Moringa oleifera SEEDS AS DISINFECTANT IN WATER TREATMENT: OPTIMIZATION AND MODELLING

BY

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ABSTRACT

The high cost of water treatment and high cost of chemicals for disinfection makes it difficult to produce high quality drinking water particularly in the developing countries. The use of chlorine which causes disinfection by-products is a major concern especially in the developed countries. As a result of this, there is the need to look for a cheap alternative such as the use of plant material as substitutes for chemical disinfectant. *Moringa oleifera* is an extensively documented plant material used for the treatment of drinking water. Its seed extracts contain active agents having excellent coagulation properties and exerting in vitro bactericidal activity. However, lack of available literature on the statistical optimization using Response Surface Methodology and Artificial Neural Network, inactivation kinetics of the seed extract using different disinfection models and effect of different seed processing for the seed extracts are major aspect never been explored. This study aims to develop operation parameters for the seed extracts to be used as disinfectant for water treatment, which might be suitable for drinking purposes. The screening of different extraction methods on *Moringa oleifera* seed extracts processing against four bacterial strains was carried out. The optimization process, based on statistical experimental design, comprises of one-factor-at-a-time (OFAT), faced centred central composite design (FCCCD) under the response surface methodology (RSM) and artificial neural network (ANN) using MATLAB 2012A were used to identify and determine the optimum process conditions. The multivariate regression analysis of the disinfection kinetic models was analysed using SPSS version 20 and the final application of the optimized process conditions with river water was evaluated. The results, based on qualitative and quantitative analysis, reveal that defatted seed cake with salt extraction was the best extraction method that inhibited bacterial strains with MBC/MIC ratio of 2 on *Escherichia coli* and *Pseudomonas aeruginosa* strains indicating that the seed extract exhibits strong bactericidal action. The statistical analysis of optimization results using RSM complemented with ANN gave a high coefficient of determination (R^2) of 0.9992 and 0.9886 for *P. aeruginosa* and *E. coli* respectively. The model developed was verified and the optimum process parameters were 124 mg/l dosage, 65 minutes of contact time, 110 rpm mixing rate for *E. coli* bacterial strain and 125 mg/l dosage, 60 minutes of contact time and 130 rpm mixing rate for *P. aeruginosa* strain. The order of reaction followed second order and the inactivation kinetics showed that modified Hom model best fits the disinfection process with R^2 of 0.9990. The findings from the application of the seed extract to river water shows that the seed extract can be used as a coagulant and disinfectant. The removal efficiency for the seed extract shows reductions in water parameters with over 99.98% reduction of total coliforms and heterotrophic bacteria after the disinfection process. The final treated water was benchmarked with the Malaysian drinking water standard and almost all of the water parameters were below the maximum acceptable range except for the total coliform. Hence, the findings of this study showed that defatted *Moringa oleifera* seed extract using the salt extraction method can be used as a disinfectant. This extract is recommended to be used in small communities in developing countries and in emergency situations. The mode of attack of the seed extract is recommended for further studies.

خلاصة البحث

تكاليف فادحة لمعالجة المياه وارتفاع تكاليف المواد الكيميائية المبيدة لجراثيم يجعل من الصعب إنتاج مياه مستعذبة جودة عالية لا سيما في الدول النامية. و استخدام الكلور الذي يسبب الآثار الجانبية مصدر قلق كبير خاصة في البلدان المتقدمة. نتيجة لهذا، هناك حاجة للبحث عن بديل أرخص مثل استخدام المواد النباتية كبدائل للمطهر الكيميائي. وعلى هذا, فان النبات المسمى" مورينغا أليفرا" مما تم التوثيق عليه كمادة تطهير لمياه مستعذبة. معتصرات بذوره تحتوي على عناصر نشطة لديه خصائص ممتازة لتختير ونشيط في انتاج عملية مضادة لجراثيم البكتيريا. ومع ذالك, قلة ما كتب في احصائيات ايجاد نتيجة الأمثل له باستخدام منهجية الاستجابة السطحية والشبكات العصبية الاصطناعية, وركود عملية انفعال معتصرات البذور باستخدام نماذج التطهير المختلفة وتأثير مختلف معالجة البذور لمعتصرات البذور يتمثل جانبا مهما الذي لم يسبق بحثه. وتهدف هذه الدراسة إلى تأصيل قواعد لعملية اجراءات في معتصرات البذور لاستخدامها كمادة تطهير لمعالجة المياه، حتى يكون مستعذبا. لقد واجرت الباحثة فحص أساليب مختلفة لإستخلاص معتصرات " مورينغا أليفرا"جارعملية لأربع سلالات بكتيرية. استنادا إلى الأسلوب التجريبي الإحصائي, فان عملية ايجاد نتيجة الأمثل تتكون من مبدأ "عامل واحد في وقت واحد" (OFAT) واجهت اسلوب احصائي مسمى "ارتكاز اسلوب مركزي مركب "(FCCCD) تحت سطح استجابة المنهجية (RSM) والشبكة العصبية الاصطناعية (ANN) باستخدام MATLAB A 2012, لتعيين وتحديد ظروف العملية المثلى. واستخدمت SPSS من نوع اصدار 20 لتحليل الإنحدار المتعدد المتغيرات للنماذج عملية تطهير, كما تم تقييم لتطبيق نهائي لعملية ظروف ايجاد نتيجة الأمثل باستخدام مياه النهر, وكانت النتيجة, بناء على التحليل النوعي والكمي, أن كعكة البذور منزوعة الشحم ملوحة المعتصرات هي أفضل طريقة الإعتصار التي تحول دون السلالات البكتيرية مع MBC MIC/ بنسبة 2 على سلالة جنس البكتيرية المساة *Pseudomonas aeruginosa* and *Escherichia coli* مما يدل على أن معتصرات البذور تنتج الإنفعال القوي المضاد للبكتيرية. أعطى التحليل الإحصائي لنتائج الأمثل باستخدام RSM تستكمل مع ANN معامل عالية الجدية 0.9992 of (R^2) and 0.9886 ل *P. aeruginosa* and E. *coli* على التوالي. تم التحقق من النموذج المخترع وكانت قواعد العملية المثلى 124ملغ /الجرعة الواحدة، 65دقيقة من وقت الاتصال، 110دورة في الدقيقة معدل خلط جنس البكثيرية السماة *P. aeruginosa*. ويجري نظام الإنفعال على نظام الثانية وأظهرت أن عدم إنفعال الكيمياء يدل ان نموذج هوم المعدل أفضل نموذج يناسب عملية التطهير مع R2من .0.9990 والنتائج من تطبيق معتصرات البذور لمياه النهر تبين أن مستخلص البذور يمكن أن تستخدم كمادة تخلط والتطهير .وتظهر كفاءة مستعصرات البذور لإزالة الجراثيم انخفاضا في مؤشرات مدى تأثر المياه بجراثيم مع خفض أكثر من 99.98% من نسبة مأوية لنوعي البكتيرية, coliforms and heterotrophic بعد عملية التطهير . وقداعتمد التطهير النهائي على معيار مياه الشرب الماليزي, وكلها تقريبا كانت دون أقصى الحد المقبول من المؤشرات ما عدا اجمالي بكتيرية coliforms. اذا, قد أثبتت نتيجة هذه الدراسة أن معتصرات بذور مورينغا oleifera منزوع الشحم باستخدام طريقة اعتصار بالملح يمكن أن تستخدم كمادة تطهير مياه الشرب. ويوصى باستخدام هذا المعتصر في مجتمعات صغيرة في البلدان النامية وفي حالات الطوارئ .ويوصى بمزيد من الدراسات لكيفية تأ ثير هذا المعتصر.

APPROVAL PAGE

The thesis of Munirat Abolore Idris has been examined and is approved by the following:

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DECLARATION

I hereby declare that this thesis is the result of my own investigations, except where otherwise stated. I also declare that it has not been previously or concurrently submitted as a whole for any other degrees at IIUM or other institutions.

Munirat Abolore Idris

Signature …….. Date …….....

INTERNATIONAL ISLAMIC UNIVERSITY MALAYSIA

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***Moringa oleifera* SEEDS AS DISINFECTANT IN WATER TREATMENT: OPTIMIZATION AND MODELLING**

Affirmed by: Munirat Abolore Idris.

…… ..…….

Signature Date

Dedicated to my families, both nuclear and extended

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All praise belongs to Allah (SWT), the sole Fashioner of my existence, the Sustainer and Cherisher of the universe. And may His blessings, mercy and peace be upon the Prophet Muhammad (SAW), his household, and companions till the day of reckoning. Which of the favours of Allah can I deny? I am extremely grateful to Allah (SWT) for sparing my life and giving me this opportunity to contribute to humanity at large.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF SYMBOLS

L	Litre
mg	Milligram
N_t	Number of organisms at time t
N_0	Number of organisms at time 0
k	Rate constant
k'	Die-off constant
C	Concentration
n	Coefficient of dilution
Λ	Coefficient of Specific lethality
b	Lag coefficient
CT	Product of concentration and time
Λ_{CS}	Log based coefficient of lethality
m	Hom time exponent
w	weight
y_k	Output of network
f	Transfer function
w_{ki}	Weight vector
x_i	Input vector
b	Network bias
E	Network error

LIST OF ABBREVIATIONS

ANN	Artificial Neural Network
CCD	Central Composite Design
COD	Chemical Oxygen Demand
DBP	Disinfection by-Products
DOE	Design of Experiment
EPA	Environmental Protection Agency
FCCCD	Face Centred Central Composite Design
GUI	Graphical User Interface
HPC	Heterotrophic Plate Count
INWQS	Interim National Water Quality Standards
MATLAB	Mathematics Matrix
MDG	Millennium Development goals
MOH	Ministry of Health
NDWQS	National Drinking Water Quality Standards
NOM	Natural Organic Matter
OFAT	One Factor At A Time
POME	Palm Oil Mill Effluent
RSM	Response Surface Methodology
TC	Total coliform
TDS	Total Dissolved Solids
THM	Trihalomethane
TSS	Total Suspended Solids

UN	United Nations
UNICEF	United Nations International Children's Emergency Fund
WHO	World Health Organisation

CHAPTER ONE

INTRODUCTION

1.1 BACKGROUND

The availability of good drinking water has been the most critical factor for survival throughout the development of life. According to the World Health Organisation (WHO), over 1.1 billion people in the world lack access to clean water sources and about 2.4 billion people lack access to good sanitation facility. The number of people, mostly children who are below 5 years of age, that die every year due to water diseases especially diarrhoea is about 2 million. In the developing countries, most of these diseases are from people living in the rural areas (WHO, 2012).

Reports available from 1991 to 2008 show that about 1,428 diarrhoea outbreaks have occurred all over the globe particularly from Western Europe, Central Africa, Northern India and Southeast Asia (Figure 1.1) and from these reported outbreak events, 70.9% (1,012) are caused by water borne diseases. Also, 49.6% (709) of these outbreak events are from bacteria, 39.3% (561) from viruses, and 11.1% (158) from parasites (Yang et al., 2012). Water borne diseases such as diarrhoea are still major concerns for the developing world and although, there are no enough data available, the WHO estimated about four billion cases of diarrhoea every year alongside other diseases caused from drinking dirty water in the developing countries (WHO, 2000).

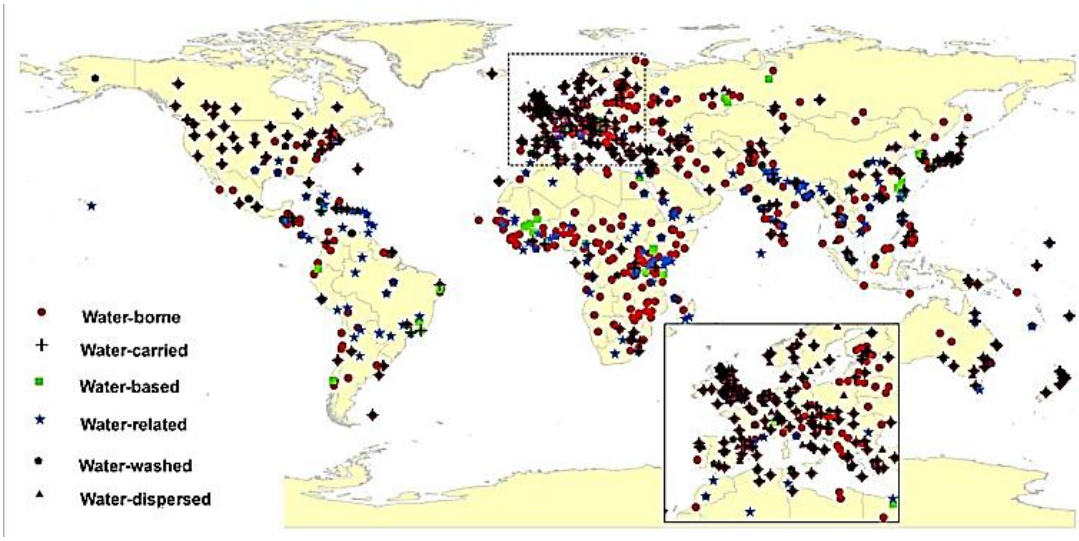

Figure 1.1 Distribution of reported outbreaks of water-associated infectious diseases from 1991 to 2008 (Yang et al., 2012)

Diarrhoea is one of the major causes of death particularly in children as they tend to dehydrate easily. It is caused by gastrointestinal infections that affect 2.2 million people yearly, especially kids in underdeveloped countries. Severe cases of diarrhoea are life threatening due to dehydration especially in infants, malnourished and immunocompromised patients (WHO & UNICEF, 2013).

Therefore, the transmission of waterborne diseases occurs by drinking contaminated water containing pathogens from human excreta. The commonly known bacteria responsible for epidemic outbreak are *Escherichia coli, Salmonella typhii, Vibrio cholera* and *Pseudomonas aeruginosa.* These microorganisms are responsible for different types of diseases such as diarrhoea, dysentery, typhoid, cholera, etc. that have caused over 6 million deaths of children every year (Ali et al., 2010).

In the developing countries, more than 80% of the people are low-income earners using surface water and groundwater for drinking purposes. These sources have been contaminated due to indiscriminate dumping of waste, poorly protected wells, seepages from sewer which percolates into water aquifers. As a result of this,

fresh waters are easily polluted making them major source of faecal microorganisms, which causes diarrhoea diseases that lead to major health problems in developing countries (Aryal et al., 2012; Jadhav, 2011; Kulshrestha & Mittal, 2003; Liew & Lepesteur, 2006).

Water disinfection is important in water treatment because it destroys disease-causing microorganisms present in water. While the importance of water disinfection cannot be over emphasized, concerns have also been raised over safety issues. The use of chemicals such as chlorine and chloramine compounds as disinfectants produces by-products that are carcinogenic. Among the by-products that have been detected are trihalomethanes, halo-acteone derivatives, halo-acetonitriles and carcinogen chloroform, etc. These compounds are formed when chlorine reacts with organic compounds in water such as amino acids (Laingam et al., 2012; Ye et al., 2011). These chlorinated compounds are very difficult to degrade and can cause the same hazards as chlorine itself. It is becoming increasingly difficult to ignore the potentially hazardous by-products that emerge when using conventional disinfectants, thus there is a need to explore organic alternatives for water treatment. According to WHO, microbial resistance to current water treatment processes is on the rise; hence, medicinal plants offer a good source of alternatives (Walter et al., 2011).

1.2 PROBLEM STATEMENT AND SIGNIFICANCE OF STUDY

About 1.1 billion people, in the developing countries lack access to good quality water and about 2.4 billion people do not have access to basic sanitation hygiene and the combination of these factors have resulted into about 1.6 million deaths yearly out of which about 90% are children below five years of age (Hamner et al., 2006; Wenhold & Faber, 2009). Although, prevention of water borne diseases can be achieved by

constructing disinfection and distribution system and effective sewage treatment facilities, however, due to high cost of construction of these facilities and high cost of chemicals for disinfection, it is difficult to produce high quality drinking water (Lantagne & Clasen, 2012; Tellen, Nkeng, & Dentel, 2010). As a result of this, there is the need to look for cheap alternative such as point-of-use treatment of water.

Disinfection is important in drinking water treatment, which is usually done at the end of the drinking water treatment train. Its importance in treating drinking water cannot be over emphasized. Chemical disinfectants such as chlorine and chlorine compounds are mostly used during the conventional disinfection process; however, they have numerous disadvantages as listed below:

1. In the disinfection of water, chlorine reacts with natural organic matter (NOM) which produces disinfection by-products that are carcinogenic and harmful to human health. Trihalomethanes including carcinogen chloroform, halo acetone derivatives that include dichloro- and bromo-acetonitrile are among the by-products detected. Although, the concentration of these chemicals are typically low in drinking water, in parts per billion, it is possible that a lifetime of chronic exposure will have significant effects on human health (Richardson, 2005). Results from a number of test systems indicate that halo acetonitriles are both mutagenic and carcinogenic. The research conducted by Hwang, Jaakkola, & Guo (2008) revealed that prenatal exposure to disinfection by-product increases the risk of ventrical or septal defects, cleft palate and anencephalus. Apart from producing disinfection by-products, it also produces odorous compounds when it reacts with NOM in water.