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Treatment of Common Effluent Treatment Plant Pollutant under Growing & Non-growing Condition of Biomass

Shipra Jha[1*] and S. N. Dikshit[2]

[1]Amity Institute of Biotechnology, Amity University, Noida, UP, India.
[2]Department of Chemistry, Jiwaji University, Gwalior, MP, India.

Authors' contributions

This work was carried out in collaboration between both authors. Author SJ designed the study, permomed the statical analysis, wrote the protocol and wrote first and final draft of the manuscript. Author SND managed the analysis of the study. Both authors read and approved the final manuscript.

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ABSTRACT

Heavy metal pollution in wastewater has always been a serious environmental problem because heavy metals are not biodegradable and can be accumulated in living tissues. Copper is widely used in various important industrial applications. The increasing level of heavy metals in the aquatic system due to incomplete treatment of industrial wastewater by existing conventional methods is of environmental concern. Therefore, there has been an increasing interest in the possibility of using biological treatments. It is important to evaluate the performance of biomass with actual industrial effluent to ensure its field applicability. Hence the experiments were conducted with actual industrial effluents collected from Effluent Treatment Plant (ETP) and tannery industry.

Keywords: Metal removal; ETP; A. lentulus; wastewater; alternate media.

**Corresponding author: E-mail: sjanvi79@gmail.com;*

1. INTRODUCTION

Strict environmental regulations compel industries to shift to cleaner production methods, demanding the development of environment-friendly, low- cost and efficient treatment technique for metal-rich effluents. Some industrial units treat the Cu-rich wastewater by conventional aerobic or anaerobic treatment plants [1]. Although, copper concentration is lowered during this treatment, it remains at a level toxic to the flora and fauna. The cost-effectiveness of the most common physicochemical processes such as oxidation and reduction, chemical precipitation, filtration, electrochemical treatment, evaporation, ion-exchange, and reverse osmosis is limited. High reagent requirements and unpredictable metal ion removal are some other disadvantages associated with such techniques [2]. Further, strong and contaminating reagents are used for desorption, resulting in toxic sludge and secondary environmental pollution. Wastewater with a low concentration of Cu (II) is usually treated with ion exchange resins, but the high cost of the resin limits its usage [3]. Biotechnological approaches can succeed in that area and are designed to cover such niches. Microbes have proven capability to take up heavy metals from aqueous solution, especially when the metal concentrations in the polluted water range from less than 1 to about 20 mg/l [4,5] Besides, flexibility to handle the range of Physico-chemical parameters in effluents, selectivity to remove only the desired metals and the cost-effectiveness are some added advantages of biological metal cleanup techniques. These factors have promoted extensive research on the biological methods of metal removal [6,7]. The ability of microbial biomass to remove metals can be described as active (energy-dependent) or passive (energy independent) processes, commonly, known as bioaccumulation and biosorption, respectively. As introduced efforts directed towards the application of microbial strains for actual effluent treatment have been lacking, nevertheless through the literature review, attempts have been made to consolidate this information [8,9].

For ensuring the applicability of efficient strains in remote small-scale industries/units, it is essential that suitable locally available alternates to the commercial expensive growth media be worked out. However, information on media formulations using locally available nutrients is very scanty. Further, presently most of media components which have been used very commonly are of animal origin which is against the environmental ethics which differ in county wise [10,11,12]. Keeping this in view there is a need to explore abundantly available plant- derived components as alternatives to animal based microbiological media. Recently, corn, sorghum, millet meal extract was used as an alternate culture supplements for Aspergillus Niger, Fusarium moniliforme, Penicillium sp., Cercospora sp. [12,13]. The A. Niger raw biomass contain chitin -chitosan units and reasonable amount of protein and amino acids which serves as matrix of COOH, $-NH_2$ and –OH group which in turn take part in binding of metal ion. Fortification of the media containing mannitol or glucose as a carbon source with Amaranthus seed meal (as a supplement providing growth factors) has been cited [14,15,16]. Majority of such studies on alternative media have been reported for solidified growth medium hence there is a large scope of research for alternative liquid growth medium or both. [17] Further, most of the existing investigations could lead to only functional supplements but not the complete substitute. It shall also be interesting to investigate whether the alternative media [it is locally available alternates to the commercial explore suitable replacements for carbon as well as nitrogen source, so that the media could be simplified.] can solely support the growth and metal removal under stress conditions [18,19].

2. METHODS

2.1 Collection of Samples

Industrial effluents were collected by dipping high density polyethylene plastic bottles (1 L) from the centre of combined effluent treatment plant in between the month of March 2014 and May 2016 in Banmore industrial area in Gwalior, (India) at 34°C. During the sample collection, pH. (pocket sized pH meter) of sample were noted .Before collecting the sample bottles were disinfected with methylated spirit and thoroughly rinsed with sample. Effluents were kept in ice during transportation and delivered on the same day to the laboratory where sample were stored at 4°C until analysis of other parameters. The effluent was characterized by standard methods of wastewater analysis (APHA) for pH, TDS, TSS, COD, BOD and heavy metals[1]. The method used in analyzing water quality parameters are shown in Table 1. The standards for respective metals prepared using standard stock solution (10,000 mg/l Merck) and Cu (II) removal from effluents was studied using growing and non-growing biomass.

2.2 Test Biomass

In this study, fungi namely; *A. lentulus* were used. The *A. lentulus* culture were obtained from the culture collection of the Department of Microbiology, Jiwaji University Gwalior, India. Before the study above fungi were sub-cultured on fresh potato dextrose agar [PDA] medium.

2.3 Preparation of Alternative Sample

The plant chosen for media substitution were pods and seeds of *L. leucocephala* and *Sesbania* sp. After collection, the plant materials were washed, dried, ground and sieved to obtained uniform-sized powder (>150 mesh) for sample analysis. The sample were used to conduct experiments with CETP effluent under growing conditions.

2.4 Under Non-growing [Pre-cultivated] Conditions

These studies were conducted using three forms of biomass i.e resting, autoclaved & immobilized biomass at 4g/l loading was contacted with effluents and kept at 180 rpm and 30°C for a designated period. Samples were drawn at regular time intervals and analyzed for Cu (II) concentration.

2.4.1 Resting biomass

Fungal strain was grown in 250 ml Erlenmeyer flasks containing 100 ml of growth media. After the exponential phase of growth, cells were harvested by centrifugation at 4000 rpm and 6°C for 10 min. The harvested cells were washed by double distilled water. This biomass was termed as resting biomass.

2.4.2 Autoclaved biomass

Autoclaved biomass was prepared by autoclaving the pre-grown resting biomass at 121°C and 15 lb for 20 minutes. The autoclaved *biomass was then used after filtration.*

2.4.3 Immobilization of biomass

Biomass was immobilized by mixing sodium alginate, gelatin with above resting biomass in the ratio of 1:1:5 Finally beads of immobilized fungal biomass were obtained.

3. RESULTS

The characteristics of the collected sample from industrial effluents are shown in Table 2. As a result, although it was expected to have a mix of metallic contaminants, the concentrations of these contaminants were low. Thus, Effluent Treatment Plant [ETP] effluents are voluminous with a comparatively lower pollutant load. Nevertheless, the concentration of Cu (II) was found to be 45 mg/l. Apart from Cu (II), this effluent contained other heavy metals such as Fe, Zn, Mn and Ni, etc.

3.1 Cu (II) Removal from Actual Effluent Using Alternative Media

Having established the need for alternative media [these are wasteland- growing plants so their cultivation does not demand high agronomical inputs] worked for effluent treatment, experiments were set up with the best alternative media worked out in the present. Based on the performance of alternative media components in Cu (II) removal from synthetic solutions, glucose and yeast extract was replaced by alternative media i.e. *L. leucocephala* and *Sesbania* sp. to find out whether Cu removal from actual effluent will be possible using these low-cost materials instead of expensive yeast extract. The results show in Fig. 1 indicate that Cu (II) removal in effluent supplemented with alternative media was at par with that in effluent supplemented with

Table 1. Laboratory procedure for analysing various physiochemical parameters of Industrial effluents

Parameter	Instruments and methods
Temperature	Thermistor
pH	Electrometric
Turbidity	Turbid meter
TDS	Electrometric
BOD	Incubation techniques with DO determination by DO meter
COD	Reflux distillation followed by titrimetric
Heavy metal analysis	Atomic Absorption spectrophotometric

the complete composite media, although the time required for complete removal in the latter case was 96 h as compared to 120 h in case of alternative media. Hence alternative media components can be used for detoxification of the ETP effluent.

Table 2. Characterization of industrial effluents

S. no.	Parameters	EETP (mg/l)
1	pH	2.8
2	TDS	3.3
3	COD	0.08
4	Cu (II)	249
5	Fe	1.33
6	Zn	0.5
7	Ni	0.5
8	Mn	20.9
9	Pb	0.4
10	Mg	12.8

3.2 Biosorption of Cu (II) by *A. lentulus* in Non-growing Mode

Pre-grown fungal biomass was used in different ways- (a) resting (b) autoclaved (c) immobilized. Among all the treatments, immobilized biomass showed the best adsorption of Cu (II). With autoclaved biomass, 91.2% Cu (II) removal was obtained as compared to 84% and 75% with immobilized and resting biomass, respectively, at pH 3 and 100 mg/l of initial Cu (II) concentration. Our result showed that immobilized beads lead to an increase in Cu (II) showed in Fig. 2.

3.3 Using Pre-cultivated Fungal Biomass

To evaluate the actual biosorption potential of *A. lentulus* under field conditions, these studies were conducted using resting, autoclaved and immobilized fungal biomass at 4 g/l loading as described in Fig. 2. When industrial effluent was kept in contact with resting biomass, complete removal of Cu (II) occurred in 4 h while with heat inactivated biomass [When the cultivated fungus pellets were washed with sterile physiological saline solution and heated at 90°C for 15 min and referred as heat inactivated fungal pellets]. These resulting products were directly used as biosorbent, the same occurred within 2 h only (Fig. 3).

The use of active cells would be more useful however, if treatment involves mixed waste with potential utilization as energy and carbon source over a longer period. Table 3 gives comparison of the two modes and points that the precultivated biomass may be useful when the effluents are extremely acidic and contain high Cu^{2+} concentration.

4. DISCUSSION

The use of growing cultures in metal removal could be advantageous as it ensures less risk of metal being released back into the environment. The constraints of metal toxicity that can kill microbial agents can be circumvented by using heavy metal resistant organisms isolated from metal contaminated areas [20-24]. The finding of

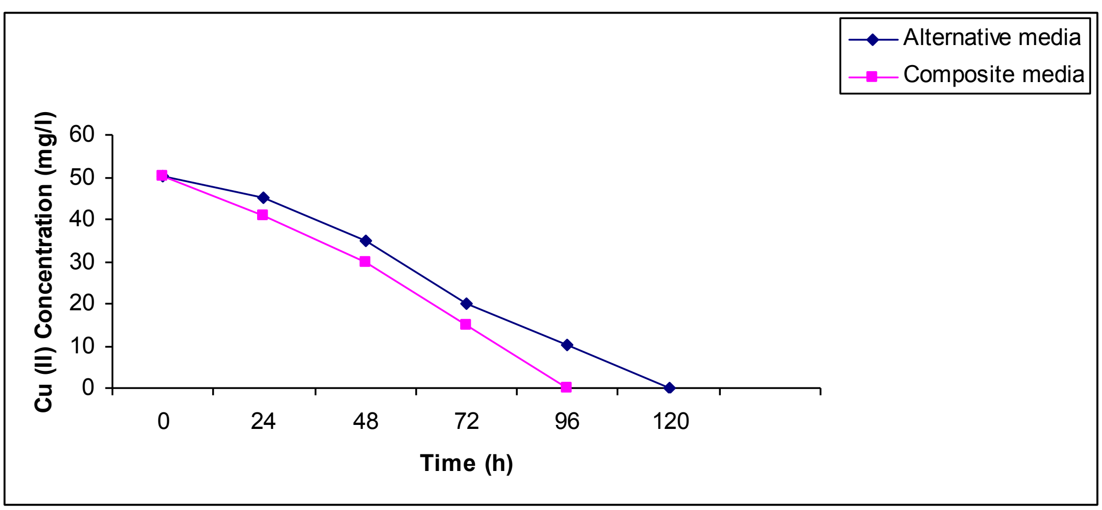

Fig. 1. Removal of Cu (II) from EFT effluent supplemented with alternative media vis- a Vis composite media using *A. lentulus* biomass

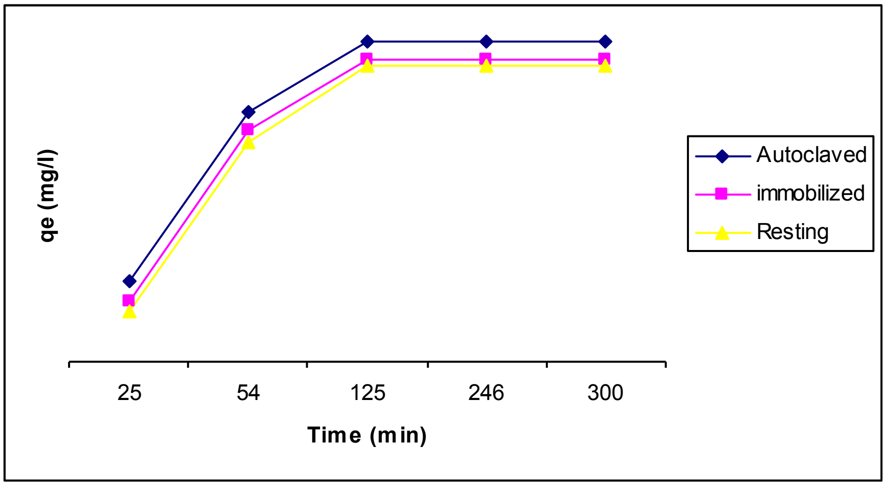

Fig. 2. Rate of biosorption using different biomass of *A. lentulus* at 100 mg/l Cu (II) concentration, pH =6 and biomass dose 4 g/l at 30°C

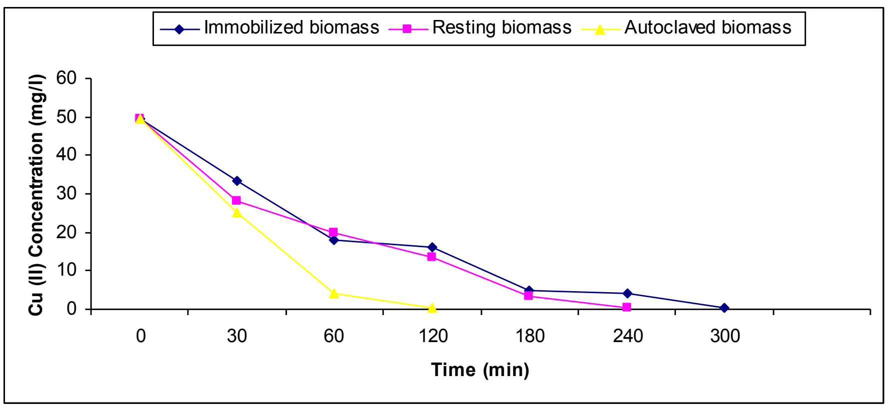

Fig. 3. Removal of Cu (II) from EFT effluent through biosorption on *A. lentulus* biomass

Table 3. Comparison of growing and non-growing biomass for metal removal

Parameter	Growing (G)	Non-growing (NG)	Comment
Process time	Long treatment time (5 days)	Short (120 min)	If cultivation and biomass preparation time included then= 2 days in non-growing
pH sensitiveness	Often yes but in case of A.lentulus not significantly affected in broad pH range (3- 11)	Yes, Removal significantly reduced in neutral range	
Pollutant Toxicity	Yes, above 550 mg/l $Cu2^+$	No	At higher pollutant load non-growing may be preferred.
Recovery of metals and recycling of biomass	Generally not attempted	Possible	
Interference from other contaminants	Yes	No	Choice of robust organism for growing

Growing-The effluent+ fungal biomass; Non-growing- Immobilized biomass the present suggest that ETP effluent supplemented with critical media components or locally available plant based alternative media can be treated without prior pH adjustment and sterilization using *A. lentulus* in the growing mode. The effluent can be brought down to appropriate discharge levels using pre-grown biomass of *A. lentulus*.

On the application side, this research finding suggests that sterilization of the particular EFT effluents shall not be required before biological treatment with *A. lentulus* making the process simple and cost-effective [25]. Very few studies are available that demarcate the role of native effluent flora in Cu removal. Manuela characterized an electroplating effluent from a mycological point of view and then different fungal biomass was tested for the removal of Cu (II) from the same effluent [26-27].

5. CONCLUSION

To validate the performance of fungal biomass in terms of field applicability, the experiments were conducted with actual industrial effluents. The strain could successfully treat nutrient supplemented effluent from Effluent Treatment Plant that indicating that effluent did not exert any additional toxicity on the organism. Overall, it reveals the possible long ecological compatibility of *A. lentulus* with native microflora. In the present investigation would help translating these results into decentralized effluent treatment system for small scale industries located in rural section.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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