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PRODUCTION OF ALCOHOL FROM SOURSOP JUICE AT DIFFERENT TEMPERATURES WITH EFFECT OF POTASSIUM SULPHATE AND AMMONIUM SULPHATE

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ABSTRACT

The production of alcohol from soursop juice at different temperatures was investigated to obtain certain useful thermodynamic parameters. This was achieved by the determination of the effects of temperature, substrate, potassium sulphate and ammonium sulphate on the rate of carbon (IV) oxide (CO_2) production. The extent of acceleration was examined by the addition of some potassium sulphate and ammonium sulphate. The results indicate that the rate of fermentation of soursop juice increased in proportion with temperature (optimum 36^oC), substrate (optimum 50%v/v), potassium sulphate (optimum 50%v/v) and ammonium sulphate (optimum 30%v/v) up to a limit and decreased. Suggesting 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}, 1.4 respectively. These values were subsequently used to obtain by calculation Arrhenius constant A, 1.51x10^{23} dm^{3}mol^{-1}s^{-1}; orientation factor P, 1.41x10^{-34} and the collision frequency, Z, 1.07x10^{11} respectively. Finally, the suggested accelerators did not bring about marked increase in the rate of fermentation and yield as expected.

INTRODUCTION

There is increase in the utilization of ethanol in industries, where it is used as solvent blended for beverage and also as fuel. Yeast alcohol is the most valuable product for the biotechnology industry with respect to both value and revenue. Approximately 80% of ethanol is produced by anaerobic fermentation of various sugar sources by *Saccharomyces cerevisiae.* Yeast alcohol technology has resulted in vast improvements during the last decade but profit margins were narrowed (Stryer, 2005). Contamination, limited availability of raw materials and fermentation process design are the major limitations causing reduced alcohol yields and quality. In view of the importance of alcohol as an alternative to fossil fuel, several investigations on ethanol fermentations are currently on-going. The price of the sugar source is an important parameter when considering the overall economy of production and it is of great interests to optimize alcohol yields to ensure an efficient utilization of carbon sources (Pisarenko, 2001). Therefore, there is need to understand the production of ethanol from local food substances and fruits. There is also need to reduce the degree of inhibition effect and improve the acceleratory factors of ethanol production, especially for the substrates selected. In order to understand fermentation and how various factors influence the fermentation processes, studies in fermentation thermodynamics are conducted (Gaden, 2000). Ethanol assumes a prominent heavy industrial raw material amongst industrial chemicals currently imported. This raw material can conveniently be mass produced by utilizing and deploying optimum process variables in a fermentation process (Levine, 2005). However, the fermentation often leads to a dismal and disappointing poor output. Nevertheless, it is known that the fermentation is limited by a few substances called inhibitors which introduce a weak link in the fermentation value chain mainly in the enzyme catalysis. It is difficult to understand and control the fermentation process as it involves various components such as substrates, inhibitors, and complex microbes etc. (Winzor, 2006). The fermentation is not only complex but also in a state of dynamics. Therefore there is need to adequately understand the variables of the fermentation process in order to put the conditions in a narrow window of optimal fermentation conditions. Fermentation involves enzyme reactions (Perez,

2005). The study of how inhibitors and accelerators influence the thermodynamics of the fermentation process would suggest possible ways by which the influence of inhibitors can be reduced or eliminated completely (Palmer, 2000). The understanding of how accelerators affect the thermodynamic consideration of the fermentation can suggest ways to enhance the positive impact of accelerators thereby promoting significantly the yield of alcohol from any fermentation processes (Winzor, 2006). Thermodynamic studies are aimed at deciding which direction such a reaction will proceed in terms of spontaneity, and the extent to which the reaction can proceed (Surov, 2017). The implementation of a continuous programme for anaerobic fermentation of soursop juice consists of possible active accelerators in the fermentation system and determining the optimal conditions required for the optimum yield of alcohol in the presence of the accelerators Moreover, the programme would provide clear quantitative data to understand the various processes involved in fermentation and to suggest possible ways to improve the process, as well as to provide the necessary baseline information required for the design, control, and regulation of fermentation systems with respect to time and energy utilization**.** In recent years, the cost of primary energy sources such as petroleum, gas and coal have astronomically increased. The desire to harness other primary sources such as sugars from varying effluents which are viably recoverable and fermented to primary alcohols is regaining global interest. Primary alcohols can easily be blended with gasoline as gasohol or combusted directly to power piston engines (Peck & Reddy, 2000).

METHOD

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. Thereafter, the required quantity (20-80%) of juice was transferred into the fermentation vessels (fermenters). Effect of potassium sulphate and ammonium sulphate) on fermentation thermodynamic 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 -21minutes 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 such as temperature, pH, yeast concentration, and fermentation time were kept constant. The substrate concentration was varied between 20–80(v/v). The effect of accelerators 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 potassium sulphate and ammonium sulphate was added. The optimum substrate and temperature 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.

The Thermodynamic Parameters: ΔH, ΔS, A, ΔG, P and Z

The temperature was varied between 30-42°C, for 30°C, substrate was varied between 20-80ml with time 30-210minutes at 30minutes interval. This was repeated for 32 - 42°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 b/c 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

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 (°C)							
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°C 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**

TABLE 4.3: VOLUME OF CO_2 PRODUCED AT DIFFERENT SUBSTRATE CONCENTRATIONS ON THE RATE OF FERMENTATION OF SOURSOP JUICE USING YEAST 1.0%(w/v), TEMPERATURE 30^{O}C AND pH 5.0

TIME (MIN)							
Volume of CO_2 produced (cm^3)							
SUBSTRATE	**30**	**60**	**90**	**120**	**150**	**180**	**210**
20	1.0±0.1	1.1±0.0	1.1±0.1	1.2±0.1	1.2±0.1	1.8±0.1	2.0±0.1
30	1.0±0.0	1.2±0.1	1.2±0.1	1.4±0.2	1.5±0.2	1.8±0.1	1.9±0.1
40	1.1±0.1	1.2±0.1	1.3±0.1	1.4±0.1	1.7±0.1	2.0±0.1	2.1±0.1
50	1.0±0.1	1.1±0.1	1.3±0.2	1.4±0.1	1.5±0.1	1.8±0.1	2.0±0.2
60	1.0±0.1	1.1±0.1	1.3±0.2	1.4±0.1	1.6±0.1	1.8±0.2	2.0±0.1
70	1.0±0.1	1.1±0.1	1.4±0.2	1.6±0.2	1.8±0.1	2.0±0.1	2.2±0.1
80	1.0±0.1	1.1±0.0	1.3±0.1	1.5±0.1	2.0±0.1	2.2±0.2	2.4±0.1

TABLE 4.4: VOLUME OF CO_2 PRODUCED AT DIFFERENT SUBSTRATE CONCENTRATIONS ON THE RATE OF FERMENTATION OF SOURSOP JUICE USING YEAST 1.0%(w/v), TEMPERATURE 32^{O}C AND pH 5.0

TIME(MIN)							
Volume of CO_2 produced (cm^3)							
SUBSTRATE	**30**	**60**	**90**	**120**	**150**	**180**	**210**
20	1.0±0.1	1.1±0.1	1.3±0.1	1.4±0.1	1.6±0.1	1.7±0.1	2.0±0.1
30	1.0±0.1	1.2±0.1	1.3±0.1	1.6±0.1	1.8±0.2	2.0±0.1	2.2±0.1
40	1.0±0.1	1.2±0.1	1.4±0.1	1.5±0.1	1.7±0.1	2.0±0.2	2.2±0.1
50	1.0±0.1	1.2±0.1	1.5±0.2	1.7±0.1	2.0±0.2	2.2±0.1	2.4±0.1
60	1.0±0.1	1.2±0.1	1.4±0.1	1.5±0.1	1.8±0.1	2.0±0.1	2.2±0.1
70	1.0±0.1	1.2±0.1	1.4±0.1	1.5±0.2	1.8±0.1	2.1±0.1	2.4±0.2
80	1.0±0.0	1.1±0.1	1.2±0.1	1.4±0.1	1.5±0.1	1.7±0.1	1.8±0.2

TABLE 4.5: VOLUME OF CO_2 PRODUCED AT DIFFERENT SUBSTRATE CONCENTRATIONS ON THE RATE OF FERMENTATION OF SOURSOP JUICE USING YEAST 1.0%(w/v), TEMPERATURE 34^{O}C AND pH 5.0

TIME (MIN)							
Volume of CO_2 produced (cm^3)							
SUBSTRATE	**30**	**60**	**90**	**120**	**150**	**180**	**210**
20	1.0±0.0	1.2±0.1	1.4±0.1	1.5±0.2	1.7±0.1	1.8±0.2	2.0±0.1
30	1.0±0.0	1.1±0.1	1.3±0.1	1.4±0.1	1.7±0.2	1.8±0.1	2.2±0.1
40	1.0±0.1	1.2±0.1	1.4±0.2	1.6±0.2	1.8±0.1	2.0±0.1	2.3±0.2
50	1.0±0.1	1.2±0.2	1.4±0.1	1.5±0.1	1.7±0.1	2.0±0.2	2.1±0.2
60	1.0±0.1	1.2±0.1	1.4±0.2	1.5±0.1	1.8±0.2	2.0±0.1	2.3±0.2
70	1.0±0.1	1.1±0.1	1.3±0.2	1.5±0.1	1.8±0.2	2.0±0.1	2.2±0.1
80	1.0±0.1	1.2±0.1	1.3±0.1	1.5±0.1	1.8±0.1	2.0±0.1	2.1±0.1

TABLE 4.5: VOLUME OF CO_2 PRODUCED AT DIFFERENT SUBSTRATE CONCENTRATIONS ON THE RATE OF FERMENTATION OF SOURSOP JUICE USING YEAST 1.0%(w/v), TEMPERATURE 36^{O}C AND pH 5.0

TIME(MIN)							
Volume of CO_2 produced (cm^3)							
SUBSTRATE	**30**	**60**	**90**	**120**	**150**	**180**	**210**
20	1.0±0.1	1.2±0.1	1.4±0.1	1.5±0.1	1.7±0.1	1.8±0.1	2.0±0.2
30	1.0±0.1	1.1±0.1	1.3±0.1	1.4±0.1	1.7±0.1	1.8±0.2	2.2±0.1
40	1.0±0.1	1.2±0.2	1.4±0.2	1.6±0.2	1.8±0.2	2.0±0.1	2.3±0.1
50	1.0±0.1	1.2±0.2	1.4±0.1	1.5±0.1	1.7±0.1	2.0±0.1	2.1±0.1
60	1.0±0.0	1.2±0.1	1.4±0.1	1.5±0.1	1.8±0.1	2.0±0.1	2.3±0.1
70	1.0±0.1	1.1±01	1.3±0.1	1.5±0.2	1.8±0.2	2.0±0.1	2.2±0.1
80	1.0±0.1	1.2±0.1	1.3±0.1	1.5±0.1	1.8±0.1	2.0±0.1	2.1±0.1

TABLE 4.6: VOLUME OF CO_2 PRODUCED AT DIFFERENT SUBSTRATE CONCENTRATIONS ON THE RATE OF FERMENTATION OF SOURSOP JUICE USING YEAST 1.0%(w/v), TEMPERATURE 38^{O}C AND pH 5.0

TIME(MIN)							
Volume of CO_2 produced (cm^3)							
SUBSTRATE	**30**	**60**	**90**	**120**	**150**	**180**	**210**
20	1.0±0.1	1.2±0.1	14±0.1	1.6±0.1	1.8±0.1	2.0±0.1	2.2±0.1
30	1.0±0.1	1.1±0.1	1.4±0.2	1.5±0.1	1.7±0.1	1.9±0.1	2.0±0.2
40	1.0±0.1	1.2±0.2	1.4±0.1	1.6±0.1	1.8±0.2	2.0±0.1	2.2±0.1
50	1.0±0.1	1.2±0.1	1.4±0.1	1.7±0.0	1.8±0.2	2.0±0.1	2.3±0.1
60	1.0±0.1	1.2±0.1	1.4±0.1	1.5±0.1	1.8±0.2	2.0±0.1	2.1±0.1
70	1.0±0.1	1.1±0.1	1.3±0.1	1.4±0.1	1.5±0.1	1.7±0.1	2.0±0.1
80	1.0±0.1	1.2±0.1	1.4±0.1	1.5±0.1	1.7±0.1	1.9±0.1	2.0±0.1

TABLE 4.7: VOLUME OF CO_2 PRODUCED AT DIFFERENT SUBSTRATE CONCENTRATIONS ON THE RATE OF FERMENTATION OF SOURSOP JUICE USING YEAST 1.0%(w/v), TEMPERATURE 40^{O}C AND pH 5.0

TIME(MIN)							
Volume of CO_2 produced (cm^3)							
SUBSTRATE	**30**	**60**	**90**	**120**	**150**	**180**	**210**
20	1.1±0.1	1.3±0.1	1.5±0.1	1.7±0.1	1.9±0.1	2.1±0.1	2.4±0.1
30	1.0±0.1	1.2±0.1	1.4±0.1	1.5±0.1	1.7±0.2	1.9±0.1	2.0±0.1
40	1.0±0.1	1.2±0.1	1.4±0.1	1.5±0.1	1.7±0.1	2.0±0.1	2.2±0.1
50	1.0±0.1	1.2±0.1	1.4±0.1	1.5±0.1	1.7±0.1	1.8±0.1	2.1±0.1
60	1.0±0.1	1.1±0.1	1.4±0.1	1.5±0.1	1.8±0.2	1.8±0.2	2.0±0.1
70	1.0±0.1	1.2±0.2	1.4±0.2	1.6±0.1	1.7±0.1	2.0±0.1	2.2±0.1
80	1.0±0.1	1.2±0.1	1.4±0.1	1.5±0.1	1.7±0.1	1.9±0.1	2.0±0.1

TABLE 4.8: VOLUME OF CO_2 PRODUCED AT DIFFERENT SUBSTRATE CONCENTRATIONS ON THE RATE OF FERMENTATION OF SOURSOP JUICE USING YEAST 1.0%(w/v), TEMPERATURE 42^{O}C AND pH 5.0

TIME(MIN)							
Volume of CO_2 produced (cm^3)							
SUBSTRATE	**30**	**60**	**90**	**120**	**150**	**180**	**210**
20	1.0±0.1	1.2±0.1	1.4±0.2	1.7±0.1	1.8±0.1	2.0±0.4	2.1±0.1
30	1.0±0.1	1.2±0.1	1.4±0.1	1.5±0.3	1.7±0.4	1.8±0.2	2.2±0.2
40	1.0±0.1	1.2±0.1	1.4±0.2	1.7±0.2	1.8±0.5	2.0±0.1	2.3±0.2
50	1.1±0.1	1.3±0.2	1.4±0.1	1.7±0.1	1.9±0.1	2.2±0.2	2.5±0.3
60	1.0±0.1	1.3±0.2	1.4±0.2	1.7±0.2	1.9±0.6	2.0±0.3	2.4±0.2
70	1.1±0.1	1.2±0.1	1.4±0.1	1.5±0.0	1.7±0.2	1.8±0.2	2.0±0.2
80	1.0±0.1	1.1±0.1	1.4±0.2	1.5±0.1	1.7±0.3	1.8±0.1	2.0±0.1

TABLE 4.9: 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 4.10: 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 4.11: DATA OF THE VARIATION OF VOLUME OF CO_2 PRODUCED WITH TIME AT DIFFERENT POTASSIUM SULPHATE CONCENTRATION OF SOURSOP JUICE USING 1.0 (w/v) YEAST, AT 30°C AND pH 5.0

POTASSIUM SULPHATE (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	4.0±0.1	5.6±0.4	5.4±0.1	5.2±0.2	4.1±0.1	4.3±0.1	3.2±0.2
60	3.4±0.3	4.5±0.4	3.4±0.7	3.2±0.2	3.5±0.3	3.4±0.3	4.1±0.2
90	2.3±0.6	4.5±0.1	2.3±0.8	2.1±0.3	3.2±0.1	3.2±0.5	4.2±0.3
120	2.1±0.1	3.7±0.5	2.8±0.6	2.1±0.1	3.2±0.5	3.7±0.3	5.6±0.1
150	2.8±0.	4.0±0.1	3.2±0.4	3.1±0.3	3.8±0.4	4.2±0.4	4.2±0.9
180	3.8±0.4	4.5±0.1	3.4±0.1	3.1±0.2	4.5±0.3	4.5±0.1	3.2±0.3
210	4.8±0.1	5.6±0.8	4.5±0.1	4.1±0.5	5.6±0.1	5.6±0.5	3.2±0.2

TABLE 4.12: DATA OF THE VARIATION OF VOLUME OF CO_2 PRODUCED WITH TIME AT DIFFERENT AMMONIUM SULPHATE CONCENTRATION OF SOURSOP JUICE USING 1.0(w/v) YEAST, AT 30°C AND pH 5.0

AMMONIUM SULPHATE (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	3.4±0.1	8.7±0.6	6.7±0.2	6.5±0.1	7.5±0.2	8.0±0.1	8.5±0.1
60	2.3±0.7	6.5±0.1	5.6±0.1	5.4±0.2	6.5±0.1	7.8±0.1	7.0±0.2
90	2.1±0.1	5.4±0.2	4.5±0.1	4.3±0.2	5.5±0.3	5.6±0.2	6.4±0.1
120	2.4±0.2	5.6±0.9	5.3±0.8	8.7±0.8	7.7±0.1	8.7±0.2	7.4±0.9
150	4.4±0.1	7.6±0.2	6.7±0.1	6.7±0.1	8.8±0.5	9.8±0.2	8.2±0.1
180	1.5±0.9	4.5±0.1	4.0±0.2	4.5±0.1	7.1±0.1	7.0±0.1	7.1±0.1
210	2.1±0.1	5.4±0.2	4.5±0.2	6.8±0.1	7.5±0.1	7.8±0.1	7.7±0.2

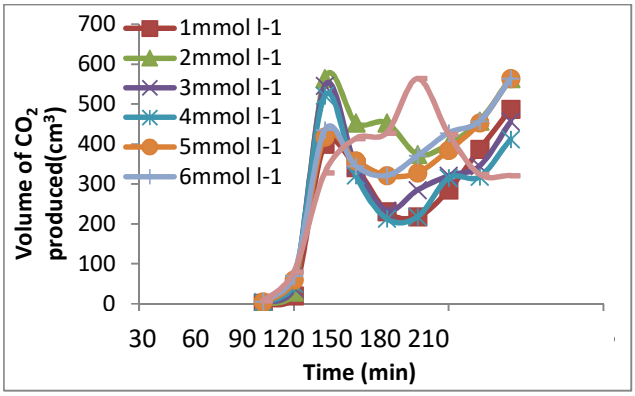

Fig.4.1: FERMENTATION OF SOURSOP JUICE - VARIATION OF VOLUME OF CO_2 PRODUCTION WITH TIME AT DIFFERENT POTASSIUM SULPHATE CONCENTRATIONS USING 1.0% (w/v) YEAST, AT 30°C AND pH 5.0

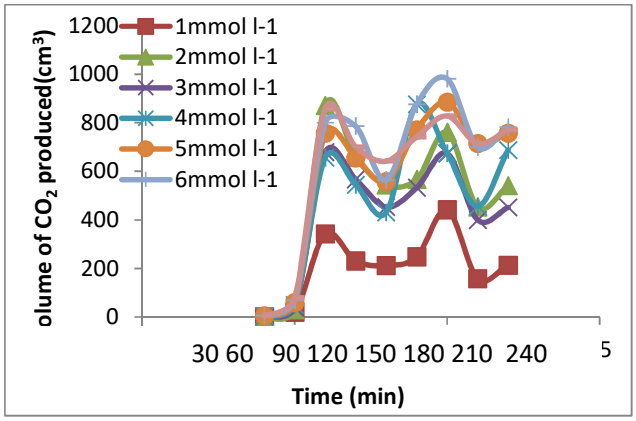

Fig.4.2: FERMENTATION OF SOURSOP JUICE - VARIATION OF VOLUME OF CO_2 PRODUCTION WITH TIME AT DIFFERENT AMMONIUM SULPHATE CONCENTRATIONS USING 1.0% (w/v) YEAST, AT 30^{O}C AND pH 5.0

TABLE: 4.13: VARIATION OF RATE OF FERMENTATION OF SOURSOP JUICE WITH ACCELERATOR CONCENTRATIONS USING, YEAST 1.0%(w/v), AT 30^{O}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**
POTASSIUM SULPHATE	44.8	52.0	55.3	56.7	38.8	36.9	32.0
AMMONIUM SULPHATE	71.9	90.7	89.0	82.3	51.9	47.1	24.7

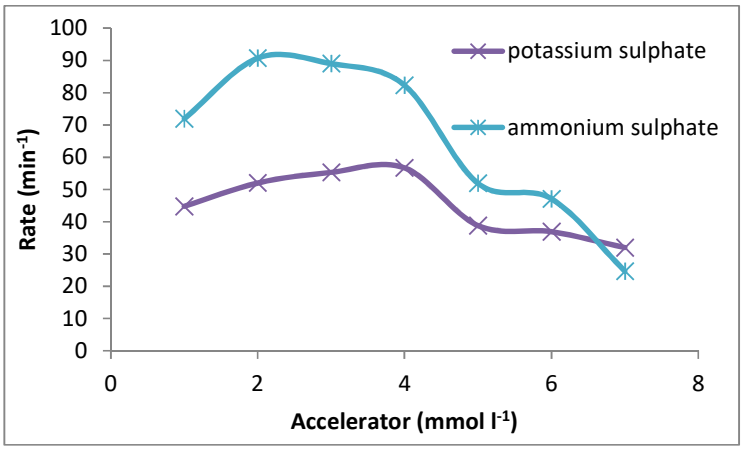

Fig.4.3: COMPARATIVE PLOT OF RATE OF FERMENTATION OF SOURSOP JUICE FOR THE INHIBITORS (AMMONIUM CARBONATE, AMMONIUM PHOSPHATE, AMMONIUM NITRATE, POTASSIUM SULPHATE AND AMMONIUM SULPHATE) USING LINEWAEVER-BURK

TABLE: 4.14: VARIATION OF LOG-RATE OF FERMENTATION OF SOURSOP JUICE WITH LOG-ACCELERATOR CONCENTRATIONS USING YEAST 1.0%(w/v), AT 30^{O}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**
POTASSIUM SULPHATE	1.65	1.71	1.74	1.75	1.58	1.56	1.50
AMMONIUM SULPHATE	1.85	1.95	1.94	1.91	1.71	1.67	1.39

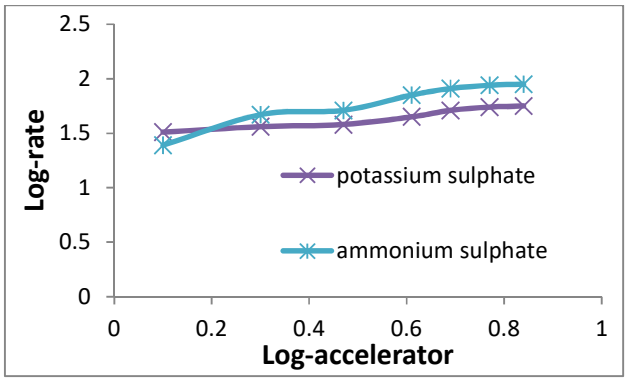

Fig.4.4: COMPARATIVE PLOT OF LOG-RATE OF FERMENTATION OF SOURSOP JUICE FOR THE INHIBITORS (POTASSIUM SULPHATE AND AMMONIUM SULPHATE) USING LINEWAEVER-BURK

TABLE: 4.14: 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**
POTASSIUM SULPHATE	0.022	0.019	0.018	0.017	0.025	0.027	0.031
AMMONIUM SULPHATE	0.013	0.011	0.011	0.012	0.019	0.021	0.040

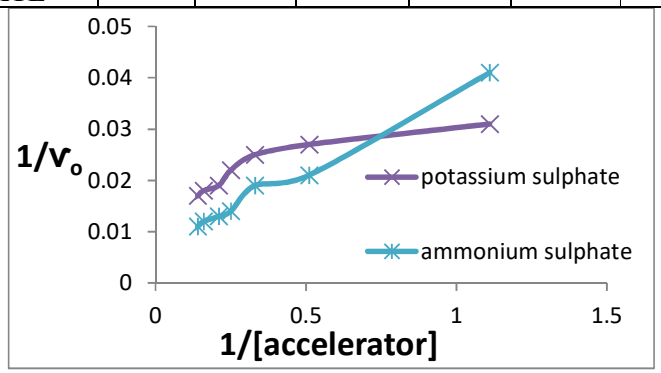

Fig.4.5: COMPARATIVE PLOT OF RATE OF FERMENTATION OF SOURSOP JUICE FOR THE INHIBITORS (POTASSIUM SULPHATE AND AMMONIUM SULPHATE) USING LINEWAEVER-BURK

TABLE: 4.15: 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 Potassium sulphate 1-7 mmol l^{-1}**	**Accelerated Ammonium sulphate 1-7 mmol l^{-1}**
20	102.8	44.8	71.9
30	103.9	52.0	90.7
40	135.7	55.3	89.0
50	148.1	56.7	82.3
60	138.3	38.8	51.9
70	98.2	36.9	47.1
80	42.4	32.0	24.7

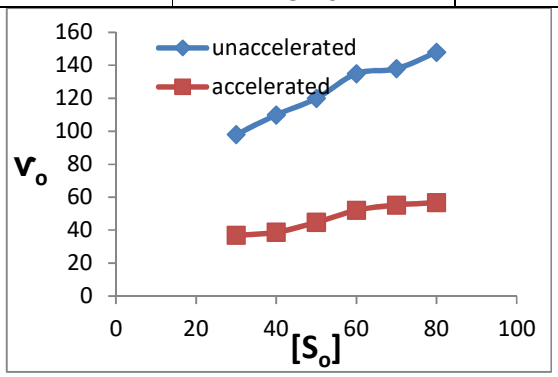

Fig.4.6: COMPARATIVE PLOT OF RATE OF FERMENTATION OF SOURSOP JUICE WITH RECIPROCAL POTASSIUM SULPHATE CONCENTRATION AND UNACCELERATED REACTIONS USING MICHAELIS-MENTEN EQUATION

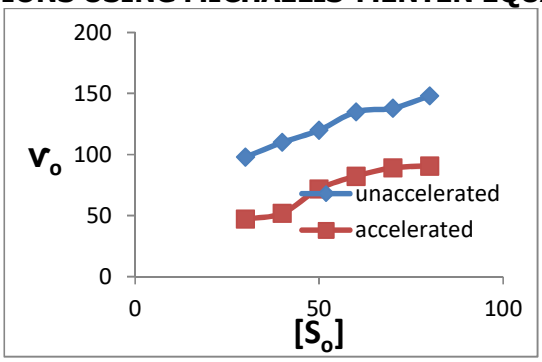

Fig.4.7: COMPARATIVE PLOT OF RATE OF FERMENTATION OF SOURSOP JUICE WITH RECIPROCAL AMMONIUM SULPHATE CONCENTRATION AND UNACCELERATED REACTIONS USING MICHAELIS-MENTEN EQUATION

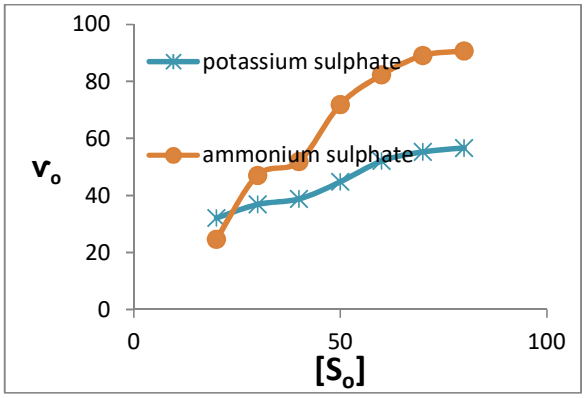

Fig.4.8: COMPARATIVE PLOT OF RATE OF FERMENTATION OF SOURSOP JUICE FOR THE INHIBITORS (POTASSIUM SULPHATE AND AMMONIUM SULPHATE) USING MICHAELIS-MENTEN

TABLE: 4.16: 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 Potassium sulphate 1-7 mmol l^{-1}**	**Accelerated Ammonium sulphate 1-7 mmol l^{-1}**
0.051	0.009	0.022	0.013
0.033	0.009	0.019	0.011
0.025	0.007	0.018	0.011
0.020	0.006	0.017	0.012
0.016	0.007	0.025	0.019
0.014	0.010	0.027	0.021
0.012	0.023	0.031	0.040

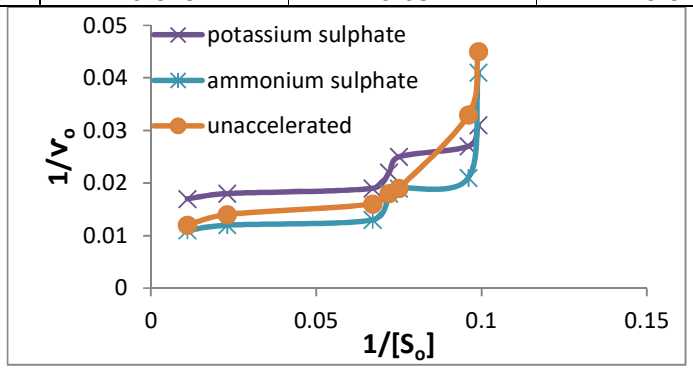

Fig.4.9: COMPARATIVE PLOT OF RATE OF FERMENTATION OF SOURSOP JUICE FOR THE INHIBITORS (POTASSIUM SULPHATE AND AMMONIUM SULPHATE) USING LINEWAEVER-BURK

TABLE 4.17: FERMENTATION OF SOURSOP - DATA FROM RECIPROCAL OF TEMPERATURE AND lnk FROM ln-RATE AND ln-SUBSTRATE PLOT

T(°C)	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
Ink	-1.507	-0.559	-0.836	-0.051	-0.217	-0.203	-0.173

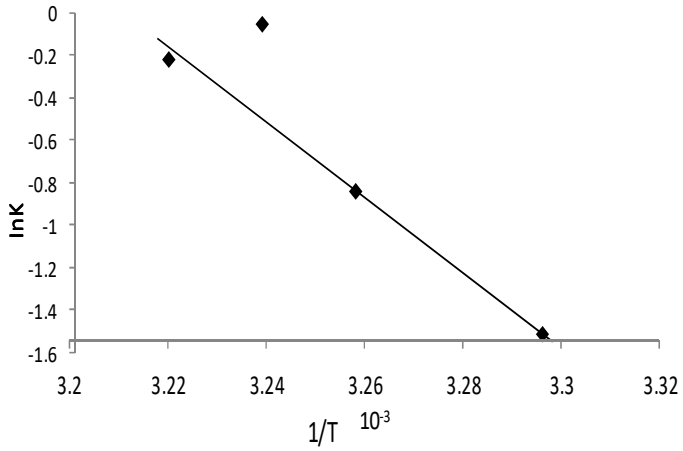

Fig.4.10: PLOT OF lnk VERSUS 1/T FOR THE *SACCHAROMYCES CEREVISIAE* CATALYZED FERMENTATION OF SOURSOP SUGAR

TABLE 4.18: THERMODYNAMIC PARAMETERS CALCULATED FOR THE *SACCHAROMYCES CEREVISIAE* CATALYZED FERMENTATION OF SOURSOP SUGAR.

Thermodynamic parameter	Values
Enthalpy ΔH^* (kJmol^{-1})	161.218
Activation Energy E_a* (kJmol^{-1})	161.218
Entropy ΔS^* (Jmol^{-1} k^{-1})	375.333
Gibbs Energy ΔG^* (kJmol^{-1})	-852.312
Equilibrium constant (K_{eq})	1.40

TABLE 4.19: 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})	$1.07x10^{11}$

The calculated thermodynamic parameters ΔH^*, E_a*, ΔS^*, ΔG^* and K, 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). The above results support the proposed two step mechanism of the enzyme catalyzed reaction. The value of the equilibrium constant indicates that though the enzyme catalyzed reaction is favoured kinetically, it is not particularly so thermodynamically. 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 shown are within the range of data obtained by biochemical microcalorimetry for glucose sugar (Chang, 2005). 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). This fact, invariably, influenced the yield of product in practice. 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 of P 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 1.4 (dm^3mol^{-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.

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