World Academy of Science, Engineering and Technology
International Journal of Environmental and Ecological Engineering
Vol:7, No:9, 2013

Statistical Optimization of Process Conditions for Disinfection of Water Using Defatted *Moringa oleifera* Seed Extract

Suleyman A. Muyibi, Munirat, A. Idris, Saedi Jami, Parveen Jamal, Mohd Ismail Abdul Karim

***Abstract*—**In this study, statistical optimization design was used to study the optimum disinfection parameters using defatted crude *Moringa oleifera* seed extracts against *Escherichia coli* (*E. coli*) bacterial cells. The classical one-factor-at-a-time (OFAT) and response surface methodology (RSM) was used. The possible optimum range of dosage, contact time and mixing rate from the OFAT study were 25mg/l to 200mg/l, 30minutes to 240 minutes and 100rpm to 160rpm respectively. Analysis of variance (ANOVA) of the statistical optimization using faced centered central composite design showed that dosage, contact time and mixing rate were highly significant. The optimum disinfection range was 125mg/l, at contact time of 30 minutes with mixing rate of 120rpm.

Keywords*—E. coli*, disinfection, *Moringa oleifera*, response surface methodology.

I. Introduction

DISINFECTION is a very important step of water treatment. Its ability to eliminate color, odor, and destroy harmful pathogen makes it an integral part of municipal drinking water treatment in order to achieve desired aesthetic water quality. The use of chemicals such as chlorine or chlorine related by-products, ozone became a vital aspect in controlling microorganisms [1]. It's been established that chemical disinfectant produces byproducts mostly referred to as disinfection-by-products. Chlorine produces disinfection by-products, which are carcinogenic and harmful. Trihalomethanes including carcinogen chloroform, halo acetone derivatives that include dichloro and bromo-acetonitrile are among the by-products detected. Results from a numbers of test systems indicate that halo-acetonitriles are both mutagenic and carcinogenic [1]-[3]. Due to these potentially hazardous by-products that arise, there is the need to explore the use of natural occurring disinfectants as an alternative to the conventional chemical disinfectant. As awareness is increasing on the use of natural disinfectants, drinking water industries require sustainable alternatives that are cheap and readily available.

Moringa oleifera is the most widely cultivated species of a monogeneric family, the *Moringaceae* that is native to the sub-Himalayan tracts of India, Pakistan, Bangladesh and Afghanistan. It is commonly referred to as the miracle tree because of its multipurpose uses. Most of its parts are useful for a good number of applications [4]-[8]. *Moringa oleifera* seed kernels contain a significant amount of oil that is commercially known as Ben oil. *Moringa oleifera* is a well-known plant material locally used for the treatment of drinking water. The crude extract of *Moringa oleifera* seed is commonly used in water treatment and purification [9]. When *Moringa oleifera* is compared with conventional chemical coagulants, it has the following advantages: Its cost effective as it is readily available, it produces bio-degradable sludge and lower sludge volume, it does not produce harmful by-products, it is easily handled as it is not corrosive and it does not affect pH of water

In the light of the above advantages, *Moringa oleifera* is environmentally friendly and available at low cost which can be good alternative to chemical coagulants with a potential application in water treatment in both developed and developing countries [10]-[14]. Its seed extracts contain active agents having excellent coagulation properties [15] and they have also been reported to exert in vitro bactericidal activity against both gram positive and gram negative bacteria in raw water. Previous reports have also revealed that *Moringa oleifera* seed extract contains a polypeptide "flo" which acts as coagulant as well as exert an antibacterial effect on harmful bacterial strains [16]. Also, an active antimicrobial agent 4 α L-rhamnosyloxy-benzyl isothiocyanate was identified from the seed extract and both defatted seed and normal shell seed contains about 8-10% of this antimicrobial agent [17], [18]. However, there have been limited studies on the application of the seed extract for disinfection purposes especially in drinking water.

In this study, the objective was to establish the optimum disinfection process conditions of defatted crude seed extract using the classical one-factor-at-at-time and central composite

Suleyman. A. Muyibi is Professor and Coordinator of Bioenvironmental Engineering Research Centre (BERC) Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, and P. O. Box 10 Jalan Gombak, Kuala Lumpur, (corresponding author; Tel: +60361964577; e-mail: suleyman@iium.edu.my).

Munirat. A. Idris is with the Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia (e-mail: muniratabloreidris@yahoo.com).

Saedi Jami is with the Bioenvironmental Engineering Research Centre (BERC) Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, and P. O. Box 10 Jalan Gombak, Kuala Lumpur (e-mail: saedi@iium.edu.my).

Parveen Jamal is with the Bioenvironmental Engineering Research Centre (BERC), Department of Biotechnology Engineering, Faculty of Engineering, International Islamic University Malaysia, and P. O. Box 10 Jalan Gombak, Kuala Lumpur (e-mail: jparveen@iium.edu.my).

Mohd Ismail Abdul Karim is with the Bioenvironmental Engineering Research Centre(BERC) Department of Biotechnology Engineering, Faculty of engineering, International Islamic University Malaysia, and P. O. Box 10 Jalan Gombak, Kuala Lumpur (e-mail: akismail@iium.edu.my).

design under the response surface methodology on *Escherichia coli (E. coli)* bacterial strain.

II. Materials and Methods

A. Sample Preparation

Some quantity of *Moringa oleifera* seed powder after defatting (removal of oil) is weighed and added to 1 liter of distilled water and mixed at high speed of 6000rpm in a centrifuge for 10 minutes then filtered to remove un-dissolved particles with the filtrate used to make stock solution of 1000mg/L.

B. Water sample

Synthetic water was prepared by seeding about 1000 bacterial cells/ml of *E. coli* inside distilled water.

C. Microorganisms and Inoculum Preparation

E. coli cells were obtained from laboratory stock solution will be inoculated into 10mL of LB broth. This was incubated with shaking overnight at 37°C. The inoculum density of the bacterial cells was determined using a haemocytometer and it was used to maintain 1000cells/ml.

D. One-Factor-at-a-Time (OFAT)

Classical one-factor-at-a-time was used to determine possible optimum range. The parameters studied were dosage, time and mixing rate. The ranges of each parameter were investigated at different levels.

E. Face Centered Central Composite Design

The process conditions determination was done using the faced centered central composite design under response surface methodology for *E. coli* bacterial strain. This design was used to illustrate the nature of the response surface in the experimental region and to elucidate the optimal concentrations of the most significant variables. Three variables were examined namely dosage, contact time and agitation. The factors were examined at three different levels (low, basal, high). The central composite design for three variables is 20 experimental runs and 6 centre points were carried out (Table I) and their observation were fitted to the following second order polynomial model

$$Y=\beta_0+\beta_1X_1+\beta_2X_2+\beta_3X_3+\beta_{12}X_1X_2+\beta_{13}X_1X_3+\beta_{23}X_2X_3+\beta_{11}X_1^2+\beta_{22}X_2^2+\beta_{33}X^2_3 \quad (1)$$

where Y is the dependent variable (Total Viable Count); X_1, X_2, X_3 are independent variables (dosage, contact time and agitation); β_0 is the intercept term; β_1, β_2, β_3 are linear coefficients; β_{12}, β_{13}, β_{23} are the interaction coefficients and β_{11}, β_{22} and β_{33} are the quadratic coefficients. The developed regression model was evaluated by analyzing the values of regression coefficient, ANOVA (analysis of variance), *p*-values and *F*-values. The quality of fit of the polynomial model equation was expressed by the coefficient of determination, R^2. The statistical software package Design Expert 6.0.8 (Stat Ease Inc., Minneapolis, USA) was used to generate a regression model to predict the effect of the operating parameters on the disinfectant property of defatted crude *Moringa oleifera* seed extracts. The fitted polynomial equation was expressed in the form of contour and surface plots in order to show the interactions between the response and the variables.

F. Validation of Experimental Model

Combinations predicted by the point prediction feature of the statistical software Design Expert 8.0.7.1 trial version were used to validate the model. Four sets of experimental combinations were performed and the predicted results were compared with the experimental results.

TABLE I
DESIGN EXPERT USING FCCCD OF THREE INDEPENDENT PARAMETERS IN THEIR ACTUAL VALUES SHOWING THE EXPERIMENTAL AND PREDICTED RESPONSE

Run order	Dosage (mg/l)	Time (minutes)	Mixing rate (rpm)	TVC (experiment)	TVC (predicted)
1	125	60	140	25	25
2	125	60	120	23	24
3	125	60	100	24	24
4	150	30	100	30	30.2
5	125	90	120	27	27.8
6	150	30	140	31	31.2
7	125	60	120	22	22.5
8	125	60	120	22	22.3
9	150	90	140	34	33.8
10	100	30	100	29	29.2
11	100	30	140	30	30.2
12	150	90	100	33	32.8
13	100	60	120	25	25
14	125	60	120	23	24
15	125	30	120	26	25.2
16	125	60	120	22	22.5
17	100	90	140	33	32.8
18	100	90	100	32	32.8
19	150	60	120	26	26
20	125	60	120	23	22.5

III. Results and Discussion

A. One-Factor-at-a-Time

The purpose of using one-factor-at-a-time design was to determine the possible optimum range of the parameters that will increase the highest disinfection property of the seed extract. There are various factors affecting disinfection process. Important factors such as disinfectant concentration, contact time, inoculum characteristic, mixing rate etc. in this study, disinfectant dosage, contact time and mixing rate were considered. The effect of *Moringa oleifera* dosage is shown in Fig. 1. Increasing the seed extract dosage led to a corresponding increase in inhibitory action against the bacterial cells as seen through the log of total viable count. This phenomenon was also observed in [19]. The inhibitory action may be due to the protein which are lipophilic in nature present in the seed that binds inside the cytoplasmic membrane of the bacterial cell [20]. At dosage between 100mg/l to 200mg/l, there was slight change in the log of total

viable count which suggests the possible optimum level which is necessary to enhance the disinfection process by applying statistical optimization.

The effect of contact time was also studied and it was examined from 30 minutes to 240 minutes as seen in Fig. 2. The seed extracts killed the bacterial strains at the shortest time limit between 30 minutes to 90 minutes. After 90 minutes, there was gradual increase in the bacterial population. This could be as a result of increasing resistance against the seed extract. The highest kill was obtained from 30 minutes to 90 minutes. The result obtained here is in close agreement with [21] who concluded that the optimum contact time on *E. coli* bacterial strain was about 31 minutes. Although, earlier reports revealed that the longer the contact time, the greater the rate of kill , however, report from this study revealed otherwise. Hence, 30 minutes to 90 minutes range was selected for the optimum contact time.

For the mixing rate, reports from earlier studies shows that mixing rate increases the rate of kill in higher magnitudes [22]. The range was varied from 100rpm to 160rpm as shown in Fig. 3. From the results, there was a significant reduction in the bacterial cells from 100rpm to 140rpm. However, as the mixing rate increases, there was no significant reduction as indicted from Fig. 3. Hence the possible optimum range selected was from 100rpm to 140rpm.

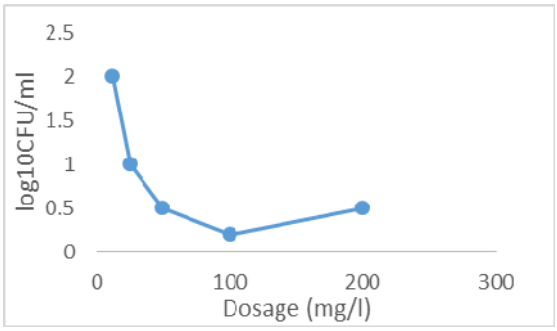

Fig. 1 Effect of dosage on the disinfection process

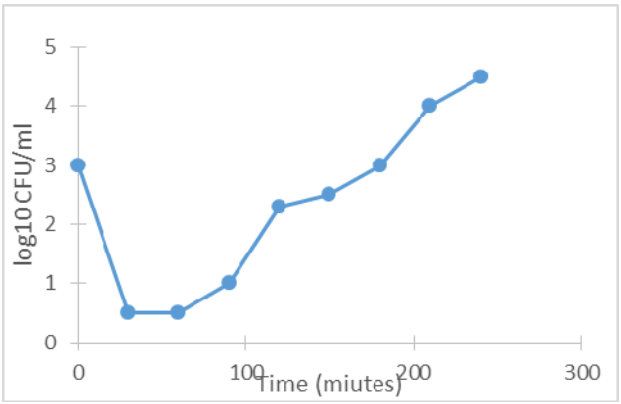

Fig. 2 Effect of contact time on the disinfection process

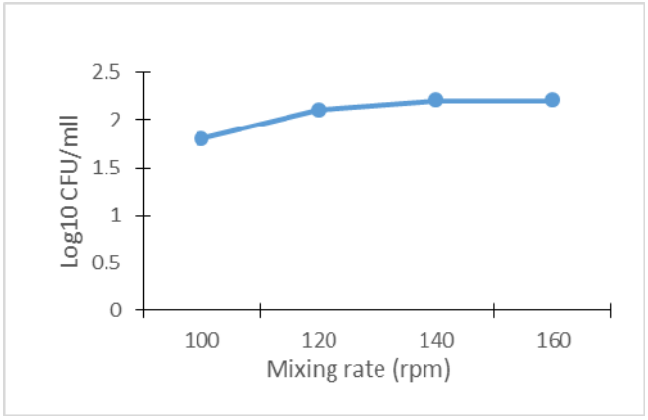

Fig. 3 Effect of mixing rate on the disinfection process

B. Optimization of Process Conditions by FCCCD

Statistical based experiment design is very advantageous for obtaining optimum values and also very useful in developing a system model with less experimental requirement [23]. Based on the experimental results obtained A second order regression equation showed the dependence of the disinfectant property on the process parameters. The parameters of the equation were obtained by multiple regression analysis of the experimental data. An empirical relationship between the response and the parameters was expressed in terms of second-order polynomial equation

$$TVC(E.coli)=22.50+0.50A+1.30B+0.50C+0.000AB+0.00A+0.000BC+3.00A^2+4.00B^2+2.00C^2 \quad (2)$$

where the Total Viable Count for *E. coli* is the response (Y) and A, B and C are dosage, contact time and agitation, respectively. The adequacy of the model was checked using analysis of variance (ANOVA) which was tested using Fisher's statistical analysis and the results are shown in Table II.

The model F value of 115.73 and p-value of <0.0001 imply that the model is significant, suggesting that there is only 0.01% chance that the model F value could occur due to noise. Model terms with Prob>F (less than 0.05) are considered significant, while those greater than 0.10 are insignificant. The non-significant lack of fit suggested that the obtained experimental responses sufficiently fit with the model. The R^2 value closer to 1 denotes better correlation between the observed and predicted values [24]. The higher values of R^2 (0.9905) and adjusted R^2 (0.9819) also indicated the efficacy of the model suggesting that 99.05% and 98.19% variation could be accounted for by the model equation respectively. Thus, for a good statistical model, the R^2 value should be in the range of 0–1.0, and the closer the value is to 1.0, the more fit the model is deemed to be.

Adequate precision measures the signal to noise ratio and a value >4 is considered appropriate for desirable models. The adequate precision value of 28.70 for the disinfection process indicates that the model can be used to navigate the design space. Also, the coefficient of variation (CV) indicates the

degree of precision with which the treatments are compared, and the low value of CV showed the reliability of experiment. In this study, a relatively lower value of the coefficient of variation (CV=2.06) suggested a good precision and reliability of the experiment. The coefficient values of the regression equation are listed in Table II. The p-values are used as a tool to check the significance of each coefficient, which also indicate the interaction strength between each independent variable; the smaller the p-values, the bigger the significance of the corresponding coefficient. The responses revealed that all the three linear coefficients (A, B, C), and all the three quadratic coefficients were significant ($p<0.05$) and had remarkable effects on the disinfection process.

The 3D response surface plot is the graphical representations of the regression equation used to investigate the interaction among variables and to determine the optimum process conditions during the disinfection process. The 3D plots shown in Fig. 4 were based on the function of two variables with the other variable being at its optimum level. Significance of the interactions between the corresponding variables is indicated by an elliptical or saddle nature of the contour plots. Fig. 4 (a) represents the interaction between mixing rate and contact time. The shape of the response surface curves showed a moderate interaction between these tested variables. While Fig. 4 (b) shows the 3D plot corresponding to contact time and dosage this plot showed somewhat an elliptical contour suggesting that not only there were well defined optimum operating conditions but also the interaction effect between the two factors was significant. In case of dosage and mixing rate (Fig. 4 (c)), the response plot was elliptical depicting interaction between them.

C. Validation of Experimental Model

In order to validate the generated second-order regression model, some sets of experiments replicated thrice were performed according to the point prediction of Design Expert and are presented in Table III.

TABLE II
ANALYSIS OF VARIANCE

Source	Sum of Square	df	Mean Square	F Value	p-value Prob > F	
Model	322.9	9	35.88	115.73	< 0.0001	significant
A-Dosage	2.5	1	2.5	8.06	0.0176	
B-Contact time	16.9	1	16.9	54.52	< 0.0001	
C-mixing rate	2.5	1	2.5	8.06	0.0176	
AB	0	1	0	0	1	
AC	0	1	0	0	1	
BC	0	1	0	0	1	
A^2	24.75	1	24.75	79.84	< 0.0001	
B^2	44	1	44	141.94	< 0.0001	
C^2	11	1	11	35.48	0.0001	
Residual	3.1	10	0.31			
Lack of Fit	1.6	5	0.32	1.07	0.4726	not significant
Pure Error	1.5	5	0.3			
Cor Total	326	19				

TABLE III
VALIDATION OF EXPERIMENTAL MODEL

Run	Dosage	Contact time	Mixing rate	Experimental	Predicted
1	100	60	120	23	26
2	125	60	100	24	24
3	125	30	120	20	25.2
4	120	33	130	24	25.3

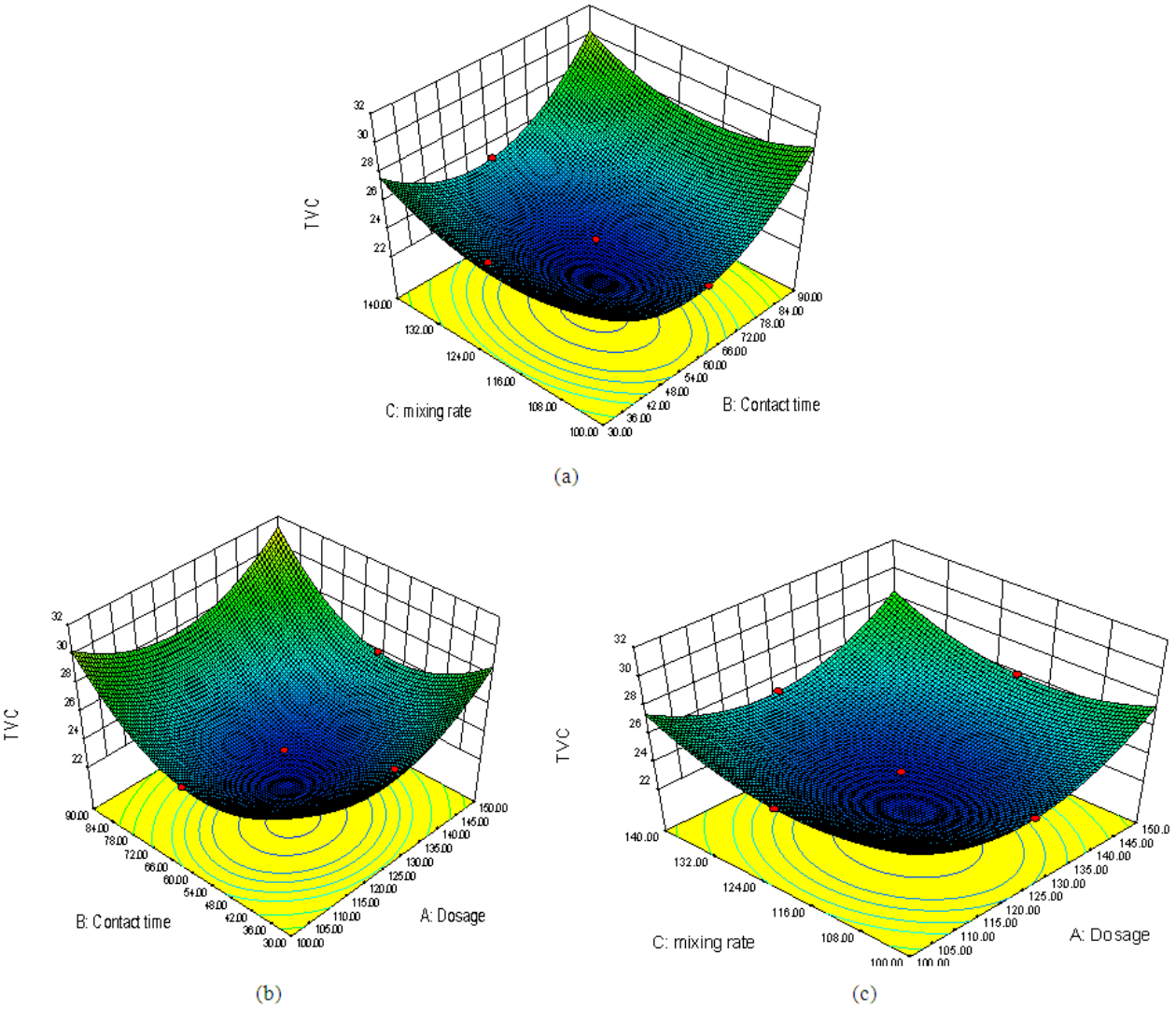

Fig. 4 The 3D curves of combined effects of dosage, contact time and mixing rate of the disinfection process conditions on *E. coli* bacteria strain: (a) contact time and mixing rate (b) contact time and dosage (c) Dosage and mixing rate

IV. Conclusion

The possible optimum level of dosage, contact time and mixing rate were determined using OFAT method. The central composite design under the response surface methodology was employed for further optimization of the process parameters through the development of second order regression model. The generated model was validated with experimental runs and there was correlation with the predicted values. All the three parameters studied were highly significant and the optimum disinfection processes obtained are dosage of 125mg/L at contact time of 60 minutes and mixing rate of 120 rpm.

Acknowledgment

The authors are grateful to the Ministry of Higher Education (MOHE) Malaysia, for financing the research project under the Fundamental Research Grant Scheme (FRGS 11-030-0178) and to Department of Biotechnology Engineering, International Islamic University Malaysia for their support.

References

[1] Musikavong, C. and S. Wattanachira, *Identification of dissolved organic matter in raw water supply from reservoirs and canals as precursors to trihalomethanes formation.* Journal of Environmental Science and Health Part a-Toxic/Hazardous Substances & Environmental Engineering, 2013. 48(7): p. 760-771.

[2] Simard, S., R. Tardif and M.J. Rodriguez, *Variability of chlorination by-product occurrence in water of indoor and outdoor swimming pools.* Water Research, 2013. 47(5): p. 1763-1772.

[3] Zhang, K.J., N.Y. Gao, Y. Deng, T.Q. Zhang and C. Li, *Aqueous chlorination of algal odorants: Reaction kinetics and formation of disinfection by-products.* Separation and Purification Technology, 2012. 92: p. 93-99.

[4] Vongsak, B., P. Sithisarn, S. Mangmool, S. Thongpraditchote, Y. Wongkrajang and W. Gritsanapan, *Maximizing total phenolics, total flavonoids contents and antioxidant activity of Moringa oleifera leaf extract by the appropriate extraction method.* Industrial Crops and Products, 2013. 44: p. 566-571.

[5] Luqman, S., S. Srivastava, R. Kumar, A.K. Maurya and D. Chanda, *Experimental Assessment of Moringa oleifera Leaf and Fruit for Its Antistress, Antioxidant, and Scavenging Potential Using In Vitro and In Vivo Assays.* Evid Based Complement Alternat Med, 2012. 2012: p. 519084.

[6] Padla, E.P., L.T. Solis, R.M. Levida, C.C. Shen and C.Y. Ragasa, *Antimicrobial isothiocyanates from the seeds of Moringa oleifera Lam.* Z Naturforsch C, 2012. 67(11-12): p. 557-64.

[7] Abou-Elezz Fouad Mohammed, K., L. Sarmiento-Franco, R. Santos-Ricalde and J.F. Solorio-Sanchez, *The nutritional effect of Moringa oleifera fresh leaves as feed supplement on Rhode Island Red hen egg production and quality.* Tropical Animal Health and Production, 2012. 44(5): p. 1035-40.

[8] Posmontier, B., *The medicinal qualities of Moringa oleifera.* Holist Nurs Pract, 2011. 25(2): p. 80-7.

[9] Muyibi, S.A., Saad A. Abass, Megat Johari M. M. Noor and F.R. Ahmadun, *Enhanced coagulation efficiency of moringa oleifera seeds through selective oil extraction.* IIUM ENGINEERING JOURNAL, 2003. 4(1): p. 1-11.

[10] Luz, L.D., M.C. Silva, R.D. Ferreira, L.A. Santana, R.A. Silva-Lucca, R. Mentele, M.L. Oliva, P.M. Paiva and L.C. Coelho, *Structural characterization of coagulant Moringa oleifera Lectin and its effect on hemostatic parameters.* Int J Biol Macromol, 2013.

[11] Mohammad, T.A., E.H. Mohamed, M.J.M.M. Noor and A.H. Ghazali, *Coagulation activity of spray dried salt extracted Moringa oleifera.* Desalination and Water Treatment, 2013. 51(7-9): p. 1941-1946.

[12] Franco, M., G.K.E. Silva and J.E.S. Paterniani, *Water Treatment by Multistage Filtration System with Natural Coagulant from Moringa Oleifera Seeds.* Engenharia Agricola, 2012. 32(5): p. 988-996.

[13] Beltran-Heredia, J., J. Sanchez-Martin, A. Munoz-Serrano and J.A. Peres, *Towards overcoming TOC increase in wastewater treated with Moringa oleifera seed extract.* Chemical Engineering Journal, 2012. 188: p. 40-46.

[14] Ferreira, R.S., T.H. Napoleao, A.F. Santos, R.A. Sa, M.G. Carneiro-da-Cunha, M.M. Morais, R.A. Silva-Lucca, M.L. Oliva, L.C. Coelho and P.M. Paiva, *Coagulant and antibacterial activities of the water-soluble seed lectin from Moringa oleifera.* Letters in Applied Microbiology, 2011. 53(2): p. 186-92.

[15] Madrona, G.S., G.B. Serpelloni, A.M.S. Vieira, L. Nishi, K.C. Cardoso and R. Bergamasco, *Study of the Effect of Saline Solution on the Extraction of the Moringa oleifera Seed's Active Component for Water Treatment.* Water Air and Soil Pollution, 2010. 211(1-4): p. 409-415.

[16] Suarez, M., J.M. Entenza, C. Doerries, E. Meyer, L. Bourquin, J. Sutherland, I. Marison, P. Moreillon and N. Mermod, *Expression of a plant-derived peptide harboring water-cleaning and antimicrobial activities.* Biotechnol Bioeng, 2003. 81(1): p. 13-20.

[17] Oluduro, O.A., B.I. Aderiye, J.D. Connolly, E.T. Akintayo and O. Famurewa, *Characterization and Antimicrobial Activity of 4-(beta-D-Glucopyranosyl-1 -> 4-alpha-L-rhamnopyranosyloxy)-benzyl thiocarboxamide; a Novel Bioactive Compound from Moringa oleifera Seed Extract.* Folia Microbiologica, 2010. 55(5): p. 422-426.

[18] Anwar, F., S. Latif, M. Ashraf and A.H. Gilani, *Moringa oleifera: a food plant with multiple medicinal uses.* Phytotherapy Research, 2007. 21(1): p. 17-25.

[19] Idris, A.M., S.A. Muyibi, M.A.k. Ismail, P. Jamal, M.H. Salleh and S. Jami, *Investigating antibacterial activity of defatted moringa oleifera seed extract on selected gram-negative and gram-positive microbes.* International journal of Academic Research, 2013. 5(1): p. 101-106.

[20] Viera, G.H., J.A. Mourao, A.M. Angelo, R.A. Costa and R.H. Vieira, *Antibacterial effect (in vitro) of Moringa oleifera and Annona muricata against Gram positive and Gram negative bacteria.* Rev Inst Med Trop Sao Paulo, 2010. 52(3): p. 129-32.

[21] Bichi, M.H., J.C. Agunwamba and S.A. Muyibi, *Optimization of operating conditions for the application of Moringa oleifera (zogale) seed extract in water disinfection using response surface methodology.* African Journal of Biotechnology, 2012. 11(92): p. 15875-15887.

[22] Metcalf and Eddy, *Wastewater Engineering: Treatment and Reuse*. 4 ed. 2004, Singapore: McGraw Hill. 1818.

[23] Salihu, A., M.Z. Alam, M.I. AbdulKarim and H.M. Salleh, *Effect of process parameters on lipase production by Candida cylindracea in stirred tank bioreactor using renewable palm oil mill effluent based medium.* Journal of Molecular Catalysis B-Enzymatic, 2011. 72(3-4): p. 187-192.

[24] Salihu, A., M.Z. Alam, M.I. AbdulKarim and H.M. Salleh, *Optimization of lipase production by Candida cylindracea in palm oil mill effluent based medium using statistical experimental design.* Journal of Molecular Catalysis B-Enzymatic, 2011. 69(1-2): p. 66-73.