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EC MICROBIOLOGY
Research Article

Assessment of Some Selected Heavy Metals in *Saccharomyces cerevisiae* Biomass Produced from Cassava Mill Effluents

Sylvester Chibueze Izah*, Sunday Etim Bassey and Elijah Ige Ohimain

Department of Biological Sciences, Faculty of Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria

***Corresponding Author:** Sylvester Chibueze Izah, Department of Biological Sciences, Faculty of Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria.

Received: September 11, 2017; **Published:** October 13, 2017

Abstract

Cassava mill effluents are discharged into the environment in cassava producing country like Nigeria without proper treatment. It has adverse effects on the receiving ecosystem and the biota found in such environment. This study evaluated the yeast biomass produced from cassava mill effluents for possible utilization for animal feed with regard to heavy metal concentration. *Saccharomyces cerevisiae* (identified using cultural, morphological, and physiological/biochemical characteristics) was inoculated into sterile cassava mill effluents filtered with double muslin cloth. 10 ml of *S. cerevisiae* broth was inoculated into 100 ml of sterile cassava mill effluents. The medium was shaked intermittently between 7.00 to 19.00 hours intervals. After 15 days of incubation at room temperature, the medium was decanted and subsequently filtered using Whatman filter paper 41. The results sludge/biomass was oven dried, digested and analyzed using flame atomic adsorption spectrometry. Results showed iron 63.780 ± 12.080 mg/kg, zinc 19.970 ± 4.005 mg/kg, copper 8.970 ± 1.936 mg/kg, manganese 28.870 ± 7.087 mg/kg, lead, cadmium, cobalt, nickel and chromium were < 0.001 ± 0.000. The produced biomass had lesser heavy metals concentration compared to the mineral tolerance level for domestic animals specified by National Research Council, but close to the limits of nutritional requirements for domesticated ruminant animals. Based on the concentration of heavy metals results of this study, biomass produced from cassava mill effluents using *S. cerevisiae* can be supplemented with other nutrient and be used as animal feed.

Keywords: *Animal Feed; Biotechnological Advances; Cassava Mill Effluent; Saccharomyces cerevisiae*

Introduction

Pollution issues are one of the major challenges confronting environmental sustainability. Environmental pollution is majorly caused by anthropogenic activities and to a lesser extent natural effects [1-3]. Several human activities resulting from economic activities (such as marketing), unsustainable waste disposal, industrial and agricultural activities (resulting from use of pesticides i.e. herbicides, fertilizers, and food processing) often release emission into the environment (soil, water and air). Some of the emissions often affect the environment and its associated biota.

In a developing country like Nigeria, some of the food processing industries that generate large wastes stream include oil palm and cassava processing. Specifically, Nigeria is the largest producer of cassava in the world [4,5] accounting for over 20% of global output. Cassava processing in Nigeria is predominantly carried out by smallholders in Nigeria. During processing large volume of water is generated from the dewatering zone and are discharged into the environment (soil which then percolates and/or may drain into pits or surface water in mills located close to the aquatic ecosystem). These effluents contain several constitutes including high chemical oxygen demand, heavy metals, total solid, anions (sulphate, nitrate, phosphate), cations (potassium, magnesium, sodium and calcium) etc.

Heavy metals have been widely reported in cassava mill effluents [6-10]. Typically, heavy metals are naturally occurring metals that have a high atomic weight and a density at least 5 times greater than that of water [11-14]. Heavy metals are typically classified into essential (elements required by living thing at certain concentration e.g. chromium, iron, copper, zinc, manganese etc) and non-essential (not required by living organisms even at low concentration e.g. cadmium, lead, arsenic, mercury etc). According to Tchounwou., *et al*. [14], heavy metals toxicity depends on some factors such as dose, route of exposure, and chemical species, age, gender, genetics, and nutritional status of the individuals exposed to the metal laden environment and/ or food. Heavy metals are highly toxic to parts of human body such as nerves, liver, kidney and bones, and also block functional groups of vital enzymes [15].

Authors have variously reported that cassava mill effluent could alter the characteristics of the receiving environment (soil and water). Some of the notable characteristics that the effluents affect include chemical, physical and microbiological parameters [9,16-31]. The effluents contain substances that are highly lethal, mobile in soil, and has the tendency to affects biodiversity such as marine lives, benthic macro-invertebrates, fisheries, microbes, plants [9], man, domestic animals (goat and sheep), fauna and flora [22].

Several studies have been carried out on biotechnological advances on the management of cassava mill effluents including bioethanol [4,32-34], biogas [35-39], bioelectricity using microbial fuel cells technology [40-45], enzymes such as amylase [46,47], protease [48] and cellulose production [46].

Saccharomyces cerevisiae biomass has found application in several sectors including food industry (bakery, brewery), probiotic in humans [49], bioremediation/biodegradation via biosorption and immobilization [50-55]. It's also used as leavening agent in bread production [56]. Bread is consumed extensively in homes, restaurants and hotels [57] irrespective of age, sex, race or religion [58]. *S. cerevisiae* also has useful role in animal diets [59-61]. For instance, Trckova., *et al*. [60] reported that dietary supplementation with live yeast (*S. cerevisiae*) to sows and piglets in the late gestation, suckling, and postweaning periods could lead to reduction in the duration and severity of postweaning diarrhea caused by enterotoxigenic *Escherichia coli*. Bruno., *et al*. [61] reported that dairy cow diets supplemented with yeast culture could enhance lactation performance (by increasing yields of milk and of solids-not-fat) when the cow is exposed to heat stress condition. *S. cerevisiae* biomass contains other nutrients such as heavy metals, vitamins, cations etc. When the concentration of heavy metal is high in the biomass it could pose health risk to animals if the biomass is to be used for animal feed or indirectly when human consumed animal products fed with heavy metal laden feed. Therefore this study is aimed at assessing heavy metals concentration in biomass produced during the treatment of cassava mill effluents using *S. cerevisiae* with intention of possible utilization in animal feed.

Materials and Methods

Sample collection

Untreated Cassava mill effluents were collected in triplicate from smallholder cassava processor using manual techniques at Ndemili in Ndokwa west Local Government Area of Delta state, Nigeria. 4 litres clean container was used to collect the sample. The samples were transported to the laboratory using ice pack. The samples were used immediately at the laboratory.

Isolation and Identification of *Saccharomyces cerevisiae* used for the study

The *S. cerevisiae* used in this study was isolated from palm wine bought from Rumoumasi, Port Harcourt, Nigeria. Pure culture of the isolate was obtained following pour plate method previously described by Pepper and Gerba [62], Benson [63] and subsequently streaking in potatoes dextrose agar supplemented with chloramphenicol. The resultant isolate was identified using conventional microbiological techniques based on their cultural, morphological, and physiological/biochemical characteristics (viz: using carbon fermentation and assimilation techniques, glucose-peptone-yeast extract broth, lacto-phenol cotton blue stain and growth based on temperature) as described by Kurtzman and Fell [64], APHA [65], Benson [63] and have been applied by Iwuagwu and Ugwuanyi [66], Abioye., *et al*. [67], Okoduwa., *et al* [68]. The resultant characteristics were compared with the guide provided by Ellis., *et al* [69].

Growth of Yeast biomass

The yeast was grown based on the method previously described by Abioye., *et al*. [67], Okoduwa., *et al*. [68] with slight modifications. Triplicate 100 ml of the prepared cassava mill effluents were measured into 250 ml Erlenmeyer's flask under aseptic condition and 10 ml of *Saccharomyces cerevisiae* inoculum was added into the flask. The flask was capped with cotton wool wrapped with aluminum foil paper. The flasks were shaked every 30 minutes between 7.00 - 19.00 hours daily. After 15 days of incubation, 60 ml of the medium were decanted into another flask and the remaining 40 ml were filtered using Whatman Number 41 filter paper. The resultant biomass was washed with distilled water and filtered again and oven dried. The resultant biomass was analyzed for heavy metals.

Biomass preparation and Heavy metal analysis

The biomass was oven dried (Model: Memmert U27) at 70oC. Then 2g of dried samples were placed into clean porcelain crucibles and dry-ashed in a muffle furnace (Oceanic SX-2 type) at 450oC until the samples were grayish-ash. The ashed samples were allowed to cool in a dessicator. 5 ml of mixture of 1N nitric acid (HNO3) and 10 ml of 1N hydrochloric acid (HCl) was added to the ashed samples to form a solution. A reagent blank containing acid mixtures used was prepared. Flame atomic absorption spectrometry (FAAS) (GBC Avanta PM A6600) was calibrated with prepared working solutions from stock solutions (AccuStandards, 1,000 mg/l). The samples and reagent were aspirated into the FAAS for each of the respective metals to be analyzed. The heavy metals were analyzed at varying wavelength of 213.9 nm, 324.70 nm, 232.0 nm, 248.3 nm, 279.5 nm, 357.90 nm, 228.8 nm, 217.00 nm and 240.70 nm for zinc, copper, nickel, iron, manganese, chromium, cadmium, lead and cobalt respectively.

Statistical Analysis

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

Results and Discussion

The concentration of heavy metal in *S. cerevisiae* biomass produced from cassava mill effluents and nutritional requirement for some domestic animal limit established for cattle and sheep is presented in table 1. The limits for cattle by National Research Council (NRC) have been applied for other animals such as poultry [70]. This was also adopted in this study. The concentration of cadmium, lead, chromium, nickel and cobalt were below detectable limits i.e. < 0.001 mg/kg in the produced yeast biomass. Typically, cadmium and lead are non-essential nutrient; its occurrence in food resources and/or biodiversity is an indication of toxicity even at low concentration. Therefore, in this study the absence of these non-essential elements suggests that they are free from their toxicity.

Heavy metals	Produced *S. cerevisiae* biomass (Mean ± Standard deviation)	[77] cited by [78]		[75] as cited by [76] , [70]		
		Domesticated ruminants		Cattle		
		Sheep	Cattle	Minerals maximum tolerable level	Health Dietary rating	Excessive exposure rating
Iron, mg/kg	63.780 ± 12.080	40	40	500 ppm	Medium	Possible
Zinc, mg/kg	19.970 ± 4.005	9 -20	9 - 20	500 ppm	Medium	Possible
Copper, mg/kg	8.970 ± 1.936	4-14	4-14	250 ppm	Low	Possible
Manganese, mg/kg	28.870 ± 7.087	20 - 25	20 - 25	2000 ppm	Low	Possible
Lead, mg/kg	<0.001 ± 0.000	-	-	10 ppm	Low	Rear
Cadmium, mg/kg	<0.001 ± 0.000	-	-	10 ppm	High	Rear
Chromium, mg/kg	<0.001 ± 0.000	-	-	500 ppm	Low	Rear
Cobalt, mg/kg	<0.001 ± 0.000	0.11-0.15	0.07 - 0.15	25 ppm	Low	Possible
Nickel, mg/kg	<0.001 ± 0.000	-	-	250 ppm	Low	Rear

Table 1: *Statistical analysis of heavy metals in produced S. cerevisiae biomass in comparison to Nutritional requirement for some animals.*

Furthermore, cobalt and chromium is essential elements that are needed by living things such as animals at certain concentration. The absence of these elements (cobalt and chromium) in the produced yeast biomass suggests that they are not present in the *S. cerevisiae* and/or effluents. Typically, *S. cerevisiae* have biosorption capacity for heavy metals. Authors have variously reported that heavy metals can be removed from environment viz: soil and effluents using *S. cerevisiae* [15,50-55,71,72]. The biosorption ability of *S. cerevisiae* depends on the characteristics of the metal ions (radius of ion, valence, etc.) in aqueous solution, age of the microbial cell, growth conditions (such as carbon source, nutrition supply, composition of growth media, etc.), biosorption conditions (viz: pH, temperature, contact time, co-ions in solution, initial concentration of metal and biomass, availability of metal ions and micro-nutrition) [52].

Some of the role of these trace essential metals present in the yeast biomass includes biosynthesis of glucose tolerance factor (chromium) and methionine metabolism (cobalt) [11,73]. Nickel has been also been considered as a possibly essential trace mineral [74]. The concentration of these heavy metals (cadmium, lead, chromium, nickel and cobalt) was lower than the limit specified by NRC [75] as cited by Weiss [76], Imran., *et al*. [70], and Freer., *et al*. [77] cited by Blackwood and Duddy [78]. This is an indication that if the biomass is used for animal feed additional source of nickel, chromium and cobalt is needed with regard to heavy metal requirement. The findings of this study is contrary to the results of previous works that reported presences of these heavy metals in variety of poultry feed in Bangladesh [79], Eastern Nigeria [80] and Pakistan [70].

In the produced *S. cerevisiae* biomass iron concentration were 63.780 mg/kg. The concentration of iron in the produced *S. cerevisiae* biomass were lesser compared to the limits for animal feed as specified by [75] as cited by [70, 76] and higher than the limit of 40 mg/kg as specified by [77] cited by [78] (Table 1). Furthermore, the iron content in the biomass is lower than the value range of 76 - 116.1 ppm reported in poultry feeds (starter, grower, finisher, feed premix and crude protein) in Pakistan [70] and within the range of 50.575 - 170.075 mg/kg in some other types/variety of poultry feed in Eastern Nigeria [80]. This suggests that the conventional poultry feed has similar iron content compared to the biomass produced. Iron plays several biochemical/ metabolic processes such as oxygen transport, deoxyribonucleic acid synthesis, electron transport chain and regulation of cell growth and differentiation in animals [11,81-83]. The low concentration compared to standard could lead to impairment in the normal function of iron in the animals that may consume such product. Therefore, if the biomass is to be used for animal feed, additional source of iron is required.

The concentration of zinc in produced *S. cerevisiae* biomass was 19.970 mg/kg. Again, zinc concentration in the produced *S. cerevisiae* biomass was lower compared to the limits for animal feed as specified by [75] as cited by [70,76] and within the range of 9 - 20 mg/kg specified by [77] cited by [78] (Table 1). However, the zinc level in the biomass is lesser than the value of poultry feeds from different countries which ranged of 23.6 - 50.2 ppm in Pakistan [70], 34.038 - 49.950 mg/kg in Eastern Nigeria [80], but within the range of 0.0232 - 422.3023 ppm reported in Bangladesh [79]. This is an indication that poultry feed had superior zinc content compared to the biomass produced. Zinc is essential metal that play several role in living organisms including wound healing, cell growth, development, differentiation, homeostasis, connective tissue growth and maintenance, DNA synthesis, RNA transcription, cell division, cell activation, and regulatory, catalytic, co-catalytic and structural roles in enzyme molecules [11,84,85]. Specifically, in livestock zinc are needed for some enzymes to function which play essential role growth and reproduction [80] and carbohydrate metabolism and protein synthesis [78]. As such, its low level could lead to bone formation impairment and affect normal growth and development [70].

The concentration of copper in produced *S. cerevisiae* biomass was 8.970 mg/kg. Copper level in the produced *S. cerevisiae* biomass was lower compared to the limits for animal feed as specified by [75] as cited by [70, 76] and within the range of 4 - 14 mg/kg specified by [77] cited by [78], but higher than the value range of 1.22 - 5.98 ppm reported in poultry feed in Pakistan [70], and fall within the range of 6.52 - 14.20 mg/kg reported in some brand of poultry feed in Eastern Nigeria [80], 0.0463 - 37.5725 ppm in Bangladesh [79]. This is an indication that copper from cassava mill effluents had comparable quality compared to the conventional poultry feed. Like in human, copper play essential role in animals especially poultry products. The mean value in study is within the nutritional requirement level (8 ppm) for broiler chicken [70]. This value suggested that if poultry product consumes the biomass, they may likely not to suffer from copper deficiency that could affect healthy growth. Typically, copper is an anti-oxidant and play essential role in preventing cell structure damage by removing the free radicals [86,87]. Copper also play essential role in bone formation, skeletal mineralization, and the integrity of the connective tissue [78,87,88]. According to Palacios [88], copper-containing enzyme such as Lysyl oxidase also play important role in the cross-linking of collagen fibrils, thereby enhancing the mechanical strength of the protein and forming strong, flexible connective tissue. Specifically, copper in livestock is required for the proper functioning of certain enzymes that influences growth and reproduction [80].

Manganese level in produced *S. cerevisiae* biomass was 28.870 mg/kg. This value in this study is far lesser than the limits for animal feed as specified by [75] as cited by [70,76], but higher than the limit of 9 - 20 mg/kg specified by [77] cited by [78]. However, the manganese concentration in the biomass is within the values widely reported for variety of poultry feed in different countries including Eastern Nigeria (26.913 to 76.738 mg/kg) [80], 0.0695 - 302.201 ppm in Bangladesh [79] and far higher than the range of 0.524 - 0.97 ppm reported in Pakistan [70]. Like copper, manganese had superior quality compared to conventional poultry feed with regard to heavy metal concentration. Unlike copper, manganese concentration in the produced biomass is far lower than nutrient requirement level (60 ppm) for broiler chicken [70]. Palacios [88] reported that manganese is required for the biosynthesis of mucopolysaccharides in bone matrix formation. The author further reported that manganese is also a cofactor for many enzymes in the bone tissue. Prashanth., *et al*. [73], Izah., *et al*. [11] also reported that manganese is essential for the activation of enzyme and as a component of metalloenzymes. Furthermore, deficiency of manganese could inhibit proper growth. Therefore additional source of manganese need to be added if the biomass is to be used for animal feed so as to enhance normal growth.

Since the detected heavy metals (iron, zinc, copper and manganese) concentration in this study were below mineral tolerance level as specified by [75] for animal feed, therefore biomass/residue from cassava mill effluents treated with *S. cerevisiae* may not produce unsafe residue among the final products. These detected mineral elements viz: iron, manganese, copper and zinc have been reported to be essential dietary nutrients for poultry and livestock [70, 79, 80]. The trend of this study having low concentration of zinc, iron, manganese and copper is in line with the findings of Okoye., *et al*. [80] that reported low concentration of this metals in poultry feed (layer, finisher, grower and starter).

Figure 1 present the distribution of heavy metals under study in the biomass produced. The heavy metals were in the order: lead = cadmium = nickel = chromium = cobalt < copper < zinc < manganese < iron. The trend reported in the detected heavy metals is in agreement with the findings of Okoye., *et al*. [80] on heavy metals level in poultry feed. This is an indication that iron accounts for large amount of heavy metals found in the biomass. It also had some similarity with the work of Imran., *et al*. [70] that reported heavy metals in the order; iron > zinc> nickel > lead > copper > chromium > manganese > cadmium in poultry feed in Pakistan. This trend of iron accounting for higher concentration among heavy metals have widely reported in several environmental and food samples including poultry feed [70], vegetables [89], cassava mill effluents [6,8,9], ready to eat snacks [87]. This suggests that abundance of iron in the environment and its role in biodiversity.

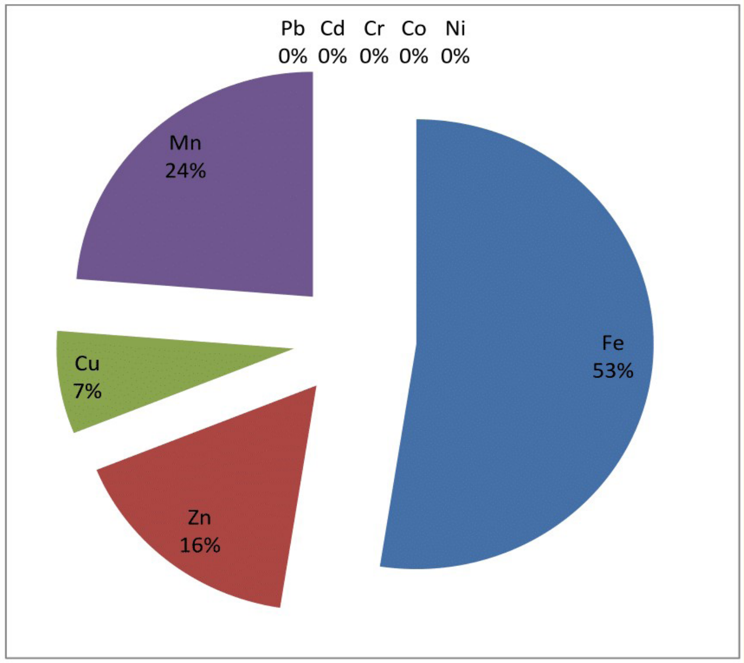

Figure 1: *Heavy metal distribution in yeast biomass produced from cassava mill effluents.*

Conclusion

Cassava mill effluents typically cause adverse effects on the receiving environment (soil and surface water). This study investigated the heavy metals concentration in yeast biomass produced from cassava mill effluents for potential utilization in animal feed industry. The study found that heavy metals such as cadmium, chromium, lead, nickel and cobalt are not present in the yeast biomass. Furthermore, zinc, iron, copper and manganese concentration were lower in the produced yeast biomass compared to the limits specified by NRC, and close the limits allowed in animal feed based on the Nutrient requirements for domesticated ruminants animals. Therefore, if the biomass is to be incorporated into animal feed, additional source of chromium, zinc, iron, copper, manganese, nickel and cobalt is required with regard to the heavy metals content.

Acknowledgement

This paper is based on part of PhD project work of S.C. Izah supervised by Dr S.E. Bassey and Prof. E.I. Ohimain at the Niger Delta University, Wilberforce Island, Nigeria. The authors wish to thank Dr. B.O. Eledo of Madonna University, Nigeria for proofreading this manuscript.

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