

## Modeling of mortality rate of heterotrophic bacteria due to chromium in waste stabilization pond

Masoud Gamiel Ahmed<sup>1</sup> and A. W. Mayo<sup>2</sup>

1-Civil Engineering Department, Sudan University of Science and Technology, Khartoum, Sudan

2- Civil Engineering Department, University of Dar es Salaam, Dar es Salaam, Tanzania

**ABSTRACT:** Bacteria play an important role in oxidation and stabilization of organic and inorganic matter in wastewater treatment plant. This paper presents the development of mathematical model to study the effect of chromium on heterotrophic bacteria in waste stabilization pond. The governing equation of the model is based on the Haldane equation. The model developed reflects the mortality rate of heterotrophic bacteria at variable pH and concentration of chromium. To verify the computed results a series of laboratory experiments has been carried out, the predicted mortality rate was obtained from these models and compared with experimental data. The correlation coefficient  $R^2$  was 0.87.

**KEYWORDS:** heterotrophic bacteria, Chromium, Waste Stabilization Ponds

المستخلص: تلعب البكتيريا دورا مهما في أكسدة و استقرار المواد العضوية و غير العضوية في المخلفات السائلة في محطات المعالجة. تعرض هذه الورقة لإنشاء نموذج رياضي لدراسة تأثير الكروم على البكتيريا الهيتروتروفية في أحواض الأكسدة. المعادلة الرئيسية للنموذج هي معادلة هالدين. يحسب النموذج معدل وفاة البكتيريا مع تغير كل من التركيز الأيوني للهاييدروجين ( $pH$ ) وتركيز الكروم. لأختبار النتائج المحسوبة من النموذج أجريت سلسلة من الأختبارات المعملية و بمقارنة النتائج المحسوبة من النموذج والمقاسة وجد أن هناك علاقة جيدة بمعامل ارتباط  $R^2=0.87$

### INTRODUCTION:

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 <sup>(1)</sup>. 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 of wastewater treatment facilities use biological treatment processes for stabilization of organic and inorganic substance 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 <sup>(2)</sup>.

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 formulate a mathematical model describing the reduction of heterotrophic bacteria in waste stabilization pond due to chromium at varying pH.

### Experimental work:

Based on experimental work, the effect of chromium on heterotrophic bacteria at varying pH was investigated in batch

cultures. Samples were collected from the effluent of the primary facultative pond of the University of Dar es Salam (Tanzania) waste stabilization ponds system and were dosed with varying concentration of chromium in the laboratory (0, 5, 10, 15, 25 and 50mg/l), 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<sub>2</sub>SO<sub>4</sub> for raising or lowering pH, respectively. In this study pour plate count was used to enumerate the heterotrophic bacteria because of its ability to give the best information on the number of bacteria. All laboratory examinations were carried out in accordance to standard Methods<sup>(3)</sup>.

The enumeration of heterotrophic bacteria was found with respect to time at different pH and chromium concentration. Table 1 shows the mortality rate of heterotrophic bacteria at various pH values and concentration of chromium.

Table 1: mortality rate of heterotrophic bacteria at varying pH

Con. of chromium m (mg/l)	Mortality rate k (hr <sup>-1</sup> )					
	pH=4	pH=6	pH=7	pH=8	pH=8	pH=9
0	0.28	0.25	0.04	0.06	0.13	0.53
5	0.49	0.46	0.25	0.27	0.34	0.74
10	0.70	0.66	0.46	0.48	0.55	0.95
15	0.79	0.75	0.55	0.57	0.65	1.05
25	0.91	0.86	0.66	0.67	0.75	1.16
50	1.10	1.07	1.03	1.04	1.06	1.20

**Formulation of the mathematical equation:**

The effect of pH on the biomass growth may be directly or indirectly attributed to hydrogen ion concentration in the medium.

The effect of H<sup>+</sup> on the specific rate may be described by the Haldane equation<sup>(4)</sup> as shown by equation (1):

$$\mu_0 = \frac{\mu [H^+]}{[H^+] + K_{OH} + [H^+]^2 / K_H} \tag{1}$$

Where  $\mu_0$  is the specific maximum growth rate at a certain substrate concentration, and it is function of the environmental factors.  $K_{OH}$  and  $K_H$  are rate constants and they are constant for all pH values at particular concentration. Hydrogen ion concentration can be calculated from:

$$pH = -\log_{10} [H^+]$$

$$[H^+] = 10^{-pH}$$

For decay rate we can substitute  $\mu = 1/K$  and  $\mu_0 = 1/K_0$  in equation (1). Therefore equation (1) can be transformed to:

$$K = K_0 \left[ 1 + \frac{K_{OH}}{[H^+]} + \frac{[H^+]}{K_H} \right] \tag{2}$$

Where K is mortality rate constant,  $K_0$  is the minimum mortality rate constant,  $[H^+]$  is hydrogen ion concentration,  $K_H$  and  $K_{OH}$  are rate constant.

**Modeling Process:**

Equation (2) can be used to predict the mortality rate of bacteria. The model was run at each concentration of chromium applied with reflection on the effects of variable pH on the mortality rate.

The efficiency criterion, which is widely accepted for testing of the model accuracy by Nash and Sutcliffe<sup>(5)</sup>, was used to determine the efficiency of the developed model. They defined the model efficiency R<sup>2</sup>, analogously to the coefficient of determination in regression, as the proportion of the initial variance accounted for by the sum of squares of differences F between the observed and estimated mortality rate:

Defining the initial variance F<sub>0</sub> as:

$$F_0 = \sum_{i=1}^n (Y_i - \bar{Y})^2 \tag{3}$$

Where  $\bar{Y} = \frac{1}{n} \sum_{i=1}^n Y_i$  = the mean of the observed mortality rate and n is the number of data points and F written as:

$$F = \sum_{i=1}^n (Y_i - \hat{Y}_i)^2 \tag{4}$$

$\hat{Y}_i$  is the predicted mortality rate.

The efficiency of the model may then be expressed as:

$$R^2 = 1 - \frac{F}{F_0} \quad (5)$$

The parameters  $K_H$ ,  $K_{OH}$  and  $K_0$  were fitted manually using trial and error by determining least square method (Maximum  $R^2$ ). Table 2 shows the best values of the parameters  $K_H$ ,  $K_{OH}$  and  $K_0$  which gives maximum correlation for the chromium concentration of 0, 5, 10, 15, 25 and 50 mg/l.

Table 2:  $K_H$ ,  $K_{OH}$  and  $K_0$  at different concentration at maximum  $R^2$  values

Conc. of Chromium (mg/l)	$K_0$ ( $hr^{-1}$ )	$K_H$ (m)	$K_{OH}$ (m)
0	0.15	$1.4 \times 10^{-4}$	$2.50 \times 10^{-11}$
5	0.35	$3.20 \times 10^{-4}$	$1.10 \times 10^{-11}$
10	0.51	$3.00 \times 10^{-4}$	$1.00 \times 10^{-11}$
15	0.59	$3.00 \times 10^{-4}$	$1.00 \times 10^{-11}$
25	0.76	$5.20 \times 10^{-4}$	$5.27 \times 10^{-12}$
50	1.04	$1.00 \times 10^{-3}$	$1.50 \times 10^{-12}$

The Parameters  $K_H$ ,  $K_{OH}$ ,  $K_0$  were plotted against chromium concentrations and the results are shown in the figures 1, 2 and 3. Equations 6, 7 and 8 show the relationship between these coefficients with chromium concentration.

$$K_H = 1 \times 10^{-7} C^2 + 9 \times 10^{-6} C + 0.0002 \quad (6)$$

$$K_{OH} = 2 \times 10^{-11} e^{-0.0571C} \quad (7)$$

$$K_0 = -0.0003C^2 + 0.0317C + 0.1816 \quad (8)$$

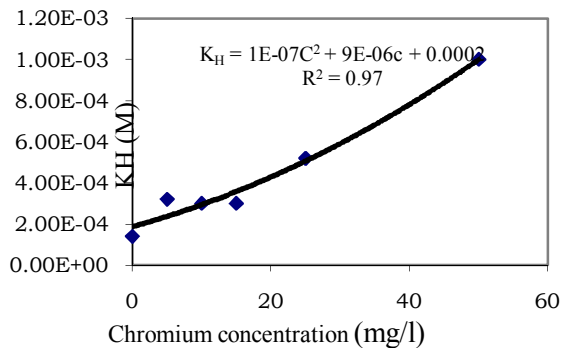


Figure 1:  $K_H$  against concentration

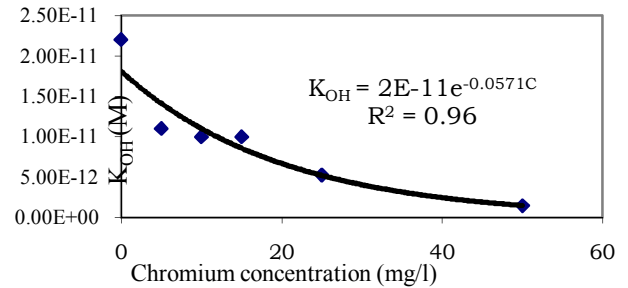


Figure 2:  $K_{OH}$  against concentration

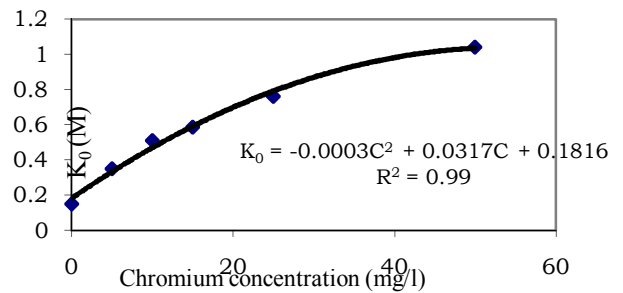


Figure 3:  $K_0$  against concentration

The coefficient of correlation was 0.97, 0.96 and 0.99 for  $K_H$ ,  $K_{OH}$ ,  $K_0$  respectively. The values of  $K_H$ ,  $K_{OH}$ , and  $K_0$  were then substituted in equation (2). The resulting model was shown by equation (9).

$$K_{pred} = (-0.0003C^2 + 0.0317C + 0.1816) \left[ 1 + \frac{2 \times 10^{-11} e^{-0.0571C}}{H^+} + \frac{H^+}{10^{-7} C^2 + 9 \times 10^{-6} C + 0.0002} \right] \quad (9)$$

Where  $C$  concentration of chromium (mg/l) and  $[H^+]$  is hydrogen ion concentration ( $= 10^{-pH}$ ), the efficiency ( $R^2$ ) of the model was found to be 0.87, which is reasonably good. The predicted and observed mortality rate constant,  $K$  was plotted in Figures 4 and 5. A regression analysis on the data results in the equation:

$$K_{pred} = 0.896 K_{observed} \quad R^2 = 0.87$$

The best-fit was very close to 45° line.

### Discussion of Results:

Figure 4 shows the effect of pH on the mortality rate of heterotrophic bacteria, a broad optimum pH range was observed, minimum heterotrophic bacteria decay rate was observed between pH 5.5 and 9.5.

Steady bacteria decay rates were observed at this range for all concentrations.

The pH is environmental factor that influence the growth rate of microorganism. Benefield and Randall<sup>(6)</sup> explain that for the most of the bacteria and most biological wastewater treatment process, the range of pH for growth is between 4 to 9. Many studies on the effect of pH on the growth of microorganism have defined the optimum pH as those at which the growth of microorganism is highest<sup>(7)</sup>.

The figure shows at neutral pH, the mortality rate was constant for all concentration of chromium used. The constant value of mortality rate was higher for higher concentration of chromium. The pH plays a big role on ion exchange and also the form at which chromium exist in the water. Usually at pH greater than 5, chromium may exist in form of precipitate. Hence if this is the case, then the influence on the mortality rate may remain steady as most of chromium ions remain in precipitate<sup>(8)</sup>. There is also a trending of chromium in the solution to free some H<sup>+</sup>, and this might have more significant effect on the mortality rate of the heterotrophic bacteria. At pH greater than 10, the model predicts an increase in mortality rate. In accordance with the model prediction, the influence of chromium on mortality rate at pH values in the acidic range was small. From figure 4, it is clear that heterotrophic bacteria was more sensitive to alkaline pH than acidic pH, the mortality rate was highest at pH 11 for all concentrations. Sharma and Forestor<sup>9</sup> found that there is significant increase in the pH of the reaction mixture containing higher chromium concentration.

Some preliminary investigations on the removal of chromium have been reported by Zhu and Wang<sup>(10)</sup>. They concluded that in treating wastewater containing heavy metal ions, the best results were obtained when the ion

concentration were low. Huang and Wu<sup>(11)</sup> studied the removal of hexavalent chromium by activated carbon and concluded that the

absorption was very pH dependent. The maximum absorption was found at pH 6. Figure 5 shows the scatter plot of the observed and model predicted mortality rate for different chromium concentration. The correlation coefficient R<sup>2</sup> was 0.87.

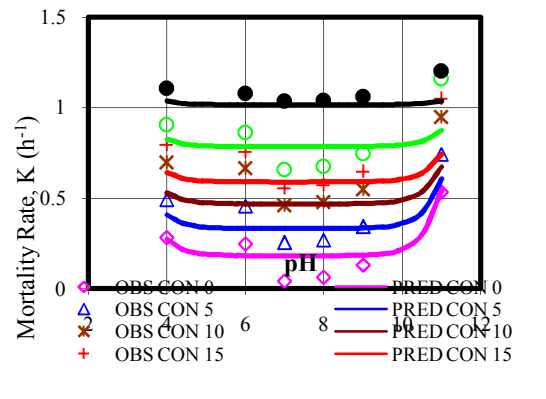


Figure 4: Effect of pH and chromium concentration on mortality rate of heterotrophic bacteria in waste stabilization ponds

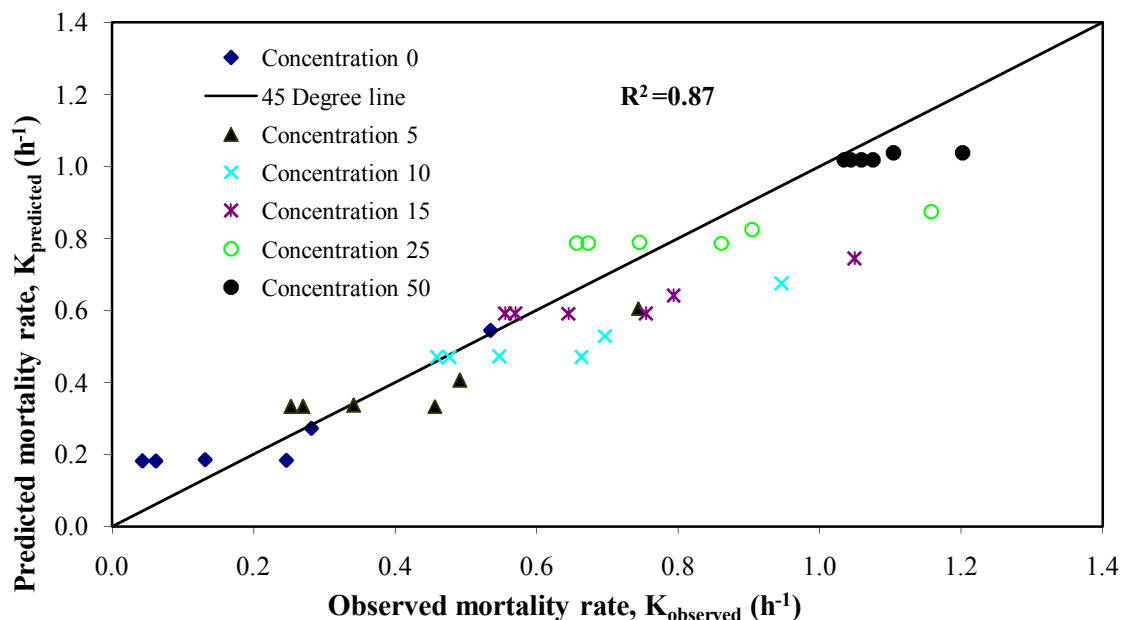


Figure 5: Scatter plot of the observed and model predicted mortality rate for different chromium concentration

#### Application and limitation of the model:

The model can be a vital tool in the design of biological treatment plants, such as oxidation ponds. This model is easy to apply, and only requires measurement of pH. The hydrogen ion concentration  $[\text{H}^+]$  may be calculated from equation (1). The heterotrophic bacteria mortality rate constant at chromium concentration  $C$  can then be calculated from the developed model as shown in equation (2). The growth of heterotrophic bacteria in waste stabilization ponds is influenced by many factors including, pH, presence of toxic heavy metals, composition of sewage, temperature and dissolved oxygen. The model developed reflects the mortality rate of heterotrophic bacteria at variable pH and concentration of chromium, other factors

#### References:

1- Okun, D.A. and Poghis, G. (1975). Community wastewater collection and disposal. World Health Organization, Geneva,

were not taken into account during development of the model.

In reality one could include other limitations due to other factors mentioned above. However, as the model stands, it provides an insight and method of quantification of mortality rate of heterotrophic bacteria at variable pH under constant influence of the chromium concentration.

#### Conclusions:

A mathematical model was developed to predict the effect of the chromium on the heterotrophic bacteria in waste stabilization pond at various pH values. For a given dose of chromium concentration, the mortality rate was minimum at pH 7. Likewise for the given pH, mortality rate of heterotrophic bacteria increased as chromium concentration increase

2- Ganguli, A. and A. K. Tripathi., (2002). Bioremediation of toxic chromium from electroplating effluent by chromate-reducing *Pseudomonas aeruginosa* in two bioreactors. Appl. Microbiol. Biotechnol., 58: 416-420.

- 3- APHA, AWWA, WPCF (1992). Standard Methods for the examination of water and wastewater 18<sup>th</sup> Edition. American Public Health Association.
- 4- Mayo, A. W. (1997). Effect of temperature and pH on the kinetic growth of unialga *Chlorella vulgaris* cultures containing bacteria. *Water Environment Research*, Vol.69, No.1, pp.64-72,
- 5- Nash, J. E. and Sutcliffe J. V. (1970). River flow forecasting through conceptual models part I, A discussion of principles, *Journal of Hydrology*, 10 (3), 282–290,
- 6- Benefield L.D. and Randall C.W. (1980). Biological process design for wastewater treatment. Prentice-Hall, Englewood Cliffs, New Jersey,
- 7- Mayo, A. W. (1997). Response of mixed cultures of *Chlorella vulgaris* and heterotrophic bacteria to variation of pH. *Wat. Sc.Tech.*, Vol. 30,No.8, pp. 285-294..
- 8- Alves et al., Alves, C. G. (1993). Chromium removal in tannery wastewaters-polishing by *Pinus Sylvestris* bark. *Wat. Res.*, Vol. 27, No.8, pp.1333-1338.
- 9- Sharma, D. C. and Forestor C.F. (1993). Removal of hexavalent chromium using sphagnum moss peat. *Water research*, Vol. 27, No. 7, pp.121-1208.
- 10- Zhu Y and Wang Z. (1984). A preliminary study of the removal of heavy metals from the wastewater with several Chinese peats. 7<sup>TH</sup> International Peat Congress proceeding, pp. 147-152.
- 11- Huang C. P. and Wu M. H. (1977). The removal of chromium from dilute aqueous solution by activated carbon. *Wat. Res.*, Vol. 11, pp. 673-679,