedure was carried out with immobilized osmotolerant *S. cerevisiae* (US3). After 48 hours at 30°C, the ethanol yields for the three concentrations were 72.5, 93, and 83 (g/L), respectively. As a result, the highest yield was obtained when the initial sugar content was 20% (200g/L).

The main parameters of fermentation batch culture obtained from experimental data are μ_{max}, Ks, Yp/s, and Yp/x. The high gravity and very high gravity involving a molasses-based medium with high concentrations of reducing sugars (209, 222, and 250 g/L) were investigated. Fermentation of 222 g/L total reducing sugars resulted in an efficiency of 89.45% and a final ethanol concentration of 104.4 g/L. The fermentation of 209 g/L total reducing sugars resulted in the highest productivity (2.98 g/L) (L.h). **(Cristiane V. C. 2021) and (Kingsley C. Agu and Mujeeb K. Oduola 2021)** discovered that the estimated values of the kinetic parameters in the developed model were m=0.04216hr-1, Xm = 6.2652g/L, Yx/s = 0.18292g/therefore, a model based on the logistic equation of yeast growth, growth-associated production of ethanol, and consumption of glucose for biomass and maintenance was found to accurately fermentation the production of ethanol from sugarcane.. The goal of this study was to determine the optimum fermentation conditions by investigating the kinetics of ethanol fermentation using *Saccharomyces cerevisiae* yeast strain in a batch system at different reducing sugar of molasses concentrations, molasses

pre-treatment, and inoculum size. Besides estimating the kinetic parameters of yeast cell growth, ethanol formation, and reducing sugar utilization.

2 Materials and Methods

2.1 Optimization of bioethanol production from sugarcane molasses with *Saccharomyces cerevisiae*

1- Seed culture preparation

Commercial instat dry yeast on this fermentation was chosen because it can be directly used as a starter to simplify the production process and reduce the bacterial contamination risk. Seed culture was prepared by growing 0.5% of instant dry yeast in shake flasks containing 400ml of sugarcane molasses 2.5%(w/v),yeast extract 0.5%(w/v),peptone 0.5%(w/v). The yeast inoculum was grown aerated at 30^{o}C for 24h.

2- Non-pretreated media

Molasse media was prepared by diluting the sugarcane molasses in tap water in a ratio of 1:1 with an initial pH of 5. Dilution was done by adding tap water to sugarcane molasses to achieve a sugar concentration of 15%, 20%, 25% and 30%. Each of the sugarcane molasses fermentation medium was enriched with 10g/L yeast extract and 10g/L peptone. Then the solution was autoclaved at 121^{o}C for 15min.

3-**Pretreated media**

Molasse media was formed by diluting sugarcane molasses in tap water in a 1: 1 ratio and starting with a pH of 5. A 96.1% concentrated H_2SO_4 solution was added until the pH of the solution reached 3.9. The mixture was then heated to 95^{o}C for 10min before being left at room temperature overnight. Filtration was used to remove the precipitates. The pretreated molasses was subsequently adjusted to give sugar concentration of 15%, 20%; 25%; and 30%. Some essential nutrition was added to pretreated

Molasses including 1.0 g/L yeast extract and 1.0 g/L peptone to ensure the proper growth of yeast during ethanol fermentation. Then the medium was autoclaved at 121°C for 15 min.

4- **Fermentation conditions**

Batch mode was used to investigate the effect of substrate concentration, pre-treatment sugarcane molasses, and inoculum size on the kinetic parameters (μmax, KS, P^0 , and Y). The content of total sugars, and inoculum size ranged from 100 to 250 g L-1 and 1 to 4 gL-1, respectively.

We employed 0.5 L shake flasks as a mini-bioreactors with a 200-mL working capacity, which were kept at 30°C, 150 rpm, for six hours, and then the flashes were incubated as a steady culture until 72 h The pH of the media was adjusted to 5 using previously sterilized NaOH 2 N and HCl 2 N solutions. Samples were taken at regular intervals (12h) for analysis. The shake flasks were autoclaved at 121 °C for 15 minutes. Aerobic fermentation was used in this work as a conventional batch, where a sugar content in the culture medium of more than 5%. (Crabtree effect).

2.2 Analytical determinations

Estimation of molasses sucrose: 2 ml of the sample was supplemented with 10 cm distilled water and added 1 ml of 2% sulfuric acid. The mixture was heated in a water bath at 70^{o}C for 5 min. and cooled. The next steps are completed as in the previous estimate above. Estimation of the amount of sucrose = the amount of inverted sugar x 0.95 (where 342 parts of sucrose were given after hydrolysis 360 parts of reducing sugars Total dissolved solids (TDS) were determined according to

Initial sugar and residual sugar were determined by DNS method according to **(Miller G. L. 1959).**Ethanol concentration was estimated by using the dichromate colorimetric method

(M.B. William, and Reese, D. 1950). The yeast cell biomass was calculated by subtracting the weight of Whatman no.1 filter paper with yeast cell pellet after drying in hot air oven at 105^{o}C from the weight of pre weighed filter paper. The Viability of yeast cell was obtained using the methylene blue staining method **(Lang et al., 1993).**

2.3 Kinetic parameters

The determination of specific rates of growth (X), production (P), and consumption (S) in fermentation processes was considered and computed using Eqs. 1 to 3:

$\mu_x = 1/x\ (dx/dt)$ 1

$\mu_s = 1/x\ (ds/dt)$ 2

$\mu_p = 1/x\ (dp/dt)$ 3

To determine the effect of substrate on microorganism development, the values of K_S and μ max were determined using the Monod equation and the Lineweaver–Burk linearization method (Eq. 4).

$$1/u = 1/umax + ks\ /umax\ (1/s)$$

$$1/up = 1/upmax + Kp\ /umax\ (1/p) \quad 4$$

The biomass yield factor based on substrate (Yx/s), the product yield factor based on substrate (Yp/s and the product yield factor based on biomass (Yp/x) were determined according to Eqs. 5 to 7

$Yx/s = Xf - Xo\ /\ So - Sf$ 5

$Yp/s = Pf - Po\ /\ So - Sf$ 6

$Yp/x = Pf - Po\ /\ Xf - Xo$ 7

The dynamic description of ethanol fermentation using unstructured models, on the other hand, may be done essentially with three differential equations for microbe growth, substrate uptake, and ethanol synthesis (Eqs. 8–10), which can be obtained from the reactor mass balance.

Fermentation process under high gravity (150 to 240 g/L of sugars) and very high gravity (≥ 250 g/L of sugars) constitutes

an option for reducing distillation costs. The VHG process leads to a higher alcohol content,]. It is therefore possible to save water, capital, and energy per liter of alcohol, while reducing the risk of bacterial contamination **(Puligundla, P 2019)** .

$$Rx = (dX/dt) \quad (8)$$

$$Rs = (dS/dt) \quad (9)$$

$$R_p = (dp/dt) \quad (10)$$

The sugar utilization, ethanol yield, ethanol productivity and fermentation efficiency were calculated by the following equations **(Rorke D etal., 2017)**.

1-Sugar utilization (%) =Initial sugar concentration−residual sugar concentration /Initial sugar concentration×100

2-Ethanol yield g(ethanol)g (glucose)=Final ethanol concentration in the broth (g/L) / Glucose consumed(g/L)

3-Fermentation efficiency (%) =Absolute ethanol yield (g/L) Theoretical yield (g/L) ×100

4-Special ethanol production rate (g/L/h) =Final ethanol concentration (g/L) Fermentation time (h)

3 Results and Discussion

Initial substrate concentration (S0), final ethanol (Pf), and biomass (Xf) concentrations, fermentation time (tT), and calculated values of ethanol yield (YP/S), biomass yield (YX/S), and maximum specific growth rate (Umax) related to each initial substrate concentration (S_o) are presented in **Tables (1-5)**

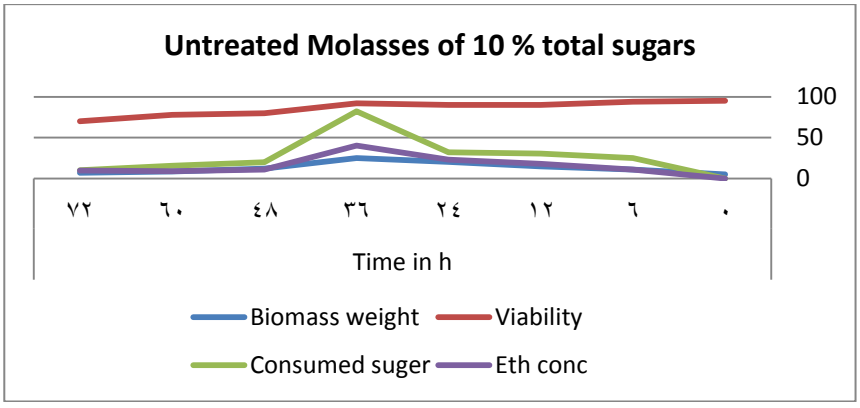
The observed results in the tables (1-5) indicated the production of ethanol in all batch runs. Moreover, *S. cerevisiae* consumed incomplete all sugar, except initial sugar concentration of 100gL-1 for both untreated and treated sugarcane molasses. The figures show that all the fermentation

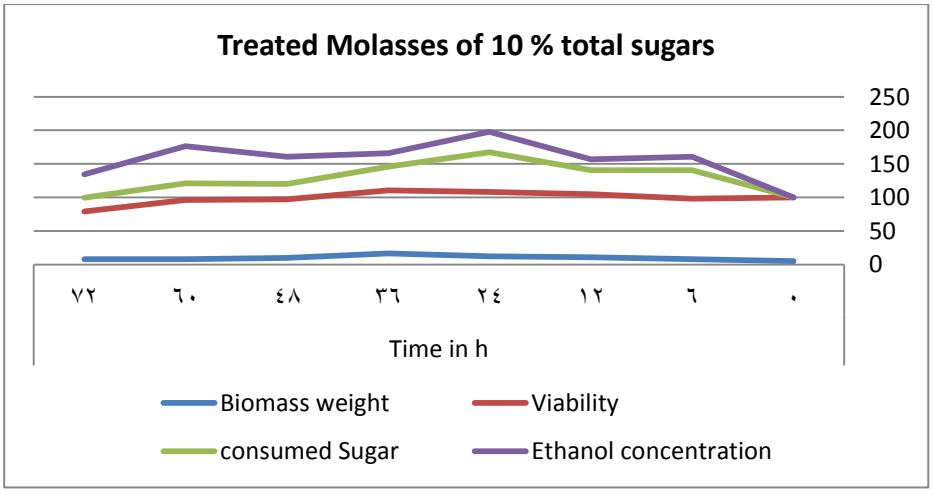
runs produced alcohol ethanol, and not all sugars were consumed in the batch cultures except for the 10 % sugar concentration group selected. The viability of the cultured cells continued until 36 h and after that it appeared to be in drop. The three measurements such as cell growth, consumption of sugars, and ethanol production took a pattern identical one. **(De Deken R.H. 1966)** reported that the yeast cells of *Saccharomyces* prefer fermentation process over oxidative phosphorylation, this means yeast cell ferment some sugar in the presence of oxygen and sugar concentration higher than 5%.

In order to obtain good quality products in plentiful quantities for a unit cost of the product, several factors must be measured from the fermentation culture to help us design the fermentation vessel and the components of the yeast media.

Table (1) Ethanol fermentation from treated- and untreated molasses of 10 % total sugars by *S. cerevisiae*

	Untreated sugarcane molasses					**Treated sugarcane molasses**				
Time in hour/ Parameter	Biomass weight (gl-1)	Viability (%)	consumed Sugar (gl^{-1})	Ethanol concentration (gl^{-1})	Eth% v/v	Biomass weight (gl-1)	Viability (%)	consumed Sugar (gl^{-1})	Ethanol concentration (gl^{-1})	Eth% v/v
00	01.51	96.00	00.00	00.00		01.00	96.00	00.00	00.00	
06	04.50	95.00	06.00	03.44		05.94	92.00	10.74	04.50	
12	10.55	96.00	14.22	09.00		09.52	90.00	10.00	08.66	
24	18.60	90.00	20.00	10.66		11.00	90,00	28.81	10.22	
36	20.84	85.00	45.00	22.00		15.00	81.00	25.86	10.00	
48	15.00	75.00	15.90	06.00		22.00	73.00	25.45	23,89	
60	16.80	71.00	00.00	00.00		10.00	60.00.	00.00	00.00	
72	06.50	67.00	00.00	00.00		05.90	50.00	00.00	00.00	
Sum	04.80		100	50.44	06.34	06.01		100	57.27	07.26

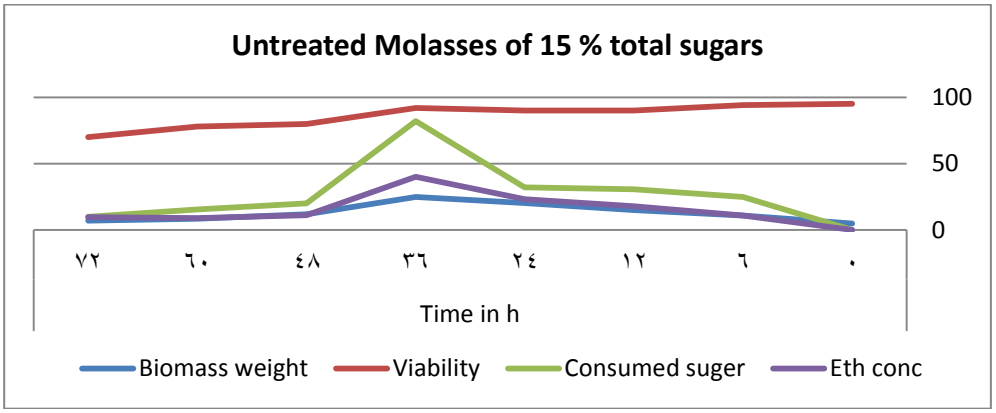


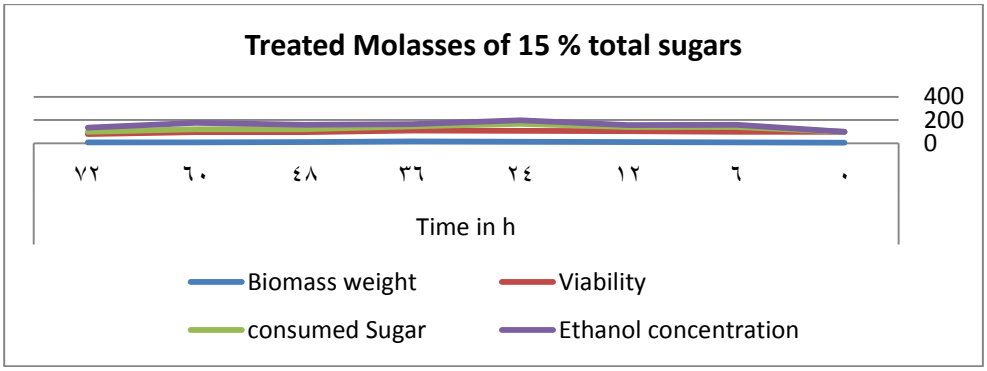


Fig(1): Ethanol fermentation from treated- and untreated molasses of 10 % total sugars by *S. cerevisiae*

Table (2) Ethanol fermentation from treated- and untreated molasses of 15 % total sugars by *S. cerevisiae*

	Untreated sugarcane molasses **Treated sugarcane molasses**									
Time-hour/ Parameter	Biomass weight (gl-1)	Viability (%)	consumed Sugar (gl-1)	Ethanol concentration (gl-1)	Eth % v/v	Biomass weight (gl-1)	Viability (%)	consumed Sugar (gl-1)	Ethanol concentration (gl-1)	Eth % v/v
00	02.00	96	00.00	00.00		02.00	96.00	00.00	00.00	
06	06.10	95	15.00	05.30		05.24	92.00	07.74	04.50	
12	14.00	94	22.50	09.00		09.52	90.00	12.00	08.66	
24	20.87	90	26.00	15.60		11.00	90,00	25.60	10.22	
36	22.51	80	16,80	10.00		15.00	81.00	15.00	10.00	
48	24.00	72	25.11	10.90		22.01	73.00	54.00	23,89	
60	08.12	65	20.00	06.00		09.00	60.00.	23.00	10.00	
72	09.60	55	18.00	06.61	8.04	02.90	55.00	12.00	07.00	9.42

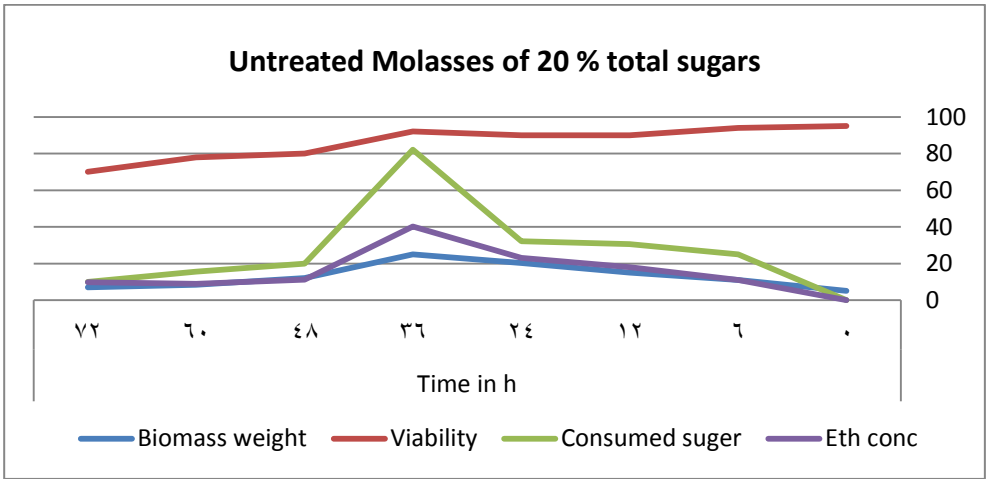


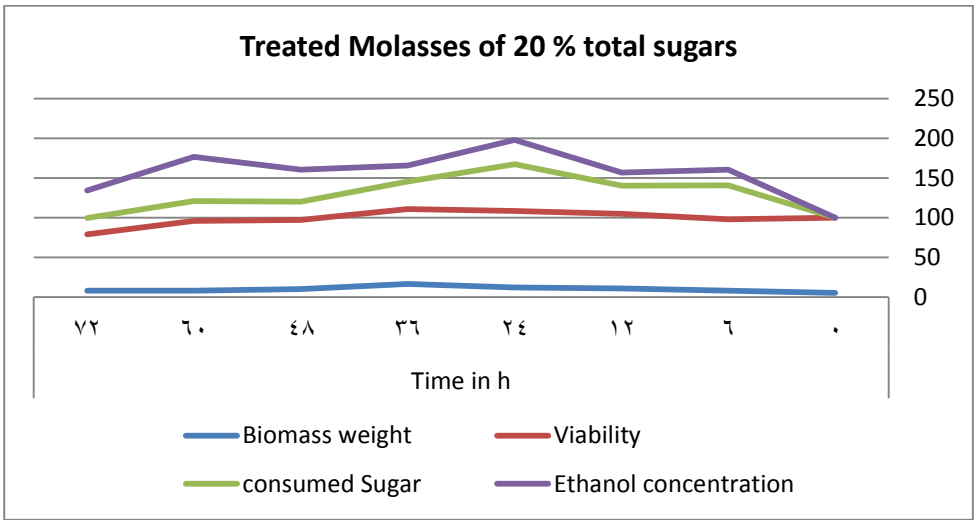


Fig(2) : Ethanol fermentation from treated- and untreated molasses of 15 % total sugars by *S. cerevisiae*

Table (3) Ethanol fermentation from treated- and untreated molasses of 20 % total sugars by *S. cerevisiae*

	UnTreated sugarcane molasses	**Treated sugarcane molasses**						
Time in hour/ Parameter	Biomass weight (gl-1)	Viability (%)	consumed Sugar (gl^{-1})	Ethanol concentration (gl^{-1})	Biomass weight (gl-1)	Viability (%)	consumed Sugar (gl^{-1})	Ethanol concentration (gl^{-1})
0	03.00	91	00.00	00.00	03.00	91	00.00	00.00
6	08.00	90	20.00	12.44	10.50	92	07.00	05.55
12	10.00	95	29.51	17.00	12.43	94	15.00	09.00
24	15.00	91	40.74	23.98	15.00	91	22.79	15.22
36	20.00	84	28.00	20.66	20.00	95	36.00	23.00
48	15.31	80	38.61	19.00	21.11	90	35.00	23.77
60	22.55	74	40.71	23.22	25.00	90	43.00	25.00
72	10.42	60	00.00	00.00	07.00	80	00.00	00.00

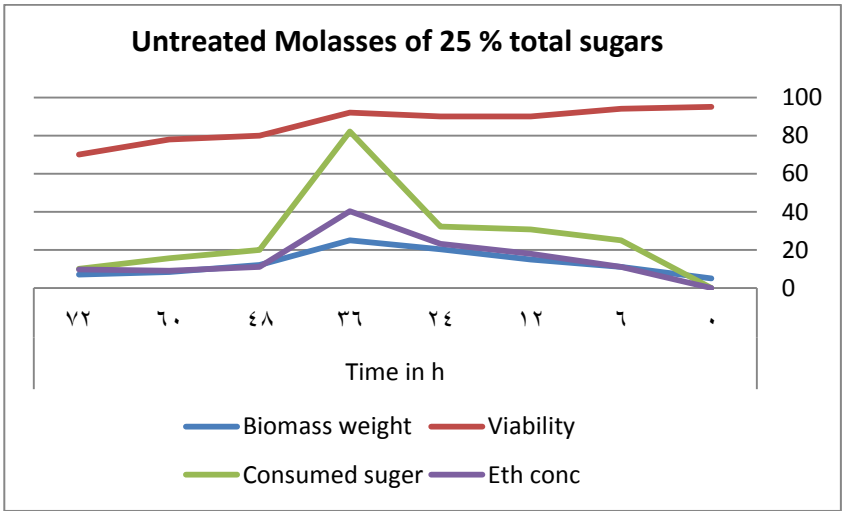




Fig(3) : Ethanol fermentation from treated- and untreated molasses of 20 % total sugars by *S. cerevisiae*

Table (4) Ethanol fermentation from treated- and untreated molasses of 25 % total sugars by *S. cerevisiae*

	Treated sugarcane molasses				**Treated sugarcane molasses**			
Time (hour) / Parameter	Biomass weight (gl-1)	Viability (%)	consumed sugar (gl^{-1})	Ethanol concentration (gl^{-1})	Biomass weight (gl-1)	Viability (%)	Consumed Sugar (gl^{-1})	Ethanol concentration (gl^{-1})
0	05.00	95	00.00	00.00	05.00	95	00.00	00.00
6	11.00	94	25.00	11.00	08.00	90	42.53	20.00
12	15.00	90	30.67	18.00	10.80	94	35.55	16.21
24	20.36	90	32.23	23.11	12.22	96	59.00	30.66
36	25.00	92	82.10	40.22	16.65	94	35.00	20.00
48	12.00	80	20.00	11.11	10.00	87	23.00	40.42
60	08.44	78	15.54	09.00	08.00	88	25.00	55.43
72	07.00	70	10.01	09.76	07.83	71	20.86	34.67



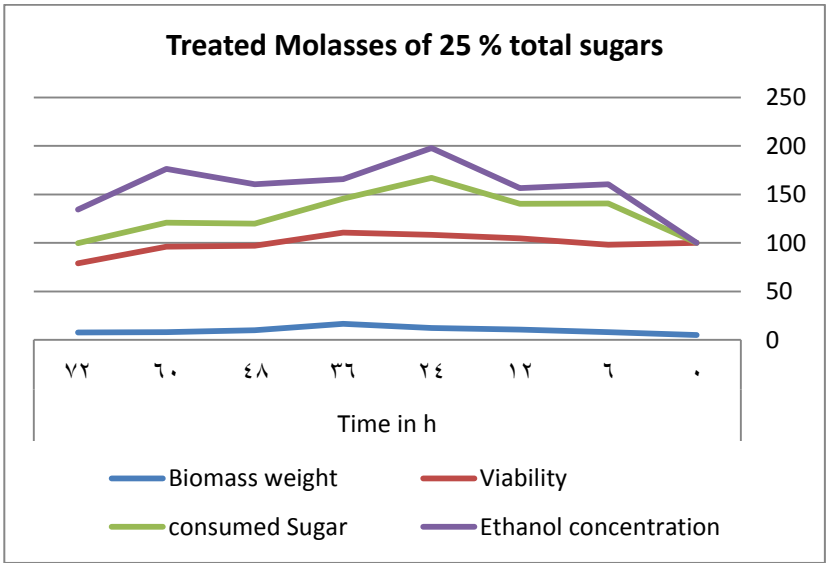


Fig (4) : Ethanol fermentation from treated- and untreated molasses of 25 % total sugars by *S. cerevisiae*

Table (5) Ethanol fermentation kinetics

	Untreated molasses					**Treated molasses**			
Initial sugar concentration									
	10	15	20	25		10	15	20	25
Xo(inoculum)	01.0٠	02.00	03.00	04.00		01..00	02.00	03.00	04.00
Xt	20.84	20.78	20.00	20.23		09.52	20.01	20.00	15.56
Xmax	20.84	24.00	22.55	20.55		18.00	22.00	25.00	16.65
Xf	68.32	139.6	104.3	101.3		93.46	67.66	131.5	151.4
Vx (average)	94.00	93.75	91.35	91.75		93.00	92.35	93.00	163.1
Rx	00.54	00.76	00.47	00.58		00.34	00.38	00.37	00.33
µx	00.07	00.83	00.05	00.05		00.05	00.0٥	00.05	00.0٧
µmax		00.83					00.٠٧		
XT	87.83	137.6	101.0	97.30		92.46	65.66	128.5	148.8
Ks		22.00					79.50		
Po-x	00.95	03.25	01.59	01.41		01.30	00.09	01.83	02.07
Tt	72.00	72.00	72.00	72.00		72.00	72.00	72.00	72.00
So	100.0	150.0.	200.0	250.0		100.0	150.0	200.0	250.0
St	45.00	28.00	28.00	82.00		25.00	22.00	43.00	35.00
Sf	100.0	١٣٩.4	147.1	215.5		100.0	138.3	158.7	241.0

SUE	100.0	٩٣.٠٠	73.50	86.20		100.0	92.20	79.35	96.40
Rs	01.04	٠١.٧١	00.78	02.28		00.52	00..46	00.72	00.97
Us	00.05	00.12	00.04	00.11		00.03	00.02	00.04	00.05
Po-s	01.39	02.65	02.04	02.99		01.37	01.92	02.20	03.35
Yx/s	00.68	01.36	00.72	00.47		00.93	00.46	00.83	00.63
TEY	120	243	127	83		164	79	146	112
Po	0	0	0	0		0	0	0	0
Pt	22.00	10.99	23.22	40.22		23.22	23.00	25.00	20.00
PTl	99.00	121	114	112		102	104	125	175
Rp	00.61	00.44	00.65	1.17		00.50	00.48	00.42	00/56
Up	00.03	00.07	00.03	00.06		00.05	00.02	00.02	00.04
Po-p	1.38	1.68	1.58	1.56		1.42	1.44	1.60	2.43
Yp/s	00.99	1.18	00.78	00.52		1.02	0.80	0.80	1.07
Yp/x	01.45								
PT (v/v) %	12.55	15.34	14.45	14.19		12.93	13.18	15.84	22.18
SCRL %	45.00	18.67	14.00	32.80		25.00	14.67	21.50	14.00
FE %	12.55	19.40	18.20	18.00		13.00	16.70	20.10	18.00
FE %	194	171	152	103		204	148	162	142

3.1 Kinetic parameters of yeast cell growth

The kinetic parameters of yeast cell growth for fermentation runs of untreated sugarcane molasses-based medium (group A) specific growth rate (μmax), saturation constant (Ks), yield factor (Yx/s) and productivity (Po-x) as shown in table (). The values of three parameters such as μ, Yx/s, and Po-x recorded 0.05-0.83, 0.47-1.36, and 0.95-3.25 respectively, for group A. In contrast, these three parameters for fermentation runs of treated sugarcane molasses-based media were,0.05-0,07,0.46-0.83, and 0.08-2.07 respectively. The highest values of four parameters were recorded for sugar concentration 15% (group A) and 25% (group B). Their figures were (0.83, 22, 1.36, and 3.25) and (0.07, 74.5,0,63, and 2.07), respectively.

3.2 Kinetic parameters of sugar consumption

Mathematical relationships can be used to predict sugar consumption and determine sugar parameters such as us, Ks, Yx/s, Po-s and SUE. Although these parameters for un-pretreatment molasses-base on medium containing an initial

sugar concentration of 150 gL-1 (group A) were determined to be 0.12, 22,1.36, 2.65, and 93 respectively, the corresponding parameter values for pretreatment molasse base medium containing sugar concentration of 250 gL-1(group B) were 0.05, 79.5, 0.69, 2.35, and 96, respectively/ From the previous results, it is clear that there is a slight superiority in the values of kinetic parameters of group. The consumption of glucose was faster than the two sugars, Fructose, and sucrose This phenomenon can be explained by kinetic parameters Yx /s and m. Glucose was a more beneficial sugar to produce biomass with higher Yx/s (1.36 for group A against 0.63 for group B.

The saturation growth constant (K_S is the substrate concentration corresponding to 1/2 $\mu_{max)}$. and saturation product constant (Kp) of group A and B were estimated according to experimental data during the growth phase with the initial substrate method by using Lineweaver–Burk plot **(Mussatto etal., 2010**) based on Monod rate equation. Their values were (22 and 79.5 gL-1) and (2 and 6.25 gL-1), respectively. The values of Ks, however, decreased with the increase in the initial reducing sugar concentration suggesting that the increment of osmotic pressure of the solution caused by the high sugar concentration had a negative effect on the activity of transport protein in yeast cells, which resulted in an increase in the value of rate constant for glucose desorption from transport protein (rdS). Furthermore, the ethanol molecule is smaller than glucose and more easily adsorbed by the transport protein, therefore the increment value of Ks will lead to a decrement in Kp due to the competition between glucose and ethanol. This phenomenon is enhanced by the increase in ethanol concentration. The values substrate utilization efficiency, SUE for group A and B were (73.5 -100%) and (49.35 - 100 %), respectively maximum SUE was recorded 100% followed by 86.2% for group A at 10 & 15% reducing sugar, while 100% followed 96% for group B at 10 %

&25 % reducing sugar. In growth associated products, the product is formed along with the growth of the microbial cells and product concentration is almost directly proportional to the microbial growth rate

3.3 Kinetic parameters of ethanol production

The estimated values of the specific ethanol production rate (μ) were (0.05, 0.12, 0.04, and 0.11 h-1) for group A and (0.03, 0.02. 0.04, and 0.05h-1) for group B at initial substrate concentrations of 100, 150, 200, and 250 g/L, respectively (Table 1), were calculated using the exponential (log) phase of ethanol production. The maximum ethanol concentration obtained with initial sugar concentration 150 g/l group A was 121 g/L and for initial sugar concentration, 250 /group B was 175 g/L after 72 h of fermentation. At the same trend Yp/s, TEY, Po-p, and PT have recorded a similar value. The highest value of Yd/s and Po-p were recorded with sugar concentrations of 150 gL-1 i.e., 1.19gg-1 and 1.68 gL-1h-1 and 107 gg-1 and 2.43 gal-1h-1 for group A and group B, respectively. As for the most important parameter, which is fermentation efficiency (FE) is found that all fermentation runs achieved more than 100 % of theoretical ethanol concentration. The highest value was recorded with a sugar concentration of 10-15% (194-171%0 group A., respectively. Note that the increased deficiency with the increase of initial sugar concentration. The increase in ethanol content was ranger 2.02 to 4 %. This is explained by the presence of other sugars such as raffinose, amino acids beside ingredients of dead yeast cell. In recent studies,found that the level of consumption of reducing sugar was 62.75 g/l, the decrease in total sugar was 14.25%, ethanol content was 9.56%, ethanol yield was 71.52%, and The fermentation efficiency was 139.95%. The ethanol content could be increased by 3.5-5.5% under ideal conditions. The concentration of fermentable sugars

and the fermentation period required for fermentation determine the promotional availability of ethanol production.

As a result, in commercial plants, fermentation at high sugar concentrations with powerful and effective yeast strains with high osmotolerance to sugar and ethanol is preferred **(Mussatto etal., 2010; Datta Mazumdar S etal., 2012 ; Shukla GK etal., 2006 and Bafrncova P etal., 1999).**Sugars had no hyperosmotic effect on yeast growth, which is encouraging for very high gravity fermentation, where high sugars can be fermented for higher ethanol yields(**[Reddy L.V.A, Reddy O.V.S, 2006) and (Sunan N.LL etal., 2011).**

In the range of 0.1–4 g/L, the inoculum size was found to have a significant effect on the model parameters μmax and Ks for both group A and group B. Because these are the key model parameters, the effect of inoculum size on these parameters had to be accounted for in the fermentation model.

The data presented in this article show that instant dry active *Saccharomyces cerevisiae* is an effective fermentor of sugarcane molasses to ethanol. This strain also efficiently co-ferments glucose-fructose-sucrose mixtures to ethanol. Kinetic studies show that the *Saccharomyces cerevisiae* is more osmo-tolerant and ethanol tolerant when glucose, rather than fructose and sucrose, is used as the fermentation substrate. The tolerance of the fermenting microorganism to ethanol is an important consideration in the design of an ethanol production process. The product-inhibition studies show that the effect of ethanol on the specific growth rate and productivity is dependent on the fermentation substrate. These parameters' magnitudes were also within the range of the highest ethanol concentrations obtained by batch fermentation studies .

The size of the inoculums is critical for achieving more efficient bioethanol production from OPT sap **(Rorke D etal., 2017)** However, a study by **(H. Zabed etal., 2014**) discovered

that inoculum concentration has no significant effect on final ethanol concentration., but it affects the consumption rate of sugar and ethanol production **(H. Zabed etal., 2014).** Besides, inoculum size also affects yeast growth, and the course of fermentation as stated in the research of **(Erten et al., in 2006)** . The researchers also concluded that yeast inoculum levels significantly affected wine fermentation. It shortened the fermentation time. The non-Saccharomyces yeasts disappeared quickly with increasing inoculum size . It is clearly seen that most bacterium has 10% v/v of its microbes as optimum inoculum size meanwhile optimum inoculum size in yeast is in a range of 3 to 10% v/v.

4 Conclusion

The results showed that the sugar concentration in % brix unit and medium pretreatment affected the production of bioethanol from sugarcane molasses by instant dry yeast. The results showed that the best treatment was obtained with a sugar concentration of 25% sugarcane molasses with acid pretreatment and a sugar concentration of 15% sugarcane molasses without acid pretreatment. The level of reducing sugar consumption was 62.75 g/l, the reduction in total sugar was 14.25%, the ethanol content was 9.56%, the ethanol yield was 71.52%, and the fermentation efficiency was 139.95%. The ethanol content could be increased by 3.5-5.5% under ideal conditions. The commercial viability of ethanol production is determined by the concentration of fermentable sugars and the fermentation time. In commercial plants, fermentation at high sugar concentrations with robust yeast strains with high osmotolerance to sugar and ethanol is preferred **(Mussatto etal., 2010; Datta Mazumdar S etal., 2012 ; Shukla GK etal., 2006 and Bafrncova P etal., 1999).**

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