FERMENTATION KINETICS OF SOURSOP JUICE USING *SACCHAROMYCES CEREVISIAE* WITH INFLUENCE OF AMMONIUM CARBONATE

C. Ogbebor
Department of Chemical Sciences,
Glorious Vision University, P.M.B 001 Ogwa, Edo State, Nigeria.

ABSTRACT
Soursop juice was fermented as substrate in the presence of ammonium carbonate using saccharomyces cerevisiae to determine kinetic parameters: overall rate constant; k, order of initial reaction; n, maximum rate of fermentation; V_{max}, catalytic constant; k_2, dissociation constant for the enzyme-substrate complex; k_s, the Michaelis constant; k_m, and the specific activity of the enzyme. These were obtained by monitoring the rate of carbon (IV) oxide production at different time intervals. It was observed that in the first order reaction, the overall rate constant k, with respect to ammonium carbonate is, 2.23. It's also seen that the curves achieved from the plots of volume of carbon dioxide produced with time approached the time axis in a shorter time and the reaction was faster upon its addition. Most importantly, the maximum rate of fermentation with the substrate, $2.58x10^2M^1min^{-1}$ and addition of ammonium carbonate is $2.49x10^2Mmin^{-1}$. The value represents the maximum velocity attainable. The maximum rate at which all the enzyme molecules were in the complex form was high and became lower upon the addition of the additive. This suggests inhibition of the fermentation process by the additive due to decrease in the maximum activity of the enzyme. The catalytic constant, k_2, $8x10^{-2}min^{-1}$ and the value upon addition of ammonium carbonate is, $2.0x10^{-2}$ min^{-1} The dissociation constant of enzyme-substrate complexes, k_s $1.64x10^2$ and for the addition of ammonium carbonate is, $5.30x10^{-1}$. The Michaelis constant, k_m $1.64x10^2$ M and $5.30x10^{-1}$ for ammonium carbonate. The specific activity which is the unit of enzyme activity per gram of protein (yeast) is found to be $1x10^{-1}$ in both. Finally, the effect was found to be uncompetitive from the extrapolated Lineweaver-Burk plots.

Keywords: Soursop, fermentation, *saccharomyces cerevisiae* and ammonium carbonate,

INTRODUCTION
Fermentation is a metabolic process that converts sugar to acids, gases, and/or alcohol. It not only occurs with yeast and bacteria, but also with oxygen-starved muscle cells as in the case of lactic acid fermentation. Fermentation is also used more broadly to refer to the bulk growth of microorganisms on a growth medium with the goal of producing a specific chemical product. It is also the slow decomposition of organic substances by enzyme of plant and animal origin. In this process starch is broken down into fermentable sugars by fungal enzymes such as alpha-Amylase and Gluco-amylase to facilitate fermentation by

mainly *Saccharomyces* species (Sebayang et al, 2016). Kinetics of Fermentation: It is stated that the rate of alcohol production by yeast is limited primarily by the rate of sugar uptake especially the rate of fructose and glucose uptake (Negi, and Anand, 2007)). In general, while both glucose and fructose are utilized simultaneously, glucose is utilized faster than fructose by yeast. *Saccharomyces cerevisiae* appears to be glucophilic, although, some strains have a clear preference for fructose (Bass et al, 2007). As a useful tool, the primary objective of a kinetic model developed for alcohol fermentation is the prediction of the kinetic behaviour of yeast fermentation performance based on the initial characteristic of the juice. The development of the corresponding mathematical models of fermentation kinetics is also important in the understanding of yeast behavior and metabolic regulation. An appropriate model of fermentation with technical, economical and physiological implication would be a powerful instrument to predict and control problem of fermentation and will be helpful to understand the fermentation process. The study of enzyme kinetics began with the work of Adrian in 1929 (Malinowski, 2001)). He studied the rate of hydrolysis of sucrose ($C_{12}H_{22}O_{11}$) by water to give glucose and its structural isomer fructose. Specifically, Adrian studied the rate of hydrolysis of sucrose as catalyzed by the yeast enzyme invertase. He showed that when the sucrose concentration was much greater than the enzymes concentration, the reaction is zero-order with respect to sucrose concentration i.e: Rate = $k[S_o] = k$. If [S] >> [E] the above reaction is zeroth-order reaction i.e the rate is independent of the concentration of the reactant. It was also found that without the enzymes, the reaction still occurred but at much slower rate. Thus, Adrian demonstrated that this enzyme invertase was not a required ingredient in the stoichiometric reaction, rather, the enzyme, serves as a catalyst. In fact, the mechanisms that Adrian developed serve as a simple starting point for many general models (Dahlia, 2009). Numerous models have been developed on kinetics. Among the models developed, the majority are biochemical-knowledge based which consists of a set of mathematical equations, describing the phenomena occurring during fermentation (De-Paula and Atkins, 2006). The advantages of these models are that they account for biological phenomena. In general, the fermentation kinetic model can be subdivided into growth model, a substrate model and a product model (Gorelik and Leitsina, 2000).

The overall scheme for glucose enzyme reaction is:

$$C_6H_{12}O_6 \xrightarrow{\text{enzyme}} enzyme \rightarrow \quad 2C_2H_5OH + 2CO_2 + ATP$$

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \quad E + P \xrightarrow{k_2}$$

Where E is the enzyme, S is the substrate, P is the product and ES is enzyme-substrate complex intermediate. k_1 is the elementary rate constant for the formation of the intermediate from E and S. k_{-1} is the elementary rate constant for the backward reaction

that leads to the formation of the reactants from ES, and k_2 is the elementary constant for the dissociation of ES to P and E. The rate of formation of product is proportional to the rate of the decomposition of the intermediate [ES].

$R_p = dp/dt = k_2 [ES]$ ----------(1)

But,
$d[ES]/dt = k_1 [E][S] – k_{-1} [ES] – k_2 [ES] = 0$ -----(2) (Palmer, 2000).

From the steady state approximation, it is necessary to obtain an expression for [ES] in terms of the concentration of E and S which can then be introduced into the rate equation. Michaelis and Menten (1913) assumed that $k_{-1} >> k_1$. If it is true, then the reversible step in the mechanism does achieve rapid equilibrium and hence, k_s is given by

$k_s = k_{-1}/k_1 = [E][S]/[ES]$ ----------(3)

Where k_s is the dissociation constant of [ES] to E and S.

Rearranging equation (2):

$[ES] = k_1 [E][S]/k_{-1} + k_2$ ----------(4)

Substituting equation (4) into (1) we have

$R_p = dp/dt = k_2 k_1 [E][S]/k_{-1} + k_2$ ----------(5)

If the total enzyme concentration is E_o and the complexed enzyme is ES,

Then, $[E_o] = [E] + [ES]$ or

$[E] = [E_o] – [ES]$ ----------(6)

Meanwhile, $d[ES]/dt = k_1 [E][S] – k_{-1} [ES] – k_2 [ES] = 0$

$[ES] = k_1 [E][S] / k_{-1} + k_2$

Multiplying through by $1/k_1$ the above equation becomes:

$[ES] = [E][S] / k_2 + k_{-1}/k_1$ i.e

$[ES] = [E][S] / k_m$ ----------(7)

Where $k_m = k_2 + k_{-1}/k_1$ ----------Michaelis constant.

Substituting equation (6) into (7) (i.e, substituting for [E])

$[ES] = ([E_o] – [ES])[S]/k_m$ ----------(8)

$[ES]k_m = ([E_o] – [ES])[S]$

$[ES]k_m = [E_o][S] – [ES][S]$

$[E_o][S] = [ES]k_m + [ES][S]$

Dividing through by ES

$[E_o][S]/[ES] = k_m + [S]$

$[E_o][S] = (S + k_m)\ [ES]$ where $[E_o]$ is the total enzyme concentration in the system.

$(S + k_m)\ [ES] = [E_o][S]$

$[ES] = [E_o][S] / S + k_m$ ----------(8b)

But rate of formation of product is:

$R_p = d\ [P]\ /dt = k_2[ES]$ substituting [ES] from equation 8b:

$R_p = k_2[E_o][S] / k_m + [S]$ ----------(9)

Given equation 9, If $[S] << k_m$ then the rate of reaction varies linearly with the enzyme and substrate concentrations, i.e the reaction will be first order in E and S. However, when $[S] >> k_m$, the initial rate of product formation v_o would be given by:

$\gamma_o = d\ [p]\ /dt = k_2[E_o][S]\ /[S]$ but, the maximum rate R_{pmax} is given by:

$R_{pmax} = V_{max} = k_2[E_o]$ ----------(10)

Where V_{max} is the rate at which all enzyme molecules are in the complex form i.e., $[E_o] = [ES]$. Thus the rate is proportional to the total concentration of the enzyme in the system. Whereas, the rate is independent of substrate concentration [S] and it is at maximum. The rate expression (equation 9) can then be written as:

$R_p = \gamma_o = V_{max}\ [S]/[S] + k_m$ ----------(11)

Where, $V_{max} = k_2[E_o]$. From equation (11), we can determine the value of k_m if we consider the substrate concentration to be half $[S]½$ which results in velocity at half the maximum velocity.

$V_{max}\ /2 = V_{max}\ [S]½ / [S]½ + k_m$

$2\ [V_{max}]\ [S]½ = V_{max}\ ([S]\ ½ + k_m)$

$2[S]½ = [S]½ + k_m$

$2\,[S]½ - [S]\,½ = k_m$

$k_m = [S]\,½$ but $k_m = k_{-1} + k_2 / k_1$ ----------(12)

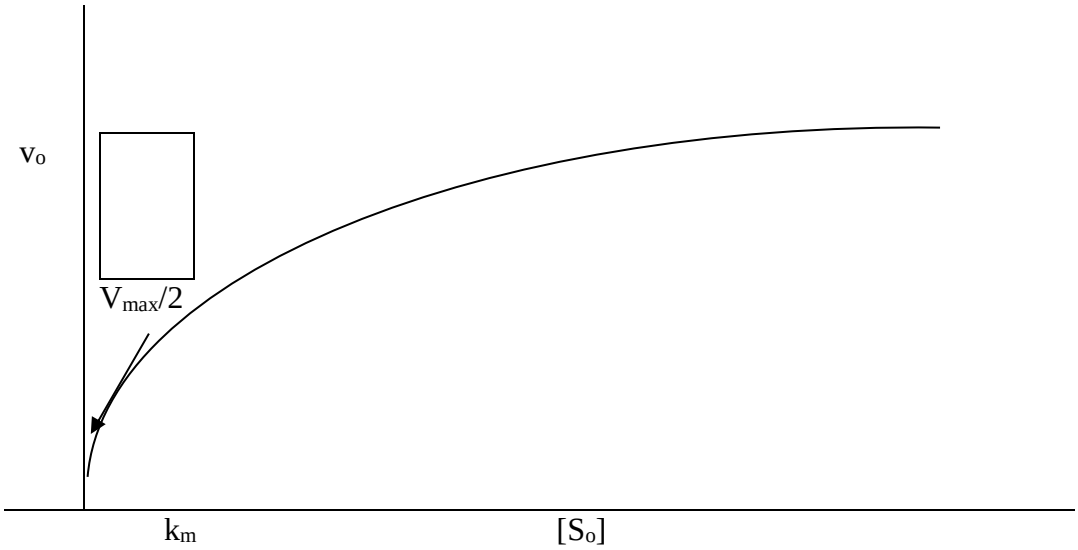

Fig.2.1: A Plot of Initial Rate versus Substrate Concentration

The turnover number k_2 of an enzyme is the number of substrate molecules converted into products per unit time, when the enzyme is fully complexed with the substrate as [ES]. In general, the turnover numbers of most enzymes range between 0.5 and 10^4 per second (Chang, 1990). But k_m lacks a simple interpretation. With respect to k_{-1} or k_2:

When $k_{-1} >> k_2$, k_m can be equated to the dissociation constant k_s.

$k_s = k_{-1}/k_1 = [E][S]/[ES]$ ----------(13)

$k_m = k_s$

In this case, k_m is a measure of the strength of the [ES] complex. A large k_m indicates weak binding; a small k_m indicates strong binding, and k_2 is obtained from

$V_{max} = k_2[E_o]$, and also

$k_2 = V_{max}/[E_o]$ ----------(14)

But $R_p = \gamma_o = V_{max}[S_o]/[S_o] + k_m$;
$1/\gamma_o = [S_o]/V_{max}[S_o] + k_m/V_{max}[S_o]$
$1/\gamma_o = k_m/V_{max} \,.\, 1/[S_o] + 1/V_{max}$ (Lineweaver-Burk equation).

As suggested by Lineweaver and Burk (1934), a double reciprocal plot of $1/\gamma_o$ versus $1/S_o$ is made

since at $\gamma_o = V_{max}/2$

$1/\gamma_o = k_m/V_{max}[S_o] + 1/V_{max}$ ----------(15)

k_m and V_{max} can be obtained from the slope and intercept of the straight line.

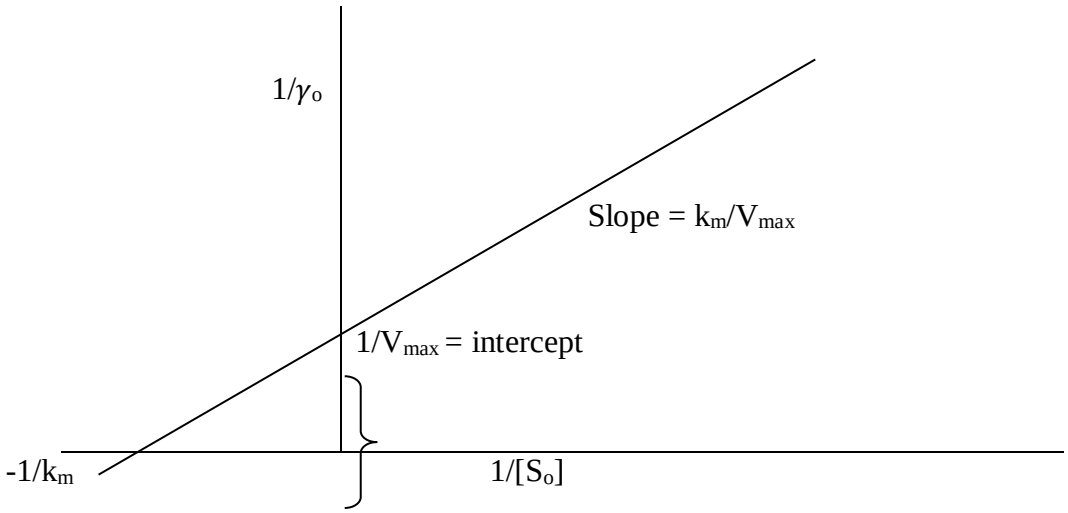

Fig. 2.2: A plot of reciprocal of initial rate $1/\gamma_o$ versus reciprocal of substrate concentration $1/[s_o]$ (lineweaver-burk plot)

MATERIALS AND METHOD

Burette, clamp and retort stand, flasks, filter cloth, fermentation vessels, measuring cylinder, pH meter, rubber tubes, refrigerator, stirring rod, stop watch, thermometer, weighing balance, and water bath. Soursop fruits were purchased from Ekpoma market, Esan West L.G.A. Edo State, Nigeria. pH meter pocket-sized, Hanna product (Hospito Mart Complex), was standardized with appropriate buffer solution at pH 4. Yeast (*Saccharomyces cerevisiae*), was supplied by Vahine professional, Mc cormick, France SAS and was used as received. sulphuric acid, sodium hydroxide, hydrochloric acid, phenolphthalein and ammonium carbonate were purchased from reputable suppliers (Hospito Mart Complex and analyst grades) and used without further purification. The analysis was carried out at Chemistry Laboratory, Samuel Adegboyega University Ogwa, Edo State Nigeria.

Extraction of Soursop

Fresh, healthy and mature soursop fruits of various sizes were collected from Ekpoma, Esan West Local Government area of Edo state. Five fully ripe soursop fruits with an average weight of 2.8154kg (Setra BL.2008 balance) were washed thoroughly with distilled water and surface sterilized with 70% ethanol. The fruits were peeled with sterile knife to remove the skin and then deseeded. The juice was then manually squeezed out with a muslin cloth by hand and preserved in a refrigerator (Thermocool). 500cm^3 of juice was obtained from 2.8154kg of soursop. The juice was filtered and treated with a 3%

Sodium metabisulphite, ($Na_2S_2O_5$) to inhibit the growth of any undesirable microorganism such as acetic acid bacteria, wild yeast and mould (Copeland, 2000). Thereafter, the required quantity (20-80%) of juice was transferred into the fermentation vessels (fermenters). Effect of succinic acid inhibitor on fermentation kinetics and parameters were examined.

Experimental Procedure

The fermentation vessels were sterilized with a 3% solution of sodium metabisulphite for 5 minutes. A litre (1000cm^3) of juice was properly conditioned and was brought to the required pH with either 0.1M HCl or 0.1M NaOH. Seven fermenters (polyethylene terephthalate bottles 75cl) containing substrate were connected with tubes to evolve the produced carbon (IV) oxide and prepared for each, seven reaction times ranging from 30 -210minutes at 30minutes intervals of time. Yeast was added to each of the fermenter. The substrate and the yeast were properly mixed by shaking and the yeast was allowed to activate for 20minutes. The escape of CO_2 was prevented by sealing the air inlet with a cresol-perfumed jelly. The CO_2 produced in each sealed fermenter was collected in water and measured by titration, with 0.IM NaOH using phenolphthalein indicator. The rate of fermentation was measured as the volume of CO_2 produced at 30 minutes intervals of time.

The effect of ammonium carbonate on the rate of fermentation was determined by varying substrate concentration between 20-80%v/v. For each volume of substrate, 1-10ml of 0.1M solution of ammonium carbonate was added.

Determination of Kinetic Parameters: V_{max}, K_m, k_2, k, n and K_s

For the kinetic parameters, modified Mechaelis-Menten equation by Linweaver-Bulk and Briggs- Halden for a single substrate-enzyme catalyzed reaction was used. The maximum velocity (V_{max}) and mechaelis constant (K_m) were obtained from the slope and intercept of the graph of reciprocal velocity versus substrate concentration using $1/v_o = k_m/V_{max}[S] + 1/V_{max}$.

For the catalytic constant (k_2) and dissociation constant (k_s) yeast was varied between 1-7grams. For 1g substrate was varied between 20-80ml and time 30-210minutes at 30minutes interval. The procedure was repeated for 2-7g respectively.

The volume of CO_2 produced was plotted against time to determine rate of fermentation. The maximum rate for yeast gram was plotted against yeast concentration and k_2 was obtained from the slope of the graph ($V_{max} = k_{2[Eo]}$) while k_s was determined by substitution using the equation $1/v_o = k_s/k_{2[Eo]}1/[S] + 1/k_{2[Eo]}$; $K_s = k_{2[Eo]}$. The order of the reaction (n) and rate constant (k) were determined using differential method: ($\log v = n\log[A] + \log k$)

RESULTS

The results of the effect of ammonium carbonate with *saccharomyces cerevisiae* on the fermentation of soursop juice are presented in Tables 1-9 and on Figures 1-11

Table 4.1: Data of the Variation of Volume of Co_2 Produced with time at different Ammonium Carbonate Concentration of Soursop Juice using 1.0(w/v) Yeast, at 30°C and pH 5.0

AMMONIUM CARBONATE (mmol l^{-1})							
Substrate concentration%(v/v)							
Volume of CO_2 produced (cm^3)							
	1.0	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
Time(min)	**20**	**30**	**40**	**50**	**60**	**70**	**80**
30	1.8±0.6	1.5±0.8	1.8±0.8	1.2±0.3	2.0±0.8	1.8±0.8	2.7±0.1
60	2.1±0.4	1.7±0.3	2.0±0.3	1.4±0.9	2.2±0.4	2.1±0.7	3.0±0.2
90	2.5±0.9	2.7±0.3	2.1±0.3	1.5±0.4	2.4±0.1	2.5±0.8	2.5±0.3
120	3.0±0.7	2.1±0.3	3.0±0.3	2.0±0.3	4.5±0.1	3.8±1.1	3.7±0.2
150	3.2±0.1	2.1±0.3	2.2±0.1	2.0±0.3	5.7±0.3	4.5±0.3	3.5±0.9
180	3.4±0.7	2.5±0.1	3.4±0.4	2.2±0.3	4.6±0.1	2.3±0.4	3.0±0.4
210	3.5±0.7	4.2±0.4	5.2±0.3	3.6±0.3	5.0±0.1	4.0±0.4	5.0±0.1

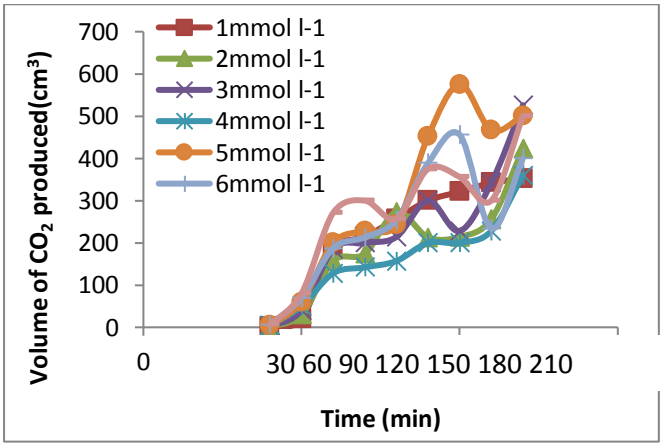

Fig.4.1: Fermentation of soursop juice - variation of volume of co_2 production with time at different ammonium carbonate concentrations using 1.0% (w/v) yeast, at 30°c and pH 5.0

Table: 4.2: variation of rate of fermentation of soursop juice with accelerator concentrations using, yeast 1.0%(w/v), at 30°c and pH 5.0

CONCENTRATION OF ACCELERATOR(mmol l^{-1})							
RATE OF FERMENTATION(mol l^{-1})							
ACCELERATORS	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
AMMONIUM CARBONATE	45.4	53.0	69.5	47.9	46.3	41.9	36.0

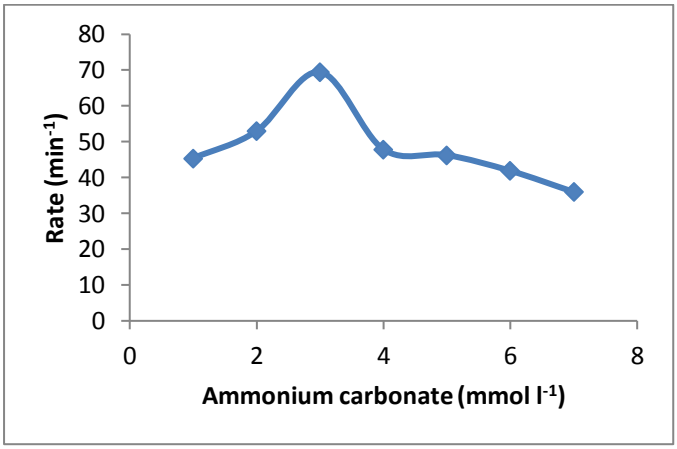

Fig.4.2: Fermentation of soursop juice - variation of rate of fermentation with ammonium carbonate concentration using yeast 1.0%(w/v), at 30°cand pH 5.0

Table: 4.3: Variation of log-rate of fermentation of soursop juice with log-accelerator concentrations using yeast 1.0%(w/v), at 30°c and pH 5.0

LOG-CONCENTRATION OF ACCELERATOR(mmol l^{-1})							
LOG-RATE OF FERMENTATION(mol l^{-1})							
ACCELERATOR	**0.00**	**0.30**	**0.40**	**0.60**	**0.69**	**0.77**	**0.84**
AMMONIUM CARBONATE	1.65	1.72	1.84	1.68	1.66	1.62	1.55

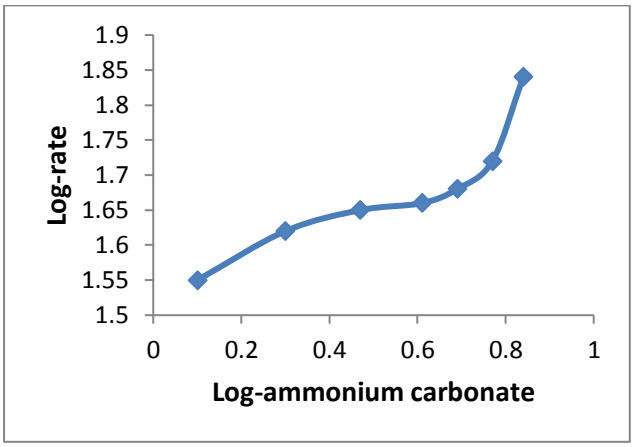

Fig.4.3: Variation of log-rate of fermentation of soursop juice with log-ammonium carbonate concentrations using yeast 1.0%(w/v), at 30°c and pH 5.0

Table: 4.4: Variation of reciprocal rate of fermentation of soursop juice with reciprocal - concentrations for the various accelerators

RECIPROCAL-CONCENTRATION OF ACCELERATOR(mmol l^{-1})							
RECIPROCAL-RATE OF FERMENTATION(mol l^{-1})							
ACCELERATOR	**1.000**	**0.500**	**0.333**	**0.250**	**0.200**	**0.166**	**0.142**
AMMONIUM CARBONATE	0.022	0.018	0.014	0.020	0.021	0.023	0.027

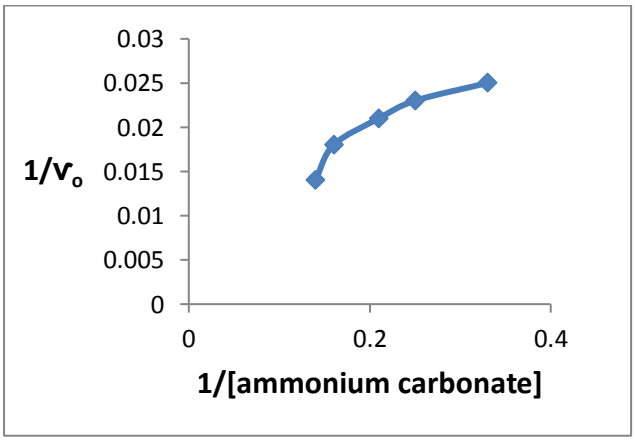

Fig.4.4: Plot of the variation of reciprocal rate of fermentation of soursop juice with reciprocal ammonium carbonate concentrations using yeast 1.0%(w/v), at 30°c and ph 5.0

Table: 4.5: Variation of rate of fermentation of soursop juice with concentrations of the various accelerator

RATE OF FERMENTATION(units per minute)		
Substrate conc. (mmol l^{-1})	**Unaccelerated**	**Accelerated Ammonium carbonate 1-7 mmol l^{-1}**
20	102.8	454
30	103.9	53.0
40	135.7	69.5
50	148.1	47.9
60	138.3	46.3
70	98.2	41.9
80	42.4	36.0

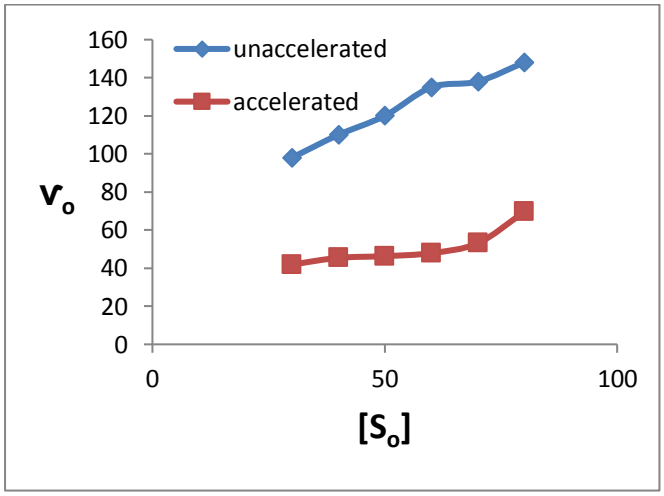

Fig.4.5: Comparative plot of rate of fermentation of soursop juice with reciprocal ammonium carbonate concentration and unaccelerated reactions using michaelis-menten equation

Table: 4.6: Fermentation of soursop juice-variation of reciprocal rate of fermentation of soursop juice with reciprocal - concentrations for the various accelerators

RECIPROCAL-RATE OF FERMENTATION(units per minute)		
Substrate conc. (mmol l^{-1})	**Unaccelerated**	**Accelerated Ammonium carbonate 1-7 mmol l^{-1}**
0.051	0.009	0.022
0.033	0.009	0.018
0.025	0.007	0.014
0.020	0.006	0.020
0.016	0.007	0.021
0.014	0.010	0.023
0.012	0.023	0.027

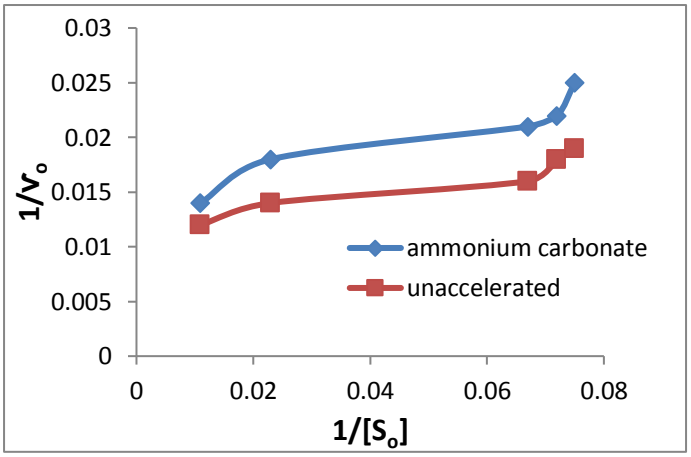

Fig.4.6: Comparative plot of reciprocal-rate of fermentation of soursop juice with reciprocal ammonium carbonate concentration and unaccelerated reactions using linewaever-burk equation

PARAMETER	MAXIMUM RATE, V_{MAX} (Mmin^{-1})	CATALYTIC CONSTANT, k_2 (min^{-1})	DISSOCIATION CONSTANT, k_S	MICHAELIS CONSTANT, k_m (M)	SPECIFIC ACTIVITY (S.A.)
SUBSTRATE	**2.58**	**0.08**	**1.64**	**1.64**	**0.10**
AMMONIUM CARBONATE	**2.49**	**0.02**	**0.53**	**0.53**	**0.10**

Table 4.7: Calculated values of maximum rate and michaelis constant for the fermentation of soursop juice using *saccharomyces cerevisiae* in the presence of inhibitors

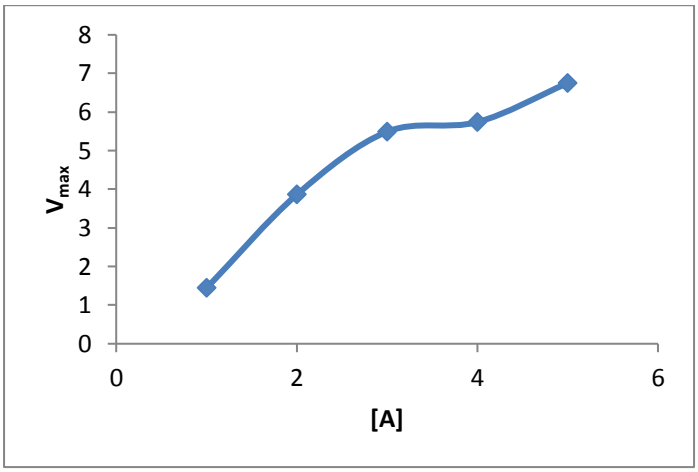

Fig.4.7: Fermentation of soursop juice - linewaever-burk plot showing the effect of accelerator on michaelis constant

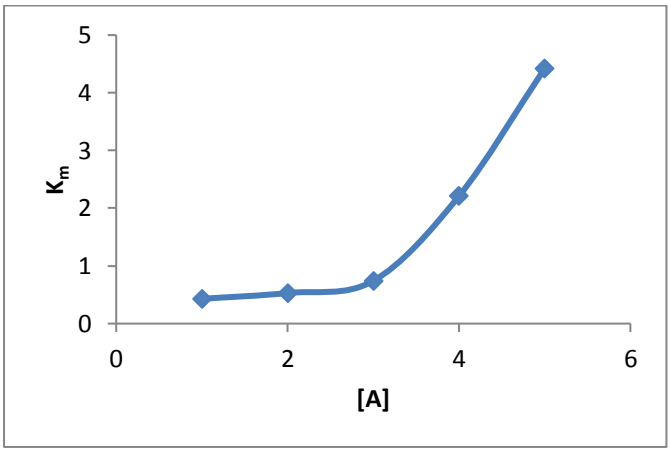

Fig.4.8: Fermentation of soursop juice - linewaever-burk plot showing the effect of accelerator on michaelis constant

Table 4.8: Calculated values of maximum rate and michaelis constant for the fermentation of soursop using *saccharomyces cerevisiae* in the presence of accelerators

ACCELERATORS	$1/V_{max}$	$1/K_m$	K_m/V_{max}
AMMONIUM CARBONATE	0.40	1.88	0.21

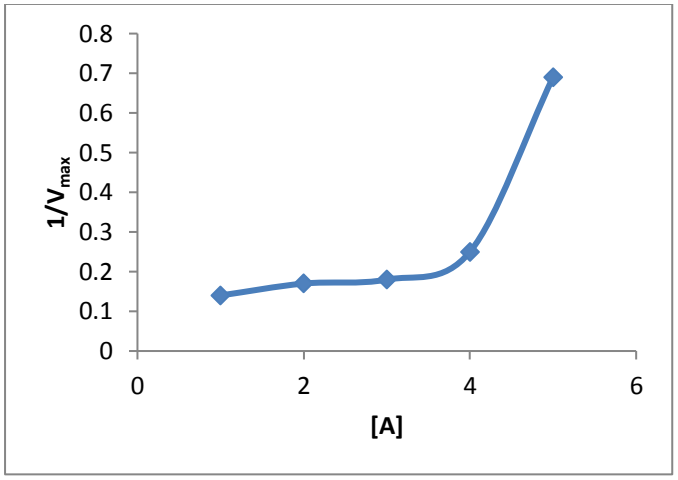

Fig.4.9: Fermentation of soursop juice - linewaever-burk plot showing the effect of accelerator on reciprocal-maximum rate

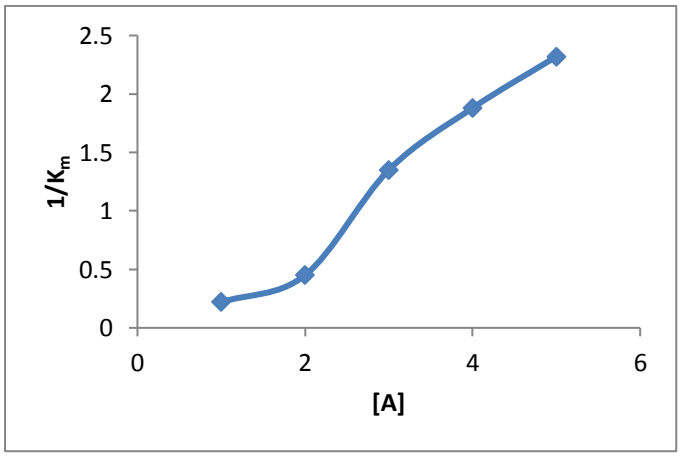

Fig.4.10: Fermentation of soursop juice - linewaever-burk plot showing the effect of accelerator on reciprocal-michaelis constant

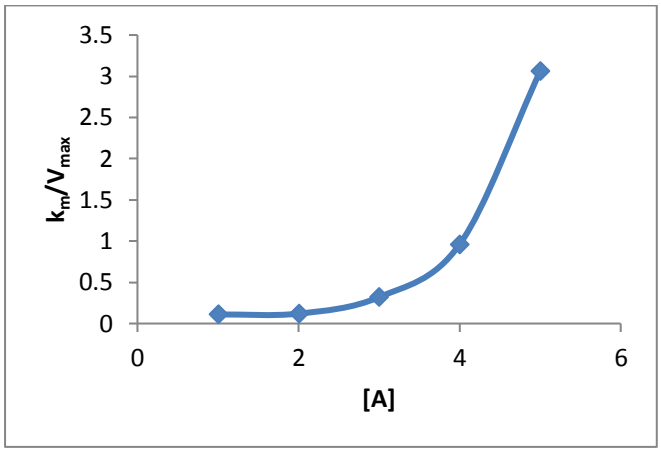

Fig.4.11: Fermentation of soursop juice - linewaever-burk plot showing the effect of accelerator on reciprocal-michaelis constant and maximum rate

Table 4.9: Calculated values of rate constant and order of reaction for the fermentation of soursop juice using *saccharomyces cerevisiae* in the presence of accelerators

ACCELERATORS	Rate constant k	Order of Reaction n
AMMONIUM CARBONATE	2.23	1.01

DISCUSSION

The kinetic parameters: overall rate constant, k, order of initial reaction, n. It is seen in Table 4.9 show that the overall rate constants k, with respect to ammonium carbonate, 2.23 and the order of initial reaction is first order for. The Tables also suggests that the curves achieved from the plots of volume of carbon dioxide produced with time approached the time axis in a shorter time in ammonium carbonate soursop juice. The reaction was faster upon its addition.

Most importantly, the kinetic parameters highly valued in fermentation process are shown in Table 4.7.

The maximum rate of fermentation, V_{max}, catalytic constant, k_2 also known as the turnover number, dissociation constant for the enzyme-substrate complex k_s, the Michaelis constant, k_m, and the specific activity of the enzyme for soursop. The maximum rate of fermentation with the addition of ammonium carbonate is, $2.49x10^{2}Mmin^{-1}$. The value represents the maximum velocity attainable. It was observed that the maximum rate at which all the enzyme molecules were in the complex form was high and became lower

upon the addition of the additive, suggesting inhibition of the fermentation process by the additive due to decrease in the maximum activity of the enzyme.

The catalytic constant, k_2, the value upon addition of ammonium carbonate is, $2.0x10^{-2}$ min^{-1} which indicates the number of substrate molecules converted into products per unit time. The catalytic constant as seen from the data in Table 4.7, as well as the formation rate constant k_1 of ES complex from E and S, that is, $k_{-1}>>k_2$, therefore, $k_m = k_s = k_{-1}/k_1$ confirms the values of k_m ($1.64x10^2$) and k_s ($1.64x10^2$). The value of k_2 ($8x10^{-2}min^{-1}$); often lies between 0.5 and 10^4S^{-1}) (Chang, 1990). It indicates that the equilibrium step involving k_{-1} is more thermodynamically favoured than step 2 involving k_2.

The dissociation constants of enzyme-substrate complexes, k_s for the addition of ammonium carbonate is, $5.30x10^{-1}$. There was no significant difference in the values calculated for Michaelis constant and dissociation constant suggesting that the rate of formation of the enzyme-substrate complex was higher than the rate of its dissociation which could have led to the formation of fewer products upon the addition of the additive.

The Michaelis constant, k_m obtained is $5.30x10^{-1}$. It is usually deployed to characterize a particular enzyme-substrate system. K_m is equal to the concentration of substrate required to give half the maximum velocity. The value of k_m in this investigation is quite large; with reference to known k_m values that lie between 10^{-1} and 10^{-6}M (Chang, 1990). The large value indicates that the binding between enzyme and substrate is very weak with respect to both substrates and *Saccharomyces cerevisiae* [E]. The presence of the additive increased the affinity of the enzyme for the substrate making product formation difficult.

The specific activity which is the unit of enzyme activity per gram of protein (yeast) is found to be $1x10^{-1}$. The specific activity of enzyme for the addition of ammonium carbonate, $1x10^{-1}$. A unit of enzyme is taken to be the amount which will catalyze the reaction of a unit of substrate per minute. Thus, specific activity in relation to enzymes is the ratio of enzyme activity to the total weight of enzyme present in the mixture. This was obtained by cross plotting the rates of fermentation with respect to enzyme and yeast concentrations.

REFERENCES

Bass, D., Howe, A., Brown, N., Barton, H., Demidova, M., Michelle, H., Li, L. et al, (2007). "Yeast forms dominate fungal diversity in the deep oceans." *Proceedings of the Royal Society B: Biological Sciences*. **(**3069–3077).

Dahlia, P. (2009). "Sugar Beet White Sugar". Food and agriculture organization. New York: McGraw-Hill. (198-200).

De-paula, J., & Atkins, P. (2006). Physical Chemistry for the Life Sciences. New York, NY: W.H. Freeman Company. (309-313).

Egharevba, F., & Ogbebor, C. (2014). A kinetic study of fermentation of cane sugar using saccharomyces cerevisiae. *Journal of Physical Chemistry, 4*(1), 26-31.

Gorelik, G. E., & Leitsina, V. G. (1995). Physical kinetics and transfer processes in phase transitions. (151-155). London: Begell House.

Malinowski, J. J. (2001). Two phase partitioning bioreactors in fermentation technology. *Journal of Biotechnology Advances, 19*, 525.

Negi, A. S., & Anand, S. C. (2007). A Textbook of Physical Chemistry. New Delhi: New Age Publication. (714 – 718).

Palmer, T. (2000). Enzymology. (5^{th} Ed.), London: Harwood. (PP. 107-108).

Sebayang, A. H., Masjuki, H. H., Ong, H. C., Dharma, S., Silitonga, A. S., Mahlia, T. M. I., et al. (2016). A perspective on bioethanol production from biomass as alternative fuel for spark ignition engine. *RSC Adv.* 6:14964–92.