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Microbial Synthesis of Polyhydric Alcohol by *Saccharomyces cerevisiae*

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Authors' contributions

This work was carried out in collaboration between all authors. Author SOJ designed the study, performed the statistical analysis, wrote the protocol and wrote the first draft of the manuscript. Authors LAO, AAA and OEA managed the analyses of the study. Authors BRI, ANA and IRA managed the literature searches. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aim: Synthesis of polyhydric alcohol from agricultural residue using *Saccharomyces cerevisiae*.
Study of Design: Submerged fermentation process, biomass yield and reducing sugar analysis, non-reducing sugar analysis, extraction and quantification of sugar monomers and polyhydric alcohols.
Place and Duration of Study: Microbiology Unit, Department of Biological Sciences, College of Natural and Applied Sciences, Fountain University Osogbo, Osun State, Nigeria between October 2015 and July 2016.
Methodology: Synthetic route for the production of polyhydric alcohol through sugar reduction under submerged fermentation using *Saccharomyces cerevisiae* MP2 isolated from fermented beverages was investigated. Biomass yield, reducing sugar concentration (DNS method) and non-reducing sugar (Anthrone method) were analysed. The crude extract obtained after fermentation

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period was subjected to derivatisation; thus sugars (reducing and non-reducing sugars) and polyhydric alcohol were quantified using Gas Chromatography Flame Ionization Detector.
Results: Biomass yield and reducing sugar concentration decreased as fermentation period increased. Sugar monomers (ribose, sucrose, xylose, rhamnose, lactose, maltose, glucose, arabinose and fructose) and polyhydric alcohol (glycerol, erythritol, arabitol, sorbitol, xylitol, galactitol, ribitol, maltitol and mannitol) with varying concentrations were obtained. The total sugar monomer concentrations obtained from pineapple peel, banana peel and orange peel media were 1612.25, 1534.79 and 1475.56 mg/100 g with corresponding polyhydric alcohol concentrations of 286.42, 250.71 and 247.94 mg/100 g respectively.
Conclusion: Based on the sugar monomers and polyhydric alcohol profile obtained in the research, *Saccharomyces cerevisiae* MP2 was able to overcome the challenging biological processes such as; delignification in order to release free cellulose and hemicellulose from the lignocellulosic material, depolymerization of the carbohydrate polymers from the cellulose and hemicellulose to generate free sugars, and fermentation of mixed hexose and pentose sugars to finally produce polyhydric alcohol.

Keywords: Erythritol; arabitol; sorbitol; fermentation; Saccharomyces cerevisiae; agricultural residues.

1. INTRODUCTION

Polyhydric alcohol (sugar alcohols, polyalcohol or glycitol) are polyols that possess sugar's carbonyl (aldehyde or ketone) which is reduced to the corresponding primary or secondary hydroxyl group. They had characteristics similar to sugar (white, water-soluble solids) and used to improve the nutritional profile of food products (as thickeners and sweeteners) owing to health-promoting properties such as lower caloric content, noncariogenicity, and low glycemic index and insulin response [1]. Sugar alcohols are usually incompletely absorbed into the bloodstream from the small intestines which eventually leads to slight changes in blood glucose than the regular sugar (sucrose) thus becomes favourite sweeteners among diabetics and people on low carbohydrate diets [2].

Polyols such as sorbitol (E420), mannitol (E421), isomalt (E953), maltitol (E965), lactitol (E966), xylitol (E967), erythritol and hydrogenated starch hydrolysates (HSHs) belong to bulk sweeteners, and consumption does not promote tooth decay [3]. This research aims to synthesise polyhydric alcohol from agricultural residue using local strain of *Saccharomyces cerevisiae*.

2. METHODOLOGY

2.1 Preparation of Fermentation Medium

Agricultural residues ($1\%^{w}/_{v}$) such as pineapple, banana and orange peels were washed and pulverised separately with 100 mL of distilled water using an electric blender. Each fermentation medium comprised of minimal salt medium [Na_2HPO_4 (2.2 g), KH_2PO_4 (1.4 g), $MgSO_4.7H_2O$ (0.6 g), $FeSO_4.7H_2O$ (0.01 g), NaCl (0.05g), $CaCl_2$ (0.02 g), Yeast Extract (0.02 g)], trace element composition [$ZnSO4.7H_2O$ (2.32 g), H_3BO_3 (0.56 g), $CuSO_4.5H_2O$ (1.0 g), $Na_2MnO_4.2H_2O$ (0.39 g), $CoCl_2$.$6H_20$ (0.42 g), EDTA (1.0 g), KI (0.66 g)] and 100 mL of residue filtrate respectively The medium was sterilized, inoculated with *S. cerevisae* MP2; incubated on an orbital shaker at 150 rpm and 30°C for 72 hours.

2.2 Preliminary Screening of Biomass Yield and Reducing Sugar Concentration

Biomass yield of the culture medium was determined using colorimetric method at 650 nm and 24 hours interval. Reducing sugar concentration was determined using dinitrosalicyclic acid (DNS) reagent according to Miller [4]. The absorbance of the crude supernatant obtained from the fermentation medium by centrifugation at 5000 rpm was determined at 540 nm using a spectrophotometer, and the reducing sugar concentration was extrapolated from the glucose standard curve.

2.3 Determination of Non-reducing Sugar Using Anthrone Method

Anthrone method is a colorimetric method for determining the concentration of the total sugars in a sample. Anthrone reagent (2 mL) and sulfuric acid (0.5 mL) were added to 0.5 mL of crude supernatant (filtrate) obtained from the fermentation medium; boiled in water bath at 100°C for 5 minutes until the reaction was

completed, i.e. appearance of blue-green colour [5].

2.4 Confirmatory Analysis of Reducing and Non-reducing Sugar

Sugar (reducing and non-reducing) was analysed using the modified method of Roy [6]. The crude extract (an equivalent of 0.10 g) obtained from the fermentation medium after centrifugation was heated with 50 mL of 80% alcohol. The mixture was centrifuged, and 10 mL of aliquot was heated in boiling water for 4 minutes to inactivate enzymes; thus protein was precipitated and clarified by centrifugation. Starch was removed by centrifuging at 5000 rpm for 15 minutes, and 0.1 mL of the supernatant was transferred into 5 mL vial and dried at room temperature with nitrogen steam.

The extract was derivatised for 2 hours by addition of 0.5 mL of a mixture of pyridine: hexamethyldisilane: trimethylchlorosilane in the ratio of 10:2:1. The derivatised extract was concentrated to 1 mL in the vial for gas chromatography analysis using HP 6890 Powered with HP ChemStation Rev. A09.01 [1206] Software. The gas chromatography conditions for the analysis include Injection temperature (Split injection), Split ration (50:1), Carrier gas (Hydrogen), Flow rate (1.0 mL/min), Inlet temperature (250°C), Column type (BPX70), Column dimension (12 m x 0.32 mm x 0.25 µm), Oven program (Isothermal at 210°C), Detector (FID), Detector temperature (325°C), Hydrogen pressure (25 psi) and Compressed air (35 psi), respectively.

2.5 Derivatisation and Quantitative Analysis of Polyhydric Alcohol

The liquid phase was transferred into 50 mL flask and filled to the peak with ultra-sterile water. Aliquot of this solution was extracted with 80% ethanol; the extract was derivatised to methyl ester within 2 hours. The derivatised extract was concentrated to 1 mL in the vial of gas chromatography analysis and 10 µL was injected into the injector port of the GC.

3. RESULTS

3.1 Reducing Sugar Concentration and Biomass Yield

Reducing sugar concentration increased within 24 hours which later decreased gradually as fermentation period increases. The result corresponds to the biomass yield that also reduced as fermentation period increases (Figs. 1 & 2). Thus pineapple peel medium had the highest biomass yield and decreasing sugar concentration of 0.190 OD and 7.2 mg/mL after 24 hours respectively. The result implies that sugars such as lactose and maltose with a free carbonyl carbon (anomeric carbon) that can undergo oxidation to a carboxyl group by oxidizing agents such as ferric ion (Fe^{3+}) (i.e. capable of reducing ferric ion in DNS reagent) are present in the various fermentation medium.

3.2 Qualitative Analysis of Non-reducing Sugar Using Anthrone Method

Sugars react with the anthrone reagent under acidic conditions to yield a blue-green color. Variability in colors produced by the reaction of the crude extract (pineapple banana and orange media) and anthrone reagent were blue, light green and green respectively. Thus, variation in the blue-green colouration indicated the presences of non-reducing sugars such as sucrose which lack free anomeric carbon atom required for formation of the glycosidic bond.

3.3 Quantification of Sugar and Polyhydric Alcohol

Varying concentrations of reducing sugars (ribose, xylose, rhamnose, lactose, maltose, glucose, arabinose, and fructose), non-reducing sugar (sucrose) and polyhydric alcohols (glycerol, erythritol, arabitol, sorbitol, xylitol, galactitol, ribitol, maltitol and mannitol) were obtained from the crude extracts using gas chromatography flame ionization detector respectively (Tables 1 & 2). Among the agricultural residues utilized as a substrate, pineapple peel had the highest sugar and polyhydric alcohol concentrations of 1612.25 and 286.42 mg/100 g respectively.

4. DISCUSSION

Agricultural residue mainly composed of a lignocellulosic material such as cellulose, hemicelluloses and lignin (lignocellulosic complex). Microbial use of plant biomass is pivotal for life on Earth because it is responsible for large portions of carbon flux in the biosphere. The hydrolysis of lignocellulose to glucose is a major problem in cellulosic production processes [7]. The main mechanism through which yeast and other microorganisms degrade plant

biomass involves production and secretion of carbohydrate-active enzymes acting synergistically in the plant cell wall thus releasing sugar monomers that can be utilized for other purposes [2]. Variation in sugar concentrations (1475.56, 1534.78 and 1612.25 mg/100 g) (Table 1) obtained in this research implies the ability of *S. cerevisiae* MP2 to express genes responsible for synthesis of plant cell wall-degrading enzymes (CWDEs) required for polysaccharide degradation which indicated regulation of genes encoding CWDEs required for synthesis of these enzymes under conditions which the isolate requires plant polymers as carbon source [2].

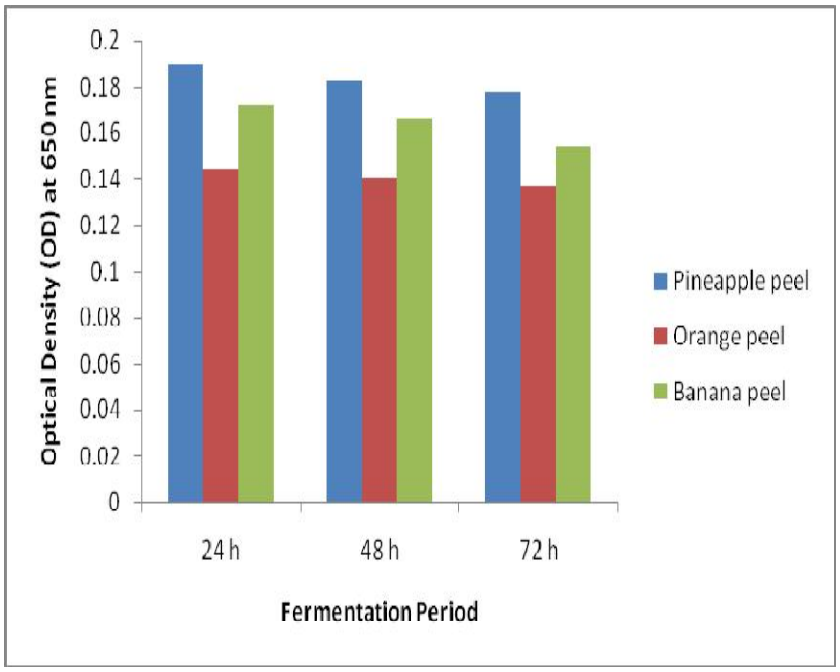

Fig. 1. Biomass yield obtained during fermentation

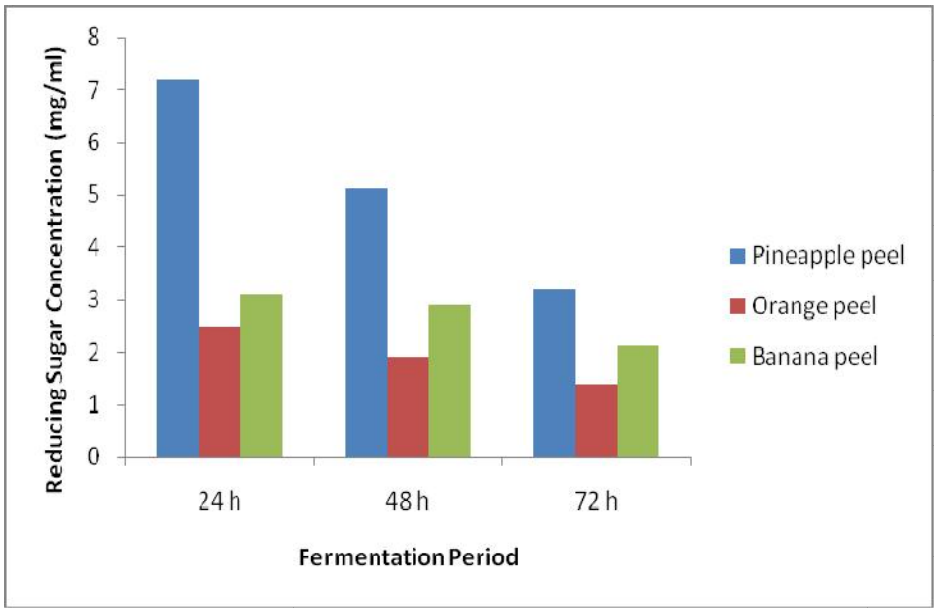

Fig. 2. Reducing sugar concentration during fermentation

Table 1. Quantification of reducing and non-reducing sugars using GCFID

Pineapple peel medium		Banana peel medium		Orange peel medium		Sugar (Reducing and non-reducing)
Retention time [min]	Amount [mg/100 g]	Retention time [min]	Amount [mg/100 g]	Retention time [min]	Amount [mg/100 g]	
8.656	8.07327e-5	8.92.1	1.06712e-5	8.905	2.01699e-4	Ribose
10.842	6.11723e-4	10.652	3.56696e-4	10.842	6.02386e-4	Xylose
12.266	3.96979e-5	12.271	3.96696e-5	12.265	3.96953e-5	Arabinose
14.152	2.48132e-4	14.155	1.12458e-4	14.152	2.32868e-4	Rhamnose
14.924	55.12412	14.925	50.10689	14.923	50.99016	Fructose
16.039	95.84540	16.039	79.78232	16.039	69.54396	Glucose
17.094	31.04530	17.161	27.74937	17.094	28.90268	Maltose
18.356	1.53157e-4	18.356	1.52845e-4	18.355	1.53096e-4	Lactose
19.523	1430.23304	19.521	1377.14733	19.521	1325.12392	Sucrose
Total Sugars	**1612.25**		**1534.79**		**1475.56**	

Table 2. Polyhydric alcohol profile obtained from fermentation medium

Pineapple peel medium		Banana peel medium		Orange peel medium		Polyhydric alcohol
Retention time [min]	Amount [mg/100 g]	Retention time [min]	Amount [mg/100 g]	Retention time [min]	Amount [mg/100 g]	
8.656	4.02824e-4	8.921	6.44232e-4	8.905	1.9867e-3	Glycerol
12.266	4.39484e-5	12.271	4.36191e-5	10.842	4.39189e-5	Erythritol
15.159	1.12968e-2	15.159	1.24771e-3	12.265	9.59160e-3	Arabitol
18.056	49.36611	18.055	31.94517	14.152	35.34955	Sorbitol
19.523	1.43858e-2	19.521	9.27528e-3	14.923	1.33347e-2	Xylitol
20.539	8.52904e-4	20.538	4.98838e-4	16.039	7.73271e-4	Galactitol
21.030	1.52688e-4	21.031	1.52555e-4	17.094	1.52661e-4	Ribitol
21.823	33.39741	21.822	29.58289	18.355	28.23767	Mannitol
22.606	203.62814	22.602	189.16743	19.521	184.32433	Maltitol
Total Polyhydric alcohol	**286.41930**		**250.70734**		**247.93743**	

Polyhydric alcohols are applicable in pharmaceuticals, personal care products and as intermediates in chemical synthesis. Production of sugar polyhols via sugar reduction by *S. cerevisiae* MP2 (Tables 1 & 2) showed the capability of the isolate to undergo enzyme-based processes which are an advantage over traditional industrial production of most sugar alcohols through hydrogenating sugars over nickel catalysts under high temperature and pressure conditions as reported by Schiweck, [8]. Xylitol is a five-carbon sugar alcohol obtained through cofactor imbalance which results in the secretion of xylitol as a xylose fermentation by-product. Presences of xylitol in the fermentation medium shows that *S. cerevisiae* MP2 naturally produce xylitol as an intermediate during D-xylose metabolism which requires the enzymatic activity of xylose reductase (XR), an NADPH-dependent enzyme [1] but the low concentration compared to other sugar polyols present implies that the yeast lacks xylose-specific transporters and is not an efficient xylose-utilizing organism [9].

Mannitol is a six-carbon sugar alcohol with a variety of clinical applications, in addition to its use as a sweetener. Variation in mannitol concentration produced by *S. cerevisiae* MP2 from plant residue is due to its ability to carry out direct reduction of fructose in an NADH dependent reaction catalyzed by mannitol dehydrogenase (MDH) enzyme [10]. Sorbitol (D-glucitol) is a stereoisomer of mannitol which is also applicable in the food industry and as a building block for pharmaceutical products [11]. The presence of sorbitol in the fermentation medium indicated the capability of the isolate to naturally convert fructose and glucose to sorbitol through glucose-fructose oxidoreductase [12].

5. CONCLUSION

This research, based on a qualitative evaluation, showed that local strain of *S. cerevisiae* MP2 could produce a higher yield of polyhydric alcohol due to its ability to adjust the rate of carbon metabolism through a regulatory mechanism by utilizing readily metabolizable carbon sources present in the fermentation medium.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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