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Full Length Research Paper

Disinfection studies of Nahar (*Mesua ferrea*) seed kernel oil using pour plate method

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As a result of disinfection byproducts (DBP) concerns from chlorine, Environmental Protection Agency (EPA) as well as the water treatment industry, place more emphasis on the use of disinfectants other than chlorine. Disinfection also plays a key role in the reclamation and reuse of wastewater for eliminating infectious diseases, and to augment domestic water supply and decrease the impact of human activities on the environment. The aims of this study were thus: to investigate the potential of the Nahar seed kernel oil (NSKO) as an alternative natural disinfectant, and to study its disinfection kinetics. Heterotrophic plate count, using CFU/ml, pour plate method, 35°C / 48 h, plate count agar were employed to evaluate the disinfection and its kinetics. The result obtained showed that NSKO has a remarkable disinfection potential and the kinetics studies suggested that NSKO fitted first-order model with a k value of -0.040.

Key words: Nahar (*Mesua ferrea*) seed kernel oil, extraction, gum Arabic, disinfection, kinetics.

INTRODUCTION

Disinfection plays a key role in the reclamation and reuse of wastewater for eliminating infectious diseases, this, in part, augments domestic water supply and decreases the impact of human activities on the environment (Bixio et al., 2008; Ying-Xue et al., 2009). However, a variety of genotoxic, mutagenic and/or carcinogenic disinfection by-products (DBPs) are formed when disinfectants, such as chlorine, react easily with dissolved organic matter (DOM) in treated wastewater (Ying-Xue et al., 2009; Costan-Longares et al., 2008; Koukouraki and Diamadopoulos, 2002; Rebhun et al., 1997; Sadiq and Rodriguez, 2004a; Stefanie and Hermann, 1998). Most disinfectants are strong oxidants and/or generate oxidants as byproducts (such as hydroxyl free radicals) that react with organic and inorganic compounds in water.

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Abbreviations: DBP, Disinfection byproduct; **EPA,** Environment Protection Agency; **NKSO,** Nahar seed kernel oil.

These contaminants of concern are grouped into four distinct categories and include disinfectant residuals, inorganic byproducts, organic oxidation byproducts and halogenated organic byproducts. Halogenated organic byproducts are formed when natural organic matter (NOM) reacts with free chlorine or free bromine. Free chlorine can be introduced to water directly as a primary or secondary disinfectant, with chlorine dioxide, or with chloramines. Free bromine results from the oxidation of the bromide ion in source water. Factors affecting formation of halogenated DBPs include type and concentration of natural organic matter, oxidant type and dose, time, bromide ion concentration, pH, organic nitrogen concentration and temperature. Organic nitrogen significantly influences the formation of nitrogen containing DBPs such as the haloacetonitriles, halopicrins and cyanogens halides (Reckhow et al., 1990; Hoigné and Bader, 1988).

As a result of DBP concerns from chlorine, Environmental Protection Agency (EPA), as well as the water treatment industry, places more emphasis on the use of disinfectants other than chlorine. Some of these alternative disinfectants, however, have also been found to produce DBPs as a result of either reactions between

Table 1. Water quality characteristics of the unfiltered surface water.

Parameter	Surface water characteristics	
	Mean	Range
pH	6.10	6.05-6.18
Temperature (°C)	22.6	21-23
Turbidity (NTU)	135	128-140
Bacterial count (CFU)	40	30-52

disinfectants and compounds in the water or as a natural decay product of the disinfectant itself (Legube et al., 1989; McGuire et al., 1990). The aims of this study were thus: to investigate the potential of the of Nahar seed kernel oil (NSKO) as an alternative natural disinfectant, and to study its disinfection kinetics.

MATERIALS AND METHODS

Seeds pretreatment and sample preparation

Fresh samples of NSK were collected and subjected to drying at 105°C for 2 h using a laboratory oven (Memmert, Germany). The dried seed kernels were then ground using a laboratory blender (Waring Products Division, Torrignton C.T., USA). The samples were stored at 4°C inside a laboratory chiller.

Extraction of NSK oil

Soxhlet apparatus using n-hexane as solvent for an extraction time of 6 h and extraction temperature of 70°C was employed. For each run, 20.0 ± 0.01 g of the ground dry seed kernels particles were loosely packed inside the cellulose thimble and placed in the soxhlet extraction chamber. After cooling, the thimble was dried in the oven at 60°C and the remaining cake was weighed to determine the amount of oil extracted, while the solvent was also dried off from the oil-solvent miscella using the rotavapor. The crude oil extract was stored inside a laboratory chiller prior to its usage in the disinfection studies (Ahmed et al., 2010).

Oil-water emulsion preparation

Gum Arabic was used as an emulsifier to prepare the oil-water emulsion. About 5 g of the crude oil was added to 500 ml of distilled water in a 1000 ml conical flask. 5 g of ground Gum Arabic was then added, and the flask was put on a magnetic stirrer for thorough stirring for 10 min to obtain a uniform oil-water emulsion which was then autoclaved at 121°C for 15 min.

Surface water characteristics and collection

Surface water samples were collected from a small pond behind the Institute of Education of the International Islamic University Malaysia (IIUM), Gombak campus. Standard water quality parameters for the surface water are summarized in Table 1. This water is used to represent worst case water quality conditions.

Water samples were collected as directed in accordance with Standard Methods 9060A (AWWA and WEF, 2005). Disinfection and analysis were initiated as soon as possible after collection to minimize changes in bacterial population. All the water required for a particular test run was taken from a single sample bottle. Each bottle was agitated to redistribute any settled matter before the sample was dispensed (AWWA and WEF, 2005; AWWA, 1991; AWWA Safe Drinking Water Advisor - Library on Internet, 1997; USEPA, 1999).

Heterotrophic plate count (HPC)

This is formerly known as the standard plate count, it is a procedure for estimating the number of live heterotrophic bacteria in water and measuring changes during water treatment and distribution or in swimming pools. Colonies may arise from pairs, chains, clusters or single cells, all of which are included in the term “colony-forming units” (CFU).

Pour plate method (9215B)

This is the simplest method, as compared to spread plate and membrane filter methods. It can accommodate volumes of sample or diluted sample ranging from 0.1 to 2.0 ml. The colonies produced are relatively small and compact, showing less tendency to encroach on each other than those produced by surface growth.

Media

Plate count agar (tryptone glucose yeast agar) was used. It is made up of: Tryptone (5.0 g), yeast extract (2.5 g), glucose (1.0 g), agar (15.0 g), reagent-grade water (1 L) at pH of 7.0 ± 0.2 after autoclaving at 121°C for 15 min.

Extract dosing

For each test, a raw water of specific volume was dispensed into a conical flask. To this water, a dose of extract solution was added, to make a final volume of 100 ml, and mixed on a shaker at 200 rpm for the intended contact time.

Bacteria enumeration

1.0 ml of the treated water sample was then taken using sterilized micropipette and dispensed into the Petri dish. Agar was then poured into the dish which was then incubated. The results were compared against a 100 ml control of the same surface water sample. Each test was repeated for a minimum of three times.

Incubation

For compliance monitoring purposes under U.S. EPA’s Surface Water Treatment Rule (40 CFR 141.74) provision on heterotrophic bacteria, pour plates was incubated at 35°C for 48 h.

Counting and recording

Manual counting was done using Quebec colony counter and bacterial count per milliliter was computed by the following equation:

$$\frac{cfu}{mL} = colonies\ counted/actual\ volume\ of\ sample\ in\ dish, mL \quad (1)$$

Kinetics study

For any reaction, γ the rate law is:

$$R_\gamma = k_\gamma [A]^\alpha [B]^\beta \quad (2)$$

Where, k_γ is the rate constant, α and β are empirical coefficients
Reaction order: Reactions may be described in terms of the overall reaction order in terms of the sum of the exponents of the rate:

$$r_A = -\frac{dC_A}{dt} = kC_A^n \quad (3)$$

Where, r_A is the rate of reaction; C_A is the concentration of component (CFU/ml); t is the time in minutes; k is the reaction rate constant and n is the overall order of reaction, negative sign indicates reduction in concentration.
It is believed that the kinetics of many biological reactions are zero-order, first-order or a combination of these which is called Michaelis-Menten kinetics (Pauline, 1995; Coker, 2001).

Order of reaction

Zero-order

From the rate equation (3), for zero order, n = 0:

$$-r_A = -\frac{dC_A}{dt} = kC_A^0 \quad (4)$$

$$-r_A = -\frac{dC_A}{dt} = k_0 \quad (5)$$

Rearranging:

$$-dC_A = k_0 dt$$

Integrating both sides, assuming that concentration of A is C_{A0} at time t_0 and C_{Af} at time t_f;

$$-\int_{C_{A0}}^{C_{Af}} dC_A = \int_{t_0}^{t_f} k_0 dt$$

$$-\int_{\ }^{C_{Af}} dC_A = k_0 \int^{t_f} dt$$

$$-\left(C_{Af} - C_{A0}\right) = k_0\left(t_f - t_0\right)$$

At $C_{A0,}$ time, $t_0 = 0$, then:

$$C_{Af} = C_{A0} - k_0 tf$$

Plotting the concentration (C_A) against time (t) gives a straight line, where C_{A0} is the intercept and k_0 is the slope.

First-order reaction

Using the same equation (3), where the order of the reaction, $n = 1$:

$$r_A = -\frac{dC_A}{dt} = k_1 dtCA^1 \quad (6)$$

Rearranging gives:

$$-\frac{dC_A}{\ } = k_1 dt$$

Integrating both sides, assuming that concentration of A is C_{A0} at time t_0 and C_{Af} at time t_f:

$$\int_{C_{A0}}^{C_{Af}} -\frac{dC_A}{C_A} = \int_{t_0}^{t_f} k_1 dt$$

$$-\int_{C_{A0}}^{C_{Af}} \frac{1}{c_A} dC_A = k_1 \int_{t_0}^{t_f} dt$$

$$-\ln\left(C_{Af} - C_{A0}\right) = k_1\left(t_f - t_0\right)$$

$$-\ln\frac{C_{Af}}{\ } = k_1\left(t_f - t_0\right)$$

At $t_0 = 0$ and $C_{A0} = C_{A0,}$ at time t_f =t and $C_{Af} = C_{Af}$, Therefore

$$-\ln\frac{C_{Af}}{C_{A0}} = k_1 t$$

Taking exponential of both sides gives:

EMBED Equation.3

$$\frac{C_{A0}}{C_{Af}} = e^{k_1 t}$$

Taking natural log of both sides gives:

EMBED Equation.3

$$\ln C_{A0} = \ln C_{Af} + \ln\left[e^{k_1 t}\right]$$

$$\ln C_{Af} = \ln C_{A0} - k_1 t \quad (7)$$

Plotting the concentration (lnC_A) against time (t) gives a straight line, where lnC_{A0} is the intercept and k_1 (min^{-1}) is the slope.

Table 2. Residual colonies (CFU/ml) after disinfection using NSKO emulsion.

pH	4			6			8		
Time (minute)	**10**	**20**	**30**	**10**	**20**	**30**	**10**	**20**	**30**
1.0 mg/ml	2	1	0	2	1	1	1	0	0
2.0 mg/ml	0	0	0	0	0	0	0	0	0
3.0 mg/ml	0	0	0	0	0	0	0	0	0
Control					50±2				

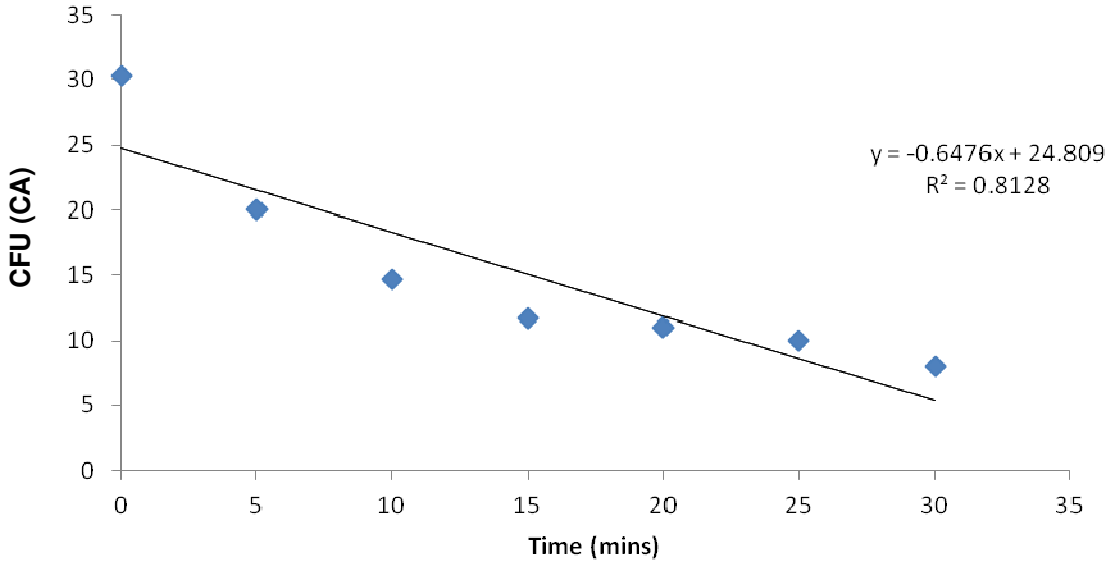

Figure 1. Zero-order kinetics plot.

To study the kinetics of disinfection of the NSK crude oil emulsion using CFU/ml, pour plate method, 35°C / 48 h, plate count agar, the pH (6.0) and dosage (1.0 mg/ml) were kept constant and the residual bacterial count was estimated for the various agitation times.

RESULTS AND DISCUSSION

Oil yield

The crude oil yields obtained from the NSK using n-hexane as solvent for an extraction time of 6 h and extraction temperature of 70°C gave about 70 to 76% on dry basis, corresponding to the values reported by us (Idris et al., 2011) from our earlier works. The slight variation might be due to the differences in variety of plant, cultivation climate, ripening stage, harvesting time of the seed and the extraction solvents used.

Effect of extract dose

NSKO crude extract demonstrated appreciable bacterial disinfection at high concentration. At concentration of 2 mg/ml and above, total disinfection was observed with little or no bacterial colonies seen after incubation. This result agreed with the earlier work done by us on the antibacterial property of NSKO (Idris et al., 2011); that the minimum bactericidal concentration (MCB) of NSK oil is 2 mg/ml. The result also showed that the oil disinfection potential, at high dosage is more or less independent on pH and agitation time as shown in Table 2.

Only first order reaction gave a straight line curve (Figure 2). The reaction constant (K) for the disinfection reaction increased from -0.647 to -0.040 as the order of reaction increased from zero to the first order (Figure 1). The higher value obtained for first order suggests that the suitable order of reaction describing the disinfection effectiveness of the extract on coliform in the wastewater stream is first order reaction. Similarly, the initial concentrations (C_{AO}) evaluated from the equations were 24.80 and 25.23 CFU/ml for the zero and first order reactions, respectively. The closeness of first order C_{AO} (25.23 CFU/ml) to the average CFU (30.33 CFU/ml) (Table 3) further suggests the suitability of first order reaction in describing the disinfection of coliform in the wastewater stream by NSKO. The higher R^2 – values (0.928) observed for the first order reaction also showed

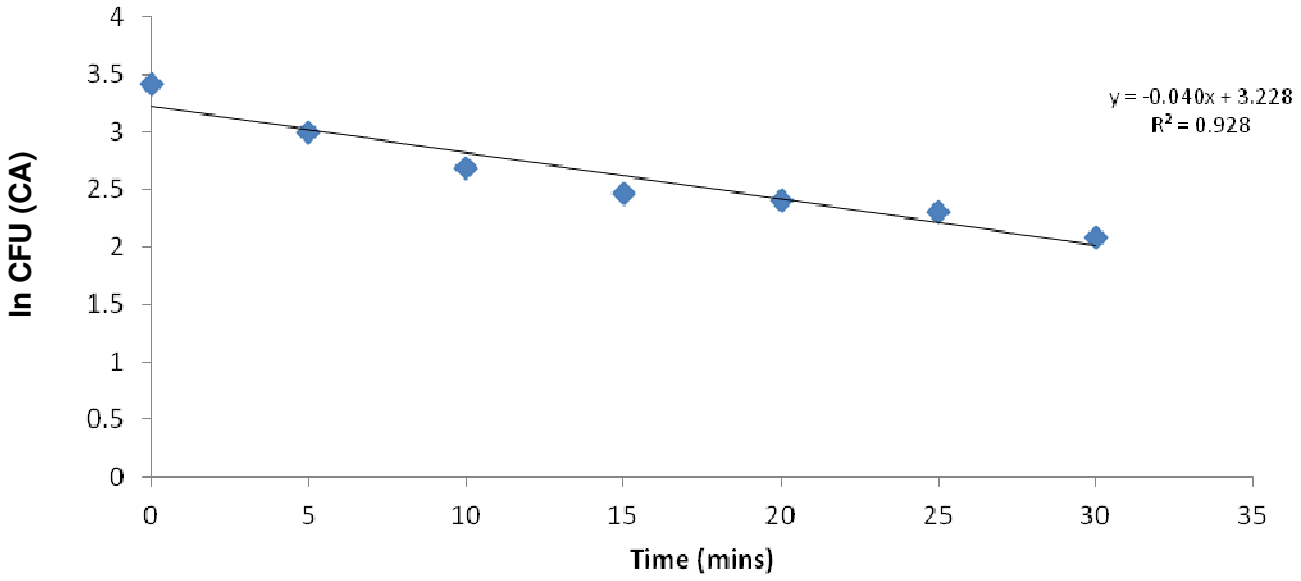

Figure 2. First-order kinetics plot.

Table 3. Residual colonies (CFU/ml) at 1.0 mg/ml at different times.

Time (minutes)	Residual bacterial count (CFU/ml)				
	Replicate 1	Replicate 2	Replicate 3	Average	ln CA
Control	30	32	29	30.33	3.41
5	22	18	20	20	3
10	17	13	14	14.67	2.69
15	10	12	13	11.67	2.46
20	10	13	10	11	2.4
25	10	8	12	10	2.3
30	6	8	10	8	2.08

Table 4. Summary of the analysis of the kinetics.

Order	Equation	K-value	C_{A0}	R^2
Zero	Y = -0.647x + 24.80	-0.647	24.80	0.812
First	Y = -0.040x + 3.228	-0.040	25.23	0.928

that the mechanism of coliform disinfection of NSKO in the wastewater stream is first order (Table 4).

Also, as the semi-log plot of the residual bacterial count concentration (CFU/ml) against time (minutes) gave a straight line with slope of k_1 then, the first-order model fits the data well. It could thus be deduced that the kinetics of disinfection of NSKO emulsion could be defined by the equation:

$$\ln C_{Af} = \ln C_{A0} - k_1 t \quad (8)$$

And with k_1 = -0.040 min^{-1} and C_{A0} = 25.23 CFU/ml, the kinetic equation could be written as:

$$C_A = C_{A0} e^{-0.040t} \quad (9)$$

Where, C_{A0} is the initial bacterial count before treatment; C_A (CFU/ml) is the final residual concentration of bacteria upon treatment with 1.0 mg/ml at a given time t (minutes)

Conclusions

The NSKO emulsion demonstrated better disinfection and inactivation of bacterial in surface water and thus has a good potential for usage as an alternative and natural disinfectants. The crude oil emulsion showed total dis-

infection at a concentration of 2 mg/ml and above, while the data generated from the disinfection at 1 mg/ml fits first-order model

The kinetics of disinfection of NSKO emulsion was defined by the equation; while the kinetic equation was written as: $C_A = C_{A0} e^{-0.040t}$

The characterization and determination of bio-active ingredients of the NSK oil are the challenges of future researches.

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REFERENCES

Ahmed IA, Elwathig MSM, Aremu MS, Jamal ID, Mikail MA (2010). Potential of Nahar (*Mesua Ferrea)* Seeds' Oil for Industrial Applications. Proceeding of The 2nd International Biotechnology & Biodiversity Conference (BIOJOHOR). pp. 75-79.

AWWA (American Water Works Association) (1991). Guidance Manual for Compliance with the Filtration and Disinfection Requirements for Public Works Systems Using Surface Water Sources.

AWWA Safe Drinking Water Advisor - Library on Internet (1997).

AWWA, WEF (2005). Standard Methods for the Examination of Water and wastewater, 21st Ed. American Pub. Health Assoc. Washingnton DC.

Bixio D, Thoeye C, Wintgens T, Ravazzini A, Miska V, Muston M, Chikurel H, Aharoni A, Joksimovic D, Melin T (2008). Water Reclamation and Reuse: Implementation. Management Issues. Desalination, 218(1–3): 13-23.

Coker KA (2001). Modeling of Chemical Kinetics and Reactor Design. Butterworth-Heinemann, Boston, USA. pp. 114-130.

Costan-Longares A, Montemayor M, Payan A, Mendez J, Jofre J, Mujeriego R, Lucena F (2008). Microbial Indicators and Pathogens: Removal, Relationships and Predictive Capabilities in Water Reclamation Facilities. Water Res. 42(17): 4439-4448.

Hoigné J, Bader H (1988). The Formation of Trichloronitromethane (chloropicrin) and Chloroform in a Combined Ozonation/Chlorination Treatment of Drinking Water. Water Res. 22(3): 3-13.

Idris AA, Mohamed ESM, Suleyman AM, Jamal ID, Maryam AM (2011). Extraction and Antibacterial Activity of Nahar (*Mesua ferrea*) Seeds' Oils. ACT-Biotechnology Res. Comm. 11: 28-32.

Koukouraki E, Diamadopoulos E (2002). THM Formation During Chlorination of Treated Municipal Wastewater. Water Sci. Technol. Water Supply. 2(3): 235-242.

Legube B, Croue JP, De Latt J, Dore M (1989). Ozonation of an Extracted Aquatic Fulvic Acid: Theoretical and Practical Aspects. Ozone Sci. Eng. 11(1): 69-91.

McGuire MJ, Ferguson DW, Gramith JT (1990). Overview of Ozone Technology for Organics Control and Disinfection. Conference proceedings, AWWA Seminar on Practical Experiences with Ozone for Organics Control and Disinfection, Cincinnati, OH.

Pauline MD (1995). Bioprocess. Engineering Principles. Academic Press. London San Diego New York Boston. 11: 272-286.

Rebhun M, Grossman LH, Manka J (1997). Formation of Disinfection Byproducts During Chlorination of Secondary Effluent and Renovated Water. Water Environ. Res. 69(6): 1154-1162.

Reckhow DA, Singer PC, Malcolm RL (1990). Chlorination of Humic Materials: Byproduct Formation and Chemical Interpretations. Environ. Sci. Technol. 24(11): 16-55.

Sadiq R, Rodriguez MJ (2004a). Fuzzy Synthetic Evaluation of Disinfection By-products-a Risk-based Indexing System. J. Environ. Manage. 73(1): 1-13.

Stefanie S, Hermann HH (1998). Generation of Halogenated Organic Compounds in Municipal Waste Water. Water Sci. Technol. 37(1): 303-309.

USEPA (U.S. Environmental Protection Agency). (1999). Alternative Disinfectants and Oxidants Guidance Manual. Office of Water (4607) EPA 815-R-99-014 April.

Ying-Xue Sun, Qian-Yuan Wu, Hong-Ying Hu, Jie T (2009). Effect of Bromide on the Formation of Disinfection By-Products During Wastewater Chlorination. Water Res. 43: 2391-2398.