Removal of heavy metals in cassava mill effluents by saccharomyces cerevisiaeisolated 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 cerevisiaeisolated 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 S. cerevisiae was inoculated into the sterile effluent and incubated for 15 days and triplicate sample were withdrawn at 3 days interval for analysis.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

Introduction

Heavy metals are metalloid whose density is about 5 times denser than that of water.1,2 Heavy metals enters the environment from natural effects (such as seepage from rocks and volcanic activity)3 and by large extent anthropogenic activities.4,4 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.5 These heavy metals lead to environmental pollution.6,10 Some of the heavy metals that have high environmental toxicity and health effects include lead, mercury, chromium, cadmium, copper, cadmium, nickel and zinc.7 Worst still, heavy metals is calcitrant to degradation and as such is a threat to biodiversity in heavy metal laden environment.8,9 Probably due to this, it’s one of the challenges facing environmental sustainability.10 Furthermore, plants have the tendency to bioaccumulation 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 accumulate 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, wastewater is generated and is called cassava mill effluents. The waste water is mostly discharged into the environment without treatment in developing country like Nigeria. Authors have variously reported that cassava mill effluent contain heavy metals.11-15 Furthermore, authors have also reported cassava mill effluent is having impact on soil characteristics with regard to general physicochemical, microbial and heavy metals characteristics.16-31 Several heavy metal pollution remediation’s are available. Some of these technology include the use of conventional adsorbents such as membrane separation, ion-exchange technologies and/or precipitation of the cation in an inorganic form31,32,33 activated carbon, the use macroporics such as algae, seaweeds,3 water hyacinths and uses of microbial techniques including biosorption process and enzymatic immobilization process. Saifuddin and Raziah34 reported the merits of enzyme immobilization technology is 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.,35 also reported the merits of biosorption process compared to bioaccumulation process to includes, growth and metabolism of microorganisms or their proteins, and the different steps involved in isolation and purification of intracellular enzymes.

S. cerevisiae is a yeast with potential for bioremediation in wastewater treatment. Okoduwa et al.,36 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 organic forms is S. cerevisiae37. According to Kelewu et al.,38 the merits of yeast over bacteria and filamentous fungi is that its inexpensive and readily available from different source, and can grow and adapt extreme conditions of pH, temperature and nutrients availability. Furthermore S. cerevisiae have been widely used in the remediation of toxicants or biodegradation of basic textile dye (Basic Green 4 and Basic Yellow 2) tetra textile dyes of carmoisine and reactive black 5 industrial effluents39 textile effluents,37 non-dairy creamer wastewater41 pharmaceutical effluents31 tannery effluents34 palm oil.
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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 purchased from palm wine vendor in Rumuomasi, Port Harcourt, Rivers state. Pour plate method previously described by Benson and Pepper, 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-phenol-yeast extract broth) as described by Kurtzman et al., APHA, Benson and have been applied by Iwuagwu et al. Abiowe et al. and Okoduwa et al. The resultant isolates were compared with the guide of Ellis et al.

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.

Effluent treatment studies

Triplicate 100ml of the prepared cassava mill effluents were measured into 250ml Erlenmeyer’s flask under aseptic condition and 10ml of S. cerevisiae inoculums (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.

Table 1

| Parameters | Initial (Day 0) | 5 Days  | 10 Days | 15 Days  | Control after 15 days  |
|------------|----------------|---------|---------|---------|------------------------|
| Cu, mg/l   | 1.460±0.460    | 1.360±0.330 | 1.030±0.030 | 0.810±0.070 | 1.263±0.305            |
| Zn, mg/l   | 4.353±0.365    | 3.037±0.165 | 2.870±0.700 | 2.120±0.230 | 2.827±0.285            |
| Mn, mg/l   | 4.637±0.195    | 3.740±0.470 | 2.440±0.340 | 2.247±0.205 | 3.993±0.606            |
| Fe, mg/l   | 28.270±1.130   | 2.680±5.125 | 2.028±2.615 | 13.340±1.450 | 22.080±5.460           |
| Cr, mg/l   | 0.180±0.020    | 0.453±0.045 | 0.407±0.035 | 0.293±0.025 | 0.407±0.105            |
| Ni, mg/l   | 1.810±0.110    | 1.187±0.295 | 1.733±0.385 | 0.630±0.050 | 1.730±0.100            |

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

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

The use of S. cerevisiae could be due to their ability to bioaccumulation heavy metals such as copper, zinc, nickel, cadmium, lead, chromium. 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. Therefore, this study aimed at evaluating heavy metal treatment of cassava mill effluents using S.cerevisiae.

Materials and methods

Effluent treatment studies

Triplicate 100ml of the prepared cassava mill effluents were measured into 250ml Erlenmeyer’s flask under aseptic condition and 10ml of S. cerevisiae inoculums (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.

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| Mn, mg/l   | 4.637±0.195    | 3.740±0.470 | 2.440±0.340 | 2.247±0.205 | 3.993±0.606            |
| Fe, mg/l   | 28.270±1.130   | 2.680±5.125 | 2.028±2.615 | 13.340±1.450 | 22.080±5.460           |
| Cr, mg/l   | 0.180±0.020    | 0.453±0.045 | 0.407±0.035 | 0.293±0.025 | 0.407±0.105            |
| Ni, mg/l   | 1.810±0.110    | 1.187±0.295 | 1.733±0.385 | 0.630±0.050 | 1.730±0.100            |

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

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

*Correlation is significant at the 0.05 level (2-tailed).
**Correlation is significant at the 0.01 level (2-tailed).

N= 15; n= 3

Copper

The initial (day 0) concentration of copper was 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 <1mg/l for effluent to be discharged into surface water as specified by FEPA. 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. The findings of the initial concentration in this study is comparable to the value 1.83mg/l reported by Orhue et al. and 1.91mg/l reported by Adejumo and Ola and lower than the value of 2.5mg/l and 2.60mg/l and higher than the value of 0.00mg/l reported by Omomowo et al.

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 <1mg/l for effluent to be discharged into surface water as specified by FEPA. Zinc decreased by26.26% after 15 days of treatment with S. cerevisiae (Figure 1). The findings of the initial level of zinc in this study is lower than the value 0.71 mg/l and 0.00mg/l and lower than the value of 7.10mg/l reported by Olorunfemi and Lolodi.

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. 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/l and 0.00mg/l and lower than the value of 7.10mg/l reported by Olorunfemi and Lolodi.

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. 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.

Figure 1 Percentage change in some selected heavy metal parameters of cassava mill effluents after 15 days of treatment with S. cerevisiae.
concentration of iron in this study is lower than the value 2.35mg/l\textsuperscript{12} 2.30mg/l\textsuperscript{11} 2.00mg/l\textsuperscript{11} and lower than the value of 30.9mg/l reported by Olorunfemi and Lolodi.\textsuperscript{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.\textsuperscript{46} 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 tannery\textsuperscript{14} and pharmaceutical effluents\textsuperscript{39} treated with Saccharomyces cerevisiae, Torul as poradellbrueckii. The findings of the initial concentration of chromium in this study are lower than the value 1.14mg/l reported by Olorunfemi and Lolodi.\textsuperscript{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* biomass\textsuperscript{31} 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.,\textsuperscript{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.,\textsuperscript{32} reported that high concentration of heavy metals could have greater impact on microbial community structure, biomass and activities. In addition, Cherfys et al.,\textsuperscript{30} 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.\textsuperscript{3} 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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