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Fermentation of Soursop Using *Saccharomyces Cerevisiae*: A Kinetic Evaluation

*[1]Ogbebor, C, [2]Akpoveta, V.O, [3]Osakwe, S.A. [4]Medjor, W.O,
[1]Department of Chemistry, Ambrose Ali University, P.M.B 14, Ekpoma, Edo state, Nigeria.
[2]Department of Chemical Sciences, Samuel Adegboyega University, P.M.B 001, Ogwa, Edo State, Nigeria
[3]Department of Chemistry, Delta State University, P.M.B 1, Abraka, Delta state, Nigeria.
[4]Department of Chemistry, Taraba State University, Jalingo, Taraba State, Nigeria
Corresponding author email: Email- akpovin2@gmail.com

ABSTRACT
A kinetic evaluation of the fermentation of soursop (Substrate) by *Saccharomyces cerevisiae* (yeast enzyme) was conducted by determining the effect of various parameters (such as Temperature, substrate, pH, and Yeast concentration) on the rate of production of CO_2. The results showed that the rate of fermentation increased in proportion with Temperature (optimum 32-36^oC), Substrate (optimum 50%v/v), pH (optimum 5.0) and Yeast concentration (optimum 3.5-4.5%w/v) up to a point and decreased. The kinetic parameters evaluated are maximum rate of reaction V_{max} ($1.79x10^2 MS^{-1}$); catalytic constant, k_2 ($8.0x10^{-2}$ dm^3 $mol^{-1}S^{-1}$); overall rate constant, k ($1.79x10^2$); order of initial reaction (first order); dissociation constant of enzyme-substrate complex, k_s ($1.64x10^2$); Michaelis constant, k_m ($1.64x10^2$M); and the specific activity of enzyme on substrate concentration ($1x10^{-1}$%w/v).
Keywords: Fermentation; Substrate Concentration; Kinetic parameters; Soursop; *Saccharomyces cerevisiae* yeast enzyme

INTRODUCTION

Fermentation originally indicated the conversion of grape juice into wine (Inekoronte and Ngoddy, 1990). It is now applied to a variety of processes of anaerobic decomposition of organic compounds by micro-organisms, or by extracts prepared from them. It is also applied for aerobic microbial processes (Gorelik, and Leitsina, 1995). The process of alcoholic fermentation is brought about through the agency of wild yeasts present on the skins of the fruit (Blanch and Clark, 1997). This yeast plants are less sensitive to inhibiting effect of alcohol but acts as catalysts or enzymes present on sucrose, a complex molecule (Goswell,1998) present in fruits as carbohydrates. Soursop (*Annona-muricata*) is a broad leaf flowering evergreen tree that is low-branching and bushy. The juice of soursop fruit contains sugar, carbohydrate, vitamins, dietary fibre, fat and protein (Liaw and Chang, 2005). It is fleshly eaten by hand when ripe. The sweet pulp is also used to make juice as well as candies, sorbet and ice cream flavoring (Laurence, 2004). The yeast of the ale fermentation is *Saccharomyces cerevisiae* and has been used since ancient times for beer, wine and bread fermentations (Copeland, 2000). *Saccharomyces cerevisiae* forms a thick, frothy yeast head on the surface of the fermenting beer; a proportion of the yeast of the fermentation is supported there by attached bubbles of CO_2. The evaluation of fermentation kinetics began with the work of Adrian, in the hydrolysis of sucrose (Adrian, 1902). He showed that when the sucrose concentration was much greater than the enzymes concentration, the reaction is zero-order with respect to sucrose concentration. In fact, the mechanisms by Adrian (Adrian, 1902) served as a simple starting point for many general models. The 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 (Leksawasdi, et al, 2001). Kinetic parameters such as maximum rate of reaction V_{max}, catalytic constant k_2, overall rate constant k, order of reaction, dissociation constant k_s, Michaelis constant k_m and specific activity of enzyme on substrate concentration were evaluated from the determined rate of reaction in this study.

MATERIALS AND METHODS

Soursop was purchased from a local market in Ekpoma town of Edo state (South-South, Nigeria). pH meter was standardized with appropriate buffer solutions (buffer 4), while yeast (*Saccharomyces cerevisiae*) manufactured by Vahine professional, Mc cormick, France SAS was used as received.

EXPERIMENTAL PROCEDURE

The soursop juice was obtained manually. Seven vials were prepared for each of seven sampling times at 30-210 minutes at 30 minutes interval. The substrate fermented in the vials with connected tubes for produced gas estimation was catalyzed by added yeast. The produced CO_2 was collected in water and measured by titration with 0.1MNaOH using phenolphthalein indicator.

Effect of Temperature: This was varied between 30-40^oC at 2^oC interval, while other factors such as substrate, pH, yeast concentration and time were kept constant. The process was conducted in a thermostated water bath.

Effect of Substrate concentration: The was varied between 20-80%(v/v), while other factors were kept constant.

Effect of pH: pH was varied between 3-6. It was regulated using 0.1MHCl and 0.1MNaOH.

Effect of Yeast concentration: It was varied between 1-7 grams.

The rate of fermentation was measured as volume of CO_2 produced at 30 minutes time intervals.

RESULTS AND DISCUSSION

The data on the effect of Temperature, Substrate concentration, pH, and Yeast concentration on the fermentation of soursop with *Saccharomyces cerevisiae* are presented in Tables 1-4.

TABLE 1: EFFECT OF TEMPERATURE ON FERMENTATION RATE USING 50%(V/V) SUBSTRATE, YEAST 1.0%(W/V), AND pH 5.0

	Volume of CO_2 produced (cm^3)						
Time (min)	**Temperature (^oC)**						
0	30	32	34	36	38	40	42
30	100	100	100	100	100	100	100
60	128	128	142	157	128	142	128
90	171	171	157	200	171	171	142
120	200	200	200	328	200	185	200
150	228	228	228	385	228	200	228
180	271	257	371	471	328	228	257
210	371	300	400	528	400	357	271
Rate	1.50	1.60	2.10	2.50	2.00	1.60	1.40

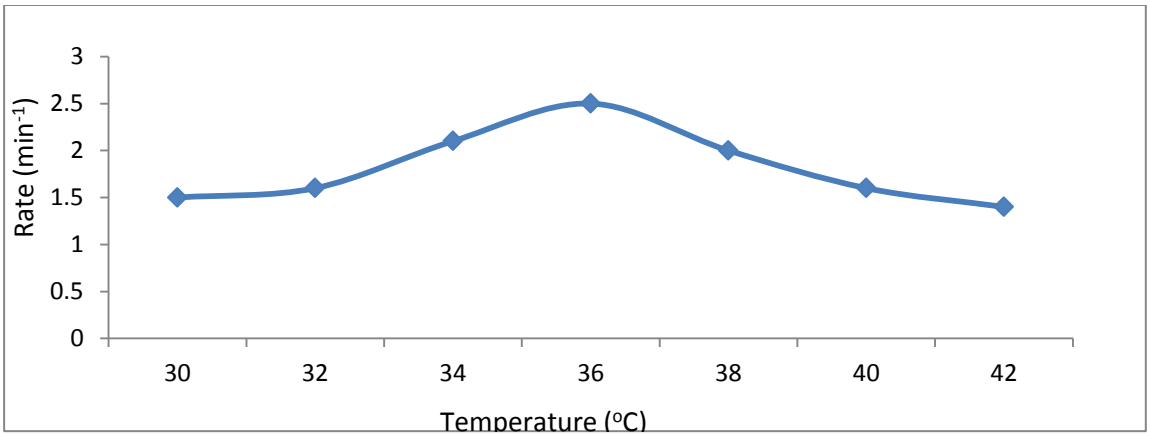

Fig 1: VARIATION OF RATE OF FERMENTATION WITH TEMPERATURE OF SUBSTRATE USING 50%(V/V) SUBSTRATE, YEAST 1.0 %(W/V), AND pH 5.0

TABLE 2: EFFECT OF SUBSTRATE CONCENTRATION ON FERMENTATION RATE USING 1.0 %(W/V) YEAST, AT 28^{O}C AND pH 5.0

	Volume of CO_2 produced (cm^3)						
Time (min)	**Substrate concentration% (v/v)**						
0	20	30	40	50	60	70	80
30	100	142	285	128	114	314	314
60	271	171	357	142	157	328	371
90	314	271	485	185	171	471	500
120	485	442	528	228	200	600	528
150	557	457	600	257	700	614	600
180	614	571	628	285	771	671	728
210	757	857	728	357	871	700	757
Rate	1.30	2.30	3.20	3.70	3.30	2.80	2.80

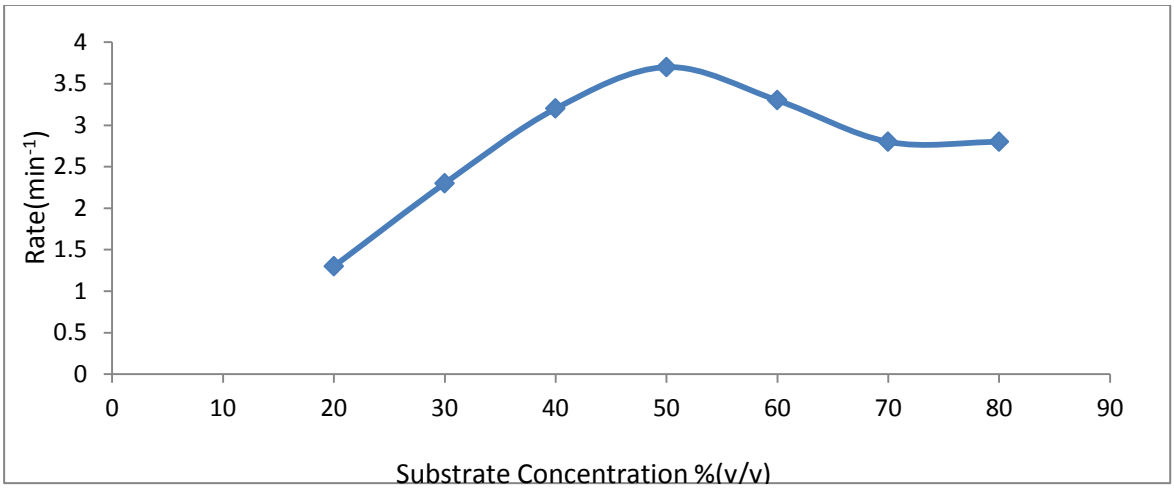

Fig 2: VARIATION OF RATE OF FERMENTATION WITH SUBSTRATE CONCENTRATION USING 1.0% (W/V) YEAST, AT 28^{O}C AND pH 5.0

TABLE 3: EFFECT OF pH ON FERMENTATION RATE USING 50% (V/V) SUBSTRATE, 1.0% (W/V) YEAST, AT 28^{O}C

	Volume of CO_2 Produced(cm^3)						
Time(min)	**pH**						
0	3.0	3.5	4.0	4.5	5.0	5.5	6.0
30	100	100	100	500	200	400	300
60	228	142	200	557	300	442	514
90	271	200	400	600	357	500	557
120	328	271	500	642	557	528	757
150	500	600	671	700	571	557	800
180	642	828	800	828	800	657	957
210	714	914	828	957	928	771	985
Rate	2.90	3.30	3.90	4.40	4.70	4.00	3.90

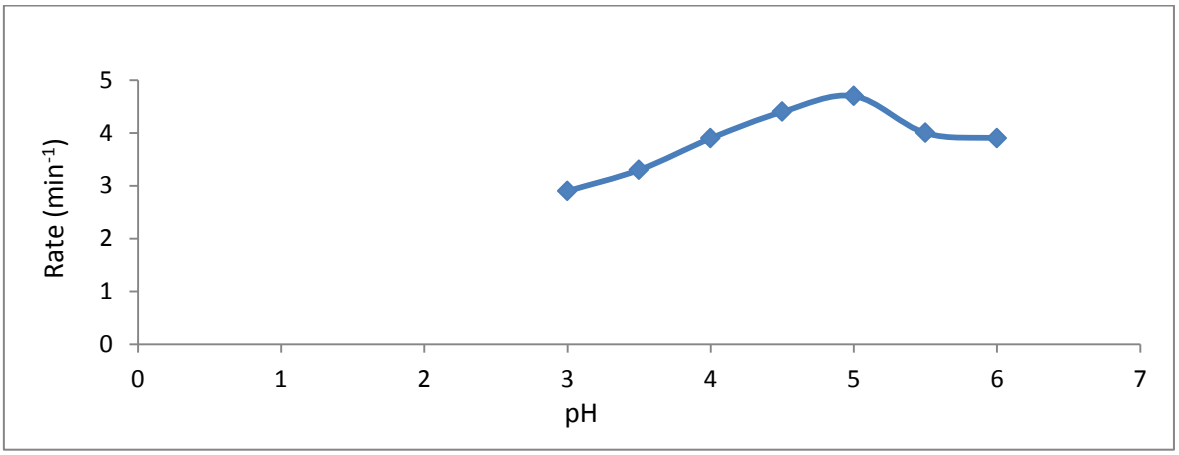

Fig 3: VARIATION OF RATE OF FERMENTATION WITH pH OF SUBSTRATE USING 50%(V/V) SUBSTRATE, 1.0 %(W/V), AT 28^{O}C

TABLE 4: EFFECT OF YEAST CONCENTRATION ON RATE OF FERMENTATION USING 50% (V/V) SUBSTRATE, AT 28^{O}C, AND pH 5.0

Time (min)	Volume of CO_2 produced (cm^3) Yeast concentration (grams)						
0	1	2	3	4	5	6	7
30	100	200	300	100	257	100	200
60	110	257	328	300	300	328	300
90	200	300	428	428	342	400	400
120	242	328	442	457	400	428	428
150	271	400	471	471	428	500	471
180	328	428	528	500	571	571	600
210	428	457	600	600	642	771	700
Rate	1.40	1.80	2.50	3.70	3.00	2.50	2.10

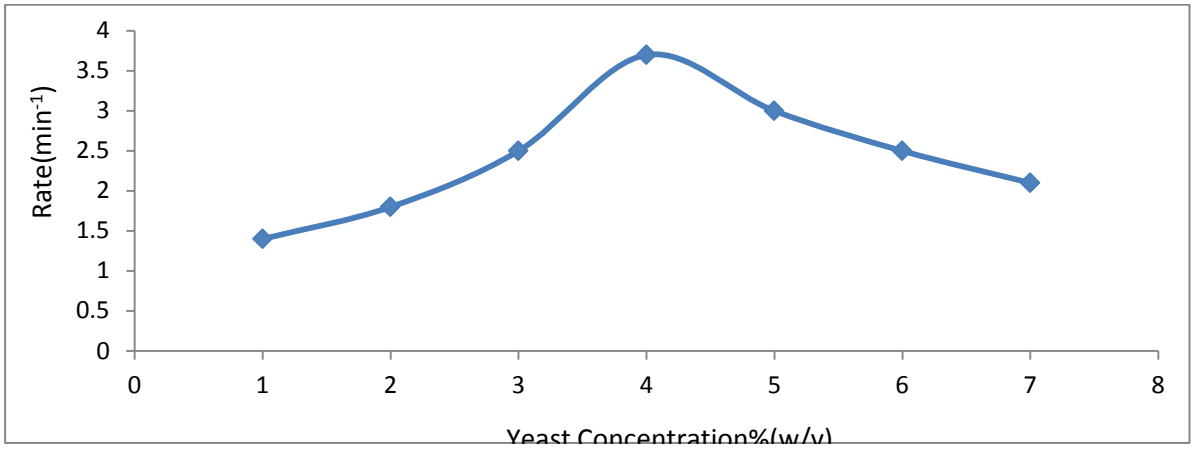

Fig 4: VARIATION OF RATE OF FERMENTATION WITH YEAST CONCENTRATION %(W/V) USING 50 %(V/V) SUBSTRATE, AT 28^{O}C, AND pH 5.0

TABLE 5: VALUES OF k AND n FOR THE FERMENTATION OF SOURSOP SUGAR USING *SACCHAROMYCES CEREVISIAE*

	RATE CONSTANT, k	ORDER OF REACTION, n
TEMPERATURE	2.42	Nil
SUBSTRATE CONCENTRATION	1.79	1.16 (approx.1st order)
YEAST CONCENTRATION	1.58	Nil
pH	1.84	Nil

TABLE 6: KINETIC PARAMETERS FOR THE FERMENTATION OF SOURSOP SUGAR USING *SACCHAROMYCES CEREVISIAE*

PARAMETER	VALUE
MAXIMUM RATE, V_{MAX} ($Mmin^{-1}$)	$1.79x10^{2}$
CATALYTIC CONSTANT, k_2 (min^{-1})	$8x10^{-2}$
DISSOCIATION CONSTANT, k_S	$1.64x10^{2}$
MICHAELIS CONSTANT, k_m (M)	$1.64x10^{2}$
SPECIFIC ACTIVITY (S.CEREVISIAE)	$1x10^{-1}$

Table 1 showed the data obtained for the effect of temperature on fermentation rate and plotted on Figure 1 as rate of fermentation with temperature. It was observed that the rate of production of CO_2 increased up to 36^{o}C and then decreased. It is seen that there was a rapid release of CO_2 at the initial period of the reaction due to increase in the average kinetic energy of the substrate molecules, and slows down as the reaction moves to completion as a result of loss of enzyme activity with time due to thermal vibration of the enzyme molecules.

The data on the effect of substrate concentration on rate of fermentation is shown in Table 2 and plotted on Figure 2. The Figure showed that the rate of fermentation varied in proportion with substrate concentration up to 50%(v/v). However, further increase in the substrate concentration showed a decline effect on the rate of fermentation. This suggests that at the initial stage of the reaction, all active sites of the enzyme were saturated and therefore further increase in substrate concentration could not lead to further increase in the rate of fermentation.

Table 3 showed the data obtained for the effect of pH on fermentation rate and plotted on Figure 3 as rate of fermentation with pH. The rate of fermentation increased in relation with pH from 3.0-5.0. This result is in conformity with the optimal pH range of *Saccharomyces cerevisiae* 4.5-5.5 (Blanch and Clark, 1997). Outside the optimum pH range, the enzyme cells were less tolerant to the pH environment and expectedly less active and less efficient in substrate conversion.

Table 4 showed the data obtained for the effect of Yeast concentration on fermentation rate and plotted on Figure 4 as rate of fermentation with Yeast concentration %(w/v). It is considered that at high yeast concentration, the substrate becomes unavailable for the large population of yeast for a particular enzyme-substrate system. This suggests that there is a fixed amount of substrate that can be complexed with the yeast.

Table 5 showed the order of reaction (approx. first order) and the value of the overall rate constant k. This is the attainable rate obtained for the study of the effect of temperature, 2.42, substrate, 1.79, pH 1.58, and yeast concentration 1.84 for the fermentation of Soursop juice. The data on the table further indicates that the rate of the reaction is highly dependent on the substrate concentration; while the other conditions such as temperature, yeast concentration and pH have seemingly low dependence on the rate and a limiting one at optimum range.

Table 6 showed the evaluated kinetic parameters: $V_{max,}$ maximum velocity, $1.79x10^{2}$, $Mmin^{-1}$ which is the maximum velocity at which all the enzymes are in the complex form ES; $k_{2,}$ catalytic constant, $8x10^{-2}$ min^{-1}, that indicates the conversion of soursop juice by the enzyme to product at 30 minutes interval of time; $k_{s,}$ dissociation constant, $1.64x10^{2}$, that measures the affinity of the enzyme for the substrate; $k_{m,}$ Michealis constant, $1.64x10^{2}M$, which is the strength of the intermediate formed ES and the specific activity, $1x10^{-1}$, which is the amount of substrate catalyzed by the enzyme in one minute.

CONCLUSION

This work recommends that alcohol production by fermentation of soursop juice should be conducted at optimum conditions of Temperature 36^{o}C, Substrate 50%(v/v), Yeast 4.5%(w/v) and pH 5.0.

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