Scientia Africana, Vol. 23 (No. 5), December, 2024. Pp 201-212
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PROCESS OPTIMIZATION OF TEMPERATURE AND SUBSTRATE CONCENTRATION ON FERMENTATION OF FRUIT JUICES FOR BREWERY AND BIO-ENERGY APPLICATIONS

Ekwe, C. O.[1], *Akpoveta, O. V.[2], Etoh, P.[3], Apuyor, K.[4], Otovbo, E. J.[5] and Obielumani, J. O.[6]
[1]Department of Chemical Sciences, Glorious Vision University, P.M.B 001 Ogwa, Edo State, Nigeria.
[*2,3,4,5]Department of Chemical Sciences, Dennis Osadebay University, Asaba, Delta State, Nigeria.
[6]Department of Chemistry Education, Federal College of Education, Technical, Asaba, Delta State, Nigeria
Corresponding Author Email- akpovin2@gmail.com; *akpoveta.oshevwiyo@dou.edu.ng*
Phone Contact-+2348141812077

Received: 24-11-2024
Accepted: 15-12-2024

https://dx.doi.org/10.4314/sa.v23i5.17
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Journal Homepage: http://www.scientia-african.uniportjournal.info
Publisher: *Faculty of Science, University of Port Harcourt.*

ABSTRACT

Optimization of fermentation processes on fruit juices as ethanol precursors is an important aspect of fermentation science for high-yield production of ethanol. The fermentation of fruit juices using soursop and sugarcane juices as substrates by *Saccharomyces cerevisiae* (yeast) was investigated to ascertain the effect of substrate concentration and temperature on the production of CO_2. Temperature and substrate concentrations were varied between 30-42^oC and 20-80% (v/v) respectively, during the fermentation process. The results show that though there was a range of temperature over which the yeast (*Saccharomyces cerevisiae*) was active, there was a narrow range of temperature (34-36^oC) over which its activity was at maximum. The initial increase in volume with temperature is expected to be a function of the increase in the average kinetic energy of the molecules. However, a further increase in temperature beyond 36^oC triggered the breakdown of the enzymatic structure due to increased thermal vibration of the enzyme molecules; decreasing further production of carbon dioxide. The increase in substrate concentration beyond 50% (v/v) with an attendant decline in the volume of CO_2 produced, suggests complete saturation of enzyme active sites at the initial stage of the reaction, such that further increase in substrate concentration could not lead to increased CO_2 production. However, there is a wide range of substrate concentrations over which the enzyme is active. The findings will therefore complement the existing database in the field of fermentation science and help in enzyme fermentation processes for breweries, bio-energy, and other applications.

Keywords: Substrate concentration, Temperature, Soursop Juice, Sugarcane Juice, Carbon (iv) oxide, Fermentation

INTRODUCTION

The production of ethanol from plant sources requires proper optimization of fermentation variables for its optimal yield. This is necessary because ethanol production through fermentation routes finds significant applications in brewery science, bio-energy

demands, and other industrial applications (Krajang *et al*., 2021; Tse *et al*., 2021). Temperature is a critical factor influencing the efficiency and kinetics of bio-ethanol production processes. The impact of temperature on bio-ethanol production can be observed at various stages, including enzymatic hydrolysis, microbial fermentation, and downstream processing (Krajang *et al*., 2021; Tenkolu *et al.*, 2022). Understanding the effects of temperature is essential for optimizing bio-ethanol production processes and maximizing bio-ethanol yield. (Esele and Byaruhanga, 2024).

Temperature plays a crucial role in enzymatic hydrolysis, where cellulolytic enzymes break down complex carbohydrates into fermentable sugars. Studies have shown that temperature significantly affects enzyme activity and substrate accessibility, with optimal temperatures typically ranging from 45°C to 50°C for most celluloses (Ogbebor *et al.*, 2016). Higher temperatures can enhance enzyme activity and substrate solubility, leading to faster hydrolysis rates and higher sugar yields. However, excessive temperatures may denature enzymes and decrease the efficiency of hydrolysis, highlighting the importance of temperature control in enzymatic saccharification processes.

Temperature also exerts a profound influence on microbial fermentation kinetics and product formation. Most fermentative microorganisms exhibit temperature-dependent growth and metabolic activity, with optimal fermentation temperatures varying among different strains. As an example, *Saccharomyces cerevisiae*, commonly used in bio-ethanol production, typically performs optimally at temperatures between 30°C and 35°C (Ogbebor *et al.*, 2016). Deviations from the optimal temperature range can alter microbial growth rates, substrate utilization patterns, and fermentation by-product profiles, affecting ethanol yield and productivity. Furthermore, temperature fluctuations during fermentation can induce cellular stress responses and compromise yeast viability, leading to reduced fermentation performance (Egharevba, *et al.*, 2014)

Downstream processing operations involving multistage unit operations carried out during bio-ethanol production are also influenced by temperature, such as distillation in ethanol recovery. The boiling point of ethanol-water mixtures decreases with increasing ethanol concentration, necessitating adjustments in distillation parameters to achieve optimal separation efficiency (Samwel, 2024). High temperatures during distillation can accelerate ethanol evaporation and reduce energy requirements, but may also promote undesirable side reactions and ethanol losses. Conversely, lower temperatures may result in prolonged distillation times and increased energy consumption, highlighting the need for careful temperature control and process optimization in downstream processing operations (Krajang *et al*., 2021; Tenkolu *et al.*, 2022; Pattanakittivorakul *et al.*, 2024). Temperature plays a critical role in bio-ethanol production processes, influencing enzymatic hydrolysis, microbial fermentation, and downstream processing operations. Optimal temperature conditions vary depending on the specific enzymes, microorganisms, and process parameters involved, necessitating careful temperature control and optimization to maximize ethanol yield and process efficiency.

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 to produce a specific

Scientia Africana, Vol. 23 (No. 5), December, 2024. Pp 201-212
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chemical product (Palmer, 2020). It is also the slow decomposition of organic substances by enzymes of plant and animal origin (Copeland, 2000). 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. Fermentation could occur under anaerobic or aerobic conditions and yields lactate, acetic acid, ethanol, carbon dioxide, or some other simple products (Voidarou, *et al*., 2020). *Saccharomyces cerevisiae* is a microorganism with a general application in food manufacturing. It is very important in many food fermentations such as bread, wine, beer, and table olives, but it also causes spoilage by re-fermentation after packaging regarding table olives, bottling of wines, beverages, and fruit concentrates (Laurence, 2021). *Saccharomyces cerevisiae* is a facultative anaerobe that employs an anaerobic fermentation approach using respiratory substrates which can also respire in small sugar concentrations (Palmer, 2020). *Saccharomyces cerevisiae* is an efficient hexose transporter possessing between 19-20 encoding genes for this purpose, with the transporters differing in abundance and hexose affinity (Perez, 2020). Simple diffusion process may be responsible for substrate uptake into yeast cells for high sugar concentrations, as the glucose transport mechanism in *Saccharomyces cerevisiae*, which is typical in industrial fermentation. There is however no known singular approach in which the yeast transports glucose (Lagunas, 1993; Perez, 2020)**.**

Apart from temperature, substrate concentration is also important in determining optimized enzymatic fermentation processes, as knowledge of the right amount of substrate necessary for enzymatic breakdown is important for obtaining optimum ethanol yield. This will maximize fermentation efficiency and minimize substrate loss. The study is therefore aimed at evaluating optimization parameters such as temperature and substrate concentration for fermentation of fruit juices using soursop and sugar cane precursors for bio-ethanol production that could find applications in breweries and bio-energy demands.

MATERIALS AND METHOD

Samples and materials collection

Soursop and sugar cane fruits which were used as precursors for bio-ethanol production were purchased from a major market in Benin City, Edo State, South-South Nigeria. The fruits were then washed to remove dirt particles and kept for further processing. The yeast (*Saccharomyces cerevisiae*) was supplied by Vahine professional, McCormick, France SAS, and was used as received.

Juice Extraction

The Soursop fruits were peeled with a sterile knife to remove the skin and then deseeded, while the sugarcane stems were chopped into bits and pounded in a mortar after their peels were removed. The fibers were manually removed by squeezing out the juice from the fruits with a muslin cloth by hand after crushing, and preserved in a refrigerator.

Experimental Procedure

The required quantities (20-80% v/v) of juice were transferred into the fermentation vessels (fermenters), each containing 1 g of yeast. Seven fermenters (75 cl polyethylene terephthalate bottles) containing substrate were connected with tubes to evolve the produced carbon (IV) oxide. Reaction times ranging from 30-210 minutes at 30 minutes intervals were set for each of the fermenters. The CO_2 produced in each sealed fermenter was collected in water and measured by titration, with 0.I M NaOH, using phenolphthalein indicator. The rate of

fermentation was measured as the volume of CO_2 produced with respect to time at 30 minutes intervals. 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^oC, using a thermostated water bath. The optimal temperature required for the fermentation was determined from the CO_2 production-time profile. Substrate concentration was also optimized by determining the volume of CO_2 produced at varying substrate concentrations at the same varied temperatures and time.

RESULTS AND DISCUSSION

The results of the effect of temperature and substrate concentration on the fermentation of soursop and sugarcane juices using *saccharomyces cerevisiae* are presented below.

Effect of temperature on fermentation rate for soursop juice

Table 1: Volume of CO_2 produced at different substrate concentrations for the fermentation of soursop juice at 30^oC and 32^oC respectively

TIME (Min)								TIME (Min)							
Volume of CO_2 produced (cm^3) at 30^OC								Volume of CO_2 produced (cm^3) at 32^OC							
Substrate Conc. % (v/v)	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**Substrate Conc. % (v/v)**	**30**	**60**	**90**	**120**	**150**	**180**	**210**
20	1.0	1.1	1.1	1.2	1.2	1.8	2.0	**20**	1.0	1.1	1.3	1.4	1.6	1.7	2.0
30	1.0	1.2	1.2	1.4	1.5	1.8	1.9	**30**	1.0	1.2	1.3	1.6	1.8	2.0	2.2
40	1.1	1.2	1.3	1.4	1.7	2.0	2.1	**40**	1.0	1.2	1.4	1.5	1.7	2.0	2.2
50	1.0	1.1	1.3	1.4	1.5	1.8	2.0	**50**	1.0	1.2	1.5	1.7	2.0	2.2	2.4
60	1.0	1.1	1.3	1.4	1.6	1.8	2.0	**60**	1.0	1.2	1.4	1.5	1.8	2.0	2.2
70	1.0	1.1	1.4	1.6	1.8	2.0	2.2	**70**	1.0	1.2	1.4	1.5	1.8	2.1	2.4
80	1.0	1.1	1.3	1.5	2.0	2.2	2.4	**80**	1.0	1.1	1.2	1.4	1.5	1.7	1.8

Table 2: Volume of CO_2 produced at different substrate concentrations for the fermentation of soursop juice at 34^oC and 36^oC respectively

TIME (Min)								TIME (Min)							
Volume of CO_2 produced (cm^3) at 34^OC								Volume of CO_2 produced (cm^3) at 36^OC							
Substrate Conc. % (v/v)	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**Substrate Conc. % (v/v)**	**30**	**60**	**90**	**120**	**150**	**180**	**210**
20	1.0	1.2	1.4	1.5	1.7	1.8	2.0	**20**	1.0	1.2	1.4	1.5	1.7	1.8	2.0
30	1.0	1.1	1.3	1.4	1.7	1.8	2.2	**30**	1.0	1.1	1.3	1.4	1.7	1.8	2.2
40	1.0	1.2	1.4	1.6	1.8	2.0	2.3	**40**	1.0	1.2	1.4	1.6	1.8	2.0	2.3
50	1.0	1.2	1.4	1.5	1.7	2.0	2.1	**50**	1.0	1.2	1.4	1.5	1.7	2.0	2.1
60	1.0	1.2	1.4	1.5	1.8	2.0	2.3	**60**	1.0	1.2	1.4	1.5	1.8	2.0	2.3
70	1.0	1.1	1.3	1.5	1.8	2.0	2.2	**70**	1.0	1.1	1.3	1.5	1.8	2.0	2.2
80	1.0	1.2	1.3	1.5	1.8	2.0	2.1	**80**	1.0	1.2	1.3	1.5	1.8	2.0	2.1

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Table 3: Volume of CO_2 produced at different substrate concentrations for the fermentation of soursop juice at 38°C and 40°C respectively

TIME (Min)								TIME (Min)							
Volume of CO_2 produced (cm^3) at 38°C								Volume of CO_2 produced (cm^3) at 40°C							
Substrate Conc. % (v/v)	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**Substrate Conc. % (v/v)**	**30**	**60**	**90**	**120**	**150**	**180**	**210**
20	1.0	1.2	1.4	1.6	1.8	2.0	2.2	**20**	1.1	1.3	1.5	1.7	1.9	2.1	2.4
30	1.0	1.1	1.4	1.5	1.7	1.9	2.0	**30**	1.0	1.2	1.4	1.5	1.7	1.9	2.0
40	1.0	1.2	1.4	1.6	1.8	2.0	2.2	**40**	1.0	1.2	1.4	1.5	1.7	2.0	2.2
50	1.0	1.2	1.4	1.7	1.8	2.0	2.3	**50**	1.0	1.2	1.4	1.5	1.7	1.8	2.1
60	1.0	1.2	1.4	1.5	1.8	2.0	2.1	**60**	1.0	1.1	1.4	1.5	1.8	1.8	2.0
70	1.0	1.1	1.3	1.4	1.5	1.7	2.0	**70**	1.0	1.2	1.4	1.6	1.7	2.0	2.2
80	1.0	1.2	1.4	1.5	1.7	1.9	2.0	**80**	1.0	1.2	1.4	1.5	1.7	1.9	2.0

Table 4: Volume of CO_2 produced at different substrate concentrations for the fermentation of soursop juice at 42°C

TIME (Min)							
Volume of CO_2 produced (cm^3) at 42°C							
Substrate Conc. % (v/v)	**30**	**60**	**90**	**120**	**150**	**180**	**210**
20	1.0	1.2	1.4	1.7	1.8	2.0	2.1
30	1.0	1.2	1.4	1.5	1.7	1.8	2.2
40	1.0	1.2	1.4	1.7	1.8	2.0	2.3
50	1.1	1.3	1.4	1.7	1.9	2.2	2.5
60	1.0	1.3	1.4	1.7	1.9	2.0	2.4
70	1.1	1.2	1.4	1.5	1.7	1.8	2.0
80	1.0	1.1	1.4	1.5	1.7	1.8	2.0

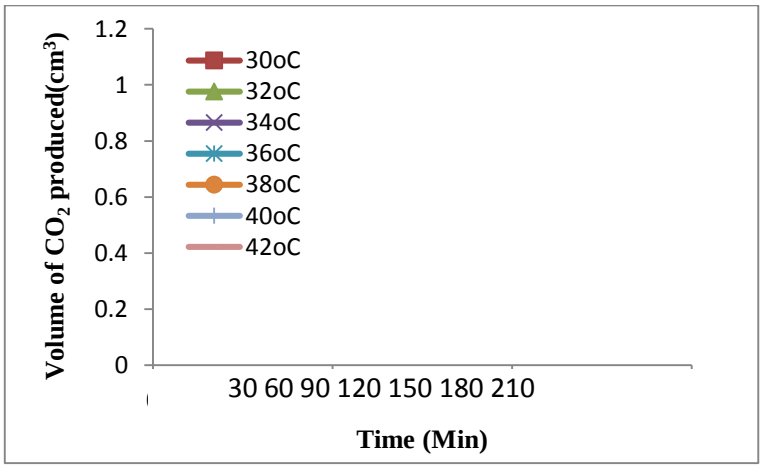

Figure 1: Variation in volume of CO_2 production with time at different temperatures for the fermentation of soursop juice

The rate of CO_2 production from the fermentation process for soursop juice at different temperatures (30-42°C) is presented in Tables 1-4 and represented as a fermentation rate profile in Fig. 1.

It is observed that the rate of production of CO_2 increased with substrate concentration over the entire range at all temperatures studied, as seen in Tables 1-4. But in most cases, the rate of CO_2 production increased over a range (20-50% v/v), and decreased minimally at higher substrate concentrations (60-80% v/v), (Tables 1-4). The narrow range of substrate concentration (20-50% v/v) at which the rate of CO_2 production increased is observed as the optimal substrate concentration range at which the fermentation rate is effective. The rate of CO_2 production from soursop juice also increases with temperature up to an optimal range of 34-40°C, where maximum yeast activity is observed (Tables 1-4). This is consistent with enzyme kinetics, where temperature boosts the kinetic energy of molecules, enhancing reaction rates. The rate of fermentation relative to temperature and substrate concentration as presented in Table 5 below, also shows the temperature dependence on the fermentation rate of soursop juice. It is seen that the rate of fermentation increases with temperature at certain ranges for each substrate concentration (Table 5). However, temperatures beyond the optimal range (36-40°C at substrate concentration of 20% v/v; 32-38°C at substrate concentration of 30-60% v/v; and 30-34°C at substrate concentration of 70-80% v/v;) (Table 5), leads to reduced rate of fermentation. This could be due to enzyme denaturation caused by excessive thermal vibrations, as indicated by the decline in activity. This agrees with the findings of Esele and Byaruhanga (2024), where they posited that temperature control is critical for maximizing yields in ethanol recovery processes. The volume of CO_2 produced also increased with time for all temperatures studied as seen in Fig. 1. The increase in CO_2 production with time is due to increased contact time between the active site of the enzyme and the substrate, allowing for effective enzymatic breakdown of the substrate and consequent increase in fermentation rate. Therefore the higher the time, the higher the chances for enzymatic surface contact with the substrate, leading to enhanced enzymatic fermentation rate.

Table5: Rate of fermentation of soursop juice at various substrate concentrations and temperatures

Temperature (°C)							
Rate of Fermentation							
Substrate Conc. % (v/v)	**30**	**32**	**34**	**36**	**38**	**40**	**42**
20	0.54	0.54	0.55	0.67	0.66	0.71	0.63
30	0.53	0.69	0.67	0.58	0.64	0.56	0.63
40	0.59	0.66	0.70	0.67	0.56	0.64	0.70
50	0.55	0.81	0.64	0.70	0.58	0.59	0.77
60	0.56	0.66	0.70	0.61	0.67	0.58	0.73
70	0.70	0.73	0.70	0.53	0.60	0.68	0.52
80	0.87	0.48	0.64	0.56	0.56	0.57	0.56

Similar trends were reported by Ogbebor *et al.* (2016), confirming that *Saccharomyces cerevisiae* typically operates best within a narrow temperature window, beyond which activity declines.

Effect of substrate concentration on CO_2production and fermentation rate of soursop juice

Table 6: Effect of substrate concentrations on the rate of fermentation of soursop juice

Substrate Conc. % (v/v)							
Volume of CO_2 produced (cm^3)							
Time (Min)	**20**	**30**	**40**	**50**	**60**	**70**	**80**
30	1.0	1.4	2.8	1.2	1.1	3.1	3.1

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60	2.7	1.7	3.5	1.4	1.5	3.2	3.7
90	3.1	2.7	4.8	1.8	1.7	4.7	5.0
120	4.8	4.4	5.2	2.2	2.0	6.0	5.2
150	5.5	4.5	6.0	2.5	7.0	6.1	6.0
180	6.1	5.7	6.2	2.8	7.7	6.7	7.2
210	7.5	8.5	7.2	3.5	8.7	7.0	7.5
Rate (molmin^{-1})	39.5	85.5	106.7	126.4	104.2	89.5	86.9

The impact of substrate concentration on the fermentation rate is presented in Table 6. There is an observed steady increase in the rate of fermentation (39.5-126.4 molmin^{-1}) within the initial substrate concentrations (20 to 50% v/v), but above this concentration (60 to 80% v/v), the rate of fermentation decreases (104.2-86.9 molmin^{-1}). The observed trend suggests a saturation of the active sites of the yeast by the substrate beyond the optimal substrate concentration range (20-50% v/v), resulting in a decline in yeast activity, thereby limiting the fermentation rate. This substrate saturation effect as observed in Table 6, supports the findings of Palmer (2020), who reported similar saturation effects in enzyme-substrate interactions during fermentation; affirming that high substrate concentrations do not always yield proportionate increases in enzymatic activity. An application of one way ANOVA on data for fermentation rate dependence on substrate concentrations showed a significant difference on fermentation rate at a 95% confidence interval, for the optimum substrate concentration ranges observed. This statistically justifies the enzymatic fermentation rate dependence on substrate concentration for bio-ethanol production from soursop juice.

Effect of temperature on fermentation rate for sugar cane juice

Table 7: Volume of CO_2 produced at different substrate concentrations for the fermentation of sugar cane juice at 30^{o}C and 32^{o}C respectively

TIME (Min)								**TIME (Min)**							
Volume of CO_2 produced (cm^3) at 30^{O}C								Volume of CO_2 produced (cm^3) at 32^{O}C							
Substrate Conc. % (v/v)	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**Substrate Conc. % (v/v)**	**30**	**60**	**90**	**120**	**150**	**180**	**210**
20	1.0	1.1	1.3	1.5	1.2	1.0	1.0	**20**	1.0	1.2	1.5	1.0	2.3	2.6	2.8
30	1.1	1.8	2.1	2.2	2.4	2.7	2.8	**30**	1.0	1.1	1.6	2.0	2.2	2.4	2.8
40	1.2	1.8	1.8	2.2	2.4	2.6	2.5	**40**	1.1	1.5	2.0	2.2	2.6	2.8	3.2
50	1.1	1.5	1.8	2.0	2.2	2.3	3.0	**50**	1.2	1.3	1.5	1.8	2.0	2.4	2.6
60	1.1	1.2	1.9	2.0	2.2	2.2	2.8	**60**	1.0	1.2	1.4	1.7	1.9	2.1	2.3
70	1.0	1.4	1.6	2.1	2.5	2.8	3.0	**70**	1.0	1.3	1.8	2.1	2.2	2.5	2.8
80	1.0	1.8	2.0	2.4	2.5	2.8	2.8	**80**	1.0	1.2	1.4	1.6	2.0	2.4	2.8

Table 8: Volume of CO_2 produced at different substrate concentrations for the fermentation of sugar cane juice at 34^{o}C and 36^{o}C respectively

TIME (Min)								**TIME (Min)**							
Volume of CO_2 produced (cm^3) at 34^{O}C								Volume of CO_2 produced (cm^3) at 36^{O}C							
Substrate Conc. % (v/v)	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**Substrate Conc. % (v/v)**	**30**	**60**	**90**	**120**	**150**	**180**	**210**
20	1.0	1.1	1.2	1.5	2.0	2.2	2.4	**20**	1.2	1.4	1.5	2.0	2.7	3.0	3.4
30	1.0	1.2	1.3	1.5	2.0	2.2	2.4	**30**	1.0	1.2	1.5	2.0	3.0	3.4	4.5

40	1.0	1.2	1.5	1.7	2.2	2.4	2.8	**40**	1.0	1.2	1.4	2.2	2.5	2.8	3.0
50	1.0	1.4	1.8	2.0	2.4	2.6	3.0	**50**	1.2	1.5	1.8	2.4	2.7	2.8	3.7
60	1.0	1.2	1.5	2.0	2.8	2.6	3.0	**60**	1.0	1.7	2.2	2.5	2.8	3.0	3.2
70	1.0	1.1	1.3	1.4	2.0	2.5	2.8	**70**	1.0	1.2	1.5	1.8	2.0	2.4	2.8
80	1.0	1.1	1.3	1.4	2.0	2.5	2.8	**80**	1.0	1.3	1.5	1.7	1.8	2.2	2.4

Table 9: Volume of CO_2 produced at different substrate concentrations for the fermentation of sugar cane juice at 38^{o}C and 40^{o}C respectively

TIME (Min)								**TIME (Min)**							
Volume of CO_2 produced (cm^3) at 38^{O}C								Volume of CO_2 produced (cm^3) at 40^{O}C							
Substrate Conc. % (v/v)	**30**	**60**	**90**	**120**	**150**	**180**	**210**	**Substrate Conc. % (v/v)**	**30**	**60**	**90**	**120**	**150**	**180**	**210**
20	1.0	1.1	1.2	1.5	1.9	2.1	2.4	**20**	1.0	1.2	1.5	1.7	2.0	2.2	2.4
30	1.0	1.1	1.3	1.4	1.5	2.2	2.5	**30**	1.0	1.1	1.3	1.5	1.7	2.0	2.4
40	1.0	1.2	1.4	2.0	2.0	2.4	2.5	**40**	1.0	1.2	1.5	1.8	2.0	2.4	2.7
50	1.0	1.2	1.4	2.1	2.1	2.3	2.8	**50**	1.0	1.2	1.3	1.7	2.1	2.3	2.5
60	1.0	1.3	1.3	2.0	2.0	2.2	2.5	**60**	1.0	1.2	1.5	2.0	2.2	2.5	2.8
70	1.0	1.1	1.5	2.0	2.0	2.1	2.4	**70**	1.0	1.2	1.4	2.1	1.8	2.0	2.2
80	1.0	1.2	1.5	2.0	2.0	2.2	2.4	**80**	1.0	1.2	1.5	2.0	1.6	2.2	2.2

Table 10: Volume of CO_2 produced at different substrate concentrations for the fermentation of sugar cane juice at 42^{O}C

TIME (Min)							
Volume of CO_2 produced (cm^3) at 42^{O}C							
Substrate Conc. % (v/v)	**30**	**60**	**90**	**120**	**150**	**180**	**210**
20	1.0	1.2	1.3	1.7	1.5	2.1	2.4
30	1.0	1.1	1.4	1.5	2.0	2.1	2.2
40	1.0	1.2	1.5	1.7	1.9	2.2	2.5
50	1.0	1.4	1.8	2.0	2.0	2.6	2.8
60	1.0	1.2	1.4	1.7	2.4	2.2	2.5
70	1.0	1.1	1.5	1.8	2.0	2.1	2.4
80	1.0	1.2	1.4	1.5	2.0	2.7	2.8

The rate of fermentation determined as the amount of CO_2 production for the fermentation of sugar cane juice at different temperatures (30-42^{O}C) is shown in Tables 7-10 and represented as the fermentation rate profile in Fig. 2. The results in Tables 7–10, demonstrate the thermal sensitivity of the yeast (*Saccharomyces cerevisiae*) to CO_2 production rate, just as similarly observed in the fermentation of soursop juice.

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 (34-40^{o}C) over which its activity was at maximum. The initial increase in rate with temperature is as expected, a function of the increase in the average kinetic energy of the molecules. However, further increases in temperature beyond the optimal range triggered the breakdown of the enzymatic structure due to increased thermal vibration of the enzyme molecules.

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Increased CO_2 production observed at optimal range of around 34-40°C, brings out the significance of temperature dependence on enzymatic catalyzed reaction rate. This underscores the balance between maximizing enzymatic activity and avoiding thermal degradation. Studies by Esele and Byaruhanga (2024) also reported similar optimal temperature windows for other microbial fermentations, emphasizing that exceeding these temperatures often causes a significant drop in enzymatic efficiency due to protein unfolding.

Temperatures above the optimal range cause a conformational change and distortion of the enzyme, leading to a decline in enzyme activity and a consequential reduction in fermentation rate, as shown in Table 11.

Table 11: Rate of fermentation of sugarcane juice at various substrate concentrations and temperatures

Temperature (°C)							
Rate of Fermentation							
Substrate Conc. % (v/v)	**30**	**32**	**34**	**36**	**38**	**40**	**42**
20	0.04	1.07	0.87	1.28	0.82	0.80	0.79
30	0.83	1.03	0.85	1.95	0.82	0.77	0.75
40	0.82	1.15	0.99	1.20	0.93	0.95	0.84
50	0.75	0.83	1.07	1.23	0.97	0.89	1.01
60	0.91	0.74	0.83	1.18	0.87	1.04	0.85
70	1.17	0.98	1.11	0.99	0.77	0.65	0.80
80	0.87	0.93	1.10	0.76	0.80	0.93	1.07

The rate of CO_2 production with respect to substrate concentration as seen in Tables 7-10, shows increased CO_2 production within a narrow optimal range of substrate concentration (20-50% v/v), at all temperatures studied, but decreased minimally at higher substrate concentrations (60-80% v/v). This is similar to the trend observed for the fermentation of soursop juice. A saturation of the active sites of the yeast by the substrate beyond the optimal substrate concentration range (20-50% v/v) resulted in a decline in yeast activity and consequently limited the rate of fermentation.

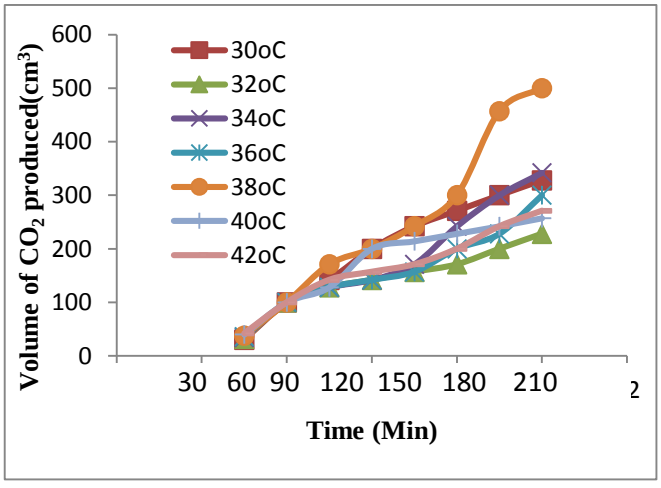

Figure 2: Variation in volume of CO_2 production with time at different temperatures for the fermentation of sugarcane juice

The CO_2 production time profile at different temperatures for the fermentation of sugarcane juice as seen in Fig. 2 is also similar to the profile obtained for the fermentation of soursop juice (Fig. 1); where the volume of CO_2 produced also increased with time for all temperatures studied. The profile defines the essence of increased contact time between substrate and enzyme active sites at all temperatures studied, as CO_2 production increases with increasing contact time. Enzyme reactivity for the fermentation process is therefore enhanced by both temperature and increased substrate-enzyme contact time.

Effect of substrate concentration on CO_2 production and fermentation rate of sugar cane juice

Table 12: Effect of substrate concentration on the rate of fermentation of sugarcane juice

Substrate Conc. % (v/v)							
Volume of CO_2 produced (cm^3)							
Time (Min)	**20**	**30**	**40**	**50**	**60**	**70**	**80**
30	1.8	2.4	1.1	1.8	2.0	1.8	1.1
60	2.7	2.5	2.1	2.4	2.2	2.5	2.1
90	3.2	2.8	3.4	4.0	2.7	2.8	2.5
120	5.2	3.1	3.7	8.0	3.0	8.0	3.7
150	5.7	8.2	4.0	8.4	3.4	8.2	4.4
180	6.0	8.5	7.0	9.4	3.1	8.4	7.1
210	8.0	9.7	7.5	9.7	4.0	9.4	7.2
Rate ($molmin^{-1}$)	102.8	103.9	135.7	148.1	138.3	98.2	42.4

Substrate concentration showed a marked effect on the rate of fermentation as seen in Table 12. The data show that the rate of fermentation varied in proportion to substrate concentration up to 50% v/v. The rate of fermentation increased steadily (102.8-148.1 $molmin^{-1}$) from 20 to 50% v/v substrate concentration, but thereafter showed a decrease in fermentation rate (138.3-42.4 $molmin^{-1}$) as substrate concentration increased beyond from 60 to 80% v/v. This shows a saturation of enzyme active site at the observed optimal substrate concentration, limiting further enzyme activity above this concentration; bringing about an attendant decrease in fermentation rate as seen in Table 12. The reports of Ogbebor *et al*. (2016) and Palmer (2020) also support this. The same trend is seen for the fermentation of soursop juice. The data indicates that there is a wide range of substrate concentrations over which the enzyme is still active, outside the optimal substrate concentration.

Statistical treatment on data for fermentation rate dependence on substrate concentrations showed a significant difference on fermentation rate at $p<0.05$ (95% confidence limit) for the optimal substrate concentration ranges observed for sugar cane; which therefore gives further credence and statistically justifies the enzymatic fermentation rate dependence on substrate concentration for bio-ethanol production from sugarcane.

Comparative evaluation of the effect of temperature and substrate concentration on fermentation rates of soursop and sugar cane juice

An evaluation of the effect of substrate type on fermentation efficiency shows that sugarcane exhibited higher CO_2 production compared to

Scientia Africana, Vol. 23 (No. 5), December, 2024. Pp 201-212
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soursop under identical conditions, as seen in the presented results (Tables 5-6 and Tables 11-12). This could be attributed to the higher glucose content in sugarcane, which facilitates easier yeast metabolism. This justification has been earlier validated by the reports of Morton (2016) and Perez *et al*. (2020). The specific fermentation behaviors of these substrates suggest that substrate composition plays a significant role in yeast activity and overall fermentation rate. The production rates obtained in this study are similar to those observed by Egharevba *et al*. (2014), where it was reported that yeast-catalyzed fermentation rates increased up to certain substrate and temperature levels before reaching a saturation point. This result also aligns with earlier reports (Dragone *et al*., 2011; Esele and Byaruhanga, 2024), showing that fruits can provide viable fermentation substrates under controlled conditions.

CONCLUSION

The findings from this study emphasize the importance of controlled substrate concentrations and temperatures to maintain optimal enzymatic function without compromising fermentation yield. The findings highlight the importance of maintaining precise temperature and substrate levels to optimize bio-ethanol production. The narrow optimal range observed in this study is valuable for industries focusing on fermentation, as it underscores the need for stringent control over fermentation conditions. The findings from this study will therefore help in enzyme fermentation processes in bio-ethanol production from fruit substrates for brewery, bio-energy, and other applications.

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