

Removal of heavy metals in cassava mill effluents by *saccharomyces cerevisiae* isolated from palm wine

Abstract

Nigeria is World leading producer of cassava account for 20% of global output. Rudimentary equipment is used for cassava processing into several products such as high quality cassava flour by smallholders that dominate the cassava processing sector in Nigeria. The properties of the wastewater, also called cassava mill effluents, often exceed the limit for effluents discharge onto environment (land and surface water) as specified by Federal Environmental Protection Agency. Cassava mill effluents induce toxicological effects on the environment and its associated biota such as fisheries, flora and fauna as well as humans. This study evaluated the capacity of *Saccharomyces cerevisiae* isolated from palm wine to remove heavy metals in cassava mill effluents. The *S. cerevisiae* was identified using conventional microbiological techniques based on their cultural, morphological and physiological/biochemical characteristics. *S. cerevisiae* was inoculated into the sterile effluent and incubated for 15 days and triplicate sample were withdrawn at 5 days interval for analysis. The samples were prepared and analyzed using flame atomic adsorption spectrometry. Results showed a decline by 44.52%, 26.26%, 51.54%, 43.20% and 65.19% for copper, zinc, manganese, iron and nickel respectively. The findings of this study showed that *S. cerevisiae* is a potential microorganism for the remediation of heavy metals in cassava mill effluents.

Keywords: cassava mill effluents, environmental pollutants, heavy metals removal, *saccharomyces cerevisiae*

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Sylvester Chibueze Izah, Sunday Etim Bassey, Elijah Ige Ohimain

Department of Biological Sciences, Niger Delta University, Nigeria

Correspondence: Sylvester Chibueze Izah, Department of Biological Sciences, Faculty of Science, Niger Delta University, Wilberforce Island, Bayelsa State, Nigeria, Tel +234 703 0192 466, Email chivestizah@gmail.com

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Introduction

Heavy metals are metalloids whose density is about 5 times denser than that of water.^{1,2} Heavy metals enter the environment from natural effects (such as seepage from rocks and volcanic activity)³ and by large extent anthropogenic activities.^{1,4-8} Some of the human activities leading to heavy metal in the environment include discharge of untreated or partially treated industrial wastes including sewage, drugs, oil, heavy metals, paints, pesticides and various chemical compounds into the environment especially aquatic ecosystem.⁹ These heavy metals lead to environmental pollution.^{9,10} Some of the heavy metals that have high environmental toxicity and health effects include lead, mercury, chromium, cadmium, copper, cadmium, nickel and zinc.³ Worst still, heavy metals are recalcitrant to degradation and as such are a threat to biodiversity in heavy metal laden environment.^{8,10} Probably due to this, it's one of the challenges facing environmental sustainability.¹⁰ Furthermore, plants have the tendency to bioaccumulate heavy metals from the environment through their different parts including roots, leaves etc. The accumulation mechanism varies on the source of the metals in the environment which can either be air or soil. Plants are often processed into different food materials. For instance, oil palm and cassava are processed into palm oil (world most traded vegetable oil) and cassava products (such as *lafun*, high quality cassava flour, *fufu* etc) respectively. Typically, during cassava processing into high quality cassava flour, waste water is generated and is called cassava mill effluents. The waste water is mostly discharged into the environment without treatment in developing countries like Nigeria. Authors have variously reported that cassava mill effluent contains heavy metals.¹¹⁻¹⁵ Furthermore, authors have also reported that cassava mill effluent has an impact on soil characteristics with regard to general physicochemical, microbial

and heavy metals characteristics.¹⁵⁻³¹ Several heavy metal pollution remediation technologies are available. Some of these technologies include the use of conventional adsorbents such as membrane separation, ion-exchange technologies and/or precipitation of the cation in an inert form.^{32,33} Activated carbon, the use of macrophytes such as algae, seaweeds,³ water hyacinths and uses of microbial techniques including biosorption process and enzymatic immobilization process. Saifuddin and Raziah³³ reported the merits of enzyme immobilization technology because the biocatalysts display better operational stability and higher efficiency of catalysis, and they are reusable. The authors further reported that immobilization of microbial cells and/or organelles eliminates the often tedious, time-consuming, and expensive steps involved in isolation and purification of intracellular enzymes. Thippeswamy et al.,³ also reported the merits of biosorption process compared to bioaccumulation process to include, growth independent and non-living cells biomass toxicity. Typically many bacterial polysaccharides have the tendency to bind heavy metals with varying degrees of specificity and affinity.³³ Among the microbes that have demonstrated huge potential for heavy metal remediation from effluents is fungi biomass.³ Okoduwa et al.,³⁴ reported that fungi are attracting interest toward biological treatment of industrial wastewater. One major yeast species that has been used to remediate heavy metals in ionic forms is *S. cerevisiae*.³⁵ According to Kelewou et al.,³⁶ the merits of yeast over bacteria and filamentous fungi is that it is inexpensive and readily available from different sources, and can grow and adapt to extreme conditions of pH, temperature and nutrient availability. Furthermore *S. cerevisiae* has been widely used in the remediation of toxicants or biodegradation of basic textile dyes (Basic Green 4 and Basic Yellow 2)³⁶ textile dyes of carmoisine and reactive black 5⁹ industrial effluents³³ textile effluents,³⁷ non-dairy creamer wastewater³⁸ pharmaceutical effluents³⁹ tannery effluents³⁴ palm oil

mill effluents.⁴⁰ The use of *S. cerevisiae* could be due to their ability to bioaccumulate heavy metals such as copper, zinc, nickel, cadmium, lead, chromium.^{3,35,41} Specifically *S. cerevisiae* has phosphate, amino, carboxyl and hydroxyl groups in its cell wall, which are responsible for the remediation of heavy metals from the environment.^{35,42} Therefore, this study aimed at evaluating heavy metal treatment of cassava mill effluents using *S. cerevisiae*.

Materials and methods

Sample collection

Triplicate raw cassava mill effluents were collected from smallholder cassava processor employing manual process at Ndemili in Ndokwa west Local Government Area of Delta state, Nigeria. Samples were collected using 4 litres clean containers and were transported to the laboratory using ice pack. The samples were used immediately it arrived laboratory.

Isolation and characterization of *Saccharomyces cerevisiae*

Palm wine was purchase from palm wine vendor in Rumuomasi, Port Harcourt, Rivers state. Pour plate method previously described by Benson⁴³ and Pepper et al.,⁴⁴ was used for the isolation following serial dilution. About 1.0ml of the serially diluted sample was plated in Potatoes Dextrose Agar supplemented with chloramphenicol. The agar medium was incubated at room temperature 30°C ± 4°C for 3-5 days. The resultant isolates were streaked in a fresh potato dextrose agar plate supplemented with chloramphenicol. The yeasts were identified using conventional microbiological techniques based on their cultural, morphological, and physiological/biochemical characteristics (using lactose-phenol, methylene blue, carbon fermentation using basal medium which comprises of 4.5g of powdered yeast extract, 7.5g of peptone, and 26.7mg of bromothymol blue indicator which was prepared with 2% sugar (maltose, glucose, sucrose, lactose) and assimilation using Yeast Nitrogen Base agar slants was supplemented with 2% sugar and based on temperature growth using glucose-peptone-yeast extract broth) as described by Kurtzman et al.,⁴⁵ APHA,⁴⁶ Benson⁴³ and have been applied by Iwuagwu et al.⁴⁰ Abioye et al.³⁹ Okoduwa et al.³⁴ The resultant isolates were compared with the guide of Ellis et al.⁴⁷

Table 1 Heavy metal concentration of cassava mill effluents treated with *S. cerevisiae*

Parameters	Initial (Day 0)	5 Days	10 Days	15 Days	Control after 15 days
Cu, mg/l	1.460±0.460 ^b	1.360±0.330 ^{ab}	1.030±0.030 ^{ab}	0.810±0.070 ^a	1.263±0.305 ^{ab}
Zn, mg/l	4.353±0.365 ^b	3.037±0.165 ^a	2.870±0.700 ^a	3.210±0.230 ^a	3.287±0.285 ^a
Mn, mg/l	4.637±0.195 ^c	3.740±0.470 ^b	2.440±0.340 ^a	2.247±0.205 ^a	3.993±0.606 ^{bc}
Fe, mg/l	28.270±1.130 ^c	26.807±5.125 ^{bc}	20.287±2.615 ^{ab}	13.340±1.450 ^a	22.080±5.460 ^{bc}
Cr, mg/l	0.180±0.020 ^a	0.453±0.045 ^c	0.407±0.035 ^c	0.293±0.025 ^b	0.407±0.105 ^c
Ni, mg/l	1.810±0.110 ^c	1.187±0.295 ^b	1.733±0.385 ^c	0.630±0.050 ^a	1.730±0.100 ^c

Data is expressed as mean±standard deviation; Different letters across the row indicate significant difference (P<0.05) according to Waller-Duncan Statistics

Effluent treatment studies

Triplicate 100ml of the prepared cassava mill effluents were measured into 250ml Erlenmeyer's flask under aseptic condition^{34,39} and 10ml of *S. cerevisiae* inoculum (*S. cerevisiae* prepared by inoculating the isolate into nutrient broth and incubated for 4 days) into the flask. The flask was capped with cotton wool wrapped with aluminum foil paper. Control was set up without *S. cerevisiae* inoculum. Growth was determined for 15 days at 5 days interval. The samples were shaken every 30 minutes between 7.00- 19.00 daily. At every day (0, 5, 10 and 15 days), 60ml of the medium were decanted into a measuring cylinder for heavy metal analysis.

Effluent heavy metal analysis

Aqua/Regia Digestion (ASTM D 3974-99) method was adopted for heavy metal determination. The flame atomic absorption spectrometry (FAAS) (GBC Avanta PM A6600) was calibrated with prepared working solutions from stock solutions (Accu Standards, 1,000mg/l) for each of the respective metals to be analyzed. The heavy metals were analyzed at varying wavelength of 213.9nm, 324.70nm, 232.0nm, 248.3nm, 279.5nm, 357.90nm, 228.8nm, 217.00nm and 240.70nm 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. Data was expressed as mean± standard deviation. One-way analysis of variance was carried out at P=0.05, and Waller-Duncan's statistics was used to discern the source of the observed differences. Spearman rho correlation matrix was used to identify the relationship between the detected heavy metals under study.

(Table 1) present the level of heavy metal concentration of cassava mill effluents treated with *S. cerevisiae*. While (Table 2) present the correlation matrix of the heavy metals. In the analyzed heavy metals cadmium, lead and cobalt was not detected in the effluent. While copper, manganese, chromium, zinc, iron, nickel and zinc were detected at varying concentration at different days of treatment.

Table 2 Spearman's rho of the detected heavy metal concentration in cassava mill effluents treated with *S. cerevisiae*

Parameters	Cu	Zn	Mn	Fe	Cr	Ni
Cu	1					
Zn	0.446	1				
Mn	0.694**	0.708**	1			
Fe	0.667**	0.39	0.704**	1		
Cr	0.034	-0.524*	-0.194	0.179	1	
Ni	0.407	0.438	0.547*	0.633*	0.115	1

**Correlation is significant at the 0.01 level (2-tailed).

*Correlation is significant at the 0.05 level (2-tailed).

N= 15; n= 3

Copper

The initial (day 0) concentration of copper were 1.460mg/l, which declined to 0.810mg/l after 15 days of treatment. Basically there was significant difference ($P < 0.05$) among the various days of treatment. Statistically, degradation of the cassava mill effluents after 15 days by *S. cerevisiae* were the source of observed significant difference. Copper showed positive significant relationship with manganese ($r = 0.694$, $P < 0.01$) and iron ($r = 0.667$, $P < 0.01$) (Table 2). The copper concentration were greater than the limit of < 1 mg/l for effluent to be discharged into surface water as specified by FEPA.⁴⁸ Copper showed a decline of 44.52% after 15 days of treatment with *S. cerevisiae*. After treatment, the level of copper was within limit of effluent to be discharged into surface water as recommended by FEPA.⁴⁸ The findings of the initial concentration in this study is comparable to the value 1.83mg/l reported by Orhue et al.¹¹ 1.91mg/l reported by Adejumo and Ola¹² and lower than the value of 2.5mg/l¹³ 2.60mg/l¹⁴ and higher than the value of 0.00mg/l reported by Omomowo et al.¹⁵

Zinc

The initial (day 0) level of zinc were 4.353mg/l, which declined to 3.210mg/l after 15 days of treatment using *S. cerevisiae*. Degradation of the cassava mill effluents by *S. cerevisiae* were not statistically different ($P > 0.05$) apart from the initial concentration (day 0) which was the source of observed significant difference. Zinc showed positive significant relationship with manganese ($r = 0.708$, $P < 0.01$) and negatively correlate with chromium ($r = 0.524$, $P < 0.05$) (Table 2). The zinc level in the initial and final (after 15 days of treatment) cassava mill effluent were higher than the limit of < 1 mg/l for effluent to be discharged into surface water as specified by FEPA.⁴⁸ Zinc decreased by 26.26% after 15 days of treatment with *S. cerevisiae* (Figure 1). The findings of the initial level of zinc in this study is comparable to the value 4.1 mg/l reported by Patrick et al.,¹³ and lower than the value of 5.90mg/l reported by Olorunfemi & Lolodi et al.¹⁴ and higher than the value of 1.07mg/l¹¹ 0.00mg/l.^{12,15}

Manganese

The initial level of manganese was 4.637mg/l, which reduced to 2.247mg/l after 15 days of treatment. Basically, there was significant variation ($P < 0.05$) among the various days of treatment. Furthermore, there was no significant difference ($P > 0.05$) exists between day 10 and

15 of treatment. Manganese showed positive significant relationship with iron ($r = 0.704$, $P < 0.01$) and nickel ($r = 0.547$, $P < 0.05$) (Table 2). The initial manganese concentration of initial cassava mill effluent was lower than the limit of 5.00 mg/l for effluent to be discharged into surface water as specified by FEPA.⁴⁸ Manganese showed a decline of 51.54% after 15 days of treatment (Figure 1). The treatment further reduced the concentration as compared to the initial level and FEPA limits for effluents to be discharged into the environment. The findings of the initial level of zinc in this study is lower than the value 0.71 mg/l¹² 0.00mg/l¹⁵ and lower than the value of 7.10mg/l reported by Olorunfemi and Lolodi.¹⁴

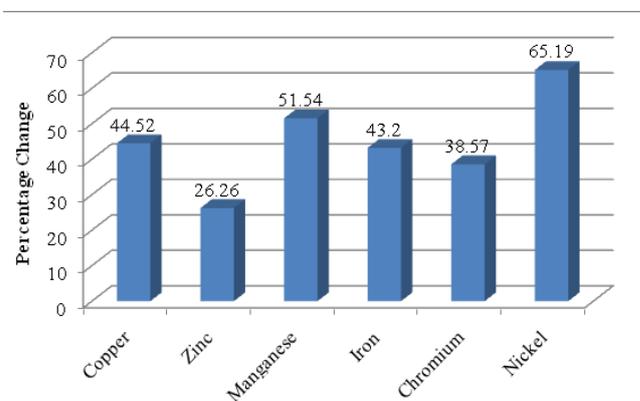


Figure 1 Percentage change in some selected heavy metal parameters of cassava mill effluents after 15 days of treatment with *S. cerevisiae*.

Iron

The initial concentration of iron was 28.270mg/l, which decreased to 13.34mg/l after 15 days of treatment. Basically, there was significant variation ($P < 0.05$) among the various days of treatment. Iron showed positive significant relationship with nickel ($r = 0.633$, $P < 0.05$) (Table 2). The initial manganese concentration of initial cassava mill effluent was lower than the limit of 5.00mg/l for effluent to be discharged into surface water as specified by FEPA.⁴⁸ Iron showed a decline of 43.20% after 15 days of treatment (Figure 1). After treatment, the level of iron was below limit of effluent to be discharged into surface water as recommended by FEPA.⁴⁸ The findings of the initial

concentration of iron in this study is lower than the value 2.35mg/l¹² 2.30mg/l¹⁵ 2.00mg/l¹¹ and lower than the value of 30.9mg/l reported by Olorunfemi and Lolodi.¹⁴

Chromium

The initial concentration of iron was 0.180mg/l, which decreased to 0.293mg/l after 15 days of treatment. Basically, there was significant variation ($P < 0.05$) among the various days of treatment. Between day 5 and 10, there was apparently decline in chromium concentration thus day 5 > day 10, though there were not statistically different ($P > 0.05$). The initial chromium concentration of initial cassava mill effluent was lower than the limit of <1mg/l (as trivalent and hexavalent) for effluent to be discharged into surface water as specified by FEPA.⁴⁸ Chromium concentration showed positive change of 38.57% as against other heavy metals (iron, manganese, copper, zinc and nickel) that showed negative change as treatment progressed using *S. Cerevisiae* (Figure 1). The treatment increased the level of chromium as compared to the initial concentration and all is within FEPA limits for effluents to be discharged into the environment. The increase in chromium concentration could be due to antagonistic behavior by the activities of the *S. cerevisiae* on other chemical constituents of the effluents. Again, the trend of the higher days having higher concentration compared to lower days and subsequently fluctuations have been reported in some physicochemical parameters of tannery³⁴ and pharmaceutical effluents³⁹ treated with *Saccharomyces cerevisiae*, Torul asporodelbrueckii. The findings of the initial concentration of chromium in this study are lower than the value 1.14mg/l reported by Olorunfemi and Lolodi.¹⁴

Nickel

The nickel concentration of the untreated cassava mill effluent at day 0 was 1.810mg/l, which reduced to 0.630mg/l after 15 days of treatment with *S. cerevisiae*. There was significant variation ($P < 0.05$) among the various days of treatment. There was a decline in the nickel concentration by 65.19% after 15 days of treatment (Figure 1). The percentage removal is higher than 47.8% nickel removal from synthetic solution using *S. Cerevisiae* biomass³⁵ The variation in the heavy metals concentration compared to previous study could be attributed to age of the cassava prior to processing, activities leading to individual heavy metal disposition in the plantation that the cassava was cultivated and possible leaching of metals from the processing equipment. The ability of the *S. cerevisiae* to degrade the heavy metals suggests that they have the tendency to withstand adverse extreme conditions. According to Adhikari et al.,³² the existence of yeast strains that can resist heavy metal ions in industrial effluents shows the ability of these strains to be resistant to stressful environmental condition. Furthermore, the authors further reported that yeast can develop survival strategies in heavy metal through tolerance, metabolism and detoxification. When the concentration of the heavy metal concentration is high, it affected the *S. cerevisiae* density. Adhikari et al.,³² reported that high concentration of heavy metals could have greater impact on microbial community structure, biomass and activities. In addition, Cherlyns et al.,³⁵ reported that low level of heavy metals can also be toxic to microbes and tolerance level of high concentration occurs when there is sufficient sulphur. Typically *S. cerevisiae* has the ability to utilize a variety of carbon and nitrogen sources however, in the absence of these nutrient sources, they could use some other synthetic chemicals.⁹ This could be the reason while there was significant reduction in most of the heavy metals concentration after 15 days of treatment.

Conclusion

S. cerevisiae is one of the commonly used yeasts. This study assessed the removal of heavy metals in cassava mill effluents using *S. cerevisiae*. The study found that most heavy metals found in cassava mill effluent often exceed permissible limit for effluent to be discharged into the environment as specified by Federal Environmental Protection Agency. The treatment of the cassava mill effluent with *S. cerevisiae* often reduces the heavy metals to specified limit. Therefore, this study showed the possibility of treating cassava mill effluent using *S. cerevisiae*.

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Conflict of interest

The author declares no conflict of interest.

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