Formulation of kinetic model to predict disinfection of water by using natural herbs
Sunil B. Somani\textsuperscript{1}, Nitin W. Ingole\textsuperscript{2}
\textsuperscript{1} Research Student, Sant Gadge Baba Amravati University, Amravati 444 602
\textsuperscript{2} Principal, IBSS College of Engineering, Ghatkhed, Amravati 444 602
somanisb@rediffmail.com
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ABSTRACT

This study investigated the enhancement of disinfection by using natural herbs other than chemical methods in rural/tribal area of India, where peoples are reluctant to use chemical as a disinfectant. The antimicrobial activity of Anjan (Hardwickia Binata), Mutha (Cyperus Rotundus), Ushir (Andropogon Muricatus) and Rajkashtaka (Luffa Cyllindrica) were tested by Disc Diffusion Method (Kirby –Bauer Method) after extracting the dried material powder of natural herbs in 50% alcohol (ethanol). An antibacterial activity was observed in all herbs used. Most effective an antibacterial activity was observed in Anjan. In all herbs maximum removal of \textit{E. coli} was found at 30 minutes optimum contact time onwards and at 1% optimum concentration of different herbs extract used in study. As the disinfection kinetic models are the basis for assessing the disinfectants performance, the experimental results were used to derive suitable kinetic model. Chick Watson model obtained for Anjan: \text{Log} \left( \frac{N}{N_0} \right) = - 0.17Ct \text{ was found very close to chlorine which is widely use as a disinfectant.}

Keywords: Disinfection, Antibacterial activity, Natural Herbs, Extract, Kinetic Model.

Nomenclature

\begin{align*}
N & \quad \text{a number of microorganism at contact time } t \\
N_0 & \quad \text{a number of initial microorganism at contact time, } t = 0 \\
k^* & \quad \text{Reaction rate constant,} \\
t & \quad \text{Contact time (min.)} \\
k & \quad \text{constant for a specific microorganism and set of condition} \\
C & \quad \text{disinfectant concentration} \\
n & \quad \text{coefficient of dilution}
\end{align*}

Notations

\begin{align*}
\text{In} & \quad \text{Logarithm to the base } e \\
\text{Log} & \quad \text{Logarithm to the base } 10
\end{align*}

1. Introduction

Water constitutes one of the important physical environments of man and has direct effect on itself. Safe and adequate drinking water should be provided to the consumer. Water supplied...
should be aesthetically clean and biologically safe. Coagulation, flocculation and filtration remove bacteria up to 99% but 1% of bacteria entering water may be pathogen and may cause some disease. So, disinfection of water is necessary before supply of water for drinking purpose (Patil et al., 2002 and Schoenen, 2002). Chlorine, which is applied to water at various points in a water treatment plant for various purposes including the main purpose of disinfection, combines with naturally occurring organic matter present in trace amounts in raw water to generate certain unwanted chemicals known as disinfection by products (DBPs) in general and halogenated DBPs in particular. Amongst the halogenated DBPs Trihalomethanes (THMs) represent the largest fraction are toxic (Clark et al., 1998 and Chapekar, 2000). So, there is need to discover alternative methods of disinfection.

These alternative methods are termed as minor methods of disinfection, since disinfection through chlorine share major proportion of disinfection all over the world. The use of minor methods are been restricted to selected areas due to lack of precise knowledge about their disinfecting power and cost effectiveness. At present these methods may not appear as a replacement to chlorination but few of them certainly (Bhagwatula et al., 2000; Fair et al., 1968 and White, 1992).

2. Materials and Methods

2.1 Collection of Plant Materials

The plant Anjan (Hardwickia Binata), Mutha (Cyperus Rotundus), Ushir (Andropogon Muricatus) and Rajkashtaka (Luffa Cyllindrica) materials (leaves /fruits) were collected and shaded dried in drying oven at the temperature 105°C to 110°C for at least 12 hours. The materials of different herbs used were converted into powdered form by using grinding machine and then stored at room temperature.
2.2. Preparation of the Extracts

The powders were subjected to successive extraction with organic solvent 50% ethanol by Soxhlet method (Figure 1). The extracts were collected and distilled off on a water bath at atmospheric pressure and the last trace of the solvent was removed in vacuo. Extracts were stored in refrigerator for antimicrobial studies. Stock solution was 20% (w/v) of dried plant materials in solvent (Ghosh *et al.*, 2008 and Singh *et al.*, 2010).

2.3. Kirby –Bauer Method

It is used to check the antimicrobial activity of disinfectant. The effectiveness of an antimicrobial in sensitivity testing is based on the size of the zone of inhibition that surrounds a disk that has been impregnated with a specific concentration of the disinfectant. The zone of inhibition, however, varies with the diffusibility of the disinfectant, the size of the inoculums, the type of medium. It is a standardized system that takes all variables into consideration. It is standard laboratory method (Benson, 2001) used to check the antimicrobial activity of the herbs used in study.

2.4. MPN Test

This test is used to determine the most probable number (MPN) of coliforms (*E. coli*) present per 100 ml of water. In this test a series of nine tubes of lactose broth are inoculated with measured amounts of water to see if the water contains any lactose–fermenting bacteria that produce gas. If, after incubation gas is seen in any of the lactose broths, it is presumed that coliforms are present in the water sample.

In this MPN test, the set up used consists of three double strength lactose broth (DSLB) tubes and six single strength lactose broth (SSLB) tubes as per the quantities given in the table – 1

**Table-1: Test set up**

| Set | No. of Tubes | Strength | ml of Media | ml of Sample |
|-----|--------------|----------|-------------|-------------|
| 1   | 3            | DSLB     | 10 ml       | 10ml        |
| 2   | 3            | SSLB     | 5 ml        | 1ml         |
| 3   | 3            | SSLB     | 5 ml        | 0.1 ml      |

After test set up, incubate the tubes at 35°C for 24 hours and examine the tubes to record the number of tubes in each set have 10% gas or more to determine MPN by using Multiple Tube Test standard table (Benson, 2001 and APHA, 1998).

2.5. Kinetics of Disinfection

Under ideal conditions all cells of a single species of organism are discrete units equally susceptible to a single species of disinfectant, both cells and disinfectants are uniformly dispersed in the water, the disinfectant stays substantially unchanged in chemical composition and substantially constant in concentration throughout the period of contact, and the water contains no interfering substances. Under such conditions the rate of disinfection is a function of the variables the contact time, the concentration of the disinfectants, and the temperature of water (Lyndon *et al.*, 1998 and Watson, 1908).
2.6. Kinetic Modeling

An essential feature of kinetic modeling is simplification and idealization of complex phenomena. Models attempt to represent interactions of disinfectants having different cellular targets and models of action with highly complex microorganisms. Kinetic inactivation models are derived based on the following assumptions being satisfied for batch reactors (i)No back mixing; (ii)Uniform dispersion of organisms and disinfectant molecules; (iii)Sufficient mixing to ensure liquid diffusion is not rate limiting; (iv)Temperature and pH are fixed; and (v)The disinfectant concentration is assumed to remain constant during the contact time (Selleck et al., 1978; Severin et al., 1987 and Yoon –Jin Lee et al., 2002).

3. Results and Discussion

A disinfection kinetic model need to be determined to compare the results obtained from different experimental data such as those involving disinfectant concentration and reaction time mainly. We attempted to determine the coefficients of selected model. The major precepts of disinfection kinetics were enunciated by Chick and recognized the close similarity between microbial inactivation by disinfectants and reactions (Chick, 1908)

\[
\ln \left( \frac{N}{N_0} \right) = k^* t
\]

By using MATLAB program using non linear regression, Curve In (N/No) verses contact time are drawn for different disinfectants used in study. Nonlinear curves for different disinfectants are shown in Figure 2, 3, 4 and 5. These nonlinear nature represents an asymptotic decay or tailing curve. The Reaction rate constant (k*) for different disinfectants are given in table (2)

| Table 2: Reaction rate constant (k*) for different disinfectants |
|---------------------------------------------------------------|
| Disinfectant | Anjan | Mutha | Ushir | Rajkashtaka |
| Reaction rate constant (k*) | 0.0949 | 0.036 | 0.01849 | 0.019 |

The logarithm of the survival ratio of E- coli based on Chick law for different disinfects used in study are illustrated in Figure 6, 7, 8 and 9.

Watson (1908) proposed an empirical logarithmic function to relate the rate constant of inactivation, k to the disinfectant concentration C. In general, disinfection systems are designed by the Ct values derived from Chick-Watson kinetics based on the data obtained from laboratory inactivation studies

\[
k^* = kC^n
\]

\[
\log \frac{N}{N_0} = -kC^n t
\]

The Watson function, (3), is based on the assumption that microorganisms are generally similar and of a single strain of synchronous development and the killing action would be a single-hit and single-site type. The assumptions are necessary in order to derive Chick-Watson model based on a chemical reaction mechanism. In many cases, the n value for...
Chick-Watson law is close to 1.0 and hence a fixed value of the product of concentration and time (Ct product) results in affixed degree of inactivation (AWWA, 1999).

\[
\log \frac{N}{N_0} = -kCt
\]  

(4)

The inactivation degrees on Ct value for different disinfectants used in studies are presented in Figure 10, 11, 12 and 13 indicates the experimental results. As seen in equation (4) our experiments the value of k found give the following Chick-Watson model for different disinfectants.

Table 3: Chick-Watson model for different disinfectants

| Sr. No. | Disinfectant | Chick-Watson model |
|---------|--------------|--------------------|
| 01      | Anjan        | \[ \log \frac{N}{N_0} = -0.17 \text{ Ct} \] |
| 02      | Mutha        | \[ \log \frac{N}{N_0} = -0.0814 \text{ Ct} \] |
| 03      | Ushir        | \[ \log \frac{N}{N_0} = -0.0568 \text{ Ct} \] |
| 04      | Rajkashtaka  | \[ \log \frac{N}{N_0} = -0.052 \text{ Ct} \] |

Figure 2: Relation between In survival ratio and contact time (Anjan)
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Figure 3: Relation between In survival ratio and contact time (Mutha)

Figure 4: Relation between In survival ratio and contact time (Ushir)

Figure 5: Relation between In survival ratio and contact time (Rajkashtaka)
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Figure 6: Relation between log survival ratio and contact time (Anjan)

Figure 7: Relation between log survival ratio and contact time (Mutha)

Figure 8: Relation between log survival ratio and contact time (Ushir)
**Figure 9:** Relation between log survival ratio and contact time (Rajkashtaka)

\[ y = -0.17x \]

**Figure 10:** Relation between log survival ratio and Ct (Anjan)

\[ y = -0.0814x \]

**Figure 11:** Relation between log survival ratio and Ct (Mutha)
4. Conclusion

In rural/tribal area, the people are using water from well or any other sources of water without any treatment. They are also reluctant to use chemicals as a disinfectant. These natural herbs used in this study can be effectively used as a disinfectant. Using these disinfectants, pathogenic bacteria from the water can be killed and water can be made safe for the user. The major population of India is living in rural/tribal area, where these natural herbs are easily available. Chick-Watson model obtained for Anjan: \( \log \left( \frac{N}{No} \right) = -0.17 \ C_t \) was found very close to chlorine disinfectant Chick-Watson model \( \log \left( \frac{N}{No} \right) = -0.16 \ C_t \) (Yoon–Jin Lee et al., 2002). Hence, Anjan was found most effective for antibacterial activity in water purification.

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