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The Use of *Candida* sp in the Biosorption of Heavy Metals from Industrial Effluent

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ABSTRACT

The ability of Candida spp to bisorp heavy metals contained in industrial effluent was studied with atomic absorbance spectrophotometer (AAS) and the conventional cultural method. AAS of the effluent revealed the presence of Mercury, Zinc, Cadmium, Lead, Iron, Copper, and Chromium, in the proportions, per 100ml of effluent, 0.008, 0091, 0.006, 0.003, 0.075, 0.82 and 0.027 ppm respectively. Enumeration of the organisms in the effluent by Millipore filter technique using Sabouraud dextrose agar medium showed counts of 2.50, 2.80 and 4.20 CFU/ml. Microscopic identification of the isolates by wet mount using lacto phenol cotton blue stain showed the presence of both branched septate hyphae and septate hyphae. Characterization of the isolates by citrate test, oxidase activity, catalase activity, starch hydrolysis, Voges Proskauer and alcohol tolerance 10% and 20%, respectively revealed that the isolates belong to the genera Candida, Fusarium and Rhizopus. Percentage disappearance of the metals according to the various treatments given to the Candida biomass with respect to time was found to be on the increase. The results revealed that, the use of Candida spp is very effective for the treatment of heavy metal polluted waste water.

Keywords: biosorption, *Candida sp*, heavy metals, industrial effluent.

INTRODUCTION

The incidence of industrialization in Nigeria has brought with it some associated consequences which are pernicious to the environment [1].One of these consequences, is the generation of industrial wastes, which come in diverse forms, and are dumped into the environment where they constitute environmental pollution [2]. An example of the forms, is industrial effluents which are normally drained into water bodies where they increase the BOD and COD of such water bodies [3] , resulting in toxicity to the aquatic lives and even poison them [4]. These indirectly affect man who is the final consumer of the aquatic organisms [5]. The environmental conditions caused by this development have led to finding solutions to ameliorate the negative impact that results from it. One of these measures is the use of microorganisms to treat the effluents before discarding into water bodies. In this wise, some groups of microorganisms like the fungi [6] and bacteria [7] have been used to demonstrate the biosorption of heavy metals from industrial effluents, with very significant positive results. The group of fungi that have mainly been used in biosorption of heavy metals have been filamentous fungi. [8,9,10].

There is no information on the use yeasts for the bisorption of heavy metals. But yeast is only a common name for single celled fungi and can belong to any of the four groups namely Ascomycetes, Basidiomycetes, Deuteromycetes and Zygomycetes. Therefore any yeast possesses the same properties as any member of the particular group to which it may belong except that it is single celled. Generally, biosorption is a property of certain types of inactive, dead, microbial biomass to bind and concentrate heavy metals from even very dilute aqueous solutions. Opposite of

biosorption is metabolically driven active bioaccumulation by living cells [11, 12]. Thus the aim of this work is to investigate the ability of the yeast, *Candida sp* to which two different forms of treatments are given, in biosorption of copper, iron and zinc and to compare it with the ability of the untreated yeast in biosorption of these metals.

MATERIALS AND METHODS

Collection of samples:
The sample used in this study was waste water from Aluminum industries, Asaba, Delta State of Nigeria. They are the manufacturers of Tower Aluminum pots. This particular waste water originated from washing the pots after beating the metal into shape.The container used for the collection was rinsed thoroughly with O.I.N Hcl prior to use, to avoid the heavy metal binding to the walls of the container. The sample after collection was sent to Anal concept Ltd. Port Harcourt, Rivers State, to determine the presence of heavy metals in the effluent by Atomic Absorbance Spectrophotometer technique. The *Candida sp* used in the study was also isolated from this effluent.

Isolation and purification of *Candida sp*
Both membrane filter(MF) and pour plate techniques were used to isolate the *Candida*. For the (MF) technique, a 100ml of the effluent was filtered through a Millipore filter paper to concentrate the fungi propagules. After filtration, the filter paper was placed on a solidified sabouraud dextose agar (SDA) with a modified pH of 5.6 [12]. This medium was previously sterilized by autoclaving at 121^0C for 15 minutes at 15Psi. It was incubated after inoculation at 28^0C for 7 days. For the pour plate technique, 0.1ml of the effluent was placed on a sterilized Petri dish using a 1 ml sterile pipette. Immediately after, the molten SDA medium was poured on it and the mixture slightly shaken to mix up, then it was allowed to solidify before incubation in the same condition the MF culture was incubated. Purification of single colonies was achieved by subculturing of the fungal cells on potato dextrose agar (PDA) medium and incubated under the same condition previously stated.

Identification of fungi isolates:
The fungal isolates were identified using cultural morphology, cellular morphology and biochemical tests. Cultural morphology to determine the colony colour, shape and texture was studied on SDA medium [13]. Cellular morphology involving Gram stain for determination of the cell shape and their Gram reaction was studied [14]. Germ tube test and the lacto phenol mount for motility were done [13]. The biochemical tests included oxidase activity, catalse activity, citrate utilization, starch hydrolysis, Voges Proskauer and alcohol tolerance.

Growth of the biosorbent Yeast
Candida sp was selected for this work because its population in the effluent was higher than that of *Fusarium sp* and *Rhizopus sp*. The growth was conducted using a modified technique [14]. The medium was a PDA broth. To a 100ml of this broth, a 0.1ml of lactic acid was added in order to inhibit bacterial growth. The medium was inoculated with 10ml of a 10^3cfu/ml of *Candida sp* and incubated at 20^0C for 7 days on a rotary shaker at 150rpm.

Analytical Assays
At the end of the 7 days of growth, the yeast culture was centrifuged, the cells washed and subjected to three different pre-treatments before being used for metal biosorption experiments. These pre-treatments are 1) alkali pre-treatment, 2) drying by oven 3) untreated.

Alkali pre-treatment
A 0.2g of the wet harvested *Candida sp* was introduced into a 50ml of 0.5N NaOH solution in a conical flask and boiled for 15mins [6]. At the end of this boiling, the cells were washed with deionized water until the pH of the solution was close to neutral (6.8-7.2) then afterwards the cells were dried and ground to powder.

Drying pre-treatment
A 0.2g of the wet yeast pellet was oven dried over night at 60^0C and afterwards , ground to powder.

Untreated yeast biomass
A 0.2g of the yeast cells were kept at room temperature till use for biosorption.

Biosorption studies:
The study was carried out using the effluent collected from the Aluminnium industries. The pH of the effluent was initially 8.01 but it was adjusted to 5.0 using 0.1N Hcl. This effluent was placed in a 100ml Erlenmeyer flask which was rinsed with a 0.1N Hcl to avoid metal from the effluent binding to the walls of the glass. A 0.2g of the ground *Candida sp* powder of the different treatments were separately introduced into separate flasks of the same effluent. The flasks were later placed on rotary shakes at a temperature of 28^0C, operating at a speed of 150 rpm and contact

time of 2 hours at which equilibrium is ensured. Samples were taken from each of the flasks at initial, 15, 60 and 120mins intervals to analyze for the metal concentration.

Analysis of the metals

Samples were collected in triplicates from each mixture for analysis to determine the concentration of unsorbed metals in the mixture using atomic absorption spectrometers.

RESULTS AND DISCUSSION

Table 1: The amount of metals in the waste water in ppm, and their percentage before treatment

Metals	Amount in ppm	% metal	pH of waste water
Cadmium (Cd)	0.006	2.05	*5.0
Chromium (Cr)	0.027	9.28	
Copper (Cu)	0.082	28.08	
Iron (Fe)	0.075	25.68	
Lead (Pb)	0.003	1.03	
Mercury (Hg)	0.008	2.74	
Zinc (Zn)	0.091	31.16	

**The water sample is only one water sample containing all these metals with a pH of* 5

Table 2: The effect of the various treatments on the ability of the *Candida* sp to biosorp metals from the effluent, after 15 minutes of the experiment

	Treatments and their effect				
Metals	**NaOH (O.5N)**	**Overnight oven dried (60°C)**	**Untreated biomass**	**Control**	**pH**
Copper (Cu	0.051	0.067	0.077	0.082	*5.8
Iron (Fe)	0.043	0.059	0.066	0.075	
Zinc (Zn)	0.062	0.074	0.082	0.091	

**pH shift after 15 minutes into the experiment.*

Table 3: The effect of the various treatments on the ability of the *Candida* sp to biosorp metals from the effluent, after 60 minutes of the Experiment

	Treatments and their effect				
Metals	**NaOH (O.5N)**	**Overnight oven dried (60°C)**	**Untreated biomass**	**Control**	**pH**
Copper (Cu)	0.038	0.043	0.049	0.082	*6.5
Iron (Fe)	0.027	0.034	0.041	0.075	
Zinc (Zn)	0.041	0.059	0.068	0.091	

**pH shift after 60 minutes into the experiment.*

Table 4: The effect of the various treatments' on the ability of the *Candida* sp to biosorp metals from the effluent, after 120 minutes of the experiment

	Treatments and their effects				
Metals	**NaOH O.5N**	**Overnight oven dried (60°C)**	**Untreated biomass**	**Control**	**pH**
Copper (Cu)	0.002	0.020	0.018	0.082	*7.0
Iron (Fe)	0.001	0.003	0.006	0.075	
Zinc (Zn)	0.003	0.012	0.020	0.091	

**pH shift after 120 minutes into the experiment*

Table 5: Performance Analysis of the various treatments with respect to time

Metals	Treatments and their effects				Time
	NaOH (O.5N)	Overnight oven dried (60°C)	Untreated biomass	Control	
Copper (Cu)	0.031	0.051	0.005	0.082	15mins
Iron (Fe)	0.032	0.016	0.009	0.075	
Zinc (Zn)	0.029	0.017	0.009	0.091	

Table 6: Performance Analysis of the various treatments with respect to time

Metals	Treatments and their effect				Time
	NaOH (O.5N)	Overnight oven dried (60°C)	Untreated biomass	Control	
Copper (Cu)	0.044	0.039	0.033	0.082	60mins
Iron (Fe)	0.048	0.041	0.034	0.075	
Zinc (Zn)	0.05	0.032	0.023	0.091	

Table 7: Performance Analysis of the various treatments with respect to time

Metals	Treatments and their effect				Time
	NaOH (O.5N)	Overnight oven dried (60°C)	Untreated biomass	Control	
Copper (Cu)	0.079	0.062	0.064	0.082	120mins
Iron (Fe)	0.074	0.072	0.069	0.075	
Zinc (Zn)	0.088	0.079	0.071	0.091	

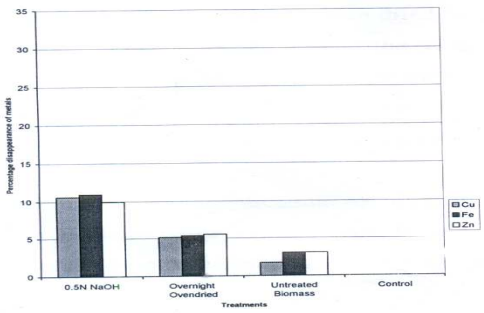

Fig. 1: Percentage disappearance of metals from the effluents after 15 mins of the experiment

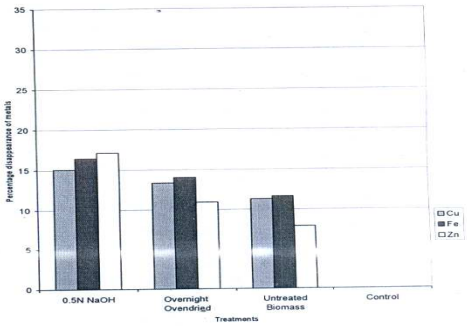

Fig. 2: Percentage disappearance of metals from the effluents after 60 mins of the experiment

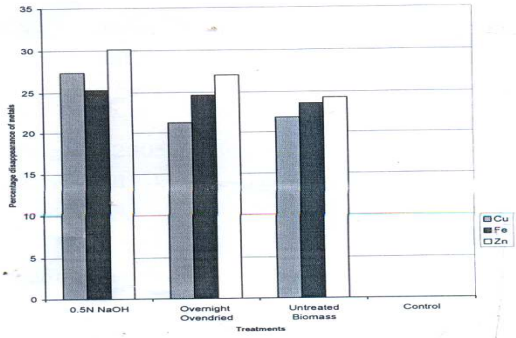

Fig. 3: Percentage disappearance of metals from the effluents after 120 mins of the experiment

The types and amounts of metals in the effluent are presented on Table 1. This shows that this effluent is heavily contaminated with heavy metals which must be removed before disposing it into water bodies to prevent pollution.

Three of these heavy metals only were analyzed for their biosorption by the *Candida sp*. Table 2 shows the amounts of copper, iron and zinc that were removed from the effluent after 15mins of the experiment, by the various treated *Candida sp*. The amount of copper ion biosorped by the untreated was 0.077ppm while the overnight oven treated recorded higher amount of 0.067ppm and even NaOH treated *Candida sp* was far after amount. The control was 0.082ppm. This means that *Candida sp* can possibly be used in the treated of copper polluted effluent much in the same way other fungal species will be used. Again, a NaOH treated *Candida sp* is best for the work when it is best for the work when it is taking place at PH 5.8 but to be cost effective, an overnight oven dried *Candida sp* can be used because it equally effective at that pH. But if only a small amount of the copper is needed to be removed from the treated, then the organism may not be treated yet, it will biosorp the copper ions to the extent needed from the effluent. The same trend of disappearance was observed in iron. The NaOH treated inoculums had the highest amount biosorped followed by overnight oven treated then the untreated biomass as against the control which remain the amount that it was in the effluent this same trend is observed in zinc. After 60mins of experiment Table 2, the same trend of removal was observed however, there is greater increase in the amounts that disappeared. This shows that time is an important factor in biosorption ability of the organism. And with time too the PH of the medium shifted from 5.8 to 6.5 Table 3). This is an obvious confirmation that the metals are disappearing. By the time it was 120 minutes (Table 4) the NaOH treated *Candida sp* biomass had removed virtually all the metals from the effluent followed by oven treated, then the untreated. And the pH had also shifted to 7.0 with respect to time he performance of the various treated *Candida* biomass are shown in Fig 1 for 15mins. Fig 2 shows if after 60mins while Fig 3 shows it after 120mins.

Comparatively, the NaOH treated *Candida sp* biomass is more effective followed by oven treated one while the untreated one is also good. These are shown on table 5, 6 and 7.

The finds are that *Candida sp* effectively biosorped copper, iron and zinc ions in industrial effluent. Iron disappearance was highest in all experiments by *Candida sp* biomass followed by copper and the zinc. Time is an important factor because if time even the untreated *Candida sp* biomass biosorped about 80% of all the metal ions in the effluent.

CONCLUSION

Candida sp biomass are an effective biosorbents for copper, iron and zinc. They are best for iron, followed by copper and then zinc.

Time is very necessary in this type of experiment because with the progress of time, untreated *Candida sp* biomass biosorped about 80% of the metal ions from the effluent. Therefore, the availability of time can guarantee cost effectiveness.

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