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Cyanide and Macro-Nutrients Content of *Saccharomyces cerevisiae* Biomass Cultured in Cassava Mill Effluents

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Abstract: This study investigated the cyanide and some macro nutrient contents of *Saccharomyces cerevisiae* biomass cultured in cassava mill effluents. The *S. cerevisiae* biomass produced was filtered and washed with distilled water and then re-filtered. The resultant sludge/biomass recovered were oven dried. The cyanide and macro nutrient contents were analyzed. The results of the macro nutrients were 331.67 mg/kg (Calcium), 679.30 mg/kg (Magnesium), 22075.30mg/kg (Potassium) and 215.04mg/kg (Sodium). The cyanide content was 0.055mg/100g, being lower than dietary cyanide limits for animal feed. The cyanide and macro nutrient suggests potential suitability of *S. cerevisiae* biomass cultured in cassava mill effluents for animal feed.

Keywords: Animal Feed, Biotechnology, Cyanide, Cassava Mill Effluents

1. Introduction

Wastes are generated from several processing and manufacturing sectors. Wastes produced are mainly solid, liquid (effluents) and gaseous emissions. The type of wastes depends on the processing/manufacturing activities being carried out. According to Chinyere et al. [1], agricultural, industrial, municipal and nuclear wastes are the major class of wastes. Food processing is one of the sectors that generate large waste streams. In developing country like Nigeria, most wastes resulting from food processing are discharged into the environment with little or no treatment especially by smallholder processors. Inadequate management of wastes often led to environmental pollution (viz: soil, water and air).

Nigeria is the world largest producer of cassava [2 – 9]. Cassava processing is a major source of livelihood to several families especially in Southern Nigeria. Cassava processing enterprise is dominated by smallholder accounting for about 80% of the entire sector [4]. During processing, cassava mill effluents, gaseous emissions and solid wastes are generated [3, 10].

Specifically, the liquid wastes are discharge into the processing environment such as soil. It also drains to the nearby pit, drainage system and/ or surface water. Authors have variously reported the effect of cassava mill effluent on the environment (soil and surface water) and the resultant effect on biodiversity such as domestic animals (sheep and goat) [10, 11], fisheries [12, 13] and plant growth and productivity [2, 10, 14-16].

Heavy metals, cyanide, ions viz: cations (such as calcium, potassium, magnesium and sodium) and anions such as phosphate, sulphate, nitrate etc) are some of the chemical constituent of cassava mill effluents. Attempt has been made to treat the effluent using *Saccharomyces cerevisiae* and results showed a decline in some of the heavy metals contents [4]. Studies have also indicated that when *S. cerevisiae* is used for the treatment of industrial effluent some of the physicochemical [5, 17, 18] and heavy metals characteristics are improved [4]. During fermentation of grated cassava, the cyanide content decreases. Authors have variously reported that decline in cyanide content of cassava is a function of time and microbial biomass [19 – 22]. During fermentation, different microbial isolates have varying effect on the cyanide content of cassava [19, 20]. This principle may be applicable to the resultant effluent generated during the processing of cassava tuber into finished products such as *gari*. Previous study work have focused on the heavy metals content of biomass produced during the treatment of cassava mill effluent using *S. cerevisiae* for potential utilization in animal feed [6]. Therefore, this present study aimed at evaluating the cyanide and some macro nutrients composition of *Saccharomyces cerevisiae* biomass produced during the treatment of cassava mill effluents for possible utilization in animal feed.

2. Materials and Methods

2.1. Sample Collection

Triplicate sample of raw cassava mill effluents containing palm oil were collected from a smallholder cassava processor at Ndemili (located at Latitude N06º01' and Longitude E006º 17') in Ndokwa west Local Government Area of Delta state, Nigeria. The samples were transported to the laboratory using ice pack. The samples were used immediately at the laboratory.

2.2. Isolation and Identification of Saccharomyces cerevisae

S. cerevisiae was isolated from palm wine bought from Rumuomasi, Port Harcourt, Nigeria. Pour plate method previously described by Benson [23], Pepper and Gerba [24] were employed in this study. Potato dextrose agar supplemented with chloramphenicol was used during isolation. The resultant isolates were streaked in another agar plate supplemented with chloramphenicol. The isolates was identified based on conventional microbiological techniques (viz: cultural, morphological, and physiological/biochemical characteristics) using carbon fermentation and assimilation, glucose-peptone-yeast extract broth, lacto-phenol cotton blue stain, methylene blue and growth based on temperature as previously described by Kurtzman and Fell [25], Benson [23], APHA [26] and have been applied by Abioye *et al.* [17], Iwuagwu and Ugwuanyi [27], Okoduwa *et al.* [18], Izah et al. [4-6]. The resultant characteristics were compared with the guide provided by Ellis *et al.* [28].

2.3. Culturing of the Biomass

Cultured method previously employed by Abioye *et al.* [17], Okoduwa *et al.* [18] was applied in this study with slight modifications. 100ml of sterile cassava mill effluents were measured into 250ml Erlenmeyer's flask under aseptic condition and 10ml of *S. cerevisiae* inoculum was added into the flask [4 - 6]. Erlenmeyer's flask was capped with cotton wool wrapped with aluminum foil paper. The flasks were shaked intermittently between 7.00 – 19.00 hours interval [4 - 6]. At the end of 15 days experimental period, 60 ml of the medium were decanted into another flask and the remaining 40ml were filtered using Whatman Number 42 filter paper [4 - 6]. The sludge/biomass trapped in the filter paper was washed with distilled water, re-filtered and oven dried. The biomass was persevered in Ziploc bag prior to analysis.

2.4. Analysis of Cyanide and Macro Nutrients (Na, K, Ca and Mg)

Cyanide determination was carried out using spectrophotometric method based on picrate paper techniques previously described by Bradbury *et al.* [29], Egan *et al.* [30]. The biomass was digested and analyzed using flame atomic absorption spectrometery (FAAS) (Model: GBC Avanta PM A6600) for sodium, potassium, calcium and magnesium at varying wavelength.

2.5. Statistical Analysis

SPSS software version 20 was used to carry out the statistical analysis. The results were expressed as mean ± standard deviation.

3. Results and Discussion

The cyanide concentration was 0.055 mg/100g. The low cyanide content could be due to the effect of fermentation. Furthermore, it could be attributed to the fact that cassava mill effluents used in this study contains palm oil, and it was boiled prior to fermentation. Irtwange and Achimba [21] also reported that cassava products such as gari toasted with palm oil contain no cyanide. Uhegbu et al. [31] reported that cooking, frying and boiling reduces cyanide content in cassava products. According to Gunawan *et al.* [19], laminarin and lotaustralin cassava's cyanogenic compounds are transformed to hydrocyanic acid by the action of the laminarase enzyme when cassava tuber is sliced. Authors have variously reported that during microbial fermentation cyanide content of cassava is reduced [19, 21, 22]. In addition, the effect of linamarase enzyme on linamarin and lotaustralin is the hydrolytic release of acetone cyanohydrin and 2-butanone which is unstable [21]. Babalola [22] reported that fermentation enhanced detoxification of cassava by releasing hydrocyanide.

Different microbes and time interval have varying effect on the cyanide content of cassava. For instance, Gunawan *et al.* [19] reported cyanide content of 7.50mg/kg declined to 1.80 mg/kg (using *Lactobacillus plantarum*), 7.60 mg/kg declined to 3.26mg/kg (using *S. cerevisiae*) and 8.10mg/kg reduced to 3.17mg/kg (using *Rhizopus oryzae*) in fermented cassava between 24 to 120 hours. Irtwange and Achimba [21] reported a decline in cyanide content from 12.67mg/kg in 0 day to 3.82mg/kg after 5 days in cassava tuber.

The cyanide content in this study is lower than the concentration of previously work in fermented cassava products by Babalola [22] that reported cyanide content in the range of 2.10 – 15.30 mg/kg in cassava product (gari) sold in different areas of Ekiti state, Nigeria. But comparable to the value of 0.5mg/kg reported in cassava fermented with *S. cerevisiae* [32]. Typically, variation among the varying cyanide content could be due microbial and fermentation time ratio [19]. Etsuyankpa *et al.* [20] reported that *S. cerevisae* had greater ability to detoxify the cassava products by about 40% than the *Lactoacillus bulgaricus*.

The cyanide content was far lower in this study than the recommended dietary cyanogens content of 100mgHCN equivalent/kg for cassava ration recommended for both ruminated and non-ruminant as reported by Okafor and Nwabuko [33]. The cyanide content in this study was also lower than the values of 59.3 – 361.90mg/dry matter reported in livestock feeds sold in Umuahia, Nigeria [33].

The macronutrients under study in comparison to the values reported in some plants and dietary requirement in some domesticated animals in presented in Table 1. The study found that calcium, magnesium, potassium and sodium content were 331.67mg/kg, 679.30mg/kg, 22075mg/kg and 215.04mg/kg respectively. Apart from potassium concentration, the macro nutrients was lower than the requirement level recommend for domestic animal as specified by Blackwood and Duddy [34], Judson et al. [35] (Table 1).

The macronutrient produced revealed that potassium is the largest among the macro nutrients under study. Thus, the nutrient were in the order; sodium< calcium <magnesium < potassium (Figure 1). This trend is not in conformity with the work of other authors on different edible parts of plant [36, 37] but comparable to the work of Umar et al. [38] on the seed of *Citrullus ecirrhosus* (wild melon) (Table 1). Furthermore, some of macro nutrient had some similarity with the values previously reported in edible part of some plants (Table 1). The variations that exist in the macro nutrients under study compare to some works on plants could be due to differences in their biochemical composition of the substrates/feedstock. The findings of this study revealed that macro nutrients concentration of the biomass produced is high to promote the role of these nutrients in the domestic animals.

Table 1. *Statistical analysis of heavy metals in produced S. cerevisiae biomass in comparison to Nutritional requirement for some animals and result in some edible part (fruit/seed) of plants.*

Heavy metals	**Produced *S. cerevisiae* biomass (Mean ± Standard deviation)**	****Freer et al. [39] cited by Blackwood and Duddy [34], Judson et al. [35]**		***Okwu [36]**	
		Domesticated ruminants		**Seed of *Garcinia kola***	**Fruit of *Synespalum dulcifcum* (miracle fruit)**
		Sheep	**Cattle**	-	-
Calcium, mg/kg	331.67±28.97	1.4 – 7.0	2.0 – 11.0	1.80	0.26
Magnesium, mg/kg	679.30±78.52	0.9 – 1.2	1.3 – 2.2	0.42	0.33
Potassium, mg/kg	22075.30±3596.90	5.0	5.0	2.50	0.65
Sodium, mg/kg	215.04±29.43	0.7 – 1.0	0.8 – 1.2	0.72	0.45

Table 1. *Continued.*

Heavy metals	**Produced *S. cerevisiae* biomass (Mean ± Standard deviation)**	***Umar et al. [38]**	**Nkwocha, [40]**	***Hassan et al. [37]**
		Seed of *Citrullus ecirrhosus* (wild melon)	***Synespalum dulcifcum* (miracle fruit)**	**Seed of *Annonas squamosa* (sugar apple)**
		-	-	-
Calcium, mg/kg	331.67±28.97	38.45	100	650.00
Magnesium, mg/kg	679.30±78.52	1315.50	-	50.00
Potassium, mg/kg	22075.30±3596.90	2962.00	-	22.00
Sodium, mg/kg	215.04±29.43	23.15	-	30.00

Data expressed as mean ± standard deviation; * expressed data in mg/100g; ** g/kg.

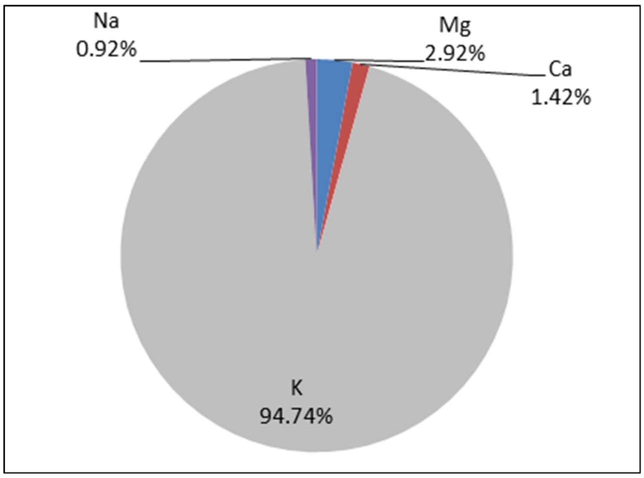

Figure 1. *Distribution of macronutrient in the S. cerevisiae biomass produced.*

For instance, macronutrients such as calcium, sodium, potassium and magnesium play essential role in living organisms. Specifically, sodium ions are vital for animals. This could be due to their role in the generation of nerve impulses and maintenance of electrolyte and fluid balance [41]. In animals, they aid in the maintenance of some organs and also involved actively in some metabolic functions.

Potassium helps in promoting alkaline environment, thereby reducing the demand for skeletal salts to balance the endogenous acid generated from acid-producing foods [42]. According to Palacios [42], potassium is indirectly involved in the maintenance normal pH and prevention of osteoporosis. Like chloride and sodium, potassium is essential for both cellular and electrical function [43]. Potassium also play essential role in energy metabolism (such as carbohydrate metabolism), synthesis of protein from amino acids in the cell and cellular biochemical reactions [43].

Calcium play essential role in bone formation. According to Palacios [42], Blackwood and Duddy [34], 99% of the calcium found in the body is located in the skeleton. Calcium is also essential for the normal functioning of the nerves, muscle contraction, blood clotting, and activation of some enzymes. Furthermore, low calcium content could lead to milk fever, lethargy, weakness bones and poor growth [34].

Like calcium, magnesium is essential for the body. Magnesium helps in the maintenance of the potassium in the cells [43]. Palacios [42], Blackwood and Duddy [34] reported that about 60 - 70% of the magnesium in the body is located in the bone/skeleton. Magnesium play essential role in ATP metabolism, and it's a cofactor for over 300 enzymes thereby indirectly involved in mineral metabolism [34, 42] and other biochemical processes involving carbohydrate, protein and lipids [34]. In animals, excess dietary magnesium intake could damage rumen causing scouring, reduced feed intake and lethargy [34].

4. Conclusion

This study evaluated the cyanide and macro nutrients (calcium, potassium, sodium and magnesium) content of *S. cerevisiae* biomass cultivated from cassava mill effluents. The results showed that cyanide is below the maximum dietary intake recommend for animal feed. While macronutrients were lower in this study compare to the recommended limit specified for animal feed except for potassium. But the concentration of the macro nutrients such as calcium, magnesium and sodium could also contribute to the role of these nutrients in animals.

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