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Bio-energy production from glucose at various Temperatures from *Citrus sinnesis* by *Trichoderma reesei*

J.A. Yabefa [1*], B.O. Aderemi[1*], D. Wankasi[2]

[1]*Department of Chemical Engineering; Ahmadu Bello University, Zaria*
[2]*Department of Applied Chemical Sciences, Niger Delter University, Wilberforce Island. Bayelsa State*

ABSTRACT

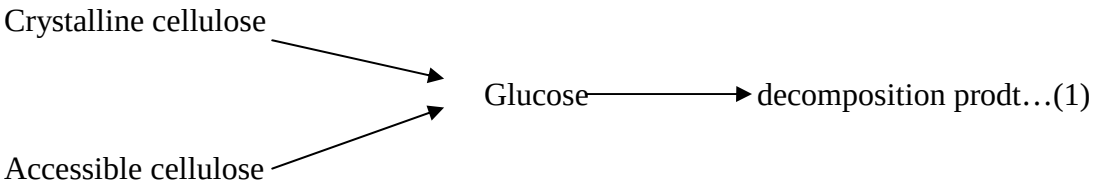
Trichoderma reesei was cultured to secret enzymes using orange mesocarp as subsrate. Untreated orange mesocarp (non-alkali treated orange mesocarp washed with distilled water) and orange mesocarp alkali-treated at 100 ºC, washed with water at pH of 6-7.When untreated orange mesocarp and alkali-treated orange mesocarp was hydrolysed at varying temperatures of 30° C,37 °C, 40 ºC,50 ºC 55 ºC, appreciable glucose yield was found(2.13, 2.34, 1.71, 2.56, 2.35) mmol/L respectively for alkali –treated mesocarp, but the yield was lower when untreated mesocarp was hydrolysed at 37 ºC(1.7mmol/L).Hence it was concluded for this particular research that untreated orange mesocarp is as good a substrate for glucose production but the optimum temperature for the alkali-treated mesocarp falls within 37-50 ºC.

Keywords: Orange Mesocarp, *Trichoderma reesei*, Temperature, Glucose, Enzyme.

INTRODUCTION

The world's population has continued to grow steadily necessitating an increase in the demand for affordable food and citrus fruits [1]. Sweet Orange (*Citrus Sinensis*) production in Nigeria is significant, due to few and small capacity processing industries which converts the fruits to juice, concentrates and canned fruits. Nigeria produces 3% (1.98 million tonnes) of fresh citrus in the world and Africa produces 5.6% (3.741 million tonnes) of varieties of citrus fruits of which Nigeria contributes 3.24 million tonnes [5,10]. Orange mesocarp, a waste product from citrus processing factories and farmers, is partly used for cattle feed. However a large part of the citrus waste produced, about 66 million tonnes annually [9] is still being discarded to nature, causing environmental problems [9]. Researches in this field have successfully converted many cellulosic materials such as saw dust ,solid animal waste, crop residues, cotton stalks, etc [1,6] to more valuable products such as fermentable sugars. Orange mesocarp contains various carbohydrate polymers ranging between 62.5-87.8% of cellulose [9,10] which makes it an interesting choice

for production of metabolites such as fermentable sugars (glucose) and ethanol by appropriate micro-organisms. Equation (1).



Aderemi [1,3,8] also, studied the kinetics of glucose production from rice straw by *Aspergilus niger.*

Enzymatic hydrolysis is an efficient method to release almost all carbohydrates present in the orange mesocarp, but its application is hampered by the high cost of enzymes and the slow rate of depolymerization reaction[9]. Thus development of a cost- effective method in which all or a high proportion of carbohydrates could be released, will help towards commercialization of the processes using orange mesocarp as raw materials.

Hitherto, little information exists in literature concerning orange mesocarp pre-treatment,optimum temperature and glucose concentration, using cells of *Trichoderma reesei.* Crude enzyme hydrolysis has the merit of direct cell secretion, hence reduces reaction time and overall cost [1].

Therefore, it was the goal of this present research to study the conversion of a chemically pre-treated [11] orange mesocarp to glucose using cells of *T. reesei* where, the optimum temperature and glucose concentration were ascertained. Glucose as an intermediate product in this process for bio-energy production stands tall in solving the present global energy crisis, for a sustainable economic development.

MATERIALS AND METHODS

Isolation of *Trichoderma reesei(T. reesei)*
Potatoes Dextrose Agar (PDA) as described by Alexopoulus and Beneke [1]with little modification using solid state fermentation was used for the isolation of the organism . The organism was identified as *T.reesei* using an Olympus Venox –T microscope based on the standard structure of *Trichoderma reesei* given by Alexopoulus and Blackwell, [2]. Sub culturing from the parent culture was done several times to obtain pure colonies. A wire loop was sterilized using flame sterilization to kill surface bacteria in order to avoid contamination The wire loop was used to take a portion of the growing fungi culture from the edge of the culture plates. This was transferred to a sterilized bottle containing fresh PDA medium. Sub-culturing was repeated for over twenty –five times to obtain a fairly pure colony and stored in the culture stock at room temperature. Fungi cells were used at their exponential growth phase for maximum activity.

Experimental Procedure
Enzymatic hydrolysis of the substrates was carried out at different temperatures, 30 ^{o}C, 37 ^{o}C, 40 ^{o}C, 50 ^{o}C, 55 ^{o}C. And the glucose produced at different temperatures was analysed using randox glucose kit and Colorimeter.

In a typical run the temperature of the shaker bath (Gallenkamp,model KQ 606, London) was set at 37 °C. A 100 millilitres (100ml) of 0.1M of sodium acetate buffer solution (pH 4.5) was introduced into an Erlenmeyer flask, along with 0.1g of *T.reesei* species and 2.0g of sodium hydroxide-treated orange mesocarp[12] were added. Samples were withdrawn after every 4 hours within 72 hours reaction time for analysis. The concentration of glucose in the substrate was determined by following the method of Lee [6] which uses a Randox glucose kit and Jenway Colorimeter, (Model 6051, Germany) at 540nm. The glucose analysis was done using the sample, enzyme blank (control), glucose standard and glucose reagent. 40µL of the sample solution was added into 400µL of the glucose reagent in a 1ml curvet and absorbance was taken. Each run was repeated three times and the mean value of each set of runs was reported.

RESULTS AND DISCUSSION

Temperature effects on the hydrolysis of orange mesocarp , ranging from 30 °C, 37 °C, 40 °C, 50 °C, 55 °C, that was studied, gave yield of 77.8%, 88.9%, 63%96.3%, and 81.5% of glucose concentration respectively. This clearly indicates that the optimum temperature lies between the ranges of 37°C to 50 °C. This result corroborates the one reported by Aderemi [1] on the hydrolysis of rice straw,the hydrolysis of soft wood reported by Tengbo *et al* [1], hydrolysis of animal lignocellulosics by Wen *et al* [1] which was found to be optimum at 50 °C. This also, is in agreement with skop (fibre waste) and cellolignin reported by Castellanose *et al* [1] which showed optimum at 50 °C.

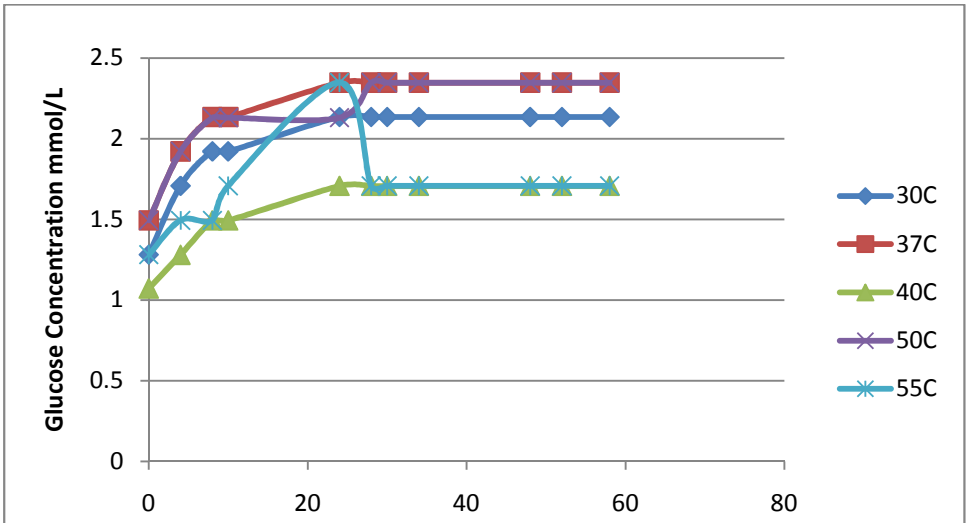


Fig.1: Effect of tmperature on glucose concentration

Although from the result in figure 1, glucose concentration was at a maximum of (2.56mmol/L) in the 34th hour at 50 °C and decreased to 1.92mmol/L in the 48th hour in the same temperature. Suddenly, there was a decline in glucose concentration to 1.70mmol/L, due probably to enzyme denaturation.

Also at 37 °C, the glucose had a maximum concentration of 2.34mmol/L in the 24th hour and maintained a steady constant value as time increases. Even in the 48th hour , at 37 °C ,the glucose concentration doubled compared to 50 °C Hence, considering maximum glucose concentration with respect to reaction time and unit cost of product , 37 ° C is preferred to 50 °C.

Generally, an increase in temperature increases the rate of reaction, since the atoms in the enzyme have greater energies and a greater tendency to move however the temperature is limited to the usual biological range. As the temperature rises, denaturation processes progressively destroy the activity of enzyme molecules. This is due to unfolding of protein chain after the breaking of weak (hydrogen) bonds, so that the overall reactions drop(fig1). Since denaturation begins to occur in many proteins at 45 to 50 °C [6].

In conclusion therefore,the production of glucose intermediate product in the orange mesocarp hydrolysis using *Trichoderma reesei* is very important .

This is due to the fact that glucose is useful as a raw material for biofuel production. Thus, this will go a long way in alleviating our global energy crisis.

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