International Journal of Engineering Research & Technology (IJERT)
ISSN: 2278-0181
Vol. 6 Issue 07, July - 2017

Kinetics of Fermentation of Local Specie of Cashew Juice using Sacharomyces *Cerevisiae* (Local Extract)

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Abstract **- The fermentation of local breed of cashew juice using a local extract (Saccharomyces cerevisae) was studied to determine the effect of temperature, concentration and pH on the rate of production of alcohol. The rate of fermentation was measured as the rate of fermentation was measured as the rate of carbon IV oxide production. The experimental results showed that the rate of production of alcohol increased with time, substrate concentration and temperature, until a specific point. The optimum pH for the fermentation was 4.5, while that of temperature was between the neighbourhood of 33-35^{0}C. Increase in substrate concentration up to 18% increased the rate of alcohol production, after which further increase in substrate concentration did not have any effect on the rate of alcohol production.**

Keywords: Fermentation process, kinetic studies to obtain optimum condition for the production of alcohol.

INTRODUCTION

Cashew (*Anacardium occidentele),* is a resilient and fast growing evergreen tree that can grow to the height of 20m (60ft) (Morton 1987). It is a nature to arid North-eastern Brazil and was taken around the world by the Portuguese and Spanish, who planted the tree in their colonies (Charm 1978). The places where cashew can be found include. Asia, Australia, North America, South America, Africa, etc. (Hindu 2002). According to Morton (1987), the edible cashew apple contains about 11.6% of fermentable sugar, 0.26% vitamin C and 0.2% protein. Further analysis of its food value per 100g of the edible apple showed that it contain 84.4-88.7g of moisture, 0.10-0.162g of protein, 0.05-0.50g, fibre, fat, 9.08-9.75g, carbonhydrate, 0.4-1.0g, fibre, ash of 0.19-0.34g,0.9-5.4mg of calcium 6.1-21.4mg of phosphorus, 0.19-0. 71mg of iron, 0.023-0.03mg thiamin, 0.13-0.4mg, riboflavin, 0.03-0.74mg riboflavin, 0.03-0.74mg carotene, Niocin 0.13-0.53gmg and ascorbic acid 146.6-372.0mg (Wimalsiri and Sinnatambly (1971) Morton 1987).

Alcoholic fermentation is the process of producing alcohol through breakdown of carbohydrate substrate as sugar, starch and cellulosic material by the action of micro-organisms or enzymes (Lewis and Young 1995). During fermentation process substrates is utilized as the production of alcohol as well as the growth of micro-organisms (Shreve 1996).

Alcohols have a wide range of application. Dihydric alcoholic such as ethanol 2-diol (glycol) is used as antifreeze in car radiators. Propane - 1,2,3-triol (glycerol) is often used in medicine and cosmetics as basis for the preparation of creams and ointment (Shceve 1996). Alcohol have many uses in the chemical industry especially as solvents and intermediates in the manufacture of esters. Esters of low alcohols are employed extensively for paint, varnishes, adhesives and ink (Asfoar, Tayeb and Mastafa 1991).

The immense importance of alcohol cannot be over emphasized, thus any effort geared towards sourcing it from untapped natural resources is a step towards the right part. Alcohols, had long been produced from petroleum products. The fact that petroleum is a non-renewable resources triggered studies on producing alcohols from renewable resources, such as agricultures sources (Ogunye, Susu and Digwo 1978). The Nigeria economy is blessed with abundance of fruits with high concentration of fermentable sugar and a study of their kinetics of fermentation will be a major step towards utilizing them in the production of alcoholic beverages with distinct taste and aroma (Hoisberg 1987). Alcohols produced from such sources can also be refined for use as motor fuel industrial solvent, extractants, antifreezes and other numerous uses of alcohol.

Many countries of the world have been able to produce ethanol from renewable sources. For example in Malaysia crops like cocoa, pineapple and sugarcane were used for ethanol production using a wild strain of *Sacaromyces cerevisiae* (Othman, Abdul-Raham, Aba-Bakar 1992). In Mexico agricultural wastes were classified with strains of *Aspergillus riger* as a commercial enzyme preparation with the fermentation of ethanol carried out with strains of *Sacharomyces cerevisiae* (Wilkins and Ramesh 1991). Wheat milling residue hydrolysate has been used for alcohol production in Egypt (Asfour, Tayeb, Mostafa 1991). In Nigeria there has been microbial conversion of agricultural materials into value added products (ethanol) (Ejike and Okereke 1991, Odoh and Azubike 1995). This present study is aimed at determining designing the optimum reaction conditions (temperature time pH, substrate concentrations for the production of alcohol from

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cashew juice via kinetic approach using strains of *Sacharomyces cerevisiae*. The economic and commercial competitiveness of a process depends on the speed, efficiency and economy at which the product can be obtained from the starting material.

MATERIAL AND METHODS

Yeast strain used: -a pure culture of *Saccharomyces cerevisae* was isolated from a fresh palm wine juice. The method of isolation from the palm wine is as described (Obisanwa Ajao and Ogunmitan 1987). Riped cashew fruit was colleted in a cashew planation in Enugu Nigeria.

Preparation of Cashew Apple Juice

Cashew apples were stored only ripped and undamaged apple were used. The fruits were sliced and ground with a blender which was finally filtered with a filter cloth to obtain the juice. This juice was sterilized by heating in an Aluminium can at temperature of 850C for 40 minutes. After cooling the juice was filtered and treated with sodium metabisulphite.

Experimental Procedure

The fermenter vessel, stirring rod, and measuring cylinder was washed with hot water and afterwards sterilized by contacting them with 2% solution of sodium metabisulphte. 150ml of properly conditioned and sterilized juice was brought to required pH and substrate concentration. This was poured into the sterilized fermenter and the temperature of juice adjusted to the required temperature. 1g of dry yeast powder was pinched into the juice and the fermentation vessel was made airtight after stirring the juice for maximum mixing. At 30 minutes interval, the volume of Co_2 generated was measured (Ekwunankama, Onwuchekwa and Onukwuli 2001).

In determining the effect of temperature on fermentation kinetics, all other factors such as substrate concentration, pH of the juice, amount of the yeast, size and shape of container, time allowed to ferment and method of measurement were kept constant. The temperature was varied between 30-40^{0}C. Temperature control was effected by circulating a temperating water around the fermentation vessel. The effect of substrate concentration and pH was monitored by varying substrate concentration and pH while keeping other factors constant. The pH of juice was varied between 3-6, while substrate concentration was between (11-21%)w/w of sucrose solution (Ekwunankama, Onwuchekwa and Onukwuli 2001).

RESULTS AND DISCUSSION

The rate of production of Co_2 and hence alcohol during the fermentation of cashew increased with increase in temperature up to 35^{0}C (Fig. I,II&III). The initial rise in rate of the reaction is expected of any chemical reaction. Increase in temperature increases the average kinetic energy of the molecules (Obisanwa, Ajao, Ogunutan 1987). This increases the chances of the formation of the product due to increased effective collision (Cham 1978) However, as the temperature rises further above 35^{0}C, the increasing thermal vibration of the enzyme molecules causes its structure to breakdown (denature) and so the 'lock' is damaged thereby making the enzyme less efficient in such a way that the substrate molecule cannot "lock in" to be changed into product (Shieve 1996). However, the temperature of most efficient generation of CO_2 is within the optimal temperature for saccharomyces cerevisiae (30-40^{0}C) (Lewis and Young 1995).

The effect of pH & time on fermentation of cashew juice is shown in Fig. I,II,III, IV & V. The growth rate of cell, rate of alcohol production and yield of alcohol displayed ph optimum in the neighbourhood of 4.5. This is within the optimal pH ranges for *saccharomyces cerevisiae* pH (4.5 to 5.0) Casida 1968) and yeast in general pH (3-6) (Donald et al 1978). The micro-organisms tend to grow over a limited pH range and frequently shift their metabolism as a result of even1-1.5pH unit change (Lewis and Young 1995). In the region outside the optimum pH, the cells of saccharomyces cerevisiae are less tolerant to the environment and hence less active and less efficient in substrate utilization.

The effect of substrate concentration on the fermentation process of cashew juice is contained in Fig. IV & V. The rate of CO_2 production and hence alcohol production increased with increase in substrate concentration up to 18%w/w. However, further increase in the substrate concentration did not affect the rate of the reaction. This is expected since higher initial substrate concentration implies availability of more fermentable sugar for conversion to alcohol and as nutrients for growth of cells. Consequently at the optimum concentration of 18%w/w, all the active site of the enzymes have been saturated and further increase in the substrate concentration (table VI), did not affect or increase the rate of fermentation any longer.

CONCLUSION

The result of this work showed that the rate of alcohol production through fermentation of cashew juice by *Saccharomyces cerevisae* increased with fermentation time up to $3^{1}/_{2}$ hours. The alcohol production was maximum at the optimal temperature between 33-35^{0}C and pH of 4.5. The substrate concentration that gave maximum production of alcohol was 8%w/w juice sample.

Findings of this study recommend that alcohol production by fermentation of cashew juice using sacharomyces cerevisiae should be conducted at a temperature of 33-350C and pH of 4.5. The substrate concentration should not exceed 20% w/w. However, further studies on fermentation kinetics of other fruits juice with high fermentable sugar should be carried out to reduce the over dependence on ethanol from palm wine and on petroleum products non-renewable source of ethanol.

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Fig [illegible] : Variation of rate of CO_2 Production with Time and Temperature of Fermentation

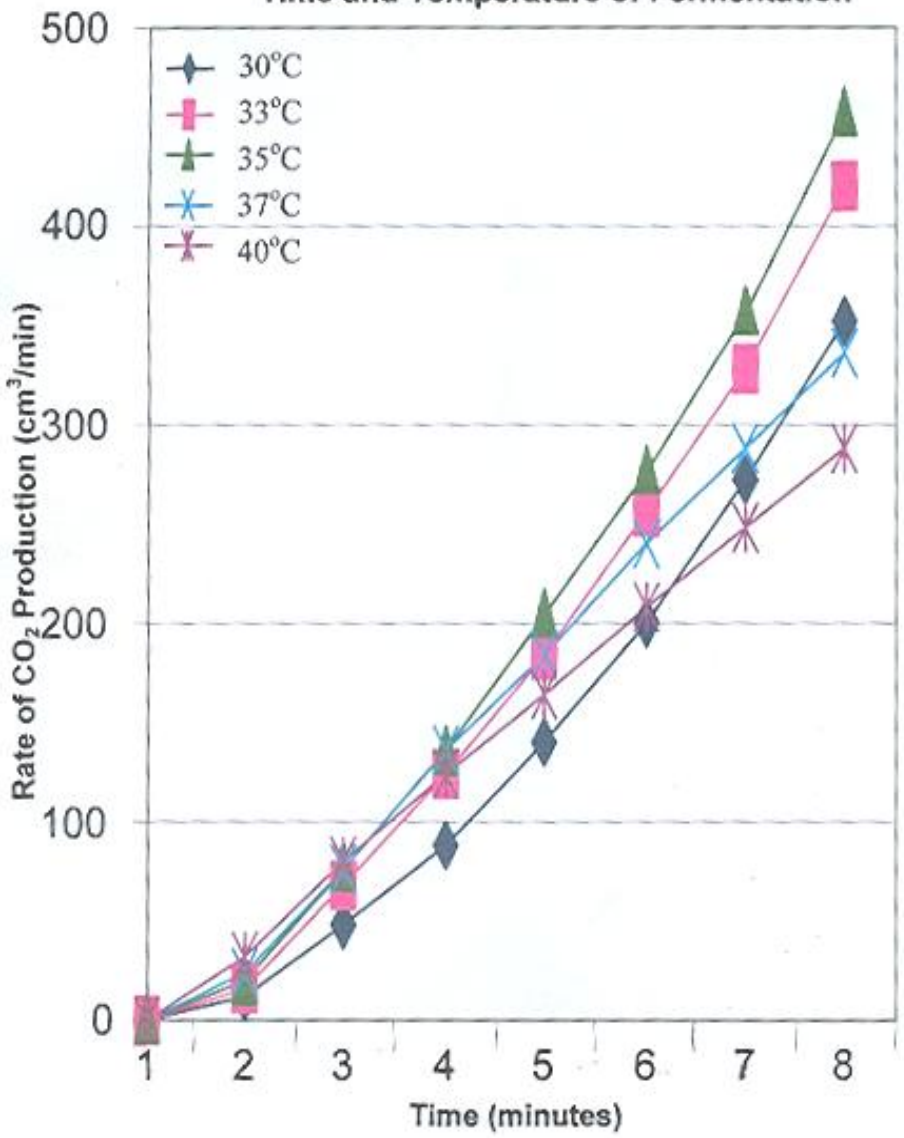

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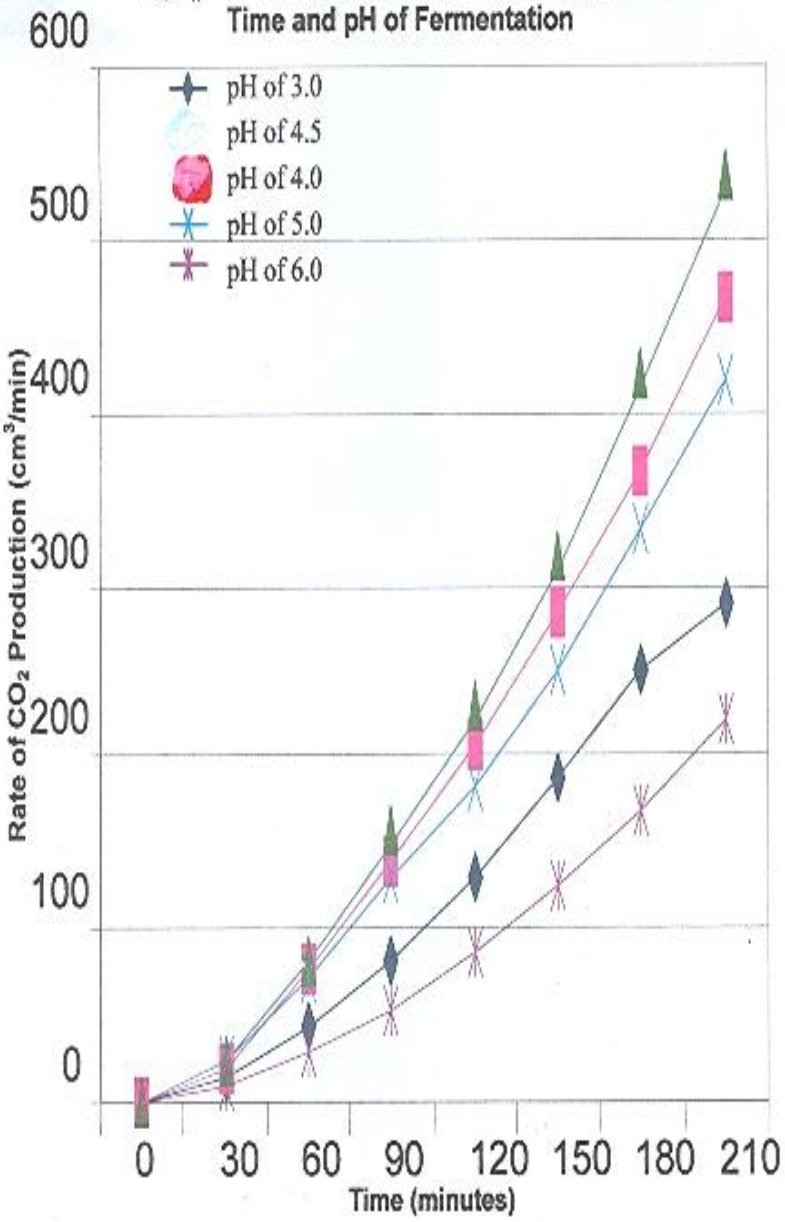
Fig ii Variation of rate of CO_2 Production with Time and pH of Fermentation



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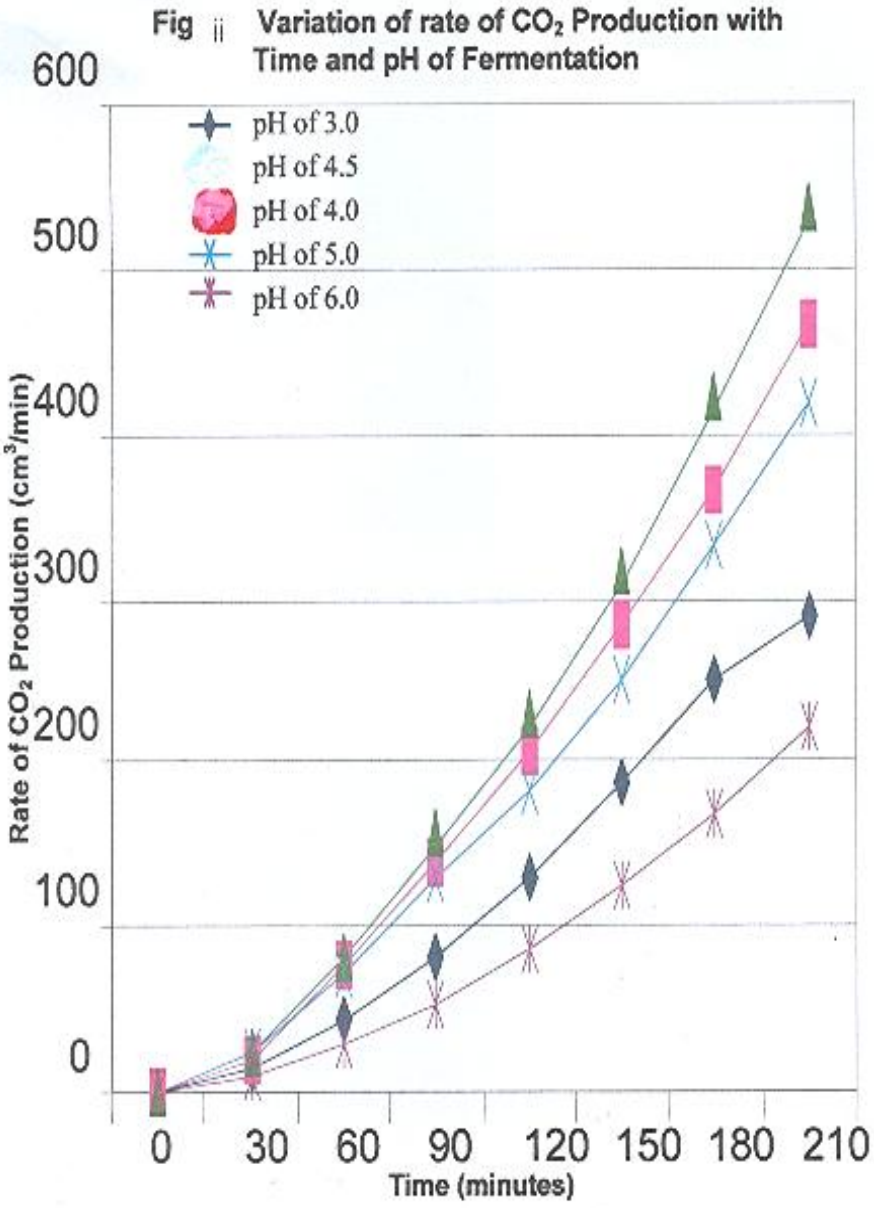

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Fig iv Variation of Volume of CO_2 Produced with Temperature of Substrate

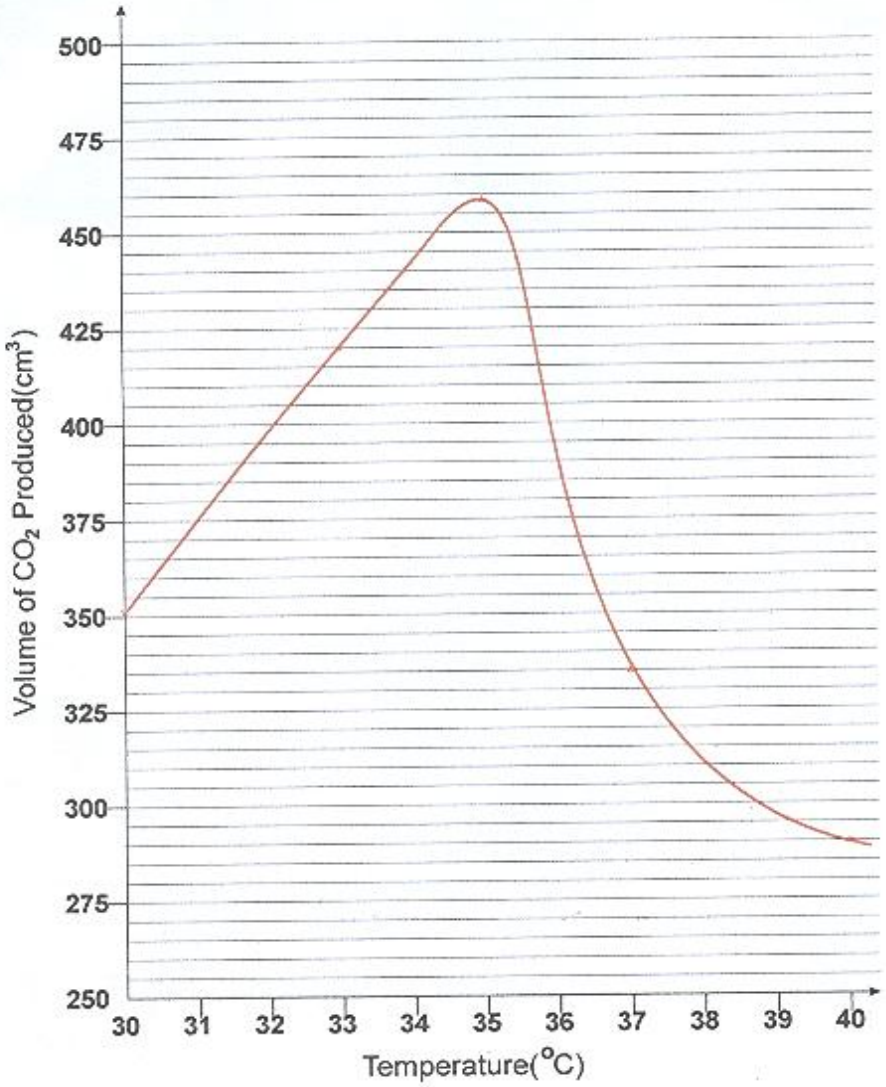

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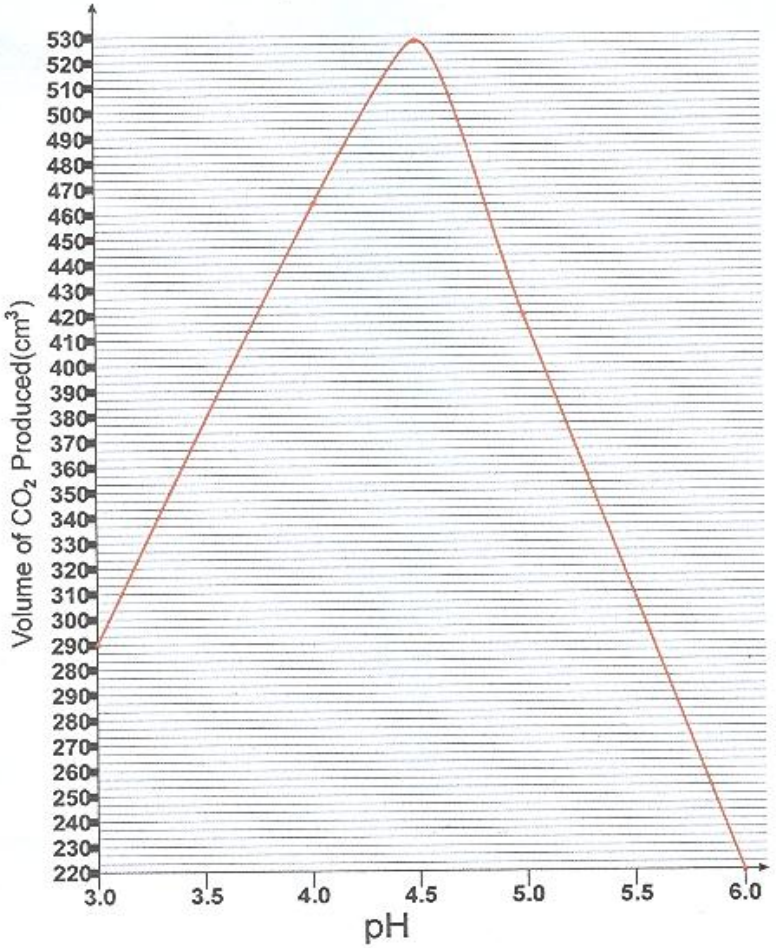
Fig v Variation of Volume of CO_2 Produced with pH of Substrate



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