THE EFFICIENCY OF *PSEUDOMONAS AERUGINOSA* TO DEGRADE THE METALLIC SALT OF COPPER

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***Abstract*- The e-waste has become the greatest threat to life. Due to more accumulation of E-waste, an increasing crisis for metal pollution arises... Especially electronic wastes like waste print circuit boards contain large amounts of copper, which not only brings greater ecological and environmental threats but also causes a serious waste of copper resources. Due to ongoing technical advancements, it eventually increases the production of e-waste; Disposing it in landfills has the potential risk of affecting human health. This research aimed at quantifying the efficiency of *Pseudomonas aeruginosa* in degrading copper. The percentage of degradation is high in 9% & 10% copper at 24 & 48 hours of culture and the lowest percentage of degradation is shown in 2% of Cu in 4 hours of culture as 5%. Then the DNA was isolated from the *Pseudomonas aeruginosa* and also from copper incorporated media. The PCR was run using the Cop A primers.**

***Keywords*: *Pseudomonas aeruginosa*, copper degradation, e-waste, heavy metals pollution, health effects**

Introduction

Waste from end-of-life electrical and electronic equipment, known as e-waste, is a rapidly growing global problem[1].The tremendous consumer demand drove manufacturers to improve the integrated circuit process with greater performance, better production yields and lower prices which gave rise to personal computers, mobile-phones, laptops and several other electronic equipment [2]. The whole world remains connected through various sophisticated electronic devices like a palmtop, mobile-phone or laptop. But the murky side of this technological roar is the accumulation of the electronic wastes. The complexity and technical advancement through the Iron Age (1200 BC), eventually giving rise to present day "Electronic Age". It is also known as the 'Digital Age" [3]. The increased necessities of mankind in Modern Era have led to a myriad of inventions from a simple calculator to a highly improvised super computer [4]. E-waste is a global, interregional, and domestic problem. Of the 20 million to 50 million tons generated yearly, it is estimated that 75% to 80% is shipped to countries in Asia and Africa for "recycling" and disposal [5].

Electronic waste, normally contains valuable as well as potentially toxic elements involved in e-waste and their unsafe methods of disposal affect human health and cause various diseases. The main environmental impacts of e-waste mainly arise due to inappropriate processing of e waste which in turn cause serious risks to human health and the environment [6]. Children and pregnant women are at higher risk than adults to contaminants released through informal e-waste recycling activities due to their unique vulnerabilities. Children have different
exposures to e-waste recycling activities. E-waste recycling activities release toxic chemicals that can cross the placenta and may contaminate breastmilk, for example mercury and copper[7].

The ratio of copper, gold, iron, and other metals is over 60 % in E-waste while 30% is plastic and 2.70% is hazardous pollutants [8]. A typical printed circuit board (PCB), for example, has roughly 3% iron and ferrite, **16% copper**, 2% nickel and 0.05% silver of its weight [9]; Which not only bring greater ecological and environmental threat, but also cause a serious waste of copper resources[10].The composition of e waste depends strongly on factors such as type of e device, model, manufacturer, date of manufacture and the age of scrap. For instance, a mobile phone contains 40 elements, base metals such as Copper (cu) & Tin (sn) and special metals such as Lithium (Li), cobalt (Co) and precious metals like Silver (Ag), Gold (Au) & Palladium (Pd)[10]. Heavy metals like Cu generates reactive oxygen species (ROE) through auto-oxidation or Fenton-like reaction[11]. On the basis of physical composition, the harmful substances found in large quantities include Cathode Ray Tubes (CRT), Printed Circuit Boards (PCB), epoxy resins, Polyvinyl Chlorides (PVC), thermosetting plastics, fibreglass, lead glass, concrete, ceramics, rubber and plywood [12]. The different methods of Degradation of E-waste include: Physical, Chemical, Biological [Microbial remediation, Phyto & Vermi remediation][13]
In the case of Microbial remediation for degradation of e waste, ***Pseudomonas aeruginosa*** helps in removal of heavy metals from the surrounding medium[14.] *Pseudomonas aeruginosa* possess seven ORFs with this family signature, many of which, including PA3920, possess putative metal binding motif [15.] So, in this study, we have focused on the role of *Pseudomonas aeruginosa* in removal of heavy metal from the surrounding medium. *Pseudomonas aeruginosa* is

member of the Gamma Proteobacteria class of Bacteria. It is a Gram negative, aerobic rod belonging to the bacterial family Pseudomonadaceae. *Pseudomonas* is characterized as a Gram-negative rod measuring 0.5 to 0.8 µm by 1.5 to 3.0 µm5 Almost all strains are motile by means of a single polar flagellum. In this we mainly focus on degradation of copper using the *Pseudomonas aeruginosa* [16. The] genes conferring copper resistance in bacteria are often present in plasmids and organized in an operon [17.] The copper resistance is encoded by the cop genes (cop A, cop B, cop C and cop D), in *Cupriavidus metallidurans* CG34, *Pseudomonas syringae* pv. tomato PT23, ***Pseudomonas aeruginosa* PA01**[18].The CopA ATPase from *Pseudomonas sp.*is responsible for copper degradation. The Cop A gene is responsible for detoxify the copper. It is the copper resistance protein A. CopA is an ATP- driven copper pump that expels copper(I) from the cytoplasm in to the periplasmic space. CopA is a protein formed as a part of a copper resistance operon in *Pseudomonas aeruginosa.* CopA, encodes a 72-kDa periplasmic protein that binds multiple copper atoms and amino acid sequence of CopA may bind four copper atoms [19].

Materials and methods

Collection of *Pseudomonas aeruginosa*:

The organism *Pseudomonas aeruginosa* was collected from soil sample.

Subculturing of *Pseudomonas aeruginosa:*

The *Pseudomonas aeruginosa* was sub cultured in nutrient agar plates. The green colour colonies were formed after incubation for overnight. It was then sub cultured in nutrient broth (LB Broth)

Copper degradation by *Pseudomonas aeruginosa*:

In this work, different concentration of copper solution was taken and the degradation of copper was estimated by the *Pseudomonas aeruginosa.* The 0.1M copper sulphate solution was prepared and the various concentration [1%, 2%, 3%,4%,5%, 6%, 7%,8%,9% and 10%] of copper was added to the nutrient broth and these were examined at various time intervals of 2 hrs, 4hrs, 8hrs,16hrs, 24hrs, 48 hrs, 60hrs & 72hrs. It is to observe the organism's growth and uptake of copper. After each incubation time, the broth was centrifuged and the supernatant was treated with ammonia to obtain the specific colour.

By collecting the treated supernatant [ammonia treated] at each incubation time and recorded at the wavelength of (480nm) in a UV Spectrophotometer.

Results and discussion

The results confirmed that *Pseudomonas aeruginosa* is capable of degrading copper. The degradation of copper by *Pseudomonas aeruginosa* with respect to time interval is high in 48hrs and beyond that, at 60hrs and 72hrs the growth of the microbe reaches the stationary phase, and as copper degradation remains constant. The degradation of copper with respect to various concentrations, the high uptake of copper is observed in 9% & 10% of copper. The degradation percentage of copper was calculated by measuring the OD of crude copper. The percentage of degradation is high in 9% & 10% copper at 24 & 48 Hrs culture. The lowest percentage of degradation is shown in 2% of Cu in 4 Hrs culture as 5%.

Comparison of results in 2 cases:

When the concentration of copper varies with constant time the OD values and the Degradation of (cu) keeps decreasing & while the concentration of copper is constant with varying time then the OD values and the Degradation of (cu)keeps increasing [ref: Table.1,2 & Fig.1,2&3]

In case of constant incubation time [2hrs & 48hrs]

The Degradation of copper is at a peak only at, while the Concentration of copper is at 2% in 2hrs incubation time and 1% in 48hrs incubation time; and in the rest of the concentrations, the degradation is lower. As a result, with the varying concentration of (cu) at constant time gives less degradation of copper.

In case of constant Concentration of copper [9% & 10%]:

The Degradation of copper is increasing along with the increasing incubation time intervals (i.e.: while the con. of. copper is 9% the degradation value is low at 2hrs and keeps increasing and attains peak at 48hrs; and while the con. of. copper is 10% the degradation value is low at the same 2hrs and keeps increasing and attains peak at 48hrs)

DEGRADATION OF Cu IN 2hrs				
VOLUME OF MEDIA (µl)	VOLUME OF CuSO4 (µl)	VOLUME OF CULTURE	PERCENTAGE OF CuSO4	OD READING AT 480 nm
1000				0
900	100			0.006
940	60	20	1	0.07
870	130	20	2	0.325
800	200	20	3	0.146
730	270	20	4	0.074
670	330	20	5	0.036
600	400	20	6	0.016
530	470	20	7	0.011
460	540	20	8	-0.007
400	600	20	9	-0.052
330	670	20	10	-0.071

Table 1: Degradation of copper in 2hrs

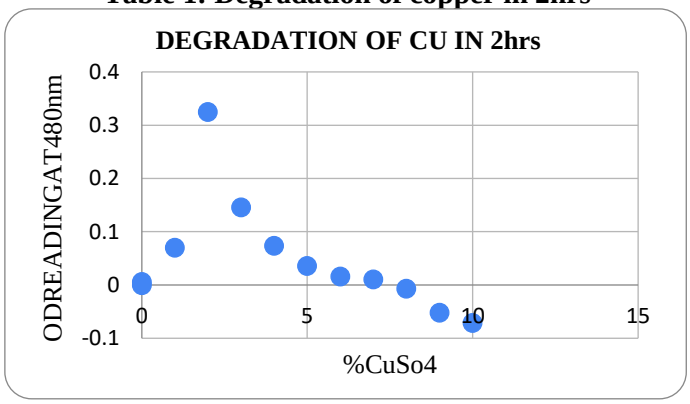

Figure 1: Degradation of copper in 2hrs

DEGRADATION OF Cu IN 48hrs					
VOLUME OF MEDIA (µl)	VOLUME OF CuSO4 (µl)	VOLUME OF CULTURE	PERCENTAGE OF CuSO4	OD READING AT 480 nm	TURBIDITY
1000				0	
900	100			0.006	1
940	60	20	1	0.051	1.05
870	130	20	2	-0.071	1.22
800	200	20	3	-0.098	1.34
730	270	20	4	-0.112	1.57
670	330	20	5	-0.133	1.69
600	400	20	6	-0.15	1.73
530	470	20	7	-0.166	1.95
460	540	20	8	-0.207	1.96
400	600	20	9	-0.274	1.98
330	670	20	10	-0.299	2

Table 2: Degradation of copper in 48hrs

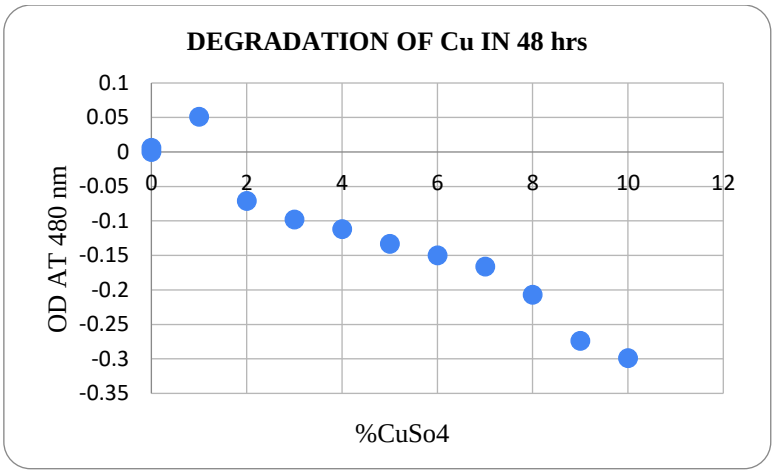

Figure 2: Degradation of copper in 48hrs

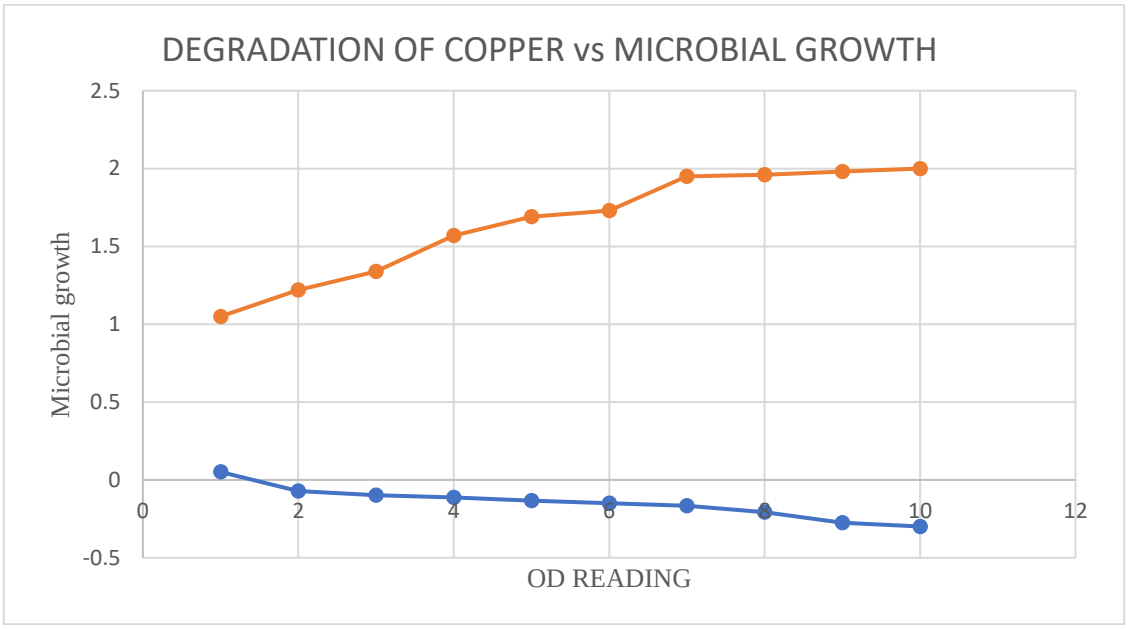

Figure 3: Plot representing degradation of copper vs microbial growth. Increasing turbidity is found to decrease Cu

Conclusion

Pseudomonas aeruginosa a gram-negative, rod bacteria which contains CopA ATPase is responsible for copper degradation. As this CopA is an ATP-driven copper pump that expels copper(I) from the cytoplasm into the periplasmic space, and it is a protein formed as a part of a copper resistance operon in *Pseudomonas aeruginosa.* Thus, this study proves that *Pseudomonas aeruginosa* is capable of degrading in copper.

Future perspective

Gene analysis of Cop A gene.

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