

IDENTIFICATION OF AMYLOLYTIC YEASTS AND FERMENTATION OF CASSAVA WASTE WATER FOR BIOMASS PRODUCTION

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ABSTRACT

Two strains of yeast were isolated from cassava waste water using Sabouraud dextrose agar (SDA). Four hundred milliliter of fresh Cassava waste water collected from Cassava grating plant containing 0.04g/ml amount of starch were divided into four portions of 100ml each.. Hundred milliliter of the cassava waste water were each measured into four conical flasks labeled batches A,B,C, and D. The batches were later duplicated and to each fifty milliliter of cassava waste water 0.1g of $MgSO_4 \cdot 7H_2O$, 0.1g of Nacl and 0.1g of KH_2PO_4 were added with different nitrogen sources (1g of peptone, Ammonium phosphate, Sodium nitrate and Yeast extract into batches A,B,C & D respectively). The yeast slurry was made by growing the yeast isolates on SDA plates, incubated for 48hours, washed with distilled water and 1.0ml containing $(2.29 \times 10^8 \text{cfu/ml}$ for isolate A and $2.36 \times 10^8 \text{cfu/ml}$ for isolate B) respectively was transferred aseptically into each of the duplicate batches and labeled. Fermentation was carried out at room temperature by static culture with occasional manual agitation for aeration for four days. Plate count carried out after the fermentation showed an appreciable increase in biomass.

Keywords; Biomass, Yeasts, Cassava, Waste water

INTRODUCTION

Yeast biomass production explores the process of producing increased yeast cells from local substrates of agricultural origin such as corn, cassava, potatoes and rice. For example, corn which was grown previously for livestock feed is being increasingly used to produce high fructose corn-syrup fuel-alcohol and other products such as alcohol, glucose and high fructose syrups [1]. Amylolytic yeasts bring about the breakdown of the starch which is the principal carbohydrate present in the cassava waste water to sugar which they utilize to multiply, thereby resulting in the increase in cell mass. The use of amylolytic yeasts obviates the liquefaction and saccharification steps usually carried out with commercial amylase enzymes which are cost intensive. Amylolytic yeasts isolated from and used in cassava waste water for their biomass production, apart from being used for single cell protein production have recorded immense applications in brewing industries [2].

LITERATURE REVIEW

The use of microorganisms in the preparation of various foods and beverages has long been known from the production of wine, bread, cheese, pickles and sauerkraut. Among these microorganisms yeasts have been extensively studied and also used for the production of biomass for single cell protein. Amylolytic activity is distributed among

bacteria including actinomycetes, fungi, protozoa and algae [3]. Some yeast species such as *Schwanniomyces occidentalis*, *Schwanniomyces castelli*, *Pichia burtonii* and *Saccharomyces distaticus* have been reported to produce amylase [4]. Also debranching enzyme activity has been detected in some strains of these yeast species [4]. Amylase has been used in various industrial processes which include saccharification of starchy adjuncts in brewing, in baking, textile designing and clarification of fruit juices [5]. The type of yeasts employed ranges from the production of baker's yeasts (*Saccharomyces crevisiae*), brewing yeast (*Saccharomyces uvarum* and *Saccharomyces crevisiae*) etc [6]. Of these, the production of Baker's yeast has received the greatest attention. The production of microbial biomass began before world war 1 when Germany was subjected to an economical blockade and lacked imported sources of protein. Max Delbrick and coworkers were the first to recommend the use of Baker's yeast as an additive to the fodder of domestic animals. In Germany, up to 60% of imported protein sources were substituted by dry or wet yeast produced from molasses, a waste from the production of cane sugar [7]. In addition to the yeasts contained in fodder mixtures, they were later added to human food. Cassava (*Manihot esculanta*) also known as tapioca is one of the major tuber crops grown in more than eighty countries of humid tropics [8]. A major portion of the cassava tubers is used in the extraction of starch which requires large quantities of water. This water is being subsequently separated and discharged but being rich in carbohydrates usually possess the problem of disposal into the environment [9].

MATERIALS AND METHODS

Sample source

Fresh cassava waste water was collected from cassava grating plant at Amaenyi, Awka, Anambra State.

Yeast Isolation

Yeasts were isolated from cassava waste water using Sabouraud dextrose agar (SDA) plates containing 0-5mg/ml chloramphenicol solution. Ten fold serial dilution of the cassava waste water was made and 10^{-7} and 10^{-8} dilutions were spread plated. The plates were incubated at room temperature for 48 hours after which the colonies that developed on the plates were subcultured for purification purposes. The isolates were purified by serial sub culturing and maintained on SDA slant at 4°C.

Determination of Amylolytic Activity of the Yeast Isolates

The ability of the yeast isolates to hydrolyse starch was tested in a starch medium of the following composition: 1% of peptone, 1.5% of soluble starch, 0.2% of KH_2PO_4 and 2% agar. Plates were inoculated and incubated at room temperature (28-30°C) for 48 hours. Plates were flooded with iodine solution thereafter and clear zones of hydrolysis indicated amylolytic activity.

Determination of the amount of starch present in the Cassava waste water

This was determined through a calibration curve of soluble starch (0.1g/10ml). These were measured; each dissolved in ten milliliter of distilled water and heated to gelatinize, to one milliliter of each, one milliliter of iodine solution was added and diluted to ten milliliter by adding eight milliliter of distilled water. The optical density of each sample was taken using the spectrophotometer Spectronic 21 and a standard curve of optical density plotted against starch concentration obtained. To one milliliter of the cassava waste water, one milliliter of iodine solution was added and diluted with distilled water. The optical density was obtained as mentioned above and the amount of starch present in the cassava waste water was extrapolated from the standard curve.

Fermentation process

One milliliter each of the isolates was aseptically transferred using sterile pipettes into each of the duplicate batches and fermentation carried out at room temperature by static culture with occasional manual agitation for aeration for four days. At the end of the fermentation, plate count was carried out and the pH and specific gravities of the samples were measured.

Fermentation test on sugar for yeast identification

This was determined with the following sugar; sucrose, galactose, lactose, maltose, D-xylose, trehalose and melibiose, using the method of Burrow (1970) [6].

RESULTS

The colonies on the Sabouraud dextrose agar plates from the duplicate batches were counted to determine the biomass increase. The results of the viable counts, pH and specific gravity of the cultures are shown in table 1.

Table1. Viable Counts, pH and specific gravity of duplicate batches

Isolates	pH	viable counts	Specific gravity
Batch A Isolate A	6.0	2.90 x 10 ⁸ cfu/ml	1.020
Batch B Isolate A	7.0	2.87 x 10 ⁸ cfu/m	1.025
Batch C Isolate A	7.0	2.75 x 10 ⁸ cfu/m	1.025
Batch D Isolate A	6.0	2.63 x 10 ⁸ cfu/m	1.020
Batch A Isolate B	6.0	2.98 x 10 ⁸ cfu/ml	1.015
Batch B Isolate B	6.0	2.70 x 10 ⁸ cfu/m	1.015
Batch C Isolate B	6.0	2.54 x 10 ⁸ cfu/m	1.030
Batch D Isolate B	6.0	2.46 x 10 ⁸ cfu/m	1.015

Key:

Batch A contains peptone as the nitrogen source

Batch B contains ammonium phosphate as the nitrogen source

Batch C contains sodium nitrate as the nitrogen source

Batch D contains yeast extract as the nitrogen source

Batches A,B,C and D contain Magnesium tetraoxosulphate (vi) heptahydrate, sodium chloride and potassium dihydrogen phosphate

Table 2: Morphological and Biochemical Characteristics of the yeast isolates

Colour Melbiose	Elevation Probable	Shape	Sucrose	Galactose	Lactose	Maltose	D-xylose	Trehatode
identity								
Milkfish -	Raised Saccharomyces	Ovoid	-, -	A, -	A, G	A, -	A, -	-, - A,
Species								
Milkish Saccharomyces	Raised	Ovoid	A, -	A, G	-, -	A, -	A, -	-, - A, -
Species								
Key: -shows a negative result to test A shows acid production G shows gas production								

DISCUSSION

The production of yeast biomass from cassava waste water proved a good method for converting carbohydrates into yeast protein. The yeasts being amyolytic were able to utilize the starch present in the cassava waste water which resulted in their cell mass increase. Mixed cultures of these yeasts have been used to produce microbial biomass from other starch materials [9]. The results obtained after the fermentation also suggest that more time is needed for efficient conversion of carbohydrates into microbial biomass since there is still the presence of residual starch in the media after the fermentation. The viable counts obtained at the end of the fermentation compared with initial counts before inoculation showed a reasonable biomass increase.

REFERENCES

1. Wolf, D. (1989). New uses of corn are explained. *Finger Lakes Times*, Jan. 23, Geneva, Newyork.
2. Berghoffer, E. and Sarhaddar, S. (1988). Production of glucose and high fructose syrup by enzymatic direct hydrolysis of Cassava root. *Process Biochemistry*, **23**(6):188-194.
3. Jarl, K. (1969). Symba yeast process. *Food Technology*, **23**:1009-1012.
4. Rose, A.H. (1979). History and Scientific basis of large scale production of microbial biomass. In *Economic Microbiology*, volume 4, PP, 1-29. Academic Press, London.
5. Faith, W.T., Neubeck, C.E. and Reese, E.T. (1971). Production and Application of enzymes. *Advances in Biochemical Engineering*, **1**:72-111.
6. Burrow, S. (1970). The yeast. In *Advances in Applied microbiology*. (Rose, A.H. and Harrison J.S., eds.). Volume 24, PP. 349-420. Academic Press, New York.
7. Philips, T.P. (1989). An overview of cassava consumption and production. In *cassava toxicity and thyroid. Research and public health issues, workshop proceedings*. International development research center (Delange, F. and Ahluwalia, R. eds). 207:83-88.
8. Sills, A.M., Russel, I. and Stewart, G.G. (1984). Production of amyolytic enzymes by *Schwanniomyces castelli*. *Journal of Institution of Brewing*, **89**:406-410.
9. Thanh, N. C. and Wu, J.S. (1975). Treatment of tapioca starch waste water by / Torula yeast. *Canadian Institute Food Science Technology Journal*, **8**:202-205.