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Effect of Chromium on the Survival of Heterotrophic Bacteria in Waste Stabilization Ponds

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ABSTRACT-Based on experimental work, the effect of pH on heterotrophic bacteria enumeration was studied in batch cultures tests conducted under different chromium concentrations. Samples were collected from the effluent of the primary facultative pond of the University of Dar es Salam (Tanzania) waste stabilization ponds system. The enumeration of heterotrophic bacteria was investigated at different pH values. The density of bacteria was higher at pH 7 than any other pH values for all concentrations. At this pH, the number varied between 1,450,000 to 280,000 at 12 and 108 hours after addition of 5 mg/l chromium concentration. In case of 50 mg/l and pH 7 the number reduced dramatically to 98 to 0 at 12 and 108 hours respectively. It was observed that, the pH of 11 was significantly detrimental to the heterotrophic bacteria. The number ranged between 4000 to 500 in case of 5 mg/l after 12 and 108 hours. Only 13 heterotrophic bacteria were counted after 12 hours when 50 mg/l of chromium concentration was added.

Keywords: *Chromium, Heterotrophic Bacteria, pH*

المستخلص-أعتمادا علي التجارب المعملية، تمت دراسة تأثير الرقم الهيدروجينيالهيدروجيني علي عدد الباكتيريا الهيتروفكية في وجود الكروم بتراكيز مختلفة. جمعت العينات من مخرج البحيرة الأولية لمعالجة المخلفات في جامعة دار السلام، تنزانيا. التعداد البكتيري تم أيجاده عند قيم مختلفة للرقمالهيدروجيني. وجد أن أعلي عدد للبكتيريا عند الرقم الهيدروجيني 7 في مختلف تراكيز الكروم. عند هذا الرقم يتراوح العدد بين 1,450,000 الي 280,000 بعد 12 و 108 ساعة من أضافة 5ملجم/لترعلي الترتيب . في حالة أضافة 50 ملجم/لتر من الكروم ورقم هيدروجيني 7 يتناقص العدد البكتيري الي 98 و 0 بعد 12 و108 ساعة من أضافة الكروم علي الترتيب. لقد تلاحظ أن العدد البكتيري يتناقص بشدة عند رقم هيدروجيني 11. تراوح الرقم بين 4000 الي 500 في حالة أضافة 5 ملجم/لتر من الكروم بعد 12 و108 ساعة من أضافة الكروم. فقط 13 من البكتيريا وجدت بعد 12 ساعة من أضافة 50 ملجم/لتر من الكروم.

INTRODUCTION

Chromium is a metallic element that functions as a co-factor to augment the action of insulin, and thus influences carbohydrate, protein, and fat metabolism. It is odorless and tasteless and occurs naturally in various types of rock, soil, ore and volcanic dust as well as in plants, animals and humans. The most common forms of chromium are trivalent (chromium 3) and hexavalent (chromium 6); In the environment, chromium usually exists in the trivalent form. It is possible for one form to change into the other when chemical conditions are right. Hexavalent chromium is recognized as a human carcinogen when it is inhaled. Chronic inhalation of hexavalent chromium has been shown[1] to increase risk of lung cancer and may also damage the small capillaries in kidneys and intestines.

Other adverse health effects associated with hexavalent chromium exposure, according to the National Institute for Occupational Safety and Health (NIOSH), include skin irritation or ulceration, allergic contact dermatitis, occupational asthma, nasal irritation and ulceration, perforated nasal septa, rhinitis, nosebleed, respiratory irritation, nasal cancer, sinus cancer, eye irritation and damage, perforated eardrums, kidney damage, liver damage, pulmonary congestion and edema, epigastric pain, and erosion and discoloration of one's teeth[1].

Microorganisms play an important role in biological treatment. Microorganisms have been responsible for the natural stabilization of human and animal wastes since the origin of these species on earth [2]. Heterotrophic bacteria are the most important species in the degradation of organic materials. It can be further classified as aerobic, anaerobic or facultative, depending on their need of oxygen. Wastewater containing chromium with high concentration, discharged into sewer system without pretreatment can cause adverse effects on biological wastewater treatment processes. Therefore, only low concentration of chromium is allowed in the effluent discharged to receiving waters, because of toxicity to aquatic and human life.

Since most municipal wastewater treatment facilities use biological treatment processes for stabilization of organic and inorganic substances in wastewater, it is necessary to have knowledge of the effect of chromium on these processes. Therefore, it is important to understand the effect of chromium on the toxicity to heterotrophic bacteria in order to limit its concentration in industrial effluents which are treated in waste stabilization ponds. Chromium enters the waste stream generally in the form of hexavalent salts such as sodium chromate, potassium chromate, and sodium dichromate.

These salts are used extensively in industries, such as electroplating, paint and pigment manufacturing, textile, fertilizer and leather tanning[3]. Most industrial wastewater must first pass through a sewage treatment plant. Since bacteria in such plants are destroyed by trace amount of chromium, the treatment plant becomes no longer effective. Temperature and pH play a vital role on survival and growth of bacteria. In general, optimal growth occurs within a fairly narrow range of temperature and pH, although the bacteria may be able to survive within much boarder limits. The objective of this paper is to study the effect of chromium on the survival of heterotrophic bacteria in waste stabilization ponds.

MATERIAL AND METHODS

Sampling

To obtain reliable results from any bacteriological analysis of water samples, one has to strictly follow the standard procedures laid down for their collection, dispatch to the laboratory, storage and sample examination. In this paper, all laboratory examinations were carried out in accordance with *Standard Methods*[4].APHA, AWWA *and* WPCF.

Sampling Apparatus

Cleanness of accessories used can affect the quality of samples and finally the results from the analysis. Sampling bottles for microbiological examination where cleansed and rinsed carefully and were given a final rinse with distilled water. All of the equipment used for the examination of heterotrophic bacteria were sterilized for more than two hours at 1700^{o} C in a hot air oven before collection of samples.

Sampling Procedure

To assess the effect of chromium on survival of heterotrophic bacteria in waste stabilization ponds, grab waste water samples were collected from the effluent of the primary facultative pond of the University of Dar es Salaam waste stabilization ponds system. A beaker was used to collect samples and the content was poured into six wide mouth glass bottles of 500 ml capacity each. An ample air space was left in the bottle (at least 2.5 cm) to facilitate mixing by shaking, prior to examination.

Preparation of chemicals

Preparation of chromium

To prepare solution to be used in the tests, 141.45 mg of $K_2Cr_2O_7$ was dissolved in 1000 ml of distilled water to make 50 mg/1 solution of chromium ions. From the 50 mg/1 of chromium solution, different dilutions of 5 mg/1, 10 mg/1, 15 mg/l, 25 mg/1 and 50 mg/1 were prepared by making use of the heavy metal solutions and the samples.

Preparation of Agar

The agar was prepared according to the specification given by the manufacturers. 35 gm of agar was diluted in 1.0 liter of the prepared distilled water. The agar was then sterilized in an autoclave for 15 minutes at a temperature of 121° C and a pressure of $1.06kg/cm^2$. The

prepared agar was left to cool down to a temperature of about 40°C in an incubator adjusted to that temperature before pouring into the plates so that it would not kill the bacteria.

Bacteriological Analysis

The effect of chromium on the survival of heterotrophic bacteria in waste stabilization ponds was investigated in batch cultures. Samples were collected from the effluent of primary facultative pond. Chromium ions were added artificially. Samples were adjusted to pH 7 and were kept at room temperature during the determination of the effect of chromium at varying concentrations. The second run was done to find the influence of chromium concentrations at fixed pH of 4, 6, 7, 8, 9 and 11 and the samples were kept at room temperature.

In this study pour plate count was used to enumerate the heterotrophic bacteria because of its ability to give best information on the number of bacteria. Two replicates in appropriate dilutions were used. Dilution water was prepared from distilled water, presterilized in an autoclave and allowed to cool at room temperature. By using pipettes, 1.0 ml of each diluted sample (plus the heavy metal ions, except for the control sample) was placed in Petri dishes.

The time lapse between making the dilutions and pouring the plates was less than 20 minutes. Then about 15 ml or the liquefied agar medium was poured in each petri dish containing the sample. The agar and sample was thoroughly mixed together for a uniform dispersion of organisms by gently tilting and rotating the dish, taking care not to splash upper portion. The mixture of diluted samples with agar was then left for about 15 minutes to allow agar to harden on a level surface. After making sure that agar has hardened enough, the plates were removed and incubated at 35°C for 72 hours in accordance with Mayo [5]. After the incubation period, colonies on the petri dishes were counted. The new samples were reincubated after every 24 hours. The duration of the whole experiment was 96 hours.

The effect of chromium on heterotrophic bacteria at varying pH

The sample was divided into thirty six portions of 300 ml each (beaker). Out of the thirty six portions of samples, six groups were selected. Each group contained six equal volume portion of sample of 300 ml. Each group was dosed with different concentration of chromium (0, 5, 10, 25 and 50 mg/1) and in each single group each sample was kept at constant pH of 4, 6, 7, 8, 9 and 11. Samples were adjusted artificially to the desired pH by addition of either NaOH or H_2SO_4 for raising or lowering pH, respectively. Analysis of heterotrophic bacteria count was done as explained.

RESULTS AND DISCUSSION

Tables 1 to 6 show the results of the effect of the pH on the enumeration of heterotrophic bacteria. The density of heterotrophic bacteria was higher at pH 7 than any other pH value for all concentrations. Table1shows the effect of pH on heterotrophic bacteria after 12 hours from chromium injection. The number ranged between 145,000 to 98 at pH 7 at concentration 5 to 50 mg/l, respectively. The pH may have a significant role on the processes associated with the change of the state of chromium ions and hence on the decay of heterotrophic bacteria. The most adverse effect of pH was observed at pH 11, the number varied between 4000 to 13 at concentrations 5 and 50 mg/l.

Table 2 shows the trend of heterotrophic bacteria count after 36 hours. Again the trend shows the mortality rate of heterotrophic bacteria is minimum at pH 7, which confirms that the optimum pH for bacteria growth is near neutral pH. Similar trend observation have been recorded but with less number of bacteria when count conducted after 60, 84 and 108 hours from chromium injection. The number goes less as time increased (Tables 3, 4 and 5) .No bacteria have been counted after 36 hours for higher concentrations(25 and 50 mg/l).

The mortality rate for control sample (without chromium) was found to be higher at pH of 11. Significant mortality rate of bacteria was also observed at pH 4 and 6. This shows that pH fluctuation in waste stabilization ponds may affect the population of heterotrophic bacteria

even in absence of heavy metals such as chromium.

Table 6 shows the mortality rate of heterotrophic bacteria at various pH values and concentration of chromium. For a given dose of chromium concentration, the mortality rate was minimum at pH 7. Likewise for the given pH, the mortality rate of heterotrophic bacteria increased as chromium concentration increased.

CONCLUSIONS

The influence of pH on the heterotrophic bacteria growth was investigated at different chromium concentrations. The optimal pH value for bacteria was found to be 7. Significant mortality rate of bacteria was observed at pH 4 and 11. The mortality rate of heterotrophic bacteria was high at pH 4 and 11 even in absence of heavy metals such as chromium.

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Table 1: Effect of pH on the enumeration of heterotrophic bacteria count after 12 hours

pH	Number of heterotrophic bacteria at different concentrations					
	0	**5**	**10**	**15**	**25**	**50**
pH=4	1020000	82000	7000	2200	460	42
pH=6	1550000	125000	10500	3500	770	59
pH=7	18000000	145000	122000	38000	9000	98
pH=8	14300000	1180000	98000	32000	7400	87
pH=9	6200000	500000	42000	13000	3100	72
pH=11	49000	4000	350	100	22	13

Table 2: Effect of pH on the enumeration of heterotrophic bacteria count after 36 hours

pH	Number of heterotrophic bacteria at different concentrations					
	0	**5**	**10**	**15**	**25**	**50**
pH=4	460000	28000	3000	700	46	0
pH=6	640000	43000	4500	1100	76	0
pH=7	4500000	500000	53000	8700	900	49
pH=8	3500000	390000	43000	8000	730	30
pH=9	1500000	171000	18000	4000	290	13
pH=11	22000	1300	145	40	0	0

Table 3: Effect of pH on the enumeration of heterotrophic bacteria count after 60 hours

pH	Number of heterotrophic bacteria at different concentrations			
	0	**5**	**10**	**15**
pH=4	140000	25000	1300	500
pH=6	200000	38000	1900	800
pH=7	2400000	440000	22000	7600
pH=8	2000000	360000	17800	6200
pH=9	850000	152000	7600	3000
pH=11	7000	1200	62	30

Table 4: Effect of pH on the enumeration of heterotrophic bacteria count after 84 hours

pH	Number of heterotrophic bacteria at different concentrations				
	0	**5**	**10**	**15**	**25**
pH=4	128000	12000	1000	400	23
pH=6	192000	18000	1500	600	39
pH=7	2000000	360000	16600	5400	450
pH=8	1800000	290000	13400	4400	370
pH=9	770000	73000	5700	2000	159
pH=11	6000	600	47	20	0

Table 5: Effect of pH on the enumeration of heterotrophic bacteria count after 108 hours

pH	Number of heterotrophic bacteria at different concentrations				
	0	**5**	**10**	**15**	**25**
pH=4	101000	9000	720	300	300
pH=6	153000	14000	1200	500	500
pH=7	1800000	280000	12000	4200	4200
pH=8	1600000	175000	9800	3300	3300
pH=9	600000	62000	4300	1700	1700
pH=11	5000	500	40	18	0

Table 6: Mortality rate of heterotrophic bacteria at varying pH

Conc. of chromium (mg/1)	Mortality rate (k) (hr^{-1})					
	4	6	7	8	9	11
0	0.2818	0.2469	0.0426	0.0617	0.1314	0.5348
5	0.4919	0.4567	0.2525	0.2696	0.3412	0.7436
10	0.6969	0.6631	0.487	0.477	0.5476	0.9466
15	0.7934	0.7547	0.5559	0.5703	0.6453	1.05
25	0.9052	0.8620	0.6574	0.6737	0.7462	1.1585
50	1.1047	1.0763	1.034	1.044	1.0597	1.2024