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Bioremoval of Heavy Metals from Effluent of Portharcourt Refinery Company Limited, Eleme, Nigeria

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Abstract

Bioremoval of heavy metals from the effluent of Port Harcourt Refinery Company limited was studied. Eight treatment options were set up. These include Natural process (positive control), poisoned and filtered sample (negative control), Pseudomonas aeruginosa, Bacillus subtilis, Rhizopus stolonifer, Aspergillus niger, Chlorella vulgaris and a mixed culture of the above microorganisms. The experiment was conducted at ambient temperature (30 ± 2^0C and pH range of 6.8 to 8.9 and incubated for 60days. Physicochemicalanalyses of the effluent on day zero revealed the presence of Cadmium (0.041mg/l), Chromium (0.5mg/l) and Zinc (0.521mg/l) and were found to be relatively high in relation to the DPR standards for petroleum industries in Nigeria. At the end of 60 days, the various treatment options (Natural process(positive control), poisoned and filtered sample(negative control), Pseudomonas aeruginosa, Bacillus subtilis, Rhizopus stolonifer, Aspergillus niger, Chlorella vulgaris and a mixed culture of the above microorganisms) were able to bioaccumulate 78%, 14.6%, 58.5%, 45%, 79.5%, 83% and 26.9% of Cadmium; 61.4%, 2.1%, 95.9%, 74.9%, 34%, 62% and 49% of Zinc and 61.4%, 13%, 50.6%, 84.8%, 87%, 87% and 38.2% of Chromium respectively. The mixed culture recovered 100% of Cadmium in 42days; 100% of Zinc and Chromium in 28days. However, not all the heavy metals recovered from the sample were bioconcentrated. Pseudomonas aeruginosa, Bacillus subtilis, Rhizopusstolonifer, Aspergillusniger, Chlorella vulgaris and the mixed culture bioconcentrated 61%, 31.7%, 53.7%, 73.1%, 22% and 70.7% of Cadmium; and 62.6%, 55.9%, 16.5%, 38.6%, 35.9% and 74.7% of Zinc. Besides, Pseudomonas aeruginosa, Bacillus subtilis and the mixed culturebioconcentrated 32, 42.2 and 50% of Chromium. This study has demonstrated the great potential of the above listed microorganisms to clean-up the refinery effluent of certain heavy metals and can be employed in treatment of environments polluted with effluents containing high levels of Cadmium, Chromium and Zinc.

Keywords: Bioremoval, heavy metals, biosorption, bioadsorption, bioconcentration, refinery effluent and bioconcentration factor.

Introduction

The release of toxic substances and their dispersal into the environment may be harmful on the exposed populations. Over the past decades, the huge increase in the use of heavy metals resulted in increased deposit of metallic substances into aquatic environment. These metals have the important characteristics of being persistent and most of their ions are toxic to living organisms. Therefore, in order to have a pollution-free environment, the toxic materials should be removed from wastewater before its disposal[1]. The various studies on heavy metal removal from solution have been launched because of the ecological effects of these toxic metals released into the environment[2,3,] . Besides, environmental pollution from man-made sources easily creates local conditions of increased metal presence and this could lead to some hazardous effects on animals and humans[3,4,5]. Heavy metals like Mercury, Lead, Cadmium, Arsenic, Antimony, Copper, Zinc, Nickel, Cobalt found themselves into the environment from electroplating, plastic and paint manufacturing, mining, metallurgical process, petrochemical process, batteries, paper and pulp[6] .

Physicochemical methods based on ion exchange, electrochemical treatment, membrane technologies, evaporation recovery, chemical oxidation or reduction, and chemical precipitation for the removal of toxic metals from aqueous solutions are usually ineffective when the metals concentration are low (range of 1-100ppm)[6] . These methods are extremely expensive[2] and may leave toxic products, biological and chemical sludge in the environment[1]. Therefore, new technologies that can concentrate and/or accumulate toxic metals at affordable costs are required hence bioremoval technology.

This study was done on effluent discharge from Port Harcourt Refinery and was studied using a few microorganisms (living cells) isolated from environmental wastes *(Pseudomonas aeruginosa, Bacillus subtilis, Aspergillus niger, Rhizopus stolonifer* and *Chlorella vulgaris)* to bioremove Zinc, Cadmium and Chromium found in Port Harcourt Refinery effluent. The study is aimed at determining the physicochemical properties of effluent discharge from Port Harcourt Refinery company and to explore the bioremoval potentials of *Bacillus subtilis, Pseudomonas aeruginosa, Rhizopus stolonifer, Aspergillus* *niger* and *Chlorella vulgaris* for the recovery of heavy metals such as Cadmium, Chromium and Zinc from the effluent discharge from Port Harcourt Refinery Company. It also studies the influence of some physicochemical parameters of the effluent (Total Organic Carbon, Nitrate and Phosphate) in the course of the bioremoval process. Finally, it examines the method employed by the test microorganisms in the recovery of these metals ie, whether it is by biosorption, bioadsorption or both.

Material and Methods

Isolation of test microorganisms (Bacterial and Fungal spp): Culture madia used were prepared according to manufacturer's instruction (oxoid manual). Culture method was used to isolate the test organisms from environmental wastes. A total of 10 samples were analyzed. Each sample was suspend in normal saline and allowed for one hour. It was shaken by mechanical means and elute of each sample was poured into 10mls universal bottle. An aliquot of each sample inoculated in nutrient agar and potato dextrose agar and incubated at 35^{0}C for 48hours. Identification of isolates was done using methods described by Stanley and Onianwah[7,8] and Holt eds.

Isolation of *Chlorella vulgaris:* BG medium was prepared according to the method of Mark and Ceane[9]. A 20ml of pond water was filter into 500mls Erlenmeyer flask in duplicate. To each flask was added 200ml of BG medium. Both flasks were incubated under sunlight for two weeks.

Purification and blooming of *Chlorella sp*.: Serial dilution of culture showing green colourationwas made and 0.1ml of 10^{-5} dilution seeded into a BG agar plate[10] fortified with 100ug/ml Nystatin and 80ug/ml Gentamicin and incubated under the above conditions. The plates were observed at weekly interval for growth. Microscopic examination was done to determine colonies showing characteristics of *Chlorella sp.* Discrete colonies were subcultured into BG medium and used as stock culture[11].

Sample Collection: A 10 litre container sterilized with 70% ethanol was filled with the effluent from the refinery drains located at Ekerekana ama on okochiri road, Okirika and taken to the laboratory for analysis. The physicochemical parameters of the sample were measured almost immediately.

Measurement of some physicochemical parameters of the effluent: The following physicochemical parameters were determine, thus pH, temperature, total organic carbon, nitrate, phosphate, biochemical oxygen demand, total petroleum hydrocarbon and heavy metals such as cadmium, chromium, zinc, lead, cobalt, nickel and mercury. The heavy metals were determined using atomic absorption spectrophotometers (AAS) (bulk scientific 200A mode). The values are expressed in mg/L of the liquid sample[7,8,12] . The total petroleum hydrocarbon was determined using the method described by American Petroleum Institute.

Bioconcentration and bioadsorption tests: The concentrations of the above heavy metals in the washed test microorganisms and the filtrate were determined respectively on the 60th day using atomic absorption spectrophotometer. The bioconcentration factor was calculated.

Experimental Design: Eight different treatment options were set up in 250mls conical flasks (Erlenmeyer) and labeled A-H.
Flask A (Positive Control) contained 200mls of the untreated sample and was monitored for natural processes. Flask B (Negative Control) contained 200mls of poisoned sample with 1% formalin solution. Flask C contained 200mls of the filtered sample seeded with 10mls of pure culture of *Pseudomonas aeruginosa.* Flask D contained 200mls of the filtered sample seeded with 10mls of pure culture of *Bacillus subtilis.* Flask E contained 200mls of the filtered sample seeded with 10mls of pure culture of *Rhizopus stolonifer.* Flask F contained 200mls of the filtered sample seeded with 10mls of pure culture of *Aspergillus niger.* Flask G contained 200mls of the filtered sample seeded with 10mls of pure culture of *Chlorella vulgaris.* Flask H contained 200mls of the filtered sample seeded with 2mls each of pure culture of *Chlorella vulgaris, Pseudomonas aeruginosa, Bacillus subtilis, Rhizopus stolonifer and Aspergillus niger.*

The samples (A-H) were incubated at ambient temperature (30±2^{0}C) for 60 days. 50mls of each sample was filtered to remove the cells and associated deposits and the concentrations of Cadmium, Chromium, and Zinc in the medium were measured fortnightly. The concentrations of the above heavy metals in the washed test microorganisms and the filtrate were determined respectively on the 60th day using atomic absorption spectrophotometer.

Results and Discussion

Physicochemical analysis of the effluent: The values of the physicochemical analyses recorded in tables-3 to 9 are mean of the duplicate analytical values. The analysis on Day zero showed that the sample contained high concentration of organic carbon (0.044mg/l), nitrate (2.6mg/l), phosphate (5.734mg/l), cadmium (0.041mg/l), chromium (0.5mg/l), zinc (0.521mg/l), BOD (396.6/mg/l) and total petroleum hydrocarbon (0.065mg/l) with temperature and pH of 36.5^{0}C and 7.86 respectively (table-3).

Percentage removal of materials from the effluent between day zero and day 60: The determination of Phosphate, Nitrate, Total Organic Carbon, Cadmium, Chromium and Zinc concentration on the 14th day for the different treatment options demonstrated animpressive performance (table-4). High concentration of the heavy metals where removedfrom the medium in the treatment options A, C, E, F and H. Option D did not show any evidence of Cadmium removal in the first 14 days while options B and G showed little evidence of heavy metal removal. Treatment option A (natural processes) and B (poisoned sample) served as positive and negative controls respectively. In options A, C, E, F and H, there were rapid rise in the bioremoval of Cadmium on the 14^{th} day (0.009mg/l, 0.007mg/l, 0.014mg/l, 0.009mg/l and 0.024mg/l respectively).

Table-4 showed a steady rise in chromium bioremoval for all the treatment options except for option B (negative control). Options A, C, D, E, F, G and H were able to bioremove0.149mg/l, 0.089mg/l, 0.189mg/l, 0.183mg/l, 0.172mg/l, 0.093mg/l and 0.398mg/l of chromium respectively on the 14^{th} day. Also, there was a uniform rise in the potential of all the treatment options for the bioremoval of zinc on the 14^{th} day with the exception of option B where there was no Zinc removal. There was a corresponding rise in the percentage of organic carbon, nitrate and phosphate utilization.

On day 28 (table-5, figure-1), there were corresponding increase in percentage of Cadmium bioremoval for options C, D, F, and G. The increase continued in options A, B, E and H though at reduced percentage. However, greater concentration of chromium was bioaccumulated by options B, C, D, E, and F on the 28^{th} day. There was reduced chromium removal percent for options A, G and H (figure-2). The zinc removal percent was rapid on day 28 for options C, D, E, and G. The increase continued at reduced rate for options A, F, and H; with H exhibiting its maximum potential for zinc and chromium (0.521mg/l and 0.500mg/l; 100%) on day 28. No significant rate was observed for B (figure-3).

Table-1
Physicochemical Parameters of effluent on day Zero

Parameter	Mean values
pH	7.86 (6.5-9.2)*
Temperature	36.5^{o}c (21.8-33.4)*
Total organic carbon	0.044mg/l
Nitrate	2.6mg/l (50mg/l)*
Phosphate	5.734mg/l
Zinc	0.521 (3mg/l)*
Cadmium	0.041 (0.003mg/l)*
Lead	Nil
Mercury	Nil
Chromium	0.500 (0.05mg/l)*
Cobalt	Nil
Nickel	Nil
Biochemical Oxygen Demand	396.60mg/l
Total petroleum hydrocarbon	0.065mg/l

*Acceptable standards in (bracket) Nigeria (EGASPIN, 2002)

On day 42, option H was able to biorecover all the Cadmium present in the effluent (0.041mg/l; 100%). The increased rate of biorecovery continued for options A, E, F and G. Same was observed for C and D but at a reduced rate. A, C, E and F were able to bioremove 48.8%, 46.4%, 61% and 65% of the total cadmium present in the effluent (table-6). However, the rate of chromium bioremoval dropped by the 42^{nd} day for all the treatment options. Also, zinc bioremoval rate was reduced for all the treatment options on day 42 except for option B where there was a slight increase.

Table-2
Percent removal on day 14

Sample Code	TOC	NO_3	$P0_4$	Cd	Zn	Cr
A	31.8	23.1	2.6	22	31.8	29.8
B	-	-	0.01	4.8	-	2.4
C	25.0	20	6.9	17.2	23	17.8)
D	13.6	15.4	9.8	0.1	22.6	37.8
E	4.5	8.5	1.7	35.2	4.4	36.6
F	4.5	7.7	0.9	22	31	34.2
G	9.0	6.9	13.5	2.4	10.4	18.6
H	36.4	55	27.7	54.5	67.9	79.6

Table-3
Percent removal on day 28

Sample Code	TOC	NO	PO	Cd	Zn	Cr
A	52.2	28.8	37.5	29.3	48	46.1
B	-	3.8	0.6	4.9	0.2	10
C	36.4	23.1	26.6	39	76.4	47.2
D	27.3	15.4	26.6	21.9	52.2	82.2
E	29.6	9.7	16.8	46.3	16.7	84
F	31.8	9.3	17	39	42.4	83.4
G	29.6	6.2	21.8	9.7	31.7	25.0
H	65.9	79.1	63.6	82	100	100

Table-4
Percent removal on day 42

Sample Code	TOC	NO_3	PO_4	Cd	Zn	Cr
A	70.5	59.2	46.8	48.8	60.7	62.6
B	4.5	13.5	0.9	12.1	2.1	12.1
C	45.5	40	32	46.4	94.1	48
D	36.4	26.9	31	34.2	73.1	84.2
E	41	11.1	25.7	61	29.6	87
F	45.5	13.8	29.3	65.9	58.6	87
G	47.9	8.4	49.7	14.7	47.4	37.4
H	75.1	88	71.6	100	-	-

Table-5
Percent removal on day 60

Sample Code	TOC	NO_3PO_4		Cd	Zn	Cr
A	91	64.6	51.7	78	61.4	64.8
B	10	16.2	1	14.6	2.1	13
C	62.7	42.7	33.9	58.5	95.9	50.6
D	44.5	33.8	33.6	45	74.9	84.8
E	62.7	17.7	28	79.5	34	87
F	71.8	15.4	30.5	83	62	87
G	55.9	10.4	51.6	26.9	49	38.2
H	-	-	-	-	-	-

Table-6
Percent Concentration of heavy metals in washed cell Samples on day 60 (Bioconcentration Test)

Sample code	Cd	Zn	Cr
C	61	62.6	32
D	31.7	55.9	42.2
E	53.7	16.5	ND
F	73.1	38.6	ND
G	22	35.9	ND
H	70.7	74.7	50

Note: ND=Not detected

Tables-4, -5, -6 and -7 showed corresponding increase in the rate of removal of Nitrate, Phosphate and Organic carbon from the effluent. Thus on the 60th day, very high concentration of these compounds have been consumed in the different treatment options as shown in table-7. On day 60, option F had bioremoved 83% of the total Cadmium in the effluent while options E, A and C accumulated 79.5%, 78% and 58.5% respectively. The rest options recorded less than 50% performance. Also, treatment options A, C, D, E and F accumulated over 50% of Chromium in the effluent. Equally, over 50% of the total Zinc in the effluent was removed by treatment options A, C, D, E and F. The concentration of cadmium, chromium and zinc removed on day 60 is shown in table-7. The statistical analysis done on cadmium bioremoval showed that the treatment options and time were significantly different at 0.05 probability levels while the analysis of covariance showed that the treatment option H has a negative covariance (r= -1) with options A, B, C, D, E, F and G. The treatment options have positive covariance (r= +1) when paired with each other. In Chromium and Zinc removal, there was a general reduction rate for all the treatment options from day 42. For Chromium and Zinc, statistical analysis observed that while the treatments were significantly different at 0.05 probability levels, it was not so with time. Thus, a not significantly different observation at 0.05 probability levels for time. The analysis of covariance showed that the treatment option H has a negative covariance (r= -1) with options A, B, C, D, E, F and G while options A, B, c, D, E, F and G have positive covariance (r= +1) when paired with each other.

Table-7
Percent Concentration of heavy metals in Filtrate on day 60 (Bioadsorption Test)

Sample code	Cd	Zn	Cr
C	39	60.4	68
D	69	44.1	57.8
E	46.3	83.5	98.6
F	26.9	61.4	99.8
G	77.4	64.1	100
H	29.2	26	50

Table-8
Bioconcentration factor of the heavy metals analysed

Sample code	Cd	Cr	Zn
C	1.41	0.47	1.67
D	0.46	0.73	1.26
E	1.16	0	0.20
F	4.86	0	0.63
G	0.28	0	0.60
H	2.42	2.95	1.0

Bioconcentration and Bioadsorption of the metals under study: Table-8 showed the ability of the different test organisms to bioconcentate heavy metals being investigated. While all the treatment options showed ability to bioconcentate Cadmium and Zinc, only options C, D and H showed bioconcentration for Chromium. Not all the heavy metals removed were bioconcentated, certain percentage of the metals were adsorbed to the cell surface and was wash off as filtrate during the cell wash. Options E, F, and G demonstrated that the removal of Chromium was by bioadsorption (table-7).

Bioconcentrarion factor: The bioconcentration factor of the different treatment option listed in table-8 showed that option F has the highest factor for Cadmium with a recorded value of 4.86; C and D have the highest values for Zinc and Chromium (1.67 and 0.73 respectively).

Discussion: This study emphasizes the presence and bioremoval of cadmium, chiomuim and zinc by spiecs of *Pseudomonas auregniosa, Bacillus subtilis, Rhizopus stolomfer, Aspergillus niger and chlorella vnlgaris* from effluent of Port Harcourt refinery company in Nigeria. The physicochemical analisis showed the presence of cadmium, chromium and zinc at concentration of 0.041mg/L, 0.5mg/L and 0.52mg/L repectively. Other chemical components of the efficient sch as carbon, Nitrogen and Phosphorus supported the growth of the micro organisms. At the end of 60 days, *Aspergillus niger* was able to bioaccumulate more cadium than other test organisms *with Cholera vulgaris* show the list performance (figure- 1). Similarly, *Rhizopus stomifiers, Aspergithus niger* and *Bacillus subtilis* bioaccumulated high percentage of chromium on the 60th day (figure-2). The removal of zinc was best with Pseudomonas aureginosa although bacillus subtilis and aspergilusniger had impressive performances. Most of the organisms had their optimuim activity between 28 and 42 days. This may be attributed to cell growth; reduced competition for the metal binding sites. The decline in activity thereafter may be as a result of the production in history substances (metabolic biproducts), nutrients depletion, unfavorable environmental condition arising from the presence of these intermediate metabolites and consequent decline in the number of cells (cell death). *Chlorella Vulgaris* had the least performances for the three heavy metals perhaps for lack of adequate enzymes system and metal binding sites for the heavy metals or its slow growth rate as observed in this case.

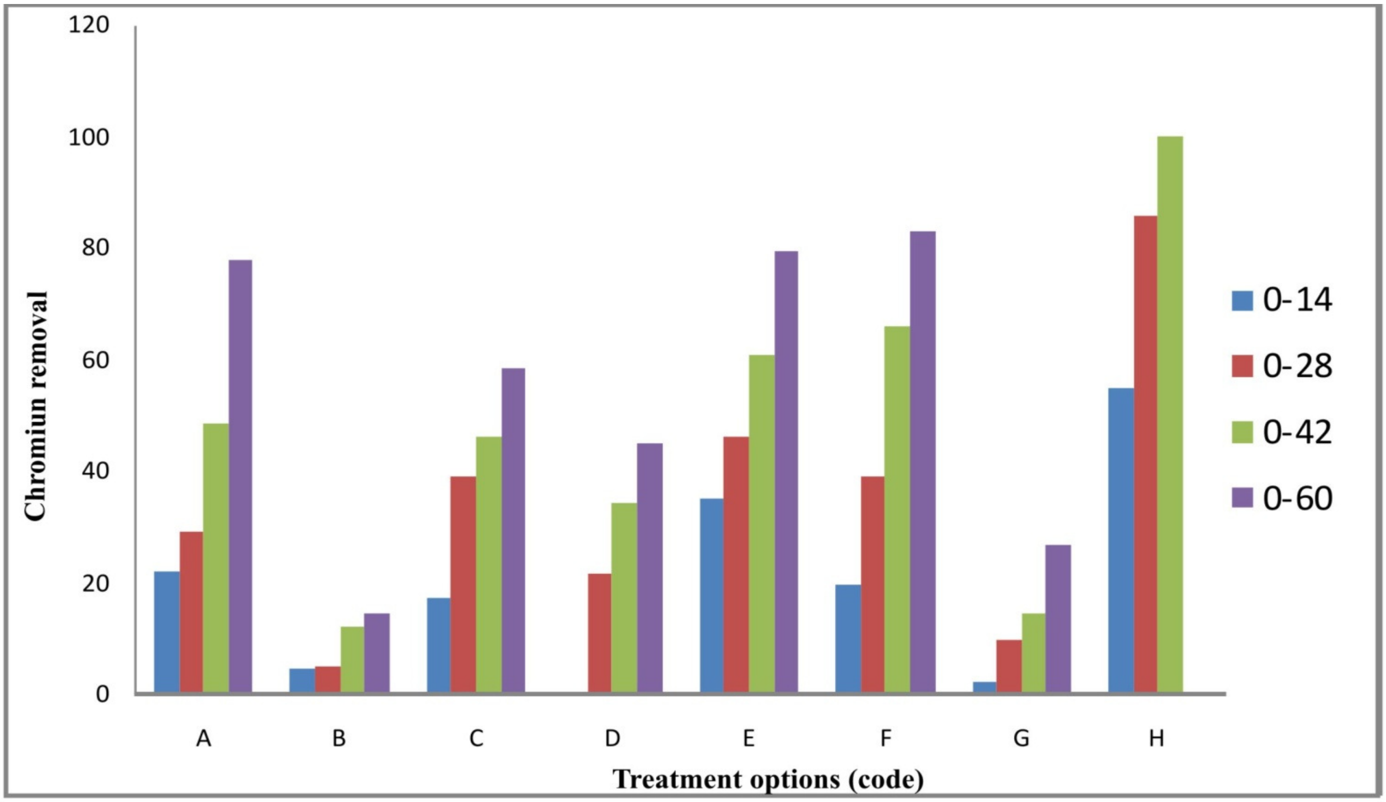

Figure-1
Cadmium removal percent

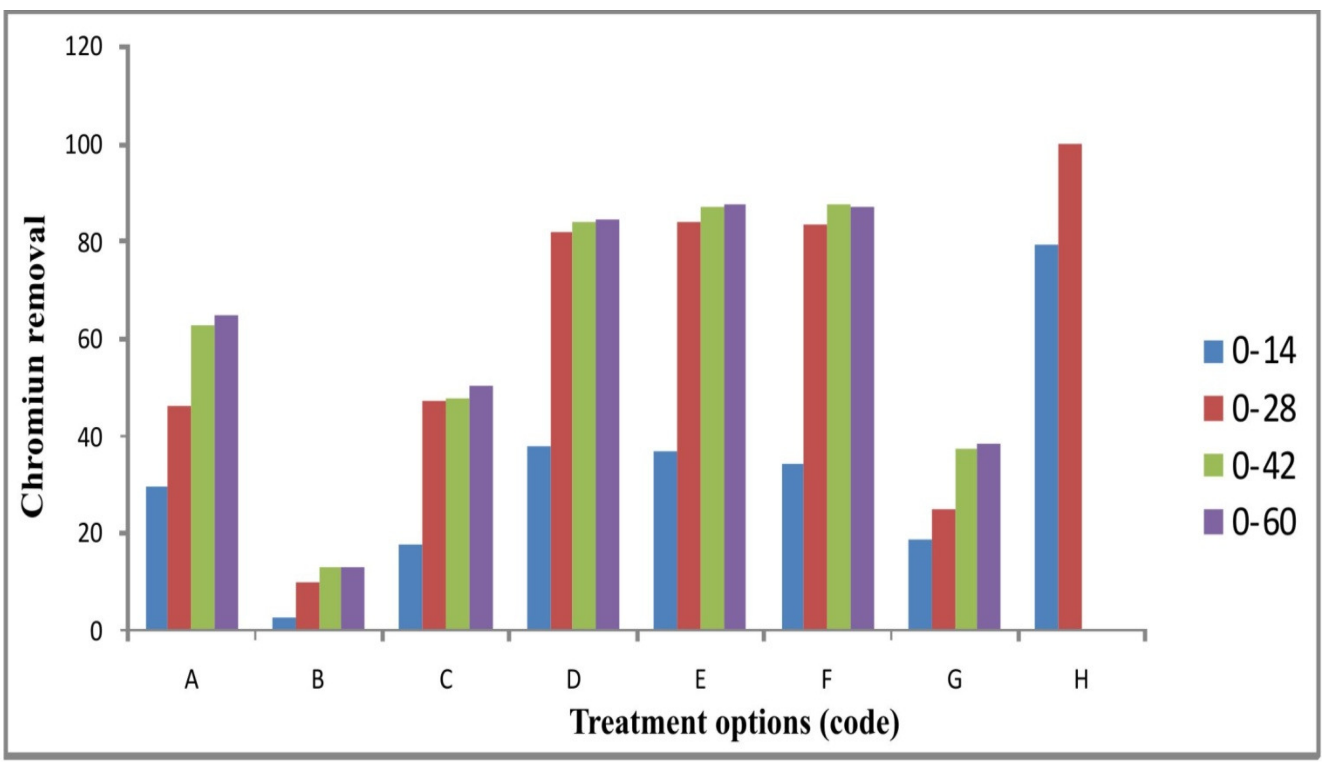

Figure-2
Chromium Removal Percent

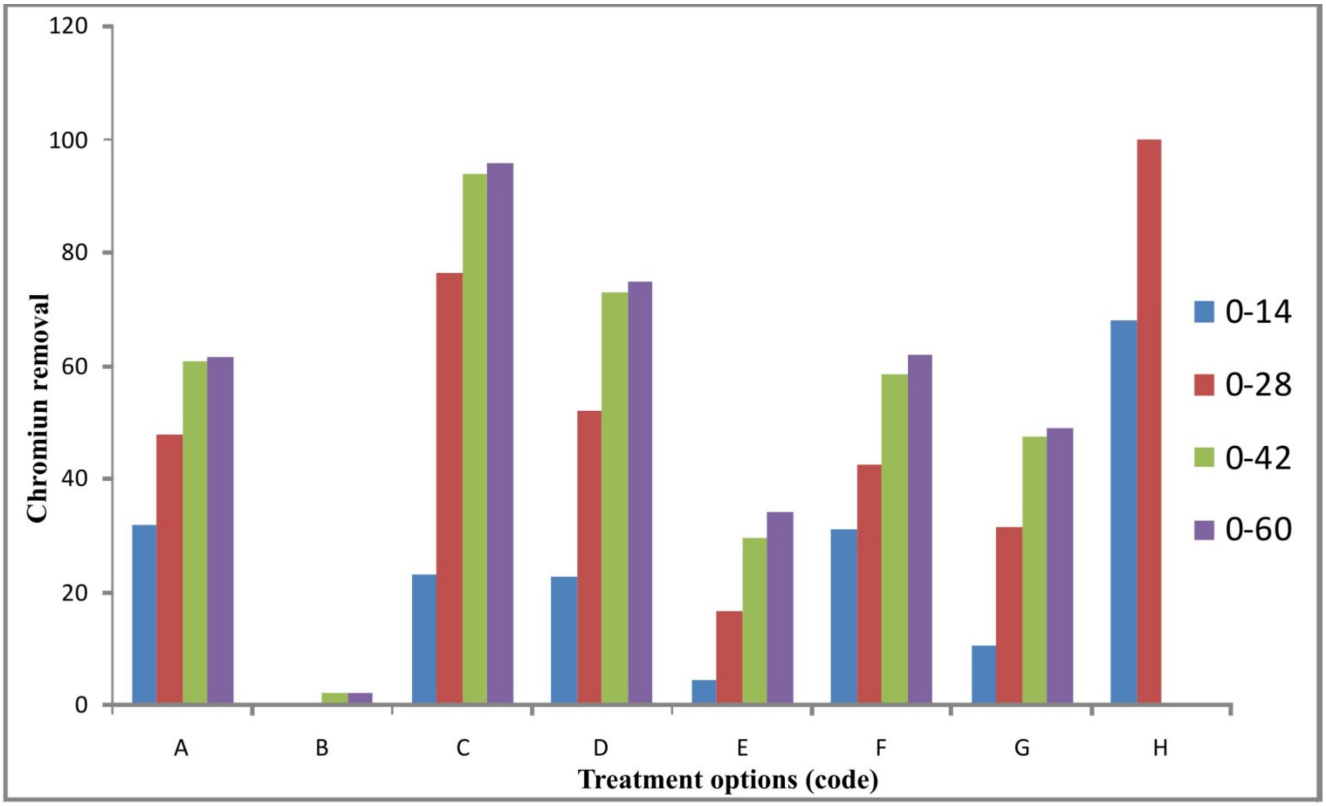

Figure-3
Zinc Removal Percent

The mixed culture of the test micro organisms proved a veritable tool for the biormoval of the heavy metals as shown in figure-1, -2 and -3. This efficiency may be that the various micro organisms were able to optimize their different biorecovery potentials, and could co-exist with minima or no toxic products from their activities (symbiosis).

This research also demonstrates that broconcentration was not the only process in place, bioabsorption took place as shown in table -7 and these metals in the later process are loosely held by the cells and can easily be washed off.basteria species showed stronger capacity to bioconcentrate there metals than their fungal and algal counterparts. Indidvidualy, *Pseudomonal auregnosa* showed the greatest potential for bioconcentration to zinc, Aspergillus niger did better for Cadmium while *Bacillus subtilis* performed better than the other test organisms for chromium biormoval. Their intracellular concentration of the different metals found within some cells involved metal in ion transport across the cell membrane which makes the release of their metals back into the environment.

The micro organisms studied demonstrated low broconcentration factor, that is the readily available these metals for biosorption to the test organisms. Cadmium and zinc appeared more readily available to the organisms than chromium, perhaps a function of concentration and toxicity of the metals. The lest microorganisms demonstrated a high capacity for the broremoval of the test metals and are recommended for use in designing a technology to be employed in remediating heavy metal impacted environment.

Conclusion

This study showed that Port Harcourt Refinery effluent contained relatively high levels of Cadmium, Chromium and Zinc. *Bacillus subtilis, Pseudomonas aeruginosa, Rhizopus stolonifer, Aspergillus niger* and *Chlorella vulgaris* have showed great ability to bioremove these metals from the effluent. However, the study demonstrated thatphysicochemical parameters such as pH, Organic carbon, Nitrate and Phosphate have direct relationship with the ability of these microbes to recover these heavy metals. The bioremoval process isby both bioadsorption and biosorption.Bioremoval is an efficient system of clean-up and can be use to remediate heavy metal imparted environment.

Recommendations: i. Bioremoval of Cadmium, Chromium and Zinc can be achieved using *Bacillus subtilis, Pseudomonas aeruginosa, Rhizopus stolonifer, Aspergillus niger and Chlorella vulgaris;* as such can complement the current treatment options for Refinery effluent. ii. The potentials of these microbes to biorecover these heavy metals from the effluent can be exploited in designing a new technology for remediating heavy metal impacted environment. iii. Since bioremoval is cost effective and does not leave toxic metabolic production, chemical or biological sludge and as such does not lead to secondary pollution, this area of research needs to be encouraged.

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