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OPTIMISATION OF *HIBISCUS SABDARIFFA* AS A NATURAL COAGULANT TO TREAT CONGO RED IN WASTEWATER

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Abstract

The process of coagulation is commonly practiced in water and wastewater treatment to reduce level of dissolved chemical, turbidity and so on with the usage of coagulant. Aluminium sulphate (alum) is the most commonly used coagulant, however, recent studies show that residual aluminium in drinking water and sludge may induce Alzheimer's disease and environmental issues. Natural coagulant which is environmental friendly and non-toxic is developed as an alternative to overcome these issues. In this work, *Hibiscus Sabdariffa* was studied as natural coagulant to treat dye wastewater containing Congo red. The seeds were extracted with different solvent such as distilled water, 0.5 M NaCl and 0.05 M NaOH to extract the coagulation agent. The working parameters were optimised using Response Surface Methodology (RSM). 0.5 M NaCl was found to have highest colour removal of 95.1 % among the solvents. In addition, *Hibiscus Sabdariffa* seed was found to be an effective coagulant that has 91.2 % colour removal at the optimal working condition of pH 2, 190 mg/L coagulant dosage at 400 ppm of dye concentration. It was also been identified that the performance of natural coagulant is comparable with conventional coagulant, aluminium sulphate with colour removal of 91.2 % and 92.3 % respectively.

Keywords: *Hibiscus Sabdariffa*, Natural coagulant, Coagulation, Wastewater treatment.

1. Introduction

Dyes are widely used in many industries such as textile dyeing industry, cosmetic industry, and fabric industry. Most of the dyes used are synthetic or chemical dye that are available in different colours [1]. During the process of dyeing and painting, it generated large amount of complex chemical constituent in the form of wastewater [2]. Direct discharge of dye wastewater into environment induces aesthetic problem such as change in colour of river and water streams [2]. This interrupts the penetration of sunlight into the river hence disturbing the biological process of aquatic life [3]. Several dyes are proven toxic to aquatic life with their decomposition derivatives [4]. As environmental protection has become a main concern in global, wastewater produced must be treated using proper treatment method before discharge to reduce the level of damage to the environment. However, synthetic origin and complex aromatic molecular structures of dye leads to difficulty in wastewater treatment. Many techniques have been developed for dye removal from wastewater such as physico-chemical treatment, adsorption, advanced oxidation, electrochemical degradation and so on [5-7].

Among the processes mentioned, coagulation is the most common physciochemical treatment practiced due to its simplicity and effectiveness [8]. Coagulation is a process to neutralize negatively charged colloid and dissolved solid present in wastewater which causes turbidity and colour by chemical coagulant. The neutralized particles will combine with each other and form large particle called floc. This floc is separable and their removal is facilitated by sedimentation [9]. Hence, level of turbidity, colour and contaminates in wastewater will be reduced. Commercially available coagulant such as aluminium sulphate, ferric sulphate and ferric chloride are most commonly used. Despite this, usage of these coagulants has several disadvantages. The treated water containing residual Aluminium from water treatment consumed by human will result in health issue. In addition, disposal of sludge generates from wastewater treatment containing aluminium leads to soil pollution.

Possible solution to these problems may be developing new coagulants which are derived from natural. Nowadays, great attention has been focused on natural coagulant to replace conventional coagulant. Natural coagulant derived from plants and animals which are high biodegradable, non-toxic is more environmental friendly and safe for human consumption. Besides, plants can be grown locally hence it is more cost effective than chemical coagulant. Several natural coagulants have been studied for water and wastewater treatment including *Moringa oleifera* [10-12], Nirmali seed [10] and *Jatropha curcas* [13,14]. Present of water-soluble protein in the seeds of these plants may contribute to coagulation process. Another potential natural coagulant which is *Hibiscus Sabdariffa* have been studied for turbidity removal for water treatment [15,16]. However, there is lack of further study of this plant as natural coagulant to treat dye wastewater.

In this project, *Hibiscus Sabdariffa* also known as Roselle is studied as a natural coagulant to treat dye wastewater. *Hibiscus Sabdariffa* is widely available in Malaysia and the production is about 240 tons yearly. This plant has been used widely for food and medical purpose. However, the capsules containing the seeds are usually discarded as a waste and by-product after the processing process [17]. Nutritional study of *Hibiscus Sabdarrifa* shows that the seeds are found to have highest protein content compared with the flower and calyces which is 31.02 % and the seed could be used as a potential source of proteins [18-21]. In addition, high amount of glutamic acid and arginine (21.10 g/100 g protein and 11.35 g/100 g protein) present in the protein from the seed of *Hibiscus Sabdariffa* are expected to have the ability for coagulation [22]. Amino acids which carry charges would give the overall charge to protein depending on the isoelectric point. This protein is expected to be cationic that is able to neutralise the negative charged of dye particle in dye wastewater.

The performance of *Hibiscus Sabdariffa* as natural coagulant to treat dye wastewater is studied in this work. The objectives of this work are to compare the performance of *Hibiscus Sabdariffa* as natural coagulant extracted by different solvents. Parameters such as pH, coagulant dosage and concentration of dye are also optimized using Response Surface Methodology (RSM). Lastly its performance is compared with conventional coagulant, aluminium sulphate (alum).

2. Methodology

2.1. Preparation of coagulant from *Hibiscus Sabdariffa* seeds.

Hibiscus Sabdariffa was obtained from Raub, Pahang. The seed bub was removed and the good quality seeds were washed with water and dried at 60 ± 2 ^{o}C for 2 hours in an oven [23]. The dried seeds were then pulverised using a grinder into powder form and was used for each experiment. 5 g of seed powder was mixed with 100 ml of solvent which were distilled water, 0.5 M sodium chloride (NaCl) and 0.05 M sodium hydroxide (NaOH) to extract its coagulant agent. The extraction was done by using a household food blender for 2 minutes. These solvents were chosen based on previous researches [10,11]. The coagulant was filtered through muslin cloth to remove impurities and used in the subsequent jar test. In order to prevent microbial decomposition of organic compound present in the coagulant, the coagulant was prepared and used on the same day for optimum performance.

2.2. Preparation of synthetic dye wastewater

Stock solution of synthetic dye wastewater was prepared by dissolving accurately weighed Congo Red ($C_{32}H_{22}N_6Na_2O_6S_2$; molecular weight: 696.66 g/mol) in distilled water to concentration of 1000 ppm. The stock solution was diluted with distilled water to achieve different concentrations of dye wastewater. The pH of the wastewater was adjusted using concentrated hydrochloric acid (95 %) or 1.0 M sodium hydroxide (NaOH) to the desired pH value.

2.3. Jar test

The coagulation process was performed to evaluate the performance of natural coagulant using jar test. 500 ml of dye wastewater was added with coagulant and the mixture was mixed for 4 minutes at 100 rpm for rapid mixing enhance floc formation. The sample was stirred at 40 rpm to allow flocculation for 25 minutes and then settle for 30 minutes. The colour removal after the coagulation was measured using a UV-Spectrophotometer (Model HALO RB-10-5110026) with wavelength of 500 nm [23]. The colour removal percentage was calculated using the following equation:

$$Colour\ removal\ (\%) = \frac{Abso - Abs}{Abso}\ x\ 100 \quad (1)$$

where Abs_o is the absorbance value before treatment and *Abs* is the absorbance value after treatment process. Each experiment was conducted in triplicate and calibration graph of absorbance value and concentration of dye wastewater is shown in Fig. 1.

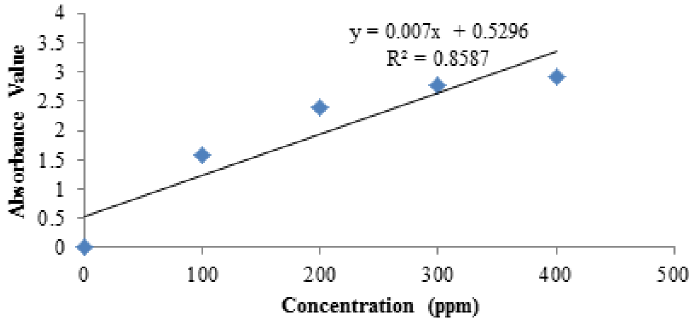

Fig. 1. Calibration graph of absorbance value against concentration of dye wastewater (ppm).

2.4. Effects of using different solvents for extraction of coagulation agent

Jar test for different solvent used for extraction process was done to identify the best solvent for extraction. The experiment was conducted at pH 2, coagulant dosage of 150 mg/L and concentration of dye wastewater at 100 ppm based on research done by Beltran-Heredia et al. [5]. The coagulant with highest colour removal was chosen to carry out the optimization of working condition for coagulant in next experiment.

2.5. Optimization of coagulant activity using Response Surface Methodology (RSM)

Optimisation of working condition for *Hibiscus Sabdariffa* as a natural coagulant was carried out using Response Surface Methodology (RSM). RSM is a popular and economical statistical technique in evaluating the influence of parameters on treatment response [24]. Three-factors and three level Box-Behnken response surface design (BBD) was employed in this work. The variables and the level selected are given in Table 1. Colour removal was analysed as the response.

Table 1. Experimental factors and levels of independent variables.

Factors	Range and levels		
	Low (-1)	Medium (0)	High (1)
pH	2	7	12
Dosage (mg/l)	100	200	300
Concentration (ppm)	100	250	400

Design Expert Version 6.0.8 (Stat-Ease Inc. Minneapolis) was used in this work. A total of 17 experiments were generated from Design Expert and the results were analysed and presented using 3D contour plot. Optimization was performed by the software and analysis of variance (ANOVA) was utilised to study the significant factor that affects the output of experiment.

2.6. Comparison of *Hibiscus Sabdariffa* and aluminium sulphate as a coagulant.

Optimum combination of parameters was generated from RSM and jar test was performed for both natural and chemical based coagulant with the optimised working condition. In this study, aluminium sulphate (alum) was compared as chemical based coagulant as it is widely used in conventional water and wastewater treatment. The results would indicate the ability of natural coagulant to treat dye wastewater over chemical based coagulant.

2.7. Limitations of study

In this research, purification of coagulant extracted from the seed was not performed. Purification of seed extract such as dialysis, ion-exchange, precipitation and lyophilisation is rarely done in natural coagulant. This process would increase the overall process cost during commercialisation. Most research studies employ method of drying and pulverising of plant seed into fine powder and extract using solvent to use for experiments. Without purification, coagulation performance will decrease. Hence, future study can be done to discover the effect of purified seed coagulant on treatment process.

3. Results and Discussion

3.1. Effect of using different solvents for extraction of coagulation agent from *Hibiscus Sabdariffa* seeds

Determining the best solvent to extract coagulation agent from Hibiscus Sabdariffa seeds is able to improve the coagulation process. . In order to identify the best solvent to be used, the effects of distilled water, 0.5 M NaCl and 0.05 M NaOH were compared. Figure 2 shows the colour removal of synthetic dye wastewater using three types of solvents at condition of pH= 2, dosage of 150 mg/L and concentration of dye at 100 ppm.

Based on Fig. 2, the highest percentage of colour removal obtained is 95.1 % by using 0.5 M NaCl as extraction solvent while distilled water has the lowest percentage for colour removal at 93.7 %. Protein solubility is dependent on the ionic strength of the solvent. When the ionic strength of solvent increases, dissociation of proteins will be enhanced and the solubility of protein will increase as well. During the extraction, protein interacts with the molecules of sodium chloride which allows it to form H-bonds with the surrounding molecules. When the protein surface is hydrophilic enough, the protein will dissolve [25]. After extraction, an odourless and milky coagulant solution was obtained using distilled water and 0.5 M NaCl. However, a greenish solution with rotten smell was obtained using 0.05 M of NaOH. This might be due to the denature of protein and the seed content as NaOH is a strong base with pH = 12. This observation eliminates NaOH as a solvent to be used for extraction although it shows similar result with NaCl. Similar result was reported by several researchers that 0.5 M NaCl gives highest coagulation activity using natural coagulant compared to NaOH and distilled water [10,13].

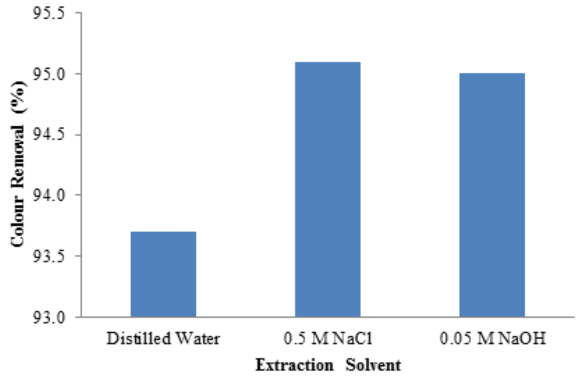

Fig. 2. Effect of using different solvent for coagulant agent extraction from *Hibiscus Sabdariffa* seeds.

3.2. Optimization of working parameter using RSM

Performance of natural coagulant can be affected by parameters such as pH, coagulation dosage and dye concentration. Hence, it is important to identify the suitable working condition for coagulant to perform optimally. The results obtained from experiment runs (colour removal) were correlated using the second-order polynomial shown in Eq. (2)

$$\text{Colour Removal (\%)} = -1.70 - 46.62A + 0.18B - 2.93C + 49.82\,A^2 - 1.03\,B^2 + 1.58\,C^2 + 0.3AB - 3.2\,AC + 0.7\,BC \quad (2)$$

where *A*, *B* and *C* are the coded values of the parameters pH, coagulant dosage and concentration of dye. Different models were compared using statistical analysis. The quadratic model was chosen based on high R^2 statistics. The R^2 value for colour removal was 0.9982 and the plot of predicted versus actual result for colour removal in Fig. 3 indicates that the experimental result is similar with the predicted result. This shows that the prediction of experiment result is satisfactory using this model.

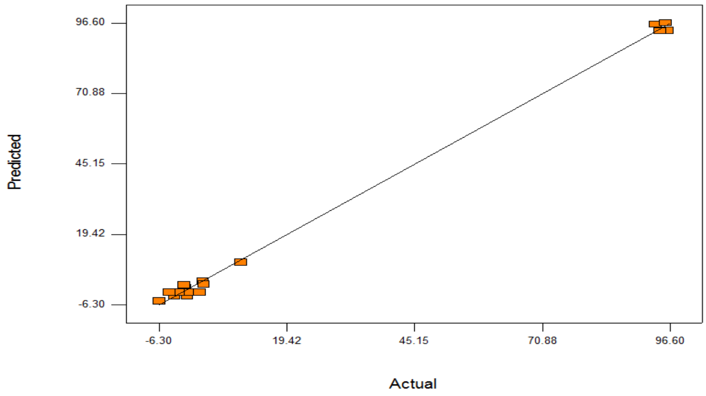

Fig. 3. Predicted vs. actual result for colour removal (%).

This model was to study the effects of the factors on the response and presented by 3D surface graphs and contour plots generated by Design-Expert software in Figs. 4 to 6.

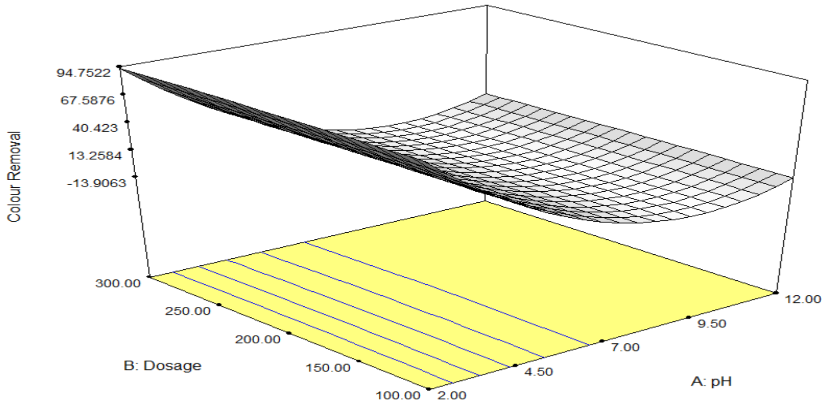

Fig. 4. Effect of coagulant dosage and pH on colour removal.

Figure 4 shows the 3D response surface plot for the effect of coagulant dosage and pH at concentration of dye at 250 ppm. Colour removal increases when the pH decreases. A significant reduction in colour removal was observed when the pH was increased beyond 2. Besides, highest colour removal was obtained at pH= 2 regardless the amount of coagulant dosage. This shows that coagulation process was highly pH-dependent and *Hibiscus Sabdariffa* works efficiently under acidic condition.

The coagulation activity happens in acidic condition due to the amino acids that make up the protein as the key component for coagulant protonated at pH= 2. Amino acid of protein present in *Hibiscus Sabdarrifa* has isoelectric point range from 3.2 to 11 [26]. When the protein is subjected to pH lower than the isoelectric point, it carries a net positive charge. Hence at pH= 2, all the amino acids carry positive charge and give protein its overall charge. This protein acts as a cationic coagulant to remove negatively charged sulfonic group of Congo Red. This can be explained as the coagulation occurred effectively at pH= 2.

According to shown in Table 2, pH is the most significant parameter that affects the colour removal. *F* value of this factor (2345.85) with *P* value less than 0.0001 indicates this factor was significant at 95 % confidence level. This proves that coagulation process using *Hibiscus Sabdariffa* as natural coagulant is highly pH-dependent.

However, identifying the optimum dosage of coagulant is important for optimum performance with minimum amount of coagulant required. This can minimize the cost of coagulant in treatment plant and reduce the sludge formation [27]. Thus, choosing a low amount of coagulant dosage with high percentage colour removal of dye is preferable. In this case, dosage required to treat 250 ppm of dye wastewater is found sufficient at range from 100 mg/l to 200 mg/l with colour removal of 94 %. Nevertheless, the dosage required depends on the initial dye concentration. The higher the concentration of dye, the more the negative charged dye particle presents hence it requires more cationic coagulant to neutralize it. The coagulation process could not be initiated if the dosage is too low while overdose of coagulant confers positive charges on the particle surface would result in re-disperse of dye particle hence low colour removal [28].

Table 2. ANOVA for Quadratic Model used for analysis of colour removal.

Source	Sum of Squares	DF	Mean Square	F Value	Prob>F
Model	28044.43	9	3116.05	420.32	< 0.0001
A, pH	17391.12	1	17391.12	2345.85	< 0.0001
B, dosage	0.24	1	0.24	0.033	0.8609
C, Concentration	68.44	1	68.44	9.23	0.1890
A^2	10452.76	1	10452.76	1409.95	< 0.0001
B^2	4.42	1	4.42	0.60	0.4651
C^2	10.44	1	10.44	1.41	0.2740
AB	0.36	1	0.36	0.049	0.8319
AC	40.96	1	40.96	5.53	0.0510
BC	1.96	1	1.96	0.26	0.6230
Residual	51.89	7	7.41		
Lack of Fit	28.45	3	9.48	1.62	
Pure Error	23.44	4	5.86		
Cor Total	28096.33	16			

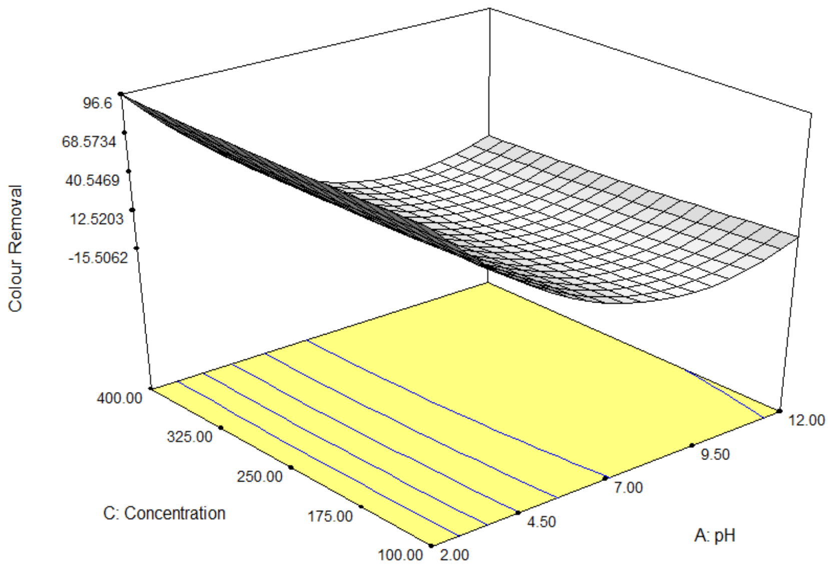

Fig. 5. Effect of initial concentration of dye and pH on colour removal.

Figure 5 shows the effect of different concentration of dye and pH at coagulant dosage of 200 mg/L. Coagulation was performed efficiently with dye concentration varied from 100 ppm to 400 ppm. This indicates that 200 mg/L of coagulant was sufficient to treat dye wastewater up to 400 ppm with approximate 96.6 % colour removal. This suggests that *Hibiscus Sabdariffa* can be used to treat dye wastewater with this range of concentration.

On the other hand, maximum colour removal was again observed at pH = 2 regardless of the initial concentration of dye due to the coagulant is highly pH-dependent. Colour removal reduced significantly when pH increases beyond 2 to 9.5. This is due to the formation of positive and negative charged amino acid when subjected to this pH. This mixture of different charges of amino acid would have decreased the cationic ability of the coagulant, hence reduce the performance of coagulation. The colour removal was slightly increased to 10 %-12 % at pH range from 11 to 12. This may be due to the present of arginine in protein with isoelectric point of 11 [26]. Arginine which has high percentage in total protein present in seed of *Hibiscus Sabdariffa* might have the responsibility in the coagulation process at this pH. However, the ability of colour removal is low and not significant. Hence it is not consider as optimum pH for coagulant process.

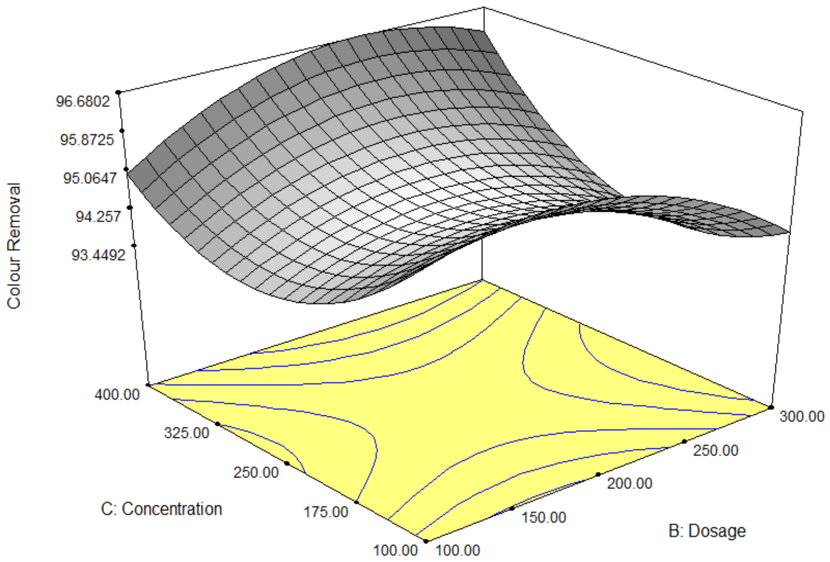

Fig. 6. Effect of initial concentration of dye and dosage on colour removal.

pH was determined as the significant factor that affect the output of the experiment. The relationship between concentration of dye and dosage of coagulant is also important to study. The effect of initial concentration and coagulant dosage at pH= 2 was shown in Fig. 6. The plot shows that colour removal increases with increment of dosage from 100 mg/l to 200 mg/L. Wastewater consists of more negative charged sulfonic group requires higher dosage of coagulant in order to achieve complete neutralization. In spite of this, colour removal decreased when dosage increased from 200 mg/l to 300 mg/l. This is explained as overdosing of coagulant. Excess amount of coagulant causes the floc formed to re-disperse thus colour removal decreased [29]. This should be avoided to ensure the performance of coagulation.

The working condition was optimized by using the numerical optimization of Design-Expert based on the experimental results obtained and the model selected. The maximum colour removal with 96.1% was predicted at pH=2, initial concentration of 400 ppm and dosage at 190 mg/L. This can be seen from Fig. 6. maximum colour removal was achieved at the range of 325 ppm to 400 ppm of dye concentration and dosage at 150 mg/L to 200 mg/L. Experiment was conducted at this condition and the actual value obtained was 91.2 % with 4.2 % deviation. This result showed that the model developed from Eq. (2) was well fitted to the experimental result. Thus, the optimum value of the factors was finalized at pH= 2, initial concentration 400 ppm and dosage of 190 mg/L. Based on its optimum working performance, *Hibiscus Sabdariffa* is relevant to be used to treat wastewater in acidic condition for example in peat soil [14].

3.3. Comparison of *Hibiscus Sabdariffa* and aluminium sulphate as coagulant.

Optimum working condition for *Hibiscus Sabdariffa* was obtained using RSM as pH= 2, initial concentration 400 ppm and dosage 190 mg/L. Jar test was performed using *Hibiscus Sabdariffa* and aluminium sulphate at this condition for comparison purpose. Performance of *Hibiscus Sabdariffa* was found comparable to alum based on Fig. 7. 91.2 % colour removal was achieved by *Hibiscus Sabdariffa* while alum recorded a percentage colour removal of 92.3 %. This shows that the cationic protein of *Hibiscus Sabdarrifa* has the ability for coagulation similar to alum. Hence, *Hibiscus Sabdariffa* can be used to replace alum as a commercial coagulant. By using natural coagulant, the drawback of using alum related to health and environmental can prevented.

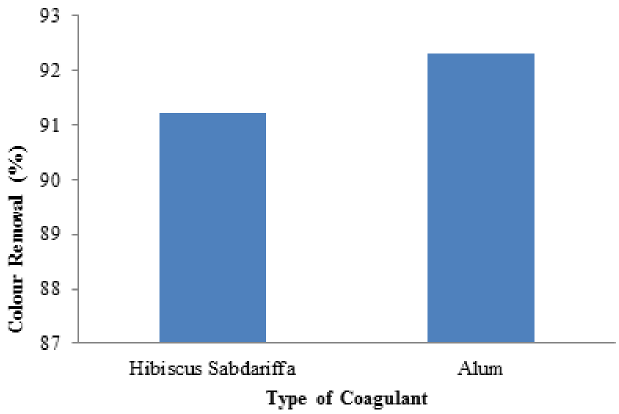

Fig.7. Comparison of *Hibiscus Sabdariffa* and Aluminium Sulphate.

4. Conclusions

In this study, *Hibiscus Sabdariffa* seed was found as a highly effective colour removal agent for coagulation of Congo Red dye. Present of cationic protein in this plant is the key component contributed to coagulation process. Extraction of coagulation agent from the seed using 0.5 M NaCl solution was found to have highest colour removal of 95.1 % compared to seed extracted by distilled water and NaOH. Working conditions such as pH, intial dye concentration and coagulant dosage was evaluated using Response Surface Methodology and pH was found as the significant factor that affects the output for colour removal. The coagulation process was also modelled and optimized and the optimum working condition was found at pH= 2, 400 ppm of initial dye concentration and 190 mg/L of dosage. Lastly, *Hibiscus Sabdariffa* also showed a relatively good and comparable performance with aluminium sulphate to treat synthetic dye wastewater. This can be concluded that *Hibiscus Sabdariffa* can be used to replace aluminium sulphate as a coagulant in the industry, which is safe, biodegradable and environmental friendly.

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