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Extent of Inhibition of Malonic Acid on the Fermentation of Soursop Juice: A Thermodynamic Evaluation

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ABSTRACT: *The fermentation of soursop juice as substrate by Saccharomyces cerevisiae (yeast) was investigated to obtain certain useful thermodynamic parameters. This was achieved by the determination of the effects of temperature, substrate and inhibitor on the rate of carbon (IV) oxide (CO_2) production. The extent of inhibition was examined by the addition of malonic acid inhibitor. The results indicate that the rate of fermentation of soursop juice increased in proportion with temperature (optimum 36^oC), substrate (optimum 50%v/v) and malonic acid (optimum 30%v/v) up to a limit and decreased. This suggests that the reaction takes place in two steps (equilibrium and decomposition steps). The thermodynamic parameters determined are: enthalpy, ΔH^* 151.14 kJmol^{-1}; entropy of activation, ΔS^* 275.359 Jmol^{-1}k^{-1}; Gibb's free energy of activation, ΔG^* -752.21 kJmol^{-1} and Equilibrium constant K_{eq}, 1.33 respectively. The results show that the enthalpy value is positive indicating that the fermentation process is endothermic while the activation energy is same as the enthalpy. The entropy value is positive corroborating the positive enthalpy value. The fermentation process is spontaneous as shown by the negative change in free energy. These values were subsequently used to obtain by calculation Arrhenius constant A, 1.51x10^{23}; orientation factor P, 1.41x10^{-34} and the collision frequency, Z, 1.07x10^{11} respectively. The calculated A and the P values obtained on the basis of collision theory and absolute reaction rate theory (or transition state theory), respectively show that the collision factor is of the order of 10^{11} min^{-1}, about half that of gaseous molecules that can only be attained by an induced reduction in activation energy by a catalyst. The equilibrium constant value K suggests that the intermediate complex is rather interacting more with the substrate and also showing that only a limited amount of the substrate is converted to product. The equilibrium constant K is observed to be 1.33dm^{3}mol^{-1} and not significantly far from unity implying that ΔG^* can be conveniently equated with the change in free energy at standard conditions. Finally, the type of inhibition was found to be uncompetitive from the extrapolated Lineweaver-Burk plots and did not bring about marked decrease in the rate of fermentation as expected.*

KEYWORDS: inhibition, malonic acid, fermentation, soursop juice, thermodynamic evaluation

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INTRODUCTION

Thermodynamics is concerned with the changes in energy and similar factors as a chemical process takes place, and not with the mechanism or speed of the process. The first law of thermodynamics states that energy can neither be created nor destroyed, but can be converted into other forms of energy or used to perform work.

The second law of thermodynamics states that the entropy or degree of disorder of the universe is always increasing. The third law is an extension of the second law, which states that the entropies of substances at zero kelvin can be assigned the value of zero (Chang, 2005). In general, thermodynamic studies are aimed at deciding which direction such a reaction will proceed in terms of spontaneity, subsumed in the second law of thermodynamics and the equilibrium constant which invariably determines the extent to which any particular reaction will proceed under any given conditions. The thermodynamic assessment of an enzyme catalyzed fermentation process provides a key instrument for efficiently controlling fermentation based reactions (Danson, 2000). This however requires a thorough understanding of the application of basic laws of thermodynamics especially in scale up processes. However, models that will all-time be applicable to all possible substrate and catalysts are yet to be provided (Haq et al., 2010). Such model will take into account the spontaneity of the fermentation, the nature of the substrate, the sugar content (glucose etc.), extent and direction of fermentation, the driving force, the catalyst (enzymes), the nature of the intermediate and products such as alcohol, aldehydes, kinetics etc. and the inhibitory factors and possibilities (Eisenthal et al., 2007). A continuous search for possible models to account for enzyme catalyzed reactions by a step by step but steady approach will assist in accumulating the necessary data towards achieving the aim (Negi and Anand, 2007). However, Arrhenius, Errying and van't Hoff's (Duy and Fitter, 2005) models were deployed to characterize the fermentation of soursop juice using *saccharomyces cerevisiae.* It is hoped that this work will be able to establish quantitatively some process variables as they relate to each other. It was previously held that fermentation catalyzed by enzyme could not be treated in the usual classical molecular reaction models nor the equilibrium attained between reactants and products or intermediates handled as if it were like the familiar thermodynamic system (Haq et al., 2010). It has also been noted that the free energy ΔG^* that drives a chemical reaction is also not frequently calculated for fermentation on account of several possible reaction pathways (Duy and Fitter, 2005).Yet the second law of thermodynamics is deployed to determine which products will accumulate in such a system. Besides, thermodynamic concepts have been used to predict microbial growth, which is a key consideration in many industrial biotechnology processes (Eisenthal et al., 2007). Be that as it may, we will consider the first step in the two-step reaction involved in the fermentation as a rapid equilibrium leading to a complex of the enzyme and substrate reminiscent of a lock and key system in which the enzyme provides a lower activation energy exclusive of any tunneling action of the reactants. However, since the enzyme merely provides a catalytic action offering lower energy pathway, we shall in this research adapt the Eyring (1935) absolute reaction rate theory (transition

state theory) since there are adequate facilities nowadays to monitor or measure the products of fermentation. The quantity K_BT/h in the Eyring's equation is independent of the nature of reactants or activated complex and would be same for all changes occurring at the same temperature (Negi and Anand, 2007).

Soursop (*Annona muricata*) is well-known for its pleasant, highly aromatic juicy flesh and distinctive flavour. The soursop fruit has a relatively short shelf life because it gets bruised easily during handling and transportation. It is also difficult to consume fresh due to its mushy flesh. To prolong its shelf life, it may be processed into other forms like puree, juices, nectars syrups, concentrates, jams jellies, ice creams, powders, fruit bars and flakes. Although the soursop juice is popularly produced for its convenience to be consumed, the yield of its juice is little due to its cotton pulpy flesh (Liaw, 2002).
In our previous studies (Egharevba and Ogbebor, 2014), we critically investigated the fermentation of soursop and sugarcane as substrates by *Saccharomyces cerevisiae* to obtain certain useful kinetic and thermodynamic parameters and also to determine the effect of temperature, substrate concentration, pH and yeast on the rate of fermentation. The rate of fermentation was measured as rate of CO_2 production, and established the optimal condition for producing soursop and sugarcane ethanol. In this study, the focus is to examine the influence of some inhibitor (malonic acid) on the thermodynamics of the fermentation of soursop juice using *Saccharomyces cerevisiae*.

The Enthalpy, Entropy and Gibb's Free Energy
In the concept of thermodynamics, a closed system is one which can exchange energy but not matter with its surroundings. The exchange of energy must involve thermal transfer for the performance of work. If, in a closed system at constant temperature and pressure, a process takes place which involves a transfer of heat to or from the surroundings and there is a change in volume of the system, it follows from the first law of thermodynamics that,
$\Delta E^* = \Delta H^* - P\Delta V$ ----------(1)
Where ΔE^* is the increase in intrinsic energy (internal energy) of the system, ΔH^* is the increase in enthalpy (thermal energy) and $P\Delta V$ is the work done by ΔV at constant pressure, P and temperature, T. The enthalpy change ΔH^* is defined simply as the quantity of heat absorbed by the system under constant pressure and temperature. At constant temperature and pressure, the increase in entropy of the surrounding is:
$-\Delta H^* /T$----------(2)
If under thermodynamic reversibility, i.e infinitely slow, the increase in entropy of the system is $\Delta H^*/T$ hence, $\Delta \mathbf{S^*}$ is $- \Delta H^*/T > 0$. This support therefore, the possible thermodynamics that would apply to enzyme reactions (Equation 2) from a consideration of: (a) Arrhenius equation (Equation 4) in which k is the rate constant for the decomposition of the enzyme-substrate complex, ES, to product P.
It is assumed that an equilibrium is established between enzyme and the inhibitor almost

$$E + S \underset{k_{-1}}{\overset{k_1}{\rightleftharpoons}} ES \xrightarrow{k_2} E + P \quad ..(3)$$

$k = A\, e^{-Ea/RT}$ ----------(4)

Where A is a constant known as the frequency factor (pre-exponential coefficient), E_a - activation energy, R, gas constant, and T, absolute temperature.

(b) Eyring equation, in which the rate constant k for the decomposition of the complex ES, a non-stable intermediate is given as

$k = K_B T /h\, K$ ----------(5)

where K_B, is the Boltzmann's constant, h, the planck's constant, K, the equilibrium constant of the first step, and

(c) van't Hoff's equation (1884) in which the equilibrium constant, K, is expressed as

$K = e^{\Delta S^*/R}\, e^{-\Delta H^*/RT}$ ----------(6)

where ΔS^*, is the change in entropy of activation and ΔH^*, the change in enthalpy of activation.

Equating 4 and 5 gives

$A\, e^{-Ea/RT} = K_B\, T/h\, K$ ----------(7)

Substituting equation 5 into 6 gives

$A\, e^{-Ea/RT} = K_B\, T/h\, .e^{\Delta S^*/R}\, e^{-\Delta H^*/RT}$ ----------(8)

As in a unimolecular reaction, when $\Delta n = 0$, there is no change in the number of moles, in solution, there is also no appreciable change in volume; therefore,

$\Delta H^* = \Delta E^*$ with minimal error (Negi and Anand, 2007; Laidler and King, 1999).

In the light of this, equation 8 reduces to

$A = K_B\, T/h\, .\, e^{\Delta S^*/R}$ ----------(9)

This accounts for the frequency or pre-exponential factor, an important factor in enzyme reactions. Since the reaction takes place in solution, the rate of the reaction at unit concentration of the reactant is known as specific rate or rate constant. We will be able to evaluate the rate dependence on temperature by substituting the Gibb's free energy equation,

$\ln K = -\Delta G^*/RT = -(\Delta H^* - T\Delta S^*)/RT$ ----------(10)

in equation 31 (the Eyring equation, Eyring, 1935 in Winzor, 2006) to obtain

$\ln k = \ln K_B\, T/h + \Delta S^*/R - \Delta H^*/RT$ ----------(11)

From this a plot of Ink (observed rate constant) versus reciprocal of the absolute temperature 1/T, gives $\Delta H^*/R$ as slope, and ΔS^* as the intercept. When ΔH^* and ΔS^* are known, ΔG^* can be calculated and finally, the equilibrium constant K.

MATERIALS AND METHOD

Materials

Apparatus used are: beakers, 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 fruit was 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 malonic acid were purchased from reputable suppliers (Hospito Mart Complex and analyte grades) and used without further purification. The analysis was carried out at Chemistry Laboratory, Samuel Adegboyega University Ogwa, Edo State Nigeria.

Extraction of Soursop Juice

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 fibres were then manually removed by squeezing out the juice. 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 malonic acid and thermodynamic parameters were examined.

EXPERIMENTAL PROCEDURE

Determination of effect of Temperature and Substrate Concentration

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 -210 minutes 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 temperature on fermentation was determined by keeping other factors such as substrate concentration, pH of the juice, yeast concentration, and fermentation time constant. The temperature was varied between 30-42^{o}C, using a thermostat water bath. In determining the effect of substrate concentration on fermentation, all other factors were also kept constant. The substrate concentration was varied between 20–80(v/v). The optimum temperature and substrate concentration required for the fermentation were determined from the maximum

heights achieved from the plot of the CO_2 produced versus time curves, measurements were in triplicates.

Determination of the effect of Inhibitor

The effect of inhibitor 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 inhibitor (malonic acid) was added.

Determination of Thermodynamic Parameters: ΔH, ΔS, A, ΔG, P and Z

For the effect of temperature, it was varied between 30-42^{o}C, for 30^{o}C, substrate was varied between 20-80ml with time 30-210minutes at 30minutes interval. This was repeated for 32, 34, 36, 38, 40 and 42^{o}C respectively. The rate of fermentation at different temperatures was determined from the maximum heights achieved from the plot of volume of CO_2 produced versus time curves. Temperature was converted to kelvin. Reciprocal temperature was plotted against Ink to obtain heat content and randomness of the system. Thermodynamic formulation transition state equation (**$Ink = -\Delta H^*/RT + k_BT/h.\Delta S^*/R$**). From the plot of Ink and 1/T to calculate the enthalpy (**ΔH)** and entropy **(ΔS)** of activation from the slope and intercept of the graph. Change in free energy **(ΔG)** for a system at constant temperature process ($\Delta G^*=\Delta H^* - T\Delta S^*$) was used to determine standard free energy of activation. The actual free energy of the system was determined using ($\Delta G =\Delta G^* + RTInK_{eq}$) because the reactants and products were not in their standard states to know the spontaneity of the process. The equilibrium constant was determined by substitution using $\Delta G^* = -RTInK_{eq}$. It tells the direction of the process. Eyring equation ($Z = K_BT/h$**)** was used to calculate the rate constant of the reaction because it gives considerable accuracy. Arrhenus equation ($k = A\ e^{-Ea/RT}$) was used to calculate the frequency factor (A) to know the frequency (number) of encounters between the reactant molecules**.** The relationship between orientation factor (P), frequency factor (**A)** and probability factor (**Z)** which is equal to $k_BT/h\ {}_e\text{-}E_a/RT$ was determined using A= PZ.

RESULTS

The results of the effect of temperature, substrate concentration, (*saccharomyces cerevisiae)* inhibitor (malonic acid) on the fermentation of soursop juice are presented in Tables 4.1-4.15. and on Figures 4.1-4.13

Soursop Juice Fermentation

TABLE 4.1: DATA OF THE VARIATION OF VOLUME OF CO_2 PRODUCED WITH TIME AT DIFFERENT TEMPERATURES AND RATE OF FERMENTATION OF SOURSOP JUICE USING 50%(v/v) SUBSTRATE, YEAST 1.0%(w/v) AND pH 5.0

Temperature (^OC)							
Volume of CO_2 produced (cm^3)							
Time(min)	**30**	**32**	**34**	**36**	**38**	**40**	**42**
30	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1	1.0±0.1
60	1.2±0.1	1.2±0.3	1.4±0.1	1.5±0.1	1.2±0.1	1.4±0.4	1.2±0.2
90	1.7±0.2	1.7±0.5	1.5±0.2	2.0±0.2	1.7±0.2	1.7±0.3	1.4±0.2
120	2.0±0.0	2.0±0.3	2.0±0.1	3.2±0.2	2.0±0.2	1.8±0.3	2.0±0.3
150	2.2±0.3	2.2±0.6	2.2±0.2	3.8±0.1	2.2±0.2	2.0±0.7	2.2±0.1
180	2.7±0.1	2.5±0.1	3.7±0.3	4.7±0.3	3.2±0.1	2.2±0.4	2.5±0.1
210	3.7±0.5	3.0±0.1	4.0±0.1	5.2±0.4	4.0±0.3	3.5±0.2	2.7±0.1
Rate ($molmin^{-1}$)	**32.6**	**35.6**	**42.5**	**72.7**	**50.2**	**47.6**	**36.2**

TABLE 4.2: DATA OF THE VARIATION OF VOLUME OF CO_2 PRODUCED WITH TIME AT DIFFERENT SUBSTRATE CONCENTRATIONS OF SOURSOP JUICE USING 1.0 %(W/V) YEAST, AT 30^OC AND pH 5.0

Substrate concentration%(v/v)							
Volume of CO_2 produced (cm^3)							
Time(min)	**20**	**30**	**40**	**50**	**60**	**70**	**80**
30	1.0±1.6	1.4±1.6	2.8±0.5	1.2±1.1	1.1±2.0	3.1±1.3	3.1±1.4
60	2.7±0.4	1.7±1.3	3.5±1.9	1.4±0.9	1.5±0.3	3.2±1.2	3.7±1.2
90	3.1±0.5	2.7±1.5	4.8±0.4	1.8±0.2	1.7±1.2	4.7±0.1	5.0±0.3
120	4.8±2.1	4.4±1.4	5.2±0.3	2.2±1.0	2.0±0.4	6.0±0.1	5.2±1.9
150	5.5±2.0	4.5±2.2	6.0±0.1	2.5±1.2	7.0±0.1	6.1±0.1	6.0±0.1
180	6.1±2.4	5.7±2.2	6.2±0.1	2.8±0.1	7.7±0.1	6.7±0.1	7.2±0.8
210	7.5±1.9	8.5±0.3	7.2±0.1	3.5±0.4	8.7±1.3	7.0±0.1	7.5±0.2
Rate($molmin^{-1}$)	**39.5**	**85.5**	**106.7**	**126.4**	**104.2**	**89.5**	**86.9**

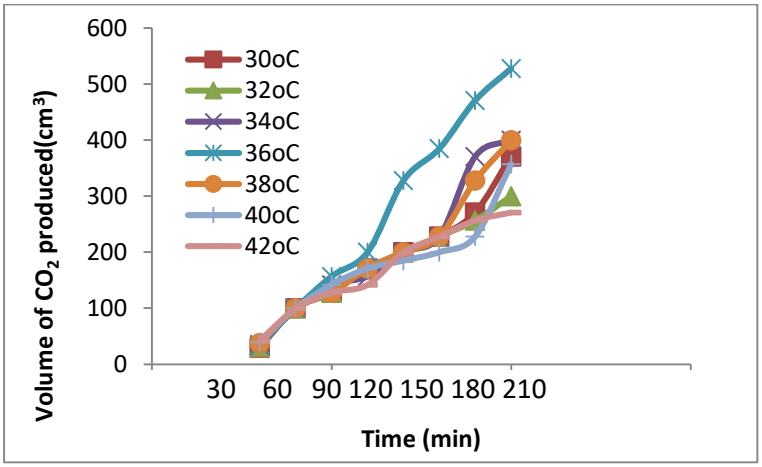

Fig.4.1: VARIATION OF VOLUME OF CO_2 PRODUCTION WITH TIME AT DIFFERENT TEMPERATURES OF FERMENTATION OF SOURSOP JUICE USING 50% (v/v) SUBSTRATE, YEAST 1.0% (w/v), AND pH 5.0

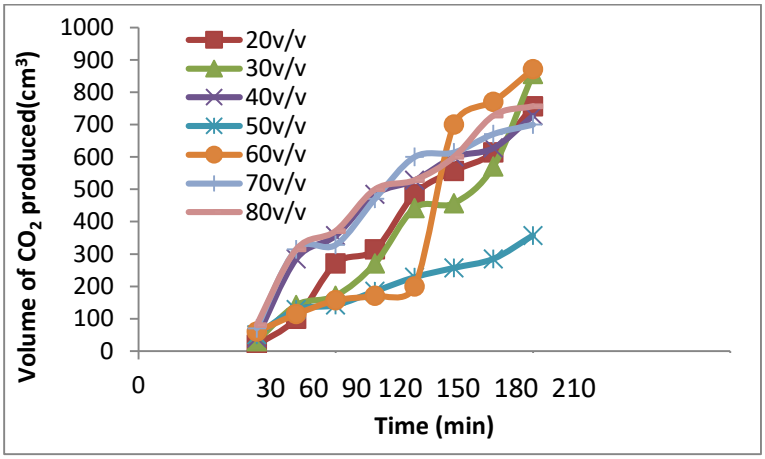

Fig.4.2: VARIATION OF VOLUME OF CO_2 PRODUCTION WITH TIME AT DIFFERENT SUBSTRATE CONCENTRATIONS OF FERMENTATION OF SOURSOP JUICE USING 1.0% (w/v) YEAST, AT 30^{O}C AND pH 5.0

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TABLE 4.3: DATA OF THE RATE OF FERMENTATION AT DIFFERENT SUBSTRATE CONCENTRATION AND TEMPERATURE OF SOURSOP JUICE

RATE OF FERMENTATION(mol l^{-1})							
Rate(molmin^{-1})	32.6	35.6	42.5	72.7	50.2	47.6	36.2
TEMPERATURE	**30**	**32**	**34**	**36**	**38**	**40**	**42**
Rate(molmin^{-1})	39.5	85.5	106.7	126.4	104.2	89.5	86.9
SUBSTRATE	**20**	**30**	**40**	**50**	**60**	**70**	**80**

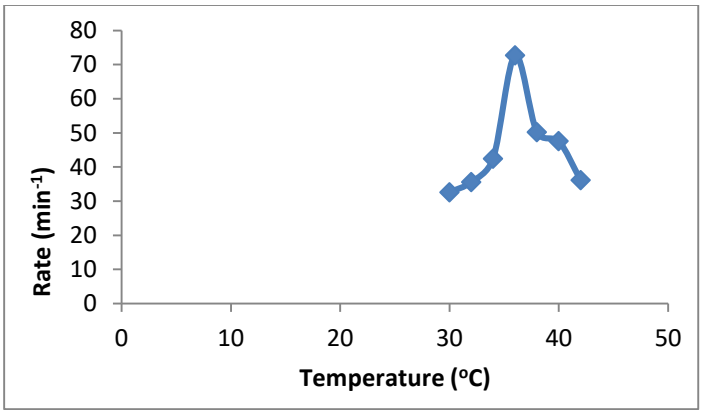

Fig.4.3: VARIATION OF RATE OF FERMENTATION OF SOURSOP JUICE WITH TEMPERATURE OF SUBSTRATE USING 50%(v/v) SUBSTRATE, YEAST 1.0 %(w/v), AND pH 5.0

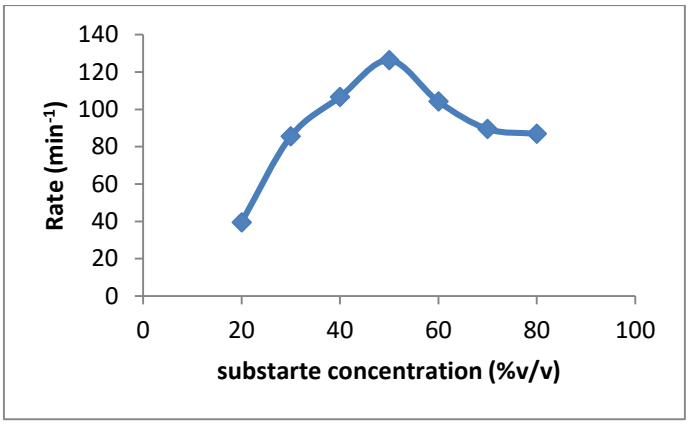

Fig.4.4: VARIATION OF RATE OF FERMENTATION OF SOURSOP JUICE WITH SUBSTRATE CONCENTRATION USING 1.0% (w/v) YEAST, AT 30^{O}C AND pH 5.0

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TABLE 4.4: EFFECT OF SUBSTRATE CONCENTRATION ON THE RATE, LOG-RATE, LOG-SUBSTRATE, RECIPROCAL-RATE AND SUBSTRATE OF FERMENTATION OF SOURSOP JUICE USING 1.0% (w/v) YEAST, AT 30^{O}C AND pH 5.0

Substrate conc.	20	30	40	50	60	70	80
Rate	39.5	85.5	106.7	126.4	104.2	89.5	86.9
Log-Rate	1.596	1.931	2.028	2.101	2.017	1.951	1.939
Log-substrate	1.301	1.477	1.602	1.698	1.778	1.845	1.903
1/Rate	0.025	0.011	0.009	0.007	0.009	0.011	0.011
1/Substrate	0.051	0.033	0.025	0.020	0.016	0.014	0.012

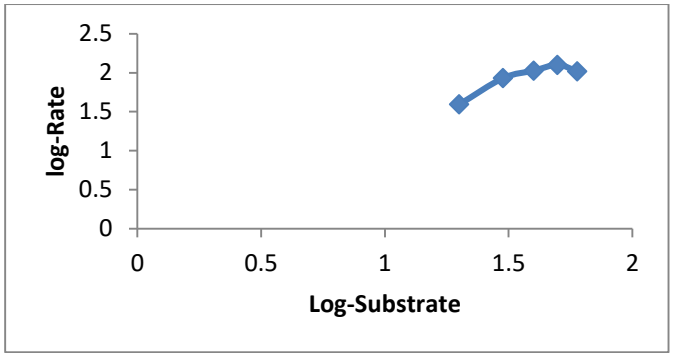

Fig.4.5: VARIATION OF LOG-RATE OF FERMENTATION OF SOURSOP JUICE WITH LOG-SUBSTRATE CONCENTRATIONS USING YEAST 1.0%(w/v), AT 30^{O}C AND pH 5.0

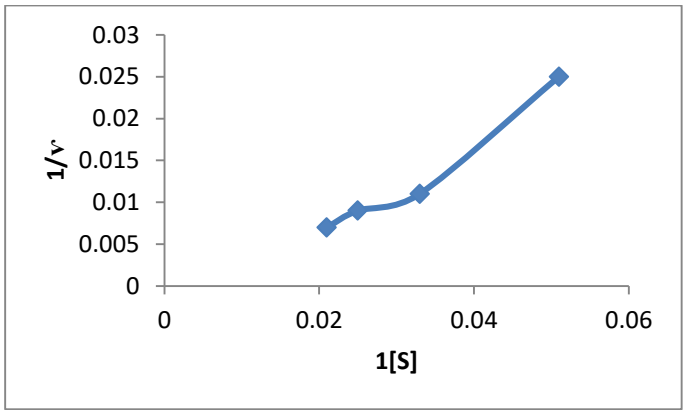

Fig.4.6: VARIATION OF RECIPROCAL-RATE OF FERMENTATION OF SOURSOP JUICE WITH RECIPROCAL-SUBSTRATE CONCENTRATIONS USING YEAST 1.0%(w/v), AT 30^{O}C AND pH 5.0

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TABLE 4.5: DATA ON THE RATE OF FERMENTATION OF SOURSOP SUGAR CATALYZED BY *SACCHAROMYCES CEREVISIAE* AT pH 5.0 AND 1.0% YEAST CONCENTRATION (W/V) AT VARIOUS SUBSTRATE CONCENTRATIONS AND TEMPERATURES.

Temperature /^{o}C							
Rate of Fermentation							
substrate concentration	**30**	**32**	**34**	**36**	**38**	**40**	**42**
20	0.538	0.535	0.551	0.666	0.656	0.708	0.626
30	0.531	0.685	0.665	0.582	0.636	0.558	0.634
40	0.589	0.660	0.702	0.667	0.558	0.641	0.696
50	0.553	0.807	0.644	0.702	0.582	0.586	0.769
60	0.556	0.659	0.702	0.611	0.666	0.575	0.727
70	0.697	0.734	0.702	0.531	0.596	0.679	0.520
80	0.867	0.483	0.636	0.556	0.556	0.566	0.558

TABLE 4.6: DATA ON THE ln-RATE OF FERMENTATION OF SOURSOP SUGAR CATALYZED BY *SACCHAROMYCES CEREVISIAE* AT pH 5.0 AND 1.0 % YEAST CONCENTRATION (W/V) AT VARIOUS ln-SUBSTRATE CONCENTRATIONS AND TEMPERATURES

Temperature /^{o}C							
In- Rate of Fermentation							
In-substrate concentration	**30**	**32**	**34**	**36**	**38**	**40**	**42**
2.995	-0.619	-0.625	-0.596	-0.401	-0.421	-0.345	-0.468
3.401	-0.632	-0.378	-0.407	-0.541	-0.452	-0.583	-0.455
3.688	-0.529	0.415	-0.353	- 0.404	-0.583	-0.444	-0.362
3.912	-0.529	-0.214	-0.441	-0.353	-0.541	-0.534	-0.262
4.094	-0.586	-0.417	-0.353	-0.492	-0.406	-0.553	-0.318
4.248	-0.360	-0.309	-0.353	-0.632	-0.517	-0.387	-0.653
4.382	-0.142	-0.727	-0.452	-0.586	-0.586	-0.569	-0.583

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TABLE 4.7: FERMENTATION OF SOURSOP - DATA FROM RECIPROCAL OF TEMPERATURE AND lnk FROM In-RATE AND ln-SUBSTRATE PLOT

T(ºC)	30	32	34	36	38	40	42
T(K)	303	305	307	309	311	313	315
1/T	0.00330	0.00328	0.00326	0.00324	0.00322	0.00320	0.00318
1/T 10^{-3}	3.30	3.28	3.26	3.24	3.22	3.20	3.18
lnk	-1.507	-0.559	-0.836	-0.051	-0.217	-0.203	-0.173

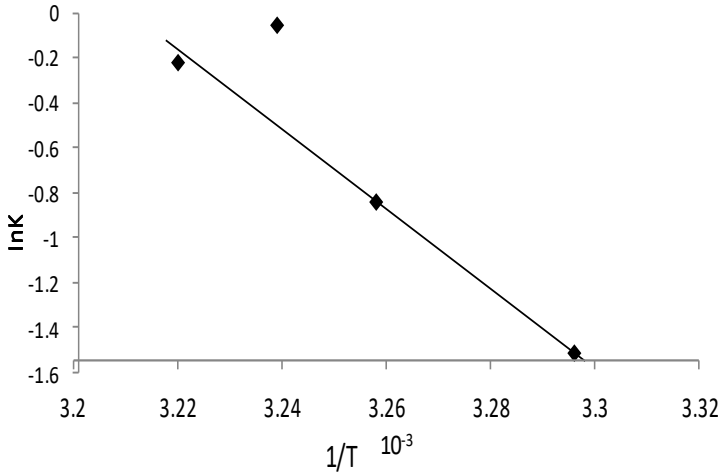

Fig.4.7: PLOT OF lnk VERSUS 1/T FOR THE *SACCHAROMYCES CEREVISIAE* CATALYZED FERMENTATION OF SOURSOP SUGAR

TABLE 4.8: THERMODYNAMIC PARAMETERS CALCULATED FOR THE *SACCHAROMYCES CEREVISIAE* CATALYZED FERMENTATION OF SOURSOP SUGAR.

Thermodynamic parameter	Values
Enthalpy ΔH^* (kJmol^{-1})	151.148
Activation Energy E_a* (kJmol^{-1})	151.148
Entropy ΔS^* (Jmol^{-1} k^{-1})	275.359
Gibbs Energy ΔG^* (kJmol^{-1})	-752.213
Equilibrium constant (K_{eq})	1.33

TABLE 4.9: CALCULATED CONSTANTS FOR THE *SACCHAROMYCES CEREVISIAE* CATALYZED FERMENTATION OF SOURSOP SUGAR

Rate constant (k)	$1.42x10^{24}$
A (min^{-1})	$1.51x10^{23}$
P	$1.41x10^{-34}$
Z (min^{-1})	$1.07x10^{11}$

TABLE 4.10: DATA FOR THE VARIATION OF VOLUME OF CO_2 PRODUCED WITH TIME AT DIFFERENT MALONIC ACID CONCENTRATION OF SUGARCANE JUICE USING 1.0(w/v) YEAST, AT 30ºC AND pH5.5

MALONIC ACID (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	2.0±0.6	1.4±0.5	1.7±0.5	2.0±0.5	1.0±0.3	2.6±0.5	1.0±0.5
60	1.8±0.5	1.0±0.5	1.2±0.5	4.0±0.5	1.8±0.5	2.7±0.5	1.0±0.4
90	2.0±0.5	1.5±0.5	1.7±0.5	1.0±0.5	1.0±0.5	4.5±0.5	1.6±0.5
120	3.0±0.5	1.3±0.5	2.4±0.5	3.0±0.5	1.3±0.5	2.1±0.5	2.5±0.5
150	2.2±0.5	1.7±0.5	1.9±0.5	2.0±0.5	1.5±0.5	2.5±1.0	1.3±0.5
180	1.0±0.5	1.4±0.5	1.7±0.5	2.0±0.5	1.3±0.5	2.7±0.5	1.3±0.5
210	2.5±0.5	1.2±0.4	1.6±0.5	3.8±0.3	1.5±0.5	3.0±0.5	1.2±0.5

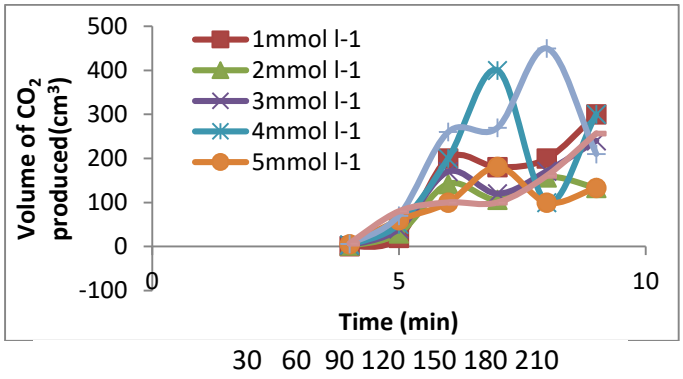

Fig.4.8: FERMENTATION OF SUGARCANE JUICE - VARIATION OF VOLUME OF CO_2 PRODUCTION WITH TIME AT DIFFERENT MALONIC ACID CONCENTRATIONS USING 1.0% (w/v) YEAST, AT 30^{O}C AND pH 5.5

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TABLE 4.11: VARIATION OF RATE OF FERMENTATION OF SUGARCANE JUICE WITH INHIBITOR CONCENTRATIONS USING YEAST 1.0%(w/v), AT 30^{O}C AND pH 5.5

CONCENTRATION OF INHIBITOR (mmol l^{-1})							
RATE OF FERMENTATION(mol l^{-1})							
INHIBITOR	**1.0**	**2.0**	**3.0**	**4.0**	**5.0**	**6.0**	**7.0**
MALONIC ACID	52.2	57.5	62.1	43.6	42.6	28.5	24.1

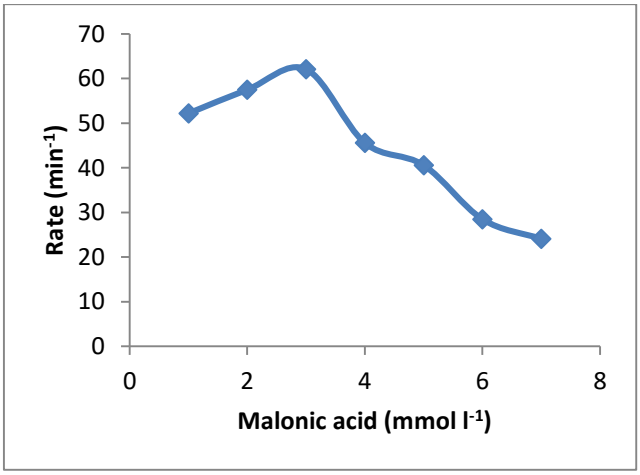

Fig.4.9: VARIATION OF RATE OF FERMENTATION OF SUGARCANE JUICE WITH MALONIC ACID CONCENTRATION USING YEAST 1.0% (w/v), AT 30^{O}C AND pH 5.5

TABLE 4.12: VARIATION OF RATE OF FERMENTATION OF SUGARCANE JUICE WITH INHIBITOR CONCENTRATIONS USING YEAST 1.0%(w/v), AT 30^{O}C AND pH 5.5

LOG -CONCENTRATION OF INHIBITOR(mmol l^{-1})							
LOG-RATE OF FERMENTATION(mol l^{-1})							
INHIBITOR	**0.0**	**0.30**	**0.47**	**0.60**	**0.69**	**0.77**	**0.84**
MALONIC ACID	1.71	1.75	1.79	1.63	1.62	1.45	1.38

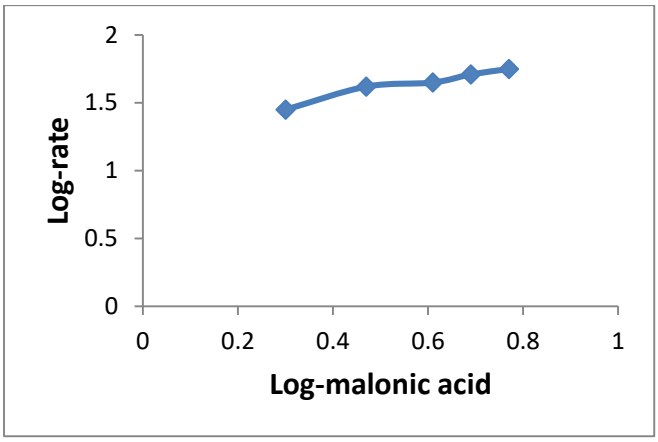

Fig.4.10: VARIATION OF LOG-RATE OF FERMENTATION OF SUGARCANE JUICE WITH LOG- MALONIC ACID CONCENTRATIONS USING YEAST 1.0%(w/v), AT 30^{O}C AND pH 5.5

TABLE 4.13: VARIATION OF RECIPROCAL- RATE OF FERMENTATION OF SUGARCANE JUICE WITH RECIPROCAL -CONCENTRATIONS OF THE VARIOUS INHIBITORS

RECIPROCAL-CONCENTRATION OF INHIBITOR(mmol l^{-1})							
	RECIPROCAL-RATE OF FERMENTATION(mol l^{-1})						
INHIBITOR	**1.00**	**0.50**	**0.33**	**0.25**	**0.20**	**0.16**	**0.14**
MALONIC ACID	0.019	0.017	0.016	0.022	0.023	0.035	0.041

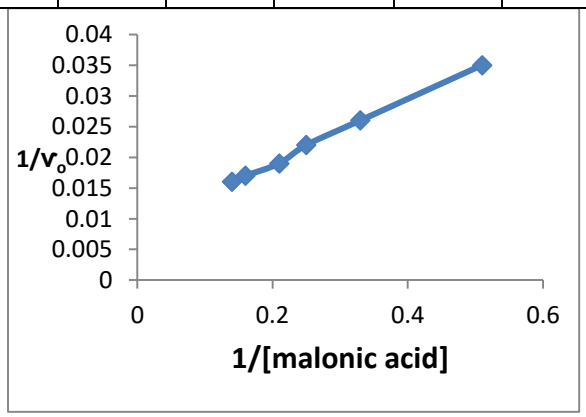

Fig.4.11: PLOT OF THE VARIATION OF RECIPROCAL-RATE OF FERMENTATION OF SUGARCANE JUICE WITH RECIPROCAL- MALONIC ACID CONCENTRATIONS USING YEAST 1.0%(w/v), AT 30^{O}C AND pH 5.5

TABLE 4.14: COMPARATIVE DATA OF RATE OF FERMENTATION WITH SUBSTRATE AND INHIBITOR CONCENTRATIONS OF SUGARCANE JUICE

RATE OF FERMENTATION(units per minute)		
Substrate conc. (mmol l^{-1})	**uninhibited**	**Inhibited Malonic acid 1-7 mmol l^{-1}**
20	102.8	52.2
30	103.9	57.5
40	135.7	62.1
50	148.1	43.6
60	138.3	42.6
70	98.2	28.5
80	42.4	24.1

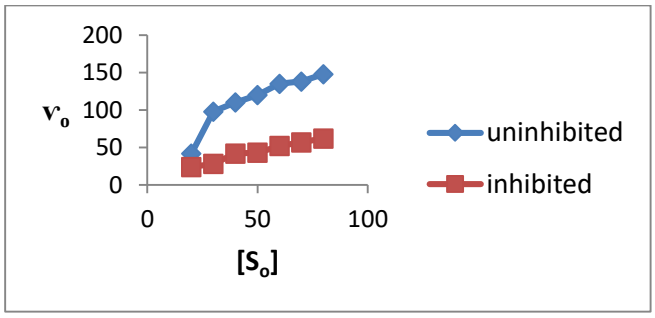

Fig.4.12: COMPARATIVE PLOT OF RATE OF FERMENTATION OF SUGARCANE JUICE FOR THE INHIBITED (MALONIC ACID) AND UNINHIBITED REACTIONS USING MICHAELIS MENTEN EQUATION

TABLE 4.15: COMPARATIVE DATA OF RECIPROCAL-RATE OF FERMENTATION WITH SUBSTRATE AND INHIBITOR CONCENTRATION OF SUGARCANE JUICE USING 1.0% (w/v) YEAST, AT 30^{O}C AND pH 5.5

RECIPROCAL-RATE OF FERMENTATION(units per minute)		
Substrate conc. (mmol l^{-1})	**uninhibited**	**Inhibited Malonic acid 1-7 mmol l^{-1}**
0.051	0.009	0.019
0.033	0.009	0.017
0.025	0.007	0.016
0.020	0.006	0.022
0.016	0.007	0.023
0.014	0.010	0.035
0.012	0.023	0.041

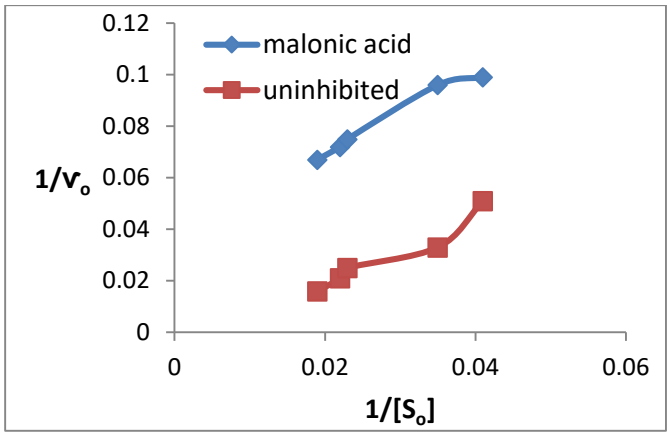

Fig.4.13: COMPARATIVE PLOT OF RECIPROCAL-RATE OF FERMENTATION OF SUGARCANE JUICE FOR THE INHIBITED (MALONIC ACID) AND UNINHIBITED REACTIONS USING LINEWAEVER-BURK EQUATION

DISCUSSION

It was observed that the rate of production of CO_2 increased up to 36^oC and decreased with time. The results show that though there was a wide range of temperature over which the yeast enzyme was active, there was also a narrow range of temperature (35-36^oC) over which its activity was at maximum. The initial increase in rate with temperature is expectedly a function of the increase in the average kinetic energy of the molecules. However, further increase in temperature beyond 36^oC triggered the breakdown of the enzymatic structure due to increased thermal vibration of the enzyme molecules. For the effect of substrate concentration on rate of fermentation, the results show that the rate of fermentation varied in proportion with substrate concentration up to 50%(v/v) suggesting that at the initial stage of the reaction, all active sites of the yeast (enzyme) were saturated. The effect of malonic acid concentration on rate of fermentation of soursop varied in proportion with substrate concentration up to 30%v/v. Further increase in the substrate concentration showed a decrease on the rate of fermentation indicating that all the active sites of the yeast are saturated by the malonic acid concentration and further increase in substrate and malonic acid concentration could not lead to increase in the rate of fermentation. The plots indicate a narrow range of malonic acid over which the enzyme was active. The thermodynamic parameters calculated are ΔH^*, E_a*, ΔS^*, ΔG^* and K. they were obtained from a plot of lnk versus 1/T on the basis of Eyring's equation. The results show that the enthalpy value is positive indicating that the fermentation process is endothermic while the calculated value for the activation energy is same as the enthalpy. The entropy value is positive corroborating the positive enthalpy value. The fermentation process is spontaneous as shown by the negative change in free energy. The equilibrium constant indicates that the conversion of substrate to products is 33% .The calculated values for k (rate constant), A (pre-exponential or frequency factor) and P (orientation parameter)

support the proposed two step mechanism of the enzyme catalyzed reaction. The enzyme-substrate complex is simply a non-isolable intermediate which though is an energized molecule is thus present in low concentration and hence, the first step which is the equilibrium step can be quantified by the Eyring and Vant Hoff's isotherms while the second or decomposition step is adequately quantified by a combination of the Arrhenius, Eyring and Van't Hoff's isotherms. The data obtained are within the range of data obtained by biochemical microcalorimetry for glucose sugar (Chang, 1990). The calculated A and the P values obtained on the basis of collision theory and absolute reaction rate theory (or transition state theory), respectively show that the collision factor is of the order of 10^{11} min^{-1}, about half that of gaseous molecules that can only be attained by an induced reduction in activation energy by a catalyst. The frequency factor A, in the Arrhenius equation is approximately equal to $K_BT/h\ e^{\Delta S^*/R}$ of the absolute reaction rate theory and the van't Hoff's equation. Therefore, the enzyme provides a favourable kinetic environment and of course does this by providing a proper-fitting orientation for the substrate molecules to undergo the change. However, in doing so, the thermodynamics is suggestively limited by other process variables such as concentration of the substrate, the purity and activity of the enzyme molecules as well as product inhibition (Riaz et al., 2007; Tanaka and Hoshino, 2003). This fact, invariably, influenced the yield of product in practice. Therefore, the enzyme provides a favourable kinetic environment for submicroscopic interactions of the enzyme-substrate complex and such interaction could be substrate interacting more with the complex or rather, the product interacting more with the intermediate complex. The equilibrium constant value K suggests that the intermediate complex is rather interacting more with the substrate and also showing that only a limited amount of the substrate is converted to product. The fraction of effective intermolecular collision increases with increase in the total number of collisions and hence the rate of reaction is proportional to the collision frequency. The P (orientation factor) value affects the interaction between substrate and enzyme. The enzyme activates the substrate molecules at particular active sites. These sites have definite size and shape or configuration. A reaction will proceed only if the size, stereochemistry and orientation of the substrate molecules fit into the active sites of the enzyme. The value shows that the orientation of molecules affects the probability factor and that simple molecule indeed have more ways of proper orientation to form complex intermediates. Hence, the probability factor is of the order applicable for simple molecules (Malinowski, 2001; Pisarenko et al., 2001). For simple sugars like glucose, the orientation factor is expected to be large and hence the P value obtained. The equilibrium constant K is observed to be $1.33 dm^3 mol^{-1}$ and not significantly far from unity implying that ΔG^* can be conveniently equated with the change in free energy at standard conditions. The equilibrium constant value support the fact that the unstable intermediate as in this case is interacting more with the substrate and in such a given situation, the yield of product should not be expected to be large. This explains partly why the yield of alcohol from most fermentable starches and sugars are low. It is of interest frequently to estimate the efficiency of a fermentation process (Chang, 1990; Leksawasdi et al., 2001) by a consideration of the free energy of combustion of glucose: $C_6H_{12}O_{6(s)} + 6O_{2(g)} \quad 6CO_{2(g)} + 6H_2O_{(l)}$ $\Delta G^* = -2879$ $kJmol^{-1.}$ On the other hand, the fermentation of sugar syrup to alcohol as summarized by the equation, $C_6H_{12}O_{6(s)} + 2H_20 \quad CO_{2(g)} + C_2H_5OH_{(l)}$, in this investigation gave $\Delta G^* = -752$

kJmol^{-1} for soursop. Therefore, the calculated fermentation efficiency is 382.84% for soursop. The high efficiency of the process is suggested to be promoted by the P value and a suitable kinetics. From the calculated thermodynamic parameters the values of enthalpy and entropy as well as that of free energy suggest that the fermentation process was spontaneous. Finally, the type of inhibition was found to be uncompetitive from the extrapolated Lineweaver-Burk plots and did not bring about marked decrease in the rate of fermentation as expected. The data obtained from this work will enable us understand the possible mechanism by which inhibitor influences fermentation processes. The understanding derived will enable us to work out sustainable procedures to achieve optimum fermentation process as regard to energy demand.

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