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MULTIVARIATE REGRESSION ANALYSIS OF DISINFECTION KINETICS USING *Moringa oleifera* DEFATTED SEED EXTRACT

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ABSTRACT

The objective of this research is to determine the disinfection inactivation kinetics of defatted *Moringa oleifera* salt seed extract on *Escherichia coli* (*E.coli)* bacterial strains using statistical multivariate regression analysis. The disinfection kinetics of the seed extract was carried out using a batch mode treatment of water in shake flasks and the bacterial count of *E.coli* bacterial cells were used as microbial indicators. The order of reaction as well as the models of different disinfection kinetics were determined. The goodness of fit for each model studied were evaluated using regression analysis in SPSS. The results revealed that modified Homs model better describes the disinfection inactivation kinetics for defatted *moringa oleifera* seed salt extract and the extract deviated from first order reaction. this is the first study on modified Hom's law to describe the inactivation of *E.coli* using defatted *Moringa oleifera* seed salt extract.

Keywords: *Moringa oleifera*, disinfection, *E. coli*, regression, models.

1. INTRODUCTION

Disinfection of water in water treatment is very important and the disinfection inactivation kinetics shows empirical relationship between process conditions. In water disinfection, there are different mathematical models proposed for the disinfection inactivation kinetics for different chemical disinfectant. They are described with analytical expression that combines process parameters such as dosage, contact time, microbial concentration and sometimes on temperature and pH etc (Marugán, van Grieken, & Pablos, 2010; Marugán, van Grieken, Sordo, Cruz, & Grieken, 2008). The commonly used models are Chick-Watsons model, Homs model, Selleck-Collins model etc. The ancient Chick-Watson model which is the product of concentration and time is the most commonly used in water treatment, while other models are used for deviations from the first order kinetics of Chick-Watson model (Azzellino *et al.,* 2011).

While the Chick-Watson model is popularly used to describe inactivation kinetics of chemicals, it has limitations in most practical disinfection process because the rate of kill does not remain constant. Rather, it decreases with time depending on the form of disinfectant, type of organism and other operating conditions (Metcalf & Eddy, 2004). The Collins-Selleck model was developed to address the declining (decelerating) rate (like a convex curve) of disinfection (MWH, 2005). Collins and Selleck began with formulation that described the declining rate of inactivation and they modified it to the lag in observed real systems. The Hom model is commonly used to describe either accelerating or declining rate of inactivation [1]. It has been used to analyse disinfection data of different organisms and to model the performance of disinfection systems and it is a linear regression of disinfectant concentration, time and survival (Lambert & Johnston, 2000).

The use of *Moringa oleifera* seed as a disinfectant for drinking water is an emerging yet useful aspect. Although, a lot of literature reviews have revealed its antibacterial property against both gram-negative and gram-positive microbes (Arun & Rao, 2011; Bichi, Agunwamba, Muyibi, & Abdulkarim, 2012; Bukar, Uba, & Oyeyi, 2010; Ferreira *et al.*, 2011; Idris et al., 2013; Nwaiwu, Ibrahim, & Raufu, 2012; Nwaiwu & Lingmu, 2011; Viera, Mourão, Ângelo, Costa, & Vieira, 2010; Walter, Samuel, Arama, & Joseph, 2011), yet its application is mostly channelled into traditional medicine and food. There are limited relevant studies available using *Moringa oleifera* seed as disinfectant for drinking water purposes. However, the research on *Moringa oleifera* seed as disinfectant in water till date was conducted in a research(2012b) who revealed that the crude seed extracts has a great potential usage as disinfectant. Water kinetics using the seed extracts was also carried out in another study (Bichi, Agunwamba, & Muyibi, 2012a) which revealed that the kinetic followed a pseudo first order kinetics hence the disinfection of water using *Moringa* seed extract obeyed the Chicks-Watson disinfection kinetics model. The coefficient of specific lethality (Λcw) obtained from the study was determined as 3.76 L mg-1 min-1 for *E.coli* inactivation using *Moringa oleifera* seeds extracts.

Similarly, in another study conducted, [11] their results revealed that the disinfection kinetic was not first order and tailing was observed when *Moringa oleifera* seed extract was used for the treatment of both medium and low turbid water. Due to different disinfection kinetic reports on the use of crude *Moringa oleifera* seed extract as a disinfectant, the results from literature differs greatly which is why this research was conducted to evaluate the different kinetics associated with the disinfection process when using the extract.

Hence for this study, defatted *Moringa oleifera* seed salt extract was investigated to get a better understanding of the disinfection inactivation mechanism for a water source that has a low microbial loading. The inactivation efficiency was evaluated with respect to microbial indicator, *Escherichia coli (E.coli)* which is the

most widely accepted indicator for the existence of faecal contamination in water.

2. MATERIALS AND METHODS

2.1 Collection of *Moringa oleifera* seed

The main material used in this study is dry *Moringa oleifera* seed which was collected from villages surrounding Bayero University (New Campus), Kano, Nigeria.

2.2 Preparation of 1Molar salt defatted seed extract

Two grams of *Moringa oleifera* seed powder after defatting was weighed and added to 1 litre of 1M solution of NaCl and mixed at high speed of 6000rpm in a centrifuge for 10 minutes then filtered. The filtrate used to make stock solution of 1000mg/L.

2.3 Order of reaction

The order of reaction was carried out by keeping the optimum dosage and the agitation constant and the residual bacterial count from the heterotrophic plate count were estimated at constant time intervals. The data collected were used to determine the order of reaction and only zero, first and second order reactions were considered. The one that gives a straight line with the highest coefficient of determination (R^2) was chosen as being the best fitted. The equations used are summarized in Table-1.

Table-1. Order of reaction equations.

Types of order of reaction	Equation used
Zero order	$C_{Af} = C_{A0} - k_0 t_f$ (1)
First order	$\ln C_{Af} = \ln C_{A0} - k_1 t$ (2)
Second order	$\frac{1}{C_{Af}} = \frac{1}{C_{A0}} + 2k_2 t$ (3)

2.4 Disinfection kinetic models using statistical analysis

The residual colonies were estimated at given time intervals at constant agitation and optimum dosage. The experimental data collected were used to fit the different disinfection inactivation models such as the Chicks, Watson, Collin-Selleck, and Homs models as shown in Table 2. The result with the highest coefficient of determination was chosen as the best fitted and non-linear multivariate regression analysis with respect to the bacterial strains was used to determine the inactivation kinetics.

The log survival ratio was chosen as the dependent variable and dosage and time taken as the predictors. The least square methods were utilized and the loss function was minimized through Quasi-Newton algorithm (uses the first and second order derivatives to achieve the minimum of the least square methods). The student t-test was used to assess the significance level of the regression coefficient (Azzellino et al., 2011). All the results obtained from this study were carried out in triplicates hence, their mean values were determined with SPSS software version 20. The significance levels of the generated data in this study was also determined.

Table-2. Commonly used disinfection kinetic models.

Chicks-Watson model	$\ln\left(\frac{N}{N_0}\right) = -\Lambda C^n t$
Collin-Sellecks model	$\ln\left(\frac{N}{N_0}\right) = -\Lambda_{CS}\left[\ln(Ct) - \ln(b)\right]$
Homs model	$\ln\left(\frac{N}{N_0}\right) = -\kappa C^n t^m$

Where C is the concentration of disinfectant, N is the number of organisms present at time t, N_0 is the number of organism present at time 0, t is time, and k is inactivation rate constant Λ is the coefficient of specific lethality representing the relative potencies of disinfectants at a unit concentration for a unit time, b is the lag coefficient in mg. min/L, Λ_{CS} is the log-based coefficient of specific lethality, Ct is the product of disinfectant concentration and time. The parameter n is the Hom dilution coefficient unit less, m is the Hom time exponent unit less. The plot of Homs model using multiple regression analysis gives values for k, n and m

3. RESULTS AND DISCUSSIONS

3.1 Order of reaction

The order of reaction reveals the functional relationship between concentration and rate. It determines how an amount of a compound or chemical speeds up or retards a reaction as well as rate of product formation (includes input reactants consumption and usage and product output formation). In order to determine the order of reaction for *E.coli* bacteria strains, the concentration of the defatted seed extract was set as 125mg/L and the total viable count (TVC) plate count of the bacterial strains was determined at every 20 minutes which lasted for two hours. The plot of the residual bacterial colonies versus the contact time is shown in Figure-1. This showed the order of reaction using the defatted seed extract as disinfectant on *E.coli* bacterial strains. Results from the graph showed that the order of reaction was second order based on the coefficient of determination R^2 of 0.7508 as compared to 0.4276 and 0.6598 of zero order and first order respectively. The second order of reaction for the disinfection process of *E.coli* using defatted salt seed extract reveals that both the dosage and concentration of the seed extract have a significant effect on the rate of reaction. Most disinfection processes are usually first or second order.

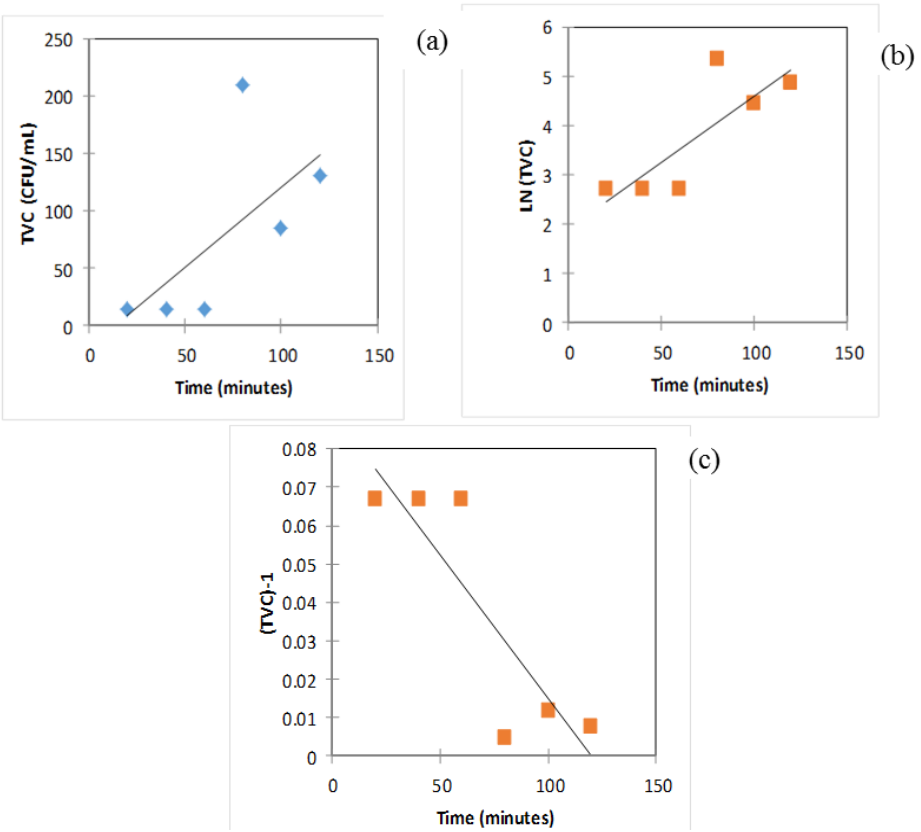

Figure-1. Order of reaction for *E.coli* cells (a) zero order reaction (b) first order reaction (c) second order reaction.

Based on the results as shown in Figure-1, the second order reaction of *E.coli* was considered because of its dependence on the concentration of the seed extract and contact time which is an important factor during disinfection process. In engineering practise, especially in disinfection process, longer time contact is favourable to allow microorganisms to be in contact with the disinfectant [4]. This was also observed in an earlier research conducted where the reaction is not a first order reaction using *moringa oleifera* seed in water treatment(Nwaiwu & Lingmu, 2011).

3.2 Inactivation kinetics of synthetic water containing *E.coli*

The plot of log survival of the *E.coli* bacterial colonies against time showed a deceleration of the process called "tail". The tailing as shown in Figure-2 may be explained either by a vitalistic hypothesis in which individual bacteria in a population are not identical (Lee & Nam, 2002) and it is also hypothesized that tailing occurs when a (small) subpopulation of a microbial population is not effectively inactivated byy the extract as a result clumping during the disinfection process exposure (Pennell, Aronson, & Blatchley, 2008). Another possible explanation might be as a result of decrease in the germicidal properties of defatted *Moringa oleifera* salt extract with time. It is also observed from the plot that the bacteria population were gradually building up over a period of time which might be as a result of natural heterogeneity in resistance among the microorganisms. This was also observed in a study conducted by a research(Nwaiwu & Lingmu, 2011) where tailing was also observed using *Moringa oleifera* seed extract for the treatment of both medium and low turbid water. This observation in terms of the efficiency if the seed extract shows that over a period of time, the disinfectant ability decreases which enhance the resistance of the microorganisms against it. The plot as seen in Figure-2 also shows the deviation from the Chick-Watson model which is expected since the order of reaction was not a first order reaction. Other disinfection models such as Hom model and Collins-Selleck empirical model are widely used to account for deviations from the first order kinetics of the Chicks-Watson model.

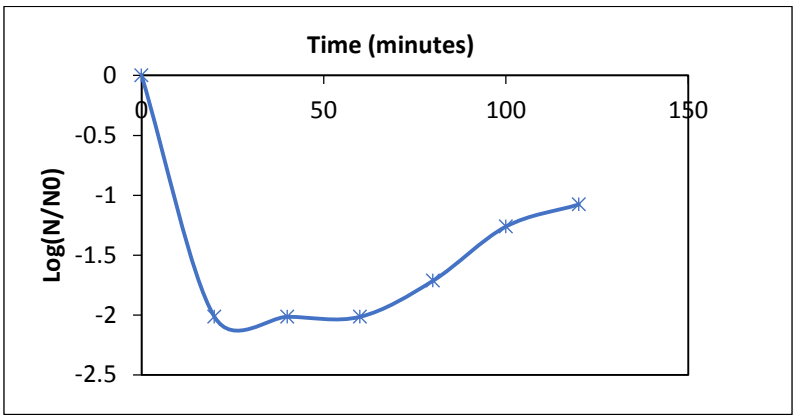

Figure-2. Plot of log survival of *E.coli* bacterial cells against time.

Hence, to determine the disinfection kinetics of *Moringa oleifera* seed extract, the goodness of fit by comparing the coefficient of determination (R^2) for Chick-Watson model, Hom's model and Selleck model were carried out using multiple linear regressions in IBM SPSS 20 statistical software. The least square methods were used and the significance level of the regression coefficient was assessed by means of the t-test. The summary of the coefficient of determination of the results are shown in Table-3.

Table-3. Coefficient determination for different disinfection kinetic models.

Disinfection kinetic model	R^2	Adjusted R^2
Chick-Watson	0.309	0.171
Selleck	0593	0.511
Hom	0.711	0.689

From the experimental data, the Hom empirical model best described the data and the constants *k*, *n* and *m* determined using multivariate regression analysis are summarized in Table-4 below:

Table-4. Summary statistics and regression estimates for the best-fit Hom's models.

	K	***N***	***m***
Estimates	3.31E14	1.586	-5.212
Standard error	1.719	0.565	0.681
T-test	8.446	2.807	-7.648
Significance (P<0.05)	0.000	0.009	0.000
N	30		
R^2	0.7111		

Since m is less than 1, tailing is observed as confirmed from Figure-2. The Homs model is a regression of disinfection performance against the two important factors, time and disinfectant concentration. Both the contact time and concentration are statistically significant as indicated in Table-4 although, time was still the most important parameter (P<0.000). The coefficient of determination R^2 obtained was 0.7111 and adjusted R^2 obtained was 0.689. Generally, the Hom model can be used to fit experimental data with either concave or convex shapes. However, the coefficient of determination using Hom model was not high enough so the inactivation kinetics of *E.coli* cells was modelled again using a modified version of the Hom model which considered the variation in concentration of the disinfectant (seed extract) over a period time using an approach used in an earlier research (Vargas, Moreira, & Jose, 2013) giving in equation 1 below:

$$\frac{dN}{dt} = -k_1 N t^m [C^n(t)] \quad (1)$$

Where C_t = concentration of the seed extract at time t, N is the number of coliforms at time t, k_1 is the rate constant of bacterial inactivation, n is the coefficient of dilution or concentration constant and m is the decomposition constant of the disinfecting agent. The rate of decrease of the defatted seed extract over time was earlier determined to be a second order reaction as shown in Figure-1 above hence the equation of second order is shown below:

$$C(t) = \frac{1}{\left(\left(1/C_0\right) + k_2 t\right)} \quad (2)$$

Where k_2 is the second order rate constant of the reaction (L/mg.min)

Combining equations 1 and 2 results in equation 3 assuming m and n is equal to 1 as reported in the study carried out by (Vargas et al., 2013)

$$N = N_0(k_2C_0t + 1)^{-\frac{k_1}{k_2}} \quad (3)$$

$$\frac{N}{N_0} = (k_2C_0t + 1)^{-\frac{k_1}{k_2}} \quad (4)$$

Where N_0 is the initial number of bacterial colonies (CFU/mL); N is the number of bacterial colonies at time t (CFU/mL); k_1 is the rate constant for bacterial inactivation (min-1); k_2 is the second-order rate constant (L/mg min); and C_0 is the initial seed extract concentration (mg/L).

The kinetic constants k_1 and k_2 were determined using equation 3 to fit the experimental data and the results were computed using non-linear multivariate regression analysis in IBM SPSS software. Where N/N_0 is the dependable variable and the concentration and time are the in predictors. The results are presented in Table 5

Table-5. Rate constants for the inactivation of *E.coli* at the optimum dosage.

	k_1 (min^{-1})	k_2 (L/mg.min)
Estimates	0.562	1
Standard error	0.078	0.012
R^2	0.9990	

A better fit was observed with the modified Hom model under the optimum condition. Hence, the results from Table-5 reveal that the modified Hom model provides a more realistic empirical model to account for the non-linear disinfection kinetics of *E. coli* using the defatted salt extract with coefficient determination of 0.9990. The final kinetic disinfection inactivation model of defatted M*oringa oleifera* salt extract at the optimum dose is given in equation 5 as

$$\frac{N}{N_0} = \left(1\frac{L}{mg.min} \times Ct + 1\right)^{\frac{0.562min^{-1}}{1\frac{L}{mg.min}}} \quad (5)$$

The results obtained in this study were contrary to findings conducted by (Bichi, Agunwamba, & Muyibi, 2012a). In their study, first order kinetic of Chick-Watson model was assumed and used for the inactivation kinetics. When the Chick-Watson model was used on the data for this study, a poor fit ($R^2 = 0.309$) was achieved (see Table-3).

A possible explanation for the deviation from the findings of research [17] could be as a result of the high microbial population used which was about 1.039×10^8 CFU/mL, therefore, it is reasonable to conclude that inactivation of *E.coli* cells may be described as a first order kinetics when the microbial population is very high. But, for this research, a low microbial population count of about 1000 CFU/mL was used. This is because the maximum acceptable level for raw surface water to be used as a drinking source as stipulated in the Malaysian Interim National Water Quality Standards (INWQS) is set to be 5000 CFU/mL for treatment of potable use. Hence, at low bacterial population the inactivation kinetics is not a first order reaction and this result is in close agreement with earlier study carried out by (Nwaiwu & Lingmu, 2011) where *Moringa oleifera* seed extract was not a first order reaction and hence did not fit into Chicks-Watson model. Based on an extensive survey of the existing literature in the field, this is the first study on modified Hom's law to describe the inactivation of *E.coli* using defatted *Moringa oleifera* seed salt extract.

4. CONCLUSIONS

Disinfection kinetic models were developed and compared with existing models for *E.coli* bacterial strains and statistical analysis applied to the inactivation experimental data shows that both the concentration and contact time are very significant in the disinfection process. The inactivation of *E.coli* cells were well fitted with the modified Hom's model which account for deviation from the first order kinetic of Chicks-Watson model. The modified Homs model better describes the disinfection inactivation kinetics for defatted *Moringa oleifera* seed salt extract. Hence, it is proposed that further research should be done by validating the disinfection inactivation model using a surface water source. Also using the salt extract of the seed will reduce cost of water treatment particularly for developing countries during water purification process.

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