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Pre-treatment of Orange Mesocarp with alkaline solutions to optimize glucose yield by *Trichoderma reesei*

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ABSTRACT

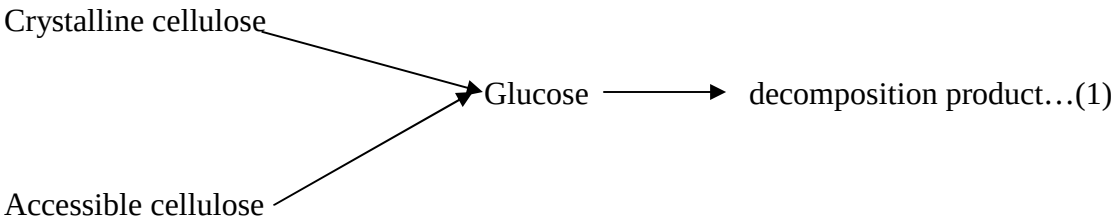
Orange Mesocarp was Pre-treated with alkali solutions in this study. This raw material was crushed to (100-150µm,200-250µm and 300-425µm) and fed into a bioreactor, where delignification was effected with sodium hydroxide and calcium hydroxide followed by washing with warm water. The optimum weight lose percent was determined to be between 63% at 15 minutes and 71%, at 25 minutes soaking time respectively. The optimum orange mesocarp to alkaline solution ratio for favourable saccharification is 1:0.1. The enzymatic hydrolysis of the untreated and pre-treated orange mesocarp by Trichoderma reesei enzymes (endo-β-1,4 glucanase, Cellobiohydrolase and β-1,4 glucosidase) gave 74% and 97% glucose yield respectively.

Key words: Orange Mesocarp, *Trichoderma reesei*, glucose yield, 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 [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 [7,9] is still being discarded to nature, causing environmental problems [7,

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] 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] 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, weight loss percent and glucose yield 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 orange mesocarp to glucose using cells of *T. reesei* ,where the optimum weight loss and glucose yield were ascertained. Glucose as an intermediate product in this process for bioenergy 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 [2;6] 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 fungal 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 at room temperature. Cultured fungi were used on the third day for maximum activity.

Pre- treatment of Orange Mesocarp

Lignocelluloses biomass contains cellulose,hemicelluloses,lignin and ash combined in a complex structure. Therefore pre-treatment reduces the crystallinity of cellulose, while removing lignin and other inhibitors, thereby enabling its enzymatic degradation. In addition, pre-treatment will increase the surface area of the cellulose thereby enhancing its reactivity with the enzyme and thus its transformation [4;5;12].

Orange Mesocarp was pre-treated at varying alkaline concentrations (0.1M,0.2M, 0.3M,and 0.4M). The orange mesocarp was crushed to 100-150µm, 200-250µm and 300-425µm particle sizes with a blender and was feed into an Erlenmeyer flask,fitted on a hot plate with magnetic stirrer at 100˚C. Hence delignification was effected through the turbulent contact of the orange

mesocarp with the alkaline solution, followed by washing with warm water and dried in an oven of model M250-VF at 50°C for 48 hours to constant dry weight. The dried mesocarp was put in transparent polyethylene bags and kept in the laboratory locker at room temperature until ready for use.

Plate.1a: 0.1M; NaOH Pretreated Orange Mesocarp (100-150)µm

Experimental procedure

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. 2.0g of sodium hydroxide- treated orange mesocarp were added. Samples were withdrawn after every 4 hours within 72 hours reaction time for analysis. The concentration of glucose in the substrates was determined by following the method of Lee [6] which uses a Randox glucose kit and Jenway Colorimeter, (Model 6051,Germany) at 540nm. Each run was repeated three times and the mean value of each set of runs was reported.

RESULTS AND DISCUSSION

The percentage loss in weight after the pre-treatments of the orange mesocarp indicated the extent of cleaning that has taken place in consequence (fig1).

Calcium hydroxide in this respect gave the highest reduction in the weight of the orange mesocarp followed by sodium hydroxide. Raw orange mesocarp contains lignin,hemicelluloses, and cellulose bound together in a cement/block structure[1;13]. The removal of the impurities (lignin and hemicellulose) is the major objective of mercerization. It is worthy of note that there is a correlation between this work and that of Yakubu [4;15], wherein sodium hydroxide was used for cotton fibre pre-treatment, to give an increased pore porosity[11], which resulted to an

optimum dye uptake. From fig 1, it is indicative that calcium hydroxide had a higher capacity of dissolving the lignin content in the mesocarp with 0.1M concentration in 25 minutes hence it is the optimum condition for pre-treatment. Therefore we want to state here, that the concentration of an alkaline solution has effect in the pre-treatment of orange mesocarp.

Effect of percentage loss in weight

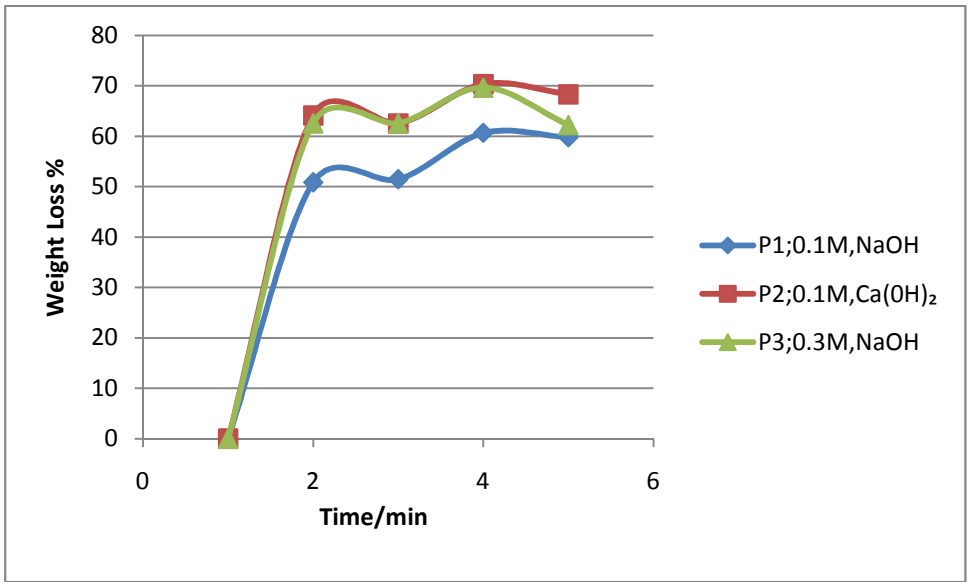


Fig 1.Effect of Concentration on weight Loss

If the concentration is less, it increases the weight loss percent, meaning an increased yield of reducing sugar; because, there is an increase in porosity of the orange mesocarp, resulting to subsequent increase in enzyme binding power with the substrate.

Glucose yield on pre-treated orange mesocarp

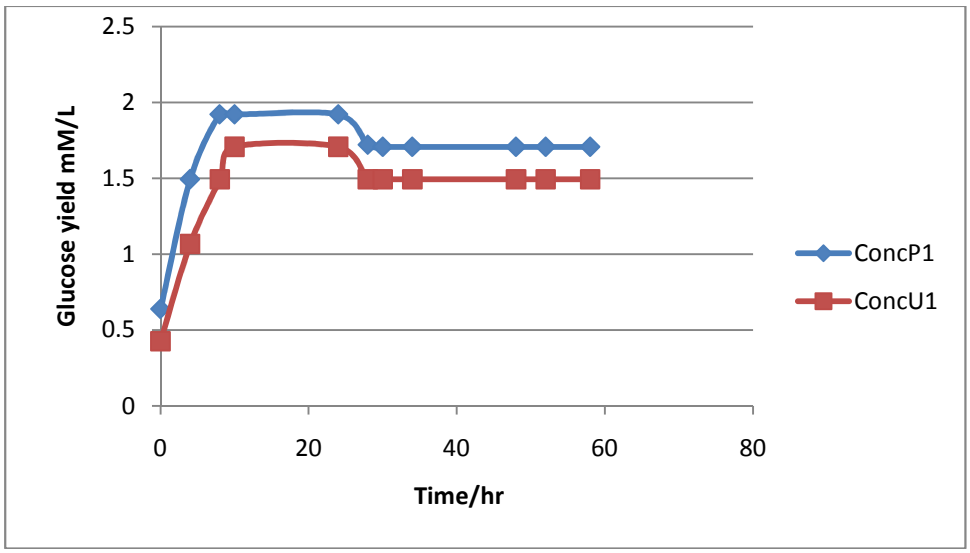


Fig.2 Effect of pre-treatment on glucose yield

Figures 2 shows the yield of glucose between untreteated and pre-treated orange mesocarp. It could be seen that a considerable amount of glucose about 97% was produced from the pretreated O.M,whereas 74% of glucose yield was realised from the untreated one. This yield could be observed within the first 8hours after which there was a constancy of glucose yield no matter the increase in time. The increase in glucose production until the optimum that was obtained was due to the availability of cellulose in the medium; while a decrease in production beyond optimum concentration is explained to be as a result of inhibitory effect of accumulated cellobiose and Cellodextrins [10;14]. It might also be due to the specific binding of the enzymes with the substrates[9,10]).Low glucose production after attaining the maximum limit probably highlights sugar depletion from the substrates into the medium [6; 1;10].Low glucose production may as well be due to the fungi feeding on the glucose produced.

In conclusion, this study reveals that orange mesocarp, which are examples of domestic and industrial agro-waste, when pre-treated in alkaline solutions produced large amounts of glucose when hydrolysed by cellulolytic micro-organism like *Trichoderma reesei.*

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