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Rambutan and fenugreek seeds for the treatment of Palm Oil Mill Effluent (POME) and its Feedforward Artificial Neural Network (FANN) modelling

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ABSTRACT

In the present research, local rambutan seed extract and fenugreek seed extract were used as a bio-coagulants for the treatment of palm oil mill effluent (POME). Jar test experiments were conducted to find the optimal operating conditions for the removal of turbidity and total suspended solids from POME. At an optimal pH of 3, bio-coagulant (rambutan combined with fenugreek at the ratio of 50/50) dosage of 600 mg/L and room temperature of 28°C, an impressive removal of 61% of total suspended solids and 78% of turbidity was achieved. Along with this, a Feedforward Artificial Neural Network (FANN) was used to model the coagulation mechanism. Three different training algorithms were tested on the FANN, namely the Levenberg-Marquardt, Bayesian Regularization and Scaled Conjugate Gradient methods. The best training algorithm was found to be Levenberg-Marquardt, based on the fact that it was in closer agreement with the experiment results and gave very low error percentage. The results of this study suggest that rambutan and fenugreek seeds have potential in being used as a bio-coagulant for POME treatment. Treatment efficiencies were reasonably high, and less sludge was produced using this natural treatment method, thus deemed to be more economical and environmentally friendly.

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Abbreviations

<i>ANFIS</i>	Adaptive Network Based Fuzzy Interference System
<i>BOD</i>	Biochemical oxygen demand
<i>BR</i>	Bayesian Regularization
<i>COD</i>	Chemical oxygen demand
<i>FANN</i>	Feedforward Artificial Neural Network
<i>FFBP</i>	Feedforward back propagation
<i>FSE</i>	Fenugreek seed extract
<i>LM</i>	Levenberg-Marquardt
<i>MAE</i>	Mean Absolute Error
<i>MSE</i>	Mean Squared Error
<i>POME</i>	Rambutan seed extract
<i>RSE</i>	Final weight
<i>SCG</i>	Scaled Conjugate Gradient
<i>TSS</i>	Total suspended solids

1. Introduction

It is predicted that the global demand of palm oil will reach 85 million metric tonnes by the year 2024 due to its wide application in everyday products (Basiron & Yew, 2016). To fulfil these demands, Malaysia, being the largest exporter in the world, will have to increase its production (Schouten & Glasbergen, 2011). According to Lee et al., (2014), while meeting the demands may not be much of a struggle, controlling the waste produced will be difficult. Palm oil mill effluent (POME) is one of the liquid waste products generated during the processing of the palm oil. It is typically an acidic, viscous discharge that has high TSS, turbidity, biological oxygen demand (BOD) and chemical oxygen demand (COD). The typical composition of POME is given in Table 1 (Malakahmad & Chuan, 2013). When POME is released into the environment, it will pollute and contaminate nearby areas. It will cause major damage to aquatic life and water resources by making their environment more acidic. Yacob, et al., (2005) reported that in the year 2004, about 30 million tonnes of POME was produced from a total of 380 palm oil mills in the country. This was also confirmed by Madaki & Seng, (2013), who reported that based on the total amount of crude palm oil produced per month, which was 990,000 m³ (2.4 million tonnes), about 740,000 m³ (1.8 million tonnes) of POME was generated. These figures of national POME production will surely rise when the industries expand to meet the increasing global demand for palm oil.

Currently, POME is treated via aerobic and anaerobic digestion through the ponding system. This method consists firstly of a preliminary treatment used to adjust the pH from acidic (pH≈4) to neutral (pH≈7) and then followed by anaerobic bacterial treatment in an open pond. This step has low energy requirements and produces biogas (methane, carbon dioxide and trace amounts of other gases) that can be captured for energy generation. After the anaerobic step, the partially treated POME will be channelled to aerobic treatment ponds for

further clarification. This treatment method using ponds is effective, but it takes a very long time (near 3 months) and needs a large piece of land. In the long run, it may become ineffective as treatment costs and environmental aspects will pose a problem. Also, the ponding system utilizes microorganisms to break down the pollutants, meaning that the environment has to be conducive for them the work (Bhatia et al., 2007b). Any alterations in the microbes' environment will cause problem in the breakdown process. Besides that, the ponding system is inefficient due to low biomass yield and its poor separation of solids. This prevents the recovery of the settled solids for further use (Sapari et al., 1996). Thus, a ponding treatment system will only be deemed productive if the microorganisms can operate at optimal conditions and the by-products (biogas and biosolids) produced are used efficiently in a timely manner.

Table 1: Properties of raw POME

Property	Value
Temperature	80°C – 90°C
TSS	18,000 mg/L
Turbidity	~170 – 200 FNU
COD	50000
BOD	25000
pH	4.0 - 5.0

This is where the process of coagulation and flocculation comes in useful. It is the quickest way to reduce the heavy emissions of POME to more tolerable levels (Ahmad et al., 2005). Coagulation and flocculation may be used at the anaerobic and aerobic treatment point, after there has been prior removal of all heavy solid materials. This treatment is targeted for the suspended solids that do not settle easily. Many scientists around the world have carried out this process using different types of coagulants and flocculants. Some of the synthetic chemical coagulants that have been used are aluminium sulphate (alum), polyaluminium chloride (PAC) and ferric salts (Selcuk et al., 2004). They are highly effective, but there are setbacks that make them undesirable, for example aluminium in synthetic coagulants is linked to the development of Alzheimer's disease (Flaten, 2001) and iron salts are known to produce rust or blood coloured stains (Gebbie, 2005). Besides that, large amounts of hazardous sludge produced through using synthetic coagulants pose a sludge handling and disposal problem. Thus, researchers have looked into using natural coagulants such as *Moringa oleifera* seeds by Bhatia et al., (2007) and (Bhatia et al., 2007a), nirmali seeds by (Deshmukh et al., 2013), aloe vera gel by Irene Kopytko et al. (2008), fenugreek seeds by Sethu et al. (2015), micro and macro algae mixtures by Kamyab et al., (2014), *Chlamydomonas* by Kamyab et al. (2015), *Lemna minor* by Kamyab et al. (2017), chickpea by Choong Lek et al. (2018) and peanut and wheat by Chung et al. (2018) to replace synthetic ones. Natural coagulants not only perform better than their synthetic counterparts but are safer and more environmentally friendly. The volume of sludge produced is also much lesser and there is an option

be used as bio-compost or fertiliser, as they are rich in nutrients and do not contain any harmful compounds.

Among the natural coagulants, *M. oleifera* is the most studied one that has produced promising results in treating water and wastewater (Amagloh & Benang, 2009; Bhatia et al., 2007a). It was also found to be better than alum in terms of performance (Sarpong & Richardson, 2010) because of the presence of water-soluble cationic proteins in the seeds (Bhatia et al., 2007a). However, *M. oleifera* is a nutritious food source in Asia and Africa, so its use in water purification may reduce its supply as a food material. Besides that, the cost to produce 1kg (3400 seeds) of *M. oleifera* was reported to be more expensive at approximately US\$2 compared to alum, which costs approximately US\$1 per kg (Goh, 2005). The higher cost of a natural coagulant is not favourable as low cost is one of the important factors that make them an attractive option. Another natural coagulant that has great potential is fenugreek seeds, where its performance is close to that of *M. oleifera* (Sethu et al., 2015). But this too is a food source and may not be a feasible option in the long run. Thus, the need to look for an alternative source of coagulant, that not only is abundant and easily available, but also not competitive with food material is crucial. One such an example is the rambutan fruit seed, a locally produced fruit waste material which holds high prospects for POME treatment.

Nephelium lappaceum or rambutan, is a tropical fruit that is indigenous to south-east Asia, specifically Malaysia, Indonesia and Thailand. Malaysia produces approximately 500,000 tonnes of rambutan fruits yearly, contributing to almost 12% of the total world production (Malakahmad & Chuan, 2013). Rambutan fruit flesh is either eaten directly or used to produce juice, jams, marmalades and jellies. Parts of the fruits that are under-utilised include the seeds and skin. They are normally discarded as waste. Since they constitute a substantial 16% weight of the whole fruit, it is important to explore their potential for other applications. One of the options would be to check its prospective use as a natural coagulant for water and wastewater treatment, which is what the current study is focused on. Very limited research has been done on rambutan seeds and its potential use as a coagulant. Only one published work so far was found, on the use of rambutan seeds to remove turbidity from synthetic kaolin wastewater (Zainal Abidin et al., 2014). The researchers managed to achieve up to 88% of turbidity removal using rambutan seeds alone and 99% turbidity removal when used along with modified aluminum (Envifloc – 401). Furthermore, Zainal Abidin et al. (2014) also concluded that the coagulation ability of rambutan seeds is highest in acidic conditions (pH 3 – 4), thus may have potential for the treatment of POME, which has about the same pH range. Hence the purpose of this study – to evaluate the potential use of rambutan seeds as a coagulant for POME treatment.

In order to model the coagulation behaviour of rambutan seeds, a Feedforward Artificial Neural Networks (FANN) is applied for the study. FANN derives its name and functioning from the human nervous system. It consists of an input module (input layer), output module (output

layer) and a processing module (hidden layer). A typical ANN is trained by utilizing experimental parameters as inputs and analysed results as the output. The results from the developed ANN model which is the predicted outputs of the ANN is compared to the actual target values and then fine-tuned so that both the predicted and target values resemble each other as closely as possible (Keijsers, 2010). Among the important water purification tasks and techniques simulated or modelled by ANN are biological reactions and processes. One of the chief biological processes is coagulation-flocculation. A few researchers have used ANN to model the coagulation-flocculation process. Selvanathan et al. (2017) carried out feedforward artificial neural network (FFBP) modelling studies for the biochars derived from rambutan (*Nephelium lappaceum*) peel through slow pyrolysis, which were characterised and investigated as potential adsorbent for the removal of copper ion, Cu(II) from aqueous solution. León-Luque et al. (2016) reported training a neural network using parameters such as turbidity, pH and alkalinity, colour, water conductivity (as an alternative for TSS measurement) and temperature. The data was obtained from jar tests to predict the amount of coagulant and flocculant required to treat water with several initial turbidities. It was found that the model created could accurately predict the dosages of coagulant required, and that the accuracy of the ANN depends entirely on the quality and quantity of data used to train and test the network. Wu & Lo (2008) also reported training the ANN and Adaptive Network Based Fuzzy Interference System (ANFIS) to predict the dosage of PAC required for water treatment.

In general, the primary aim of this research work was to investigate the coagulation potential of RSE with POME and compare to that of fenugreek (fenugreek seed extract, FSE), which is another bio-coagulant with promising potential. For this work, okra mucilage extract (OE), which is known to be a natural and efficient bio-flocculant, was used for both RSE and FSE studies. Next, the characterization of rambutan seeds and fenugreek seeds using the FTIR was carried out to identify the components responsible for the coagulant activity. The final aim was to model the optimum RSE and FSE required using the FANN model. This work is rather novel, as there have been no past studies looking into the potential of RSE with FSE for POME treatment, along with FANN modelling. Since rambutan seeds are an abundant and cheap source of local waste material, it is imperative that its potential for water/wastewater treatment be tested, so that it can be put to good use in the future.

2. Materials and Methods

2.1 Preparation of materials

2.1.1 Preparation of materials

Rambutan fruits and fenugreek seeds were bought from a local supermarket located in Semenyih, Malaysia. The rambutan fruits were deseeded and the seeds were sun-dried for 7 hours until a constant weight was reached. They were then crushed and ground to smaller particle size using a rotor beater mill (Retsch SR 300). The resulting powder was further ground to a size of approximately 80-100 µm using an ultra-centrifugal mill

(Retsch ZM200). The powder was then sieved through an 83 μm sieve to make sure all the particles were of a uniform size (less than 83 μm). To extract the active ingredient of the seeds, 1 gram of rambutan seed powder was added to 1 L of distilled water and mixed vigorously at 150 rpm using a hotplate and a magnetic stirrer for 10 minutes. The solution produced was then filtered through a Filtres Fironi 601 filter using vacuum pump filtration. The rambutan seed extract (RSE) produced was then kept refrigerated at 4°C. The same steps were used to produce fenugreek seed extract (FSE). A new set of RSE and FSE solution was prepared each time for the experiments in order to avoid natural degradation of the coagulant.

2.1.2 Preparation of bio-flocculant

Okra seedpods were purchased from a local supermarket in Semenyih, Malaysia. The seedpods were sliced into smaller pieces and soaked in distilled water overnight. The resulting mixture was filtered to remove okra residues and seeds. The supernatant was precipitated using ice cold alcohol (isopropanol) and then washed with acetone. The resulting polysaccharides were then dried overnight in an oven at 50°C for 8 hours. The dried okra mucilage was then ground and prepared in the same manner as the RSE and FSE. This okra extract (OE) was used as the bio-flocculant for all the experiments. Again, a new set of OE was produced for each set of experiment to avoid the biodegradation of the flocculant.

2.1.3 Preparation of Palm Oil Mill Effluent (POME)

The POME used throughout the duration of the experiment was obtained from a palm oil mill located in Dengkil town, Malaysia. The POME was collected from the final discharge pond on a weekly basis. The utensils used were large 30 litre drums in order to maintain homogeneity. To collect the POME, the drums were first rinsed with the POME to remove any dirt particles or impurities present in the drum. The drums were then submerged into the pond until they were filled to the brim. The drums were then sealed and transferred to the laboratory. The POME was stored overnight for one day, and the next day the supernatant was extracted for analysis. This step was carried out so that larger particles would settle down and not interrupt with the coagulation and flocculation studies.

2.1.4 Analysis of POME before and after experimental runs

The chief parameters studied were the coagulant dosage, flocculant dosage and pH. The performance of each run was evaluated by noting the changes in the turbidity and TSS. The turbidity of POME before and after the experiment was analysed using the Portable turbidity meter (ICMi415) by Martini instruments. The TSS in POME before and after the experiment was analysed through UV-Vis spectroscopy using the Lambda 35 Spectrophotometer by Perkin Elmer. pH was measured using the Mettler Toledo Seven Compact equipment.

2.2 Experimental - Jar tests

The jar test was conducted using the Lovibond Flocculator to test the coagulation activity of RSE and FSE. Before each experiment, the TSS, turbidity and the pH of POME was noted. The pH of POME was only adjusted after the initial readings to avoid the acid/alkali addition to

contribute towards the turbidity or TSS content of the POME. The entire experiment consisted of two parts. The first part consisted experimental runs conducted to test and compare the performance of RSE and FSE with OE at different conditions. The second part consisted of the experimental runs using jar tests conducted to collect data to train the FANN. All the experimental runs were carried out at room temperature and pressure (28°C, 1 atm). All subsequent experiments were conducted via the same method and conditions to maintain homogeneity. 500 mL of POME was filled in each of the six 1 L glass beakers and the impellers were submerged into the beaker. The POME was first agitated with rapid mixing at 100 rpm for 4 minutes during which the RSE (bio-coagulant) was added into the beaker, then agitation speed was reduced to 40 rpm for 30 minutes, during which the OE (bio-flocculant) added into the beaker. After this step, stirring was halted, and a sedimentation time was allowed for the flocs formed to settle. Sedimentation was carried out for about 6 hours, until no change in the mass of flocs settled were detected.

In the first part, the first few sets of experimental runs were carried out to test the optimum coagulant dosages of RSE and FSE with and without OE. For this, the pH of the POME was kept at a constant of 3 while the dosage amount was manipulated between 100 to 1000 mg/L. The dosage that gave the highest reduction in turbidity and TSS was taken as the optimum amount. Second sets of experimental runs were carried out to test the optimum pH of POME for coagulation-flocculation to occur at the optimum RSE and FSE dosage. In this set, the pH of the POME was adjusted to the desired value between ranges of 2 to its original pH with the addition of acid (0.1M HCl of analytical grade). The dosage that gave the highest reduction in turbidity and TSS was taken as the optimum pH value. Third sets of experimental runs were carried out to test the optimum flocculant dosage. The dosage of coagulant and the pH were set to the optimum levels, while the OE flocculant dosage was varied between 10 to 100 mg/L. The dosage of OE that gave the highest reduction in turbidity and TSS was taken as the optimum flocculant dosage. Fourth set of experimental runs were carried out to develop the ANN model. In this set of experimental runs, all the above-mentioned optimal parameters were considered namely the pH, coagulant dosage and flocculant dosage. The settling time after slow mixing was varied between 1 to 6 hours with a reading taken at every interval of 1 hour. The Design of Experiments (DOE) version 7.0 software was used to study the effect of all the parameters with a minimum number of experimental runs.

The processing of an ANN is relatively simple to track. This is due to their modular architecture whereby a typical FANN would contain a number of processing units (neurons) arranged in layers. These layers will be interconnected to form a network. The number of neurons in the input and output layers are defined by the number of inputs and outputs, while in the hidden layer, they are user defined. A well trained ANN can assist to decrease or even eliminate the need for jar tests to be carried out. While jar tests do help to identify the dosage of bio-coagulant or flocculants needed, they take a relatively long time to carry out and they can only be conducted

periodically. Therefore, if there are any rapid changes in the turbidity of water due to any reason (such as rain, which is common in Malaysia), the amount of coagulant needed would increase (Wu & Lo, 2008). Furthermore, it was noted from the experiments that excess coagulant or flocculant dosage can lead to an increase in turbidity of the POME. Therefore, this FANN model will be able to help instantly detect the turbidity levels of POME to work out the actual amount of coagulant or flocculant needed in real-time.

The removal of both turbidity and TSS was calculated using the formula shown below in Equation (1):

$$\text{removal (\%)} = \frac{\text{Initial value} - \text{Final Value}}{\text{Initial value}} \times 100\% \quad (1)$$

2.3 Analysis – FTIR and zeta potential

An FTIR analysis was carried out for both the rambutan and fenugreek seed powders. This is done to see the common functional groups bonds that are present in the substance and to see what functional groups contribute to the coagulation-flocculation ability of RSE and FSE. An FTIR utilizes infrared light at different wavelengths to scan the sample. The corresponding transmittance is then plotted against the frequency of the infrared used. The FTIR scan here was carried out using an Attenuated Total Reflection (ATR) FTIR cell that uses infrared beam in evanescent wave to contact the sample. The advantage of using ATR is the ease of flexibility; the samples can be analysed in liquid or solid state without any extra preparation (Arai & Sparks, 2001). Before scanning the samples, the window of contact is cleaned by pouring two drops of analytical grade acetone and wiping it with a microfibre cloth. A background scan was first carried out to eliminate any noise that may interfere with the sample scanning results. A total of 16 scans (recommended) were carried for each sample of the rambutan and fenugreek seed powders. Besides that, the zeta potential of RSE and FSE were determined using the Zetasizer equipment.

2.4 FANN modelling

In an ANN, the neurons are arranged in a layer layout. There are at least three layers that makeup a neural network, these are the input layer, output layer or at least one hidden layer. Each layer of neurons receives input information from its previous layer and is subsequently fed into the next layer. A typical neural network is defined by its number of layers, the number of neurons in those layers, the transfer functions used in those layers and most importantly, the training algorithm used. The training tools used for neural networks are the inputs and outputs (target). Inputs are used as reference for its corresponding output. The factor that helps a neural network learn and perform any task is its ability to signify a weight between different element of its layer and neuron connections which it adjusts to find a balanced value that gives the least amount of error between the actual values and the predicted values of the network.

2.4.1 Experimental Design

Experimental design allows testing of all the factors at one time, simultaneously. It helps study the level of interconnection between different factors without having to test each one (Anderson & Whitcomb, 2001).

Therefore, a D-optimal design was made considering the parameters: i) coagulant dosage (range – 100 to 1000 mg/L), ii) pH (range 3 – 6) and iii) residence time (range 1 to 6 hours). D-optimal design allows testing all the interconnected effect of specific parameters with the minimum number of experiments (Asghar et al., 2014). The response was recorded as the turbidity of water in FNU and TSS in mg/L.

2.4.2 FANN Architecture

The three-layer (input layer, single hidden layer, output layer) FANN was devised using Matlab™ coding. Log-sigmoidal transfer function was used in both hidden and output layers. The training data used to develop the FANN was split into three parts. 70% of randomly selected data was used to train the network, while 15% was used for validation and the rest of the 15% for testing of the network. The training data was fed into the system as responses to the parameters tested. The response was noted in terms of turbidity and TSS. This entire 21 run data was then normalised to level all the data fed into a range of -1 and 1. The importance of data normalization is necessary as it helps to bring all data in the range that the sigmoid activation lies in, which is between -1 and 1 for Tansig transfer function. Furthermore, normalising data also helps to avoid the network being dominantly trained by large values (higher than 1).

The training data was split using random selection by Matlab™ (Divideand Function), selection in user defined blocks (Divideblock Function, 3 runs per block) and individually selecting each run for neural network training (Divideind). Divideand exhibited the most efficient training due to a small number of runs. The training, testing and validation of the neural network were carried out using various numbers of neurons in the hidden layer. This was done because too few hidden neurons could hinder the learning process of the network due to less weight of neuron designated for each parameter while too many may give excess space for the weights to correlate making the ANN learn the noise levels. The Mean Squared Error (MSE), Mean Absolute Error (MAE) and Mean Absolute Percentage Error (MAPE), R-squared value and the entire array of predicted output values of each number of hidden neurons were fed into a Matlab™ database and plotted according to need. In this ANN, the architecture of the neural network is of utmost importance for it to be used. In this paper, the neural network architecture produced through Matlab™ coding is varied with different training algorithms to achieve an optimal structure that fits the actual experimental data as closely as possible (Asghar et al., 2014).

2.4.3 Training algorithms

The type of training algorithms used affects the learning rate of the ANN (Chen et al., 2015). There are a total of 12 back propagation training algorithms for ANN development. Three of these different types of training algorithms, namely, Levenberg–Marquardt (LM), Bayesian Regularization (BR) and Scaled Conjugate Gradient (SCG) were tested in this paper. LM was selected due to its flexibility and speed; it is the most preferred because it gives the fastest convergence, however, it also requires the largest storage requirements of any back-propagation

algorithm. BR was selected because it is programmed by default to continue validation until optimal performance (MSE) values are met. SCG was selected because they require very less processing memory and are much faster than any gradient descent training algorithm (Demuth et al., 2007).

2.4.4 Performance Evaluation

The performance and fit of the model depend on many custom based options. However, to judge the quality the model, several performance factors can be used. Some of most common performance factors that are MSE, MAE and MAPE and the lowest value. MSE factor calculates the total error produced by a network through mean of squared error. MAE calculates the network error by taking the absolute values of all the errors and taking their average. MAPE calculates the absolute error and indicates the accuracy of a neural network by expressing the error in terms of percentage. The residual plot shows the difference between the predictions made by the feedforward neural network model versus the actual response recorded from the experimental analysis. The coefficient of determination (R^2) shows the amount of variation in the dependent data that is derived from independent data (Demuth et al., 2007). The MSE, MAE and MAPE formula used are given in equations (2), (3) and (4) respectively.

$$\sum_{x=0}^n (P_x - A_x)^2 \quad (2)$$

$$\sum_{x=0}^n P_x - A_x \quad (3)$$

$$\sum_{x=0}^n \left(\frac{A_x - P_x}{A_x} \right) \times 100\% \quad (4)$$

3. Results and Discussion

3.1 Coagulation-Flocculation

3.1.1 Effect of Coagulant Dosage

Figure 1 and 2 depict the removal percentages of TSS and turbidity respectively using RSE and FSE at pH 3 and room temperature 28°C. From the figures, it can be seen that the optimal dosages for RSE and FSE are at 600 mg/L and 500 mg/L respectively. The removal of TSS is about the same for both bio-coagulants, where at optimal dosage, there seemed to be 65% removal. As for the turbidity, FSE showed better performance when compared to RSE, with a removal percentage of 85% at optimal conditions. RSE was slightly lower, at 80%. The values however, were very close and it can be concluded the performance of both bio-coagulants are comparable. Another study using RSE by Zainal Abidin et al. (2014), (2014), also showed similar trends for the removal or turbidity.

3.1.2 Effect of pH

From the pH studies at similar conditions, it was found that the optimal pH is about 3. At this point, the removal of TSS was about 68% for both RSE and FSE, and the removal of turbidity was between 80 to 82% for both RSE and FSE. Again, from the pH studies, it is observed that RSE and FSE have similar removal percentages, and are thus comparable as bio-coagulants.

Figure 1: Effect of bio-coagulant dosage on the removal percentage of TSS

Figure 2: Effect of bio-coagulant dosage on the removal percentage of turbidity

It is observed in Figure 4 that the removal of turbidity shows a drop to negative value at pH of 4. This could be due to the zeta potential of RSE being very low (-2.86, from Table 6) that at pH value of 4 and 6 the RSE does not neutralize any of the negative charge of the POME exhibiting a decline in removal efficiency and furthermore, the final turbidity of the treated POME is increased higher than its original value. This could be due to the reversed overall charge density of RSE thereby causing the coagulated particles to restabilise (Saifuddin & Dinara, 2011). From visual observations made, POME at of pH of 3 displayed a light brown colour with small flocs under inspection light. At higher pH levels, the POME appeared to be an almost black colour indicating presence of higher TSS and displayed very low removal efficiency (Bhatia et al., 2007b). This steep drop of removal efficiency at pH of

4 using RSE was also observed by (Zainal Abidin et al., 2014) whereby it was reported that at pH of 3 and lower (acidic condition) the proteins possess a positive charge (zeta potential) giving a high removal efficiency while at higher acidic pH levels, the negative charge dominates over the positive losing its ability to carry out coagulation-flocculation.

Figure 3: Effect of POME pH on the removal percentage of TSS

Figure 4: Effect of POME pH on the removal percentage of turbidity

3.1.3 Rambutan-Fenugreek Hybrid

As can be seen from Figures 5 and 6, the 50/50 ratio of RSE and FSE showed the highest removal of turbidity at 78 % but the TSS removal of 61 % was slightly lower than that of FSE and RSE alone at 66 % and 64 % respectively. However, the value was close, thus comparable with RSE and FSE alone. The 67/33 and 33/67 ratios of RSE and FSE gave the lowest removal of TSS (at

58 % and 59 % respectively) and turbidity (at 54 % and 62 % respectively). From this study, it can be said that a combination of RSE and FSE in the ratio of 50/50 gave the best removal of TSS and turbidity.

3.1.4 Effect of Flocculent Dosage

As can be seen from Figure 7, the optimum dosage of OE flocculant to be used with RSE is 100 mg/L, after which the removal efficiencies continuously drop. Adding the okra flocculant was not found to be favourable with the RSE biocoagulant. The removal of TSS and turbidity was lower when the OE flocculant was added. In fact the TSS and turbidity removal seems to drop continuously with the addition of OE.

As for the hybrids, as depicted in Figures 5 and 6, the RSE/FSE hybrid of 67/33 and 33/67 do not give higher removal efficiencies, however, the 50/50 ratio hybrid of RSE and FSE gave the highest removal efficiency for turbidity at 77.7 % and TSS at 60.7 %. This could be due to the addition of FSE which probably contributed to additional active groups for coagulation and flocculation to occur. Furthermore, as can be seen from Table 6, the zeta potential of 50/50 RSE/FSE sample was 3.72. This value was higher than the zeta potential of RSE alone at pH 3 which was only 2.86. Therefore, it can be said that the addition of FSE to RSE increased the positive charge density of RSE.

Figure 5: Effect of RSE/FSE hybrid at varying ratios on % removal of total suspended solids.

The zeta potential of FSE at pH of 3 was -6.78 (Table 2). Similar methods were carried out by (Saifuddin & Dinara, 2011) whereby the zeta potential of magnetite nanoparticles was initially -3.3 (negative), however, after adding chitosan, the zeta potential of the mixture changed to +3.88 (positive). Therefore, it can be said that the addition of FSE further increased the positive value of zeta potential of RSE, which assisted it to obtain higher removal efficiencies. It should also be noted that since the final zeta potential of RSE/FSE is positive, the 50/50 RSE/FSE hybrid carries out adsorption and charge neutralization

mechanism to aggregate the suspended particles after coagulation.

Figure 6: Effect of RSE/FSE hybrid at varying ratios on % removal of turbidity

Figure 7: Effect of Okra extract (OE) on % removal of total suspended solids and turbidity

3.1.5 Zeta potential

It can be seen from Table 2, the zeta potential of RSE and FSE at pH of 3 is 2.86 and -6.78. It can be deduced with this that RSE is a cationic photo electrolyte and aggregates the suspended particles in POME by adsorption and charge neutralization. This is because the positive charge of RSE neutralizes part of the negative charge on

POME. However, for fenugreek, the mechanism involved is interparticle bridging as noted from the zeta potential value and from previous studies (Albert, 2010). This is because the zeta potential of FSE at pH of 3 is negative. Furthermore, it can also be said that RSE behaves very much like *Moringa oleifera* as a bio-coagulant. This is because (Bhatia et al., 2007b) reports using *Moringa oleifera* as a bio-coagulant to treat POME and concluded that the dominant mechanism of coagulation was adsorption and charge neutralization due to *Moringa*'s positive charge density. According to Leone et al. (2016), the protein content of *Moringa oleifera* seed is about 32 %, which is a lot higher than the 14 % present in RSE. This may also be one of the reasons behind RSE performance being lower than that of *Moringa oleifera*.

Table 2: Zeta potential of FSE, RSE and POME obtained at specific pH values

Parameter	Zeta potential
RSE @ pH 3	2.86
RSE @ pH 4	-2.86
POME @ pH 3	-11.4
POME @ pH 4	-10.5
FSE @ pH 3	-6.78
50-50 RSE-FSE @ pH 3	3.72

3.2 FTIR

Tables 3 and 4 which correspond to Figures 8 and 9 respectively show the active groups available on the RSE and FSE. According to Ramavandi (2014) the main components present that contribute towards coagulation-flocculation are O-H, C-H and C=O groups. The O-H corresponds to the hydroxyl group (proteins/amino acids) and C-H represents the carbon-hydrogen bond (proteins/amino acids) while the C=O represents the carboxyl group. From the tables, it can be seen that RSE and FSE contains all the groups that are necessary for coagulation-flocculation. However, the C-H bond present in RSE exhibits a smaller transmittance than that of FSE. This could be the reason why adding FSE improved the performance when used in combination with RSE.

Table 3: Table showing the bonds present in RSE, extracted from the FTIR scans

Bonds present in RSE	Location (cm ⁻¹)	Transmittance (%)
O-H (hydroxyl)	3284.60	85 % (Strong and Broad)
C-H	2852.72	67 % (medium)
C=O (carboxyl)	1643.76	87 % (strong)

Figure 8: FTIR scan for characterisation of RSE

Figure 9: FTIR scan for characterization of FSE

Table 4: Table showing the bonds present in FSE, extracted from the FTIR scans

Bonds present in FSE	Location (cm ⁻¹)	Transmittance (%)
O-H (hydroxyl)	3277.70	81 % (Strong and Broad)
C-H	2856.99	87 % (medium)
C=O (carboxyl)	1643.36	85 % (strong)

3.3 FANN Models

As can be seen from Table 5, neural networks trained using all three training algorithms show very low performance and very high noise at the smaller number of neurons (1 – 4) in the hidden layer. BR exhibits the highest performance using 1 neuron in the hidden layer, giving the lowest MSE, MAE and MAPE values. However, it can also be seen that LM can reach a stable MSE value (0.001) using the least number of hidden neurons of 4, followed by BR at 5 hidden neurons. The poorest performance of the three training algorithms was that of SCG which takes at least 15 hidden neurons to reach a constant value of MSE of 0.001. With relations to that, it was found that LM reached a constant MSE performance the earliest, and BR reached constant MAE and MAPE values the earliest. This means

that the overall neural network trained using BR reached an optimum performance with lesser number of neurons in the hidden layer than with LM. Neural network trained using SCG did not reach an overall constant performance because the MAE and MAPE values do not show constant values.

3.3.1 Levenberg-Marquardt training algorithm

As demonstrated using Table 5 and as can be seen from Figures 10, 11 and 12, neural network trained using the function LM exhibited some variability in MSE, MAE and MAPE values. Similar trends are also observed in Figure 15. Here, the R-squared values up to 4 neurons in the hidden layer, after all the performance factors come to

a constant value close to zero, except R-squared which gets close to 1, shows a good fit of the theoretical data into the neural network. It can also be seen using Figure 13 that the neural network produced can accurately predict the final responses that match closely to the responses produced from the jar test experiments with small error, as seen in Figure 14. Furthermore, there is no significant noise seen training a neural network using LM with hidden neurons more than 6. LM is essentially a default training algorithm used to train kinds of feed-forward networks. It operates in such a way that requires less computational memory, takes less amount of time to train a network and produces tolerable results with small number of runs (<50) (Demuth et al., 2007).

Table 5: MSE, MAE and MAPE values obtained from testing the ANN with LM, BR and SCG algorithms

Neurons	LM			BR			SCG		
	MSE	MAE	MAPE	MSE	MAE	MAPE	MSE	MAE	MAPE
1	0.8517	0.598	0.173	1.083	0.701	0.206	0.938	0.642	0.189
2	0.8176	0.552	0.155	0.889	0.606	0.177	0.821	0.543	0.151
3	0.6713	0.418	0.109	1.084	0.700	0.205	0.774	0.548	0.157
4	0.1840	0.277	0.087	0.788	0.540	0.155	0.855	0.656	0.194
5	0.0861	0.163	0.052	1.109	0.760	0.231	0.311	0.443	0.148
6	0.9790	0.632	0.186	1.084	0.712	0.211	0.805	0.567	0.163
7	0.0802	0.129	0.039	1.083	0.675	0.194	0.793	0.546	0.153
8	0.0800	0.135	0.041	1.104	0.746	0.224	0.785	0.532	0.149
9	0.0798	0.128	0.039	1.191	0.847	0.271	0.776	0.543	0.155
10	0.0802	0.112	0.032	1.095	0.746	0.226	0.994	0.640	0.183
11	0.1709	0.278	0.089	1.077	0.685	0.198	0.792	0.516	0.142
12	0.0795	0.110	0.032	1.083	0.714	0.211	0.774	0.580	0.170
13	0.1981	0.315	0.103	1.082	0.701	0.206	0.851	0.598	0.175
14	0.1669	0.296	0.095	1.099	0.761	0.231	0.795	0.526	0.145
15	0.0791	0.120	0.035	1.082	0.719	0.214	0.789	0.552	0.158
16	0.0789	0.119	0.035	0.918	0.542	0.149	0.782	0.540	0.152
17	1.1605	0.671	0.185	1.126	0.786	0.244	0.794	0.549	0.155
18	0.0845	0.146	0.045	1.094	0.746	0.226	0.166	0.289	0.092
19	0.1645	0.268	0.083	1.078	0.694	0.204	0.589	0.507	0.150
20	0.0794	0.109	0.031	1.082	0.688	0.200	0.777	0.535	0.151

3.3.2 Bayesian Regularization training algorithm

As can be seen from Figures 10, 11 and 12, the MSE, MAE and MAPE values obtained using neural networks trained by BR do not show any significant noise at the initial number of neurons. However, against LM minimal noise is noticed. Figure 15 shows the R-squared values produced by the neural network trained using the three training algorithms and it is observed that 5 neurons onwards, the R-squared value is as close to 1 as possible. This means that the experimental data fits well to the neural network. Furthermore, as can be seen from Figure 13, the neural network using BR for training can accurately predict responses close to the actual one. This is because BR is a training algorithm that does not have validation stop during training. Therefore, the training goes on until the target performance (MSE = $1e-5$, excerpted from Table 9) is met. This gives the advantage of ideal training of each experimental data to the neural network. However, the computational time and memory required is high, furthermore, ideal training can still be managed using

validation stops as demonstrated earlier by LM. It can compensate for the long time of training if small experimental runs are used (<50), which makes this training algorithm suitable for small amounts of training quality data (Demuth et al., 2007).

3.3.3 Scaled Conjugate Gradient training algorithm

As can be seen from Figures 10, 11 and 12, the MSE, MAE and MAPE are constantly deviating over 1 to 20 number of neurons in the hidden layer. Figure 15 shows a plot of the R-squared value which shows a value quiet close to 1 however, significant amount of noise is observed too. The reason behind SCG's inability to train a neural network is the lack of large amounts of data. SCG is a fast processing training algorithm. It requires a low amount of memory and computational time; however, this means that SCG can only be used to get an approximate training of the neural network. This characteristic of SCG makes it suitable for only large amount of training data (>100) (Demuth et al., 2007).

Figure 10: Comparison MSE values produced via network training using Trains LM, BR and SCG

Figure 11: Comparison of MAE values produced via network training using Trains LM, BR and SCG

Figure 12: Comparison of MAPE values produced via network training using Trains LM, BR and SCG

Figure 13: Comparison of the errors produced via network training using Trains LM, BR and SCG

Figure 14: Comparison of the error values produced via network training using Trains LM, BR and SCG

Figure 15: Comparison of the R-square values produced via network training using Trains LM, BR and SCG

4. Conclusions

From the study carried out, it can be concluded that RSE demonstrated great potential for the treatment of POME. It performs well in acidic conditions (specifically at pH 3 – similar to POME's natural pH) giving a removal of TSS and turbidity of 65% and 70% respectively. Hybrids of RSE and FSE also showed good coagulation abilities. At a combination of 50/50 of RSE and FSE, there was a removal of 61% of TSS and 77% of turbidity from the POME. Besides that, an ANN neural network was produced to model the activity of RSE. The accuracy of the neural network was tested with three different training algorithms over different number of neurons (1 to 20) in the hidden layer. LM was observed to be the best training algorithm, giving MSE, MAE and MAPE of 0.0795, 0.11 and 0.032 respectively. The R-squared value obtained was also close to 1.

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