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Growth Pattern of *Saccharomyces cerevisiae* in Cassava Mill Effluents

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Abstract

Nigeria is the world leading producer of cassava. During processing of *gari* from cassava tuber large volume of effluents are discharged in the environment which is toxic to the environment and some of its associated biota. This study evaluated the growth pattern of *Saccharomyces cerevisiae* in cassava mill effluents. The *Saccharomyces cerevisiae* was isolated from palm wine following standard microbiological procedure. The *Saccharomyces cerevisiae* was inoculated into the sterile effluents and incubated for 15 days. At every 3days interval, 1ml of the effluents was obtained from the medium and the population density determined. Results of the growth showed that the population of *Saccharomyces cerevisiae* were 0.00 x 10^6 cfu/ml at day 0 (without inoculum), which rose to 2.88 x 10^6 cfu/ml at day 3, 272.67 x 10^6 cfu/ml at day 12 and decline slightly at day 15 (13.57 x 10^6 cfu/ml). There was significant variations ($P<0.05$) among the various period of study. The study showed that the growth of *Saccharomyces cerevisiae* in the effluent was optimum at day 12, then after the density began to decline.

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Introduction

The level of environmental degradation has been on the increasing trend probably due to human activities on the environment and to lesser extent by natural phenomenon [1-5]. The degradation of the environment (e.g air, soil, sediment and water quality) often affect different life forms in such ecosystem. Authors have reported that human activities is having an impacts on the population status of biodiversity including mammals, amphibians, fisheries, reptiles, birds, vegetation/plants among others [6-9].

Several human activities that impact on the soil including municipal solid wastes that are poorly managed in many developing countries [10], food processing effluents such as oil palm processing wastewater [11,12] and cassava mill effluents [1-3,13-21], wastes emanating from markets [22-24], slaughterhouse[25] etc. These wastes stream have the tendency to alter the characteristics of the receiving soil.

Basically Nigeria is the global leading cassava producing countries [14-21,26-30]. The cassava production in Nigeria has exceeded the combined production of second (Thailand) and third (Indonesia) largest cassava production countries in the world. In Nigeria, significant portion of the cassava tuber produced is used to produce *gari* and to lesser extent *fufu, lafun,* animal feeds and other industrial purposes such as adhesives, etc [26].

The cassava mill effluents generated during cassava processing in Nigeria are discharged into the environment with little or no treatment. Studies have shown that the effluents have some level of toxicity on the environment [2,3,14-21] and its associated biota including some domestic animals, plants, fisheries, etc [2]. Several biotechnological advances have shown the various means through which the effluents can be effectively managed [31]. Among the various methods is the production of *Saccharomyces cerevisiae* biomass from the effluents [13]. Studies have also indicated that when cassava mill effluents are treated some of its physicochemical and heavy metals characteristics are improved upon [14,15]. Therefore, this study aimed at assessing the fermentation dynamics of the *Saccharomyces cerevisiae* with regard to the microbial density.

Materials and Methods

Source of Cassava Mill Effluents

Raw cassava mill effluents used in this study were obtained from a smallholder cassava processor in triplicate at Ndemili, Delta state, Nigeria. The samples were transported to the laboratory using ice pack and it was used immediately.

Identification of Saccharomyces cerevisae

The *Saccharomyces cerevisiae* used for the study was isolated from palm wine purchased from a palm wine vendor in Rumuomasi, Port Harcourt, Nigeria. The palm wine was plated following pour plate microbial technique of Benson [32], Pepper and Gerba [33] on a potato dextrose agar containing chloramphenicol. The growths on the agar plate after incubation at room temperature were further streaked in another potato dextrose agar containing chloramphenicol. The resultant isolates was identified using standard microbiological protocol i.e. cultural, morphological, and physiological/ biochemical characteristics including carbon fermentation and assimilation, glucose-peptone-yeast extract broth, growth based on temperature [34-36], staining process using lacto-phenol cotton blue stain and methylene blue indicators [14] and phenotypic characteristics [34,35,37]. The resultant characteristics were compared with the scheme provided by Ellis *et al*. [37], Kurtzman and Fell [34], Iwuagwu and Ugwuanyi [35].

Effluents Preparation

The cassava mill effluents used for the study has pH of 3.93 at day 0 which moved toward alkalinity at day 5 (pH of 4.93), day 10 (pH of 5.33) and day 15 (pH of 6.30) [14]. Similarly the temperature was 27.67 ^{o}C, 27.57 ^{o}C, 27.60 ^{o}C and 27.20 ^{o}C at day 0, 5, 10 and 15 respectively [14]. The effluents was filtered with muslin cloth and then boiled. The boiled effluents were allowed to cool under aseptic condition and then after, 100ml of the effluents were dispensed into conical flask and 10ml of *Saccharomyces cerevisiae* inoculum [14,15] with population of 2.43 x 10^{6}cfu/ml added. The conical flask was covered cotton wool wrapped with aluminum foil paper. The flasks containing the medium were intermittently shaked between 7.00 – 19.00 throughout the study [14,15]. At every 3 day of the 15 days study period (thus growth determination period was 0, 3,6, 9, 12 and 15 days) 1ml of the effluent were pipetted and

plated in potato dextrose agar containing chloramphenicol and incubated for 3-5 days. The resultant *Saccharomyces cerevisiae* density was expressed as coliform forming using per ml (cfu/ml).

Statistical Analysis

SPSS version 20 was used for the statistical analysis. One way and analysis of variance carried out and p=0.05 and Waller-Duncan test statistics used to compare means values of the different days. The chart showing mean and standard deviation values were plotted using Paleontological statistics software package by Hammer et al. [38].

Results and Discussion

The growth pattern of *Saccharomyces cerevisiae* in cassava mill effluents is presented in Figure 1. At the initial day (day 0) the population of *Saccharomyces cerevisiae* were 0.00 x 10^6 cfu/ml, which rose to 2.88 x 10^6 cfu/ml at day 3, 14.50 x 10^6 cfu/ml at day 6, 23.0 x 10^6 cfu/ml at day 9, 272.67 x 10^6 cfu/ml at day 12 and decline slightly at day 15 (13.57 x 10^6 cfu/ml). Typically, there was significant variations (P<0.05) among the various period of study. But Waller-Duncan statistics showed that day 12 had the highest density (272.67 x 10^6 cfu/ml) which was significantly different among other days of study. This growth pattern showed that most of the nutrient in the cassava mill effluents has been utilized. Previous studies have indicated that *Saccharomyces cerevisiae* improves the physical and chemical characteristics of cassava mill effluents [14,15]. Izah et al. [14] reported a decline in total dissolved solid, conductivity, salinity, sulphate, phosphate, chemical oxygen demand, and increase in turbidity during treatment of cassava mill effluents with *Saccharomyces cerevisiae*. Izah et al. [15] also reported that *Saccharomyces cerevisiae* has the tendency to remove heavy metals (such as iron, zinc, copper, manganese) from cassava mill effluent through

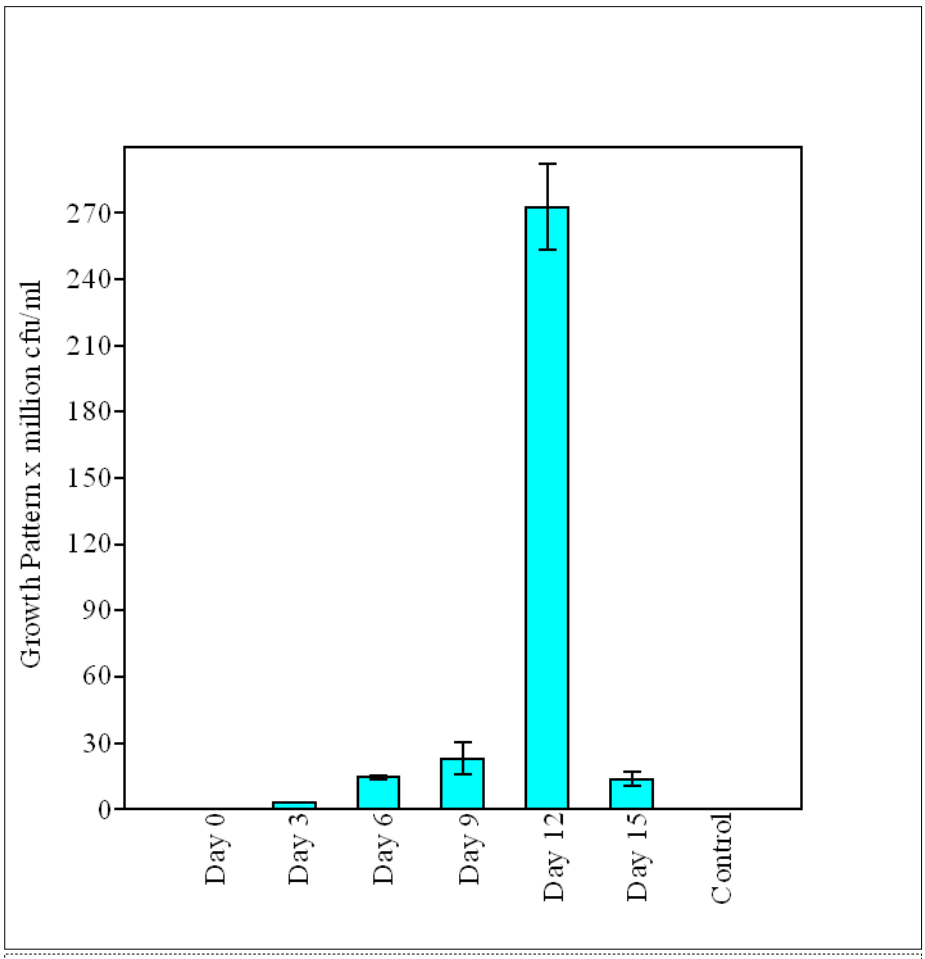

Figure 1. Growth pattern of *Saccharomyces cerevisiae* in cassava mill effluents

biosorption. Furthermore, Iwuagwu and Ugwuanyi [35] also reported that *Saccharomyces cerevisiae* could reduce the chemical oxygen demand and total dissolved solid concentration in palm oil mill effluents. Abioye et al. [39] reported that *Saccharomyces cerevisiae* aid in the degradation of pharmaceutical effluents. Okoduwa *et al*. [36] also reported the potentials of *Saccharomyces cerevisiae* in the treatment of tannery effluents. The nutrient uptake from the effluents suggests the possible increase in population of the *Saccharomyces cerevisiae.* A slight significant decline at day 15 suggest that most nutrients that are useful for the growth of *Saccharomyces cerevisiae* have been used up and therefore the cells requires addition nutrient for growth.

Conclusion

This study showed that during the treatment of cassava mill effluents using *Saccharomyces cerevisiae* the microbial cells increased from day 1 to 12 and then significantly declined at day 15. This suggest that at 12 days of fermentation of cassava mill effluents most of the nutrients that could enhance microbial growth have been used up and/ or the medium becomes toxic to the *Saccharomyces cerevisiae* cells. Therefore, there is the need for further study to focus on the implication of changing *Saccharomyces cerevisiae* population during the treatment of cassava mill effluents so as to prevent the attendant impacts associated with the untreated effluents in the environment particularly soil.

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