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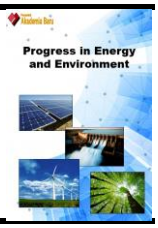

Opuntia cactus as a novel bio-coagulant for the treatment of Palm Oil Mill Effluent (POME)

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ABSTRACT

In the present study, *Opuntia* cactus, an environmentally friendly bio-coagulant that is locally available, was used for the first time for the treatment of Palm oil mill effluent (POME). The optimum conditions for applying *Opuntia* in the POME treatment process were identified by performing jar test experiments. Parameters tested were chemical oxygen demand (COD), total suspended solids (TSS) and turbidity. The results of the work indicate that *Opuntia* is an effective bio-coagulant, which can remove 91.2% COD, 94.4% TSS and 90.7% turbidity. Okra solution which was introduced as a bio-flocculant in the experiment resulted in slightly better performance. The reduction of COD, TSS and turbidity by *Opuntia* with okra solution improved to 93.9%, 96.1% and 93.6%, respectively. This was achieved at an *Opuntia* powder dosage of 8 g/L, pH 9 and reaction time of 240 minutes. The main mechanism involved in the treatment process was determined to be the polymeric bridge mechanism. From the study, it can be deduced that *Opuntia* has great potential to be used as a low-cost bio-coagulant for the treatment POME.

Keywords:
Opuntia cactus, Bio-coagulants, Palm oil mill effluent

1. Introduction

Malaysia has played a crucial role in the global palm oil industry in the modern days [1]. Large scale processing and production in this field is encouraged by the government to retain its global market competitiveness. At the same time, the significant economic growth benefited from the palm oil industry has also caused pollution issues from the generation of organic waste which is released into the environment. Palm oil mill effluent (POME) is the waste liquid generated from the extraction and purification of palm oil. It is a thick brown colloidal suspension waste with bad odour, and of low toxicity since there is no chemical addition in the process line. The COD content in POME is significant since POME contains degradable organic matters such as protein, oil and grease, suspended solids and carbohydrate constituents [1]. Thus, POME can cause severe water pollution issues if discharged without undergoing treatment [2].

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High treatment costs and difficulty in designing, operating and maintaining a proper treatment system have become the obstacles for palm oil mill owners to treat POME. These issues can be more evident and noticeable in developing countries [3]. The most common methods used by most Malaysian palm oil mills is the ponding system. Here, POME is left in ponds to undergo anaerobic, aerobic and facultative digestion by bacteria. Though effective, this process takes a very long time, sometimes up to three months before the discharge of the effluent [4]. Besides that, another challenge is that this system generates greenhouse gases such as methane and carbon dioxide, which can enhance global warming, directly into the atmosphere. The general characteristics of raw POME is given in Table 1 [5].

Table 1 General characteristics of POME

Parameters	Symbol	Unit	Range	Average value
Temperature	T	℃	–	25
pH	–	–	5.5 – 7.5	6.5
Suspended solids	SS	mg/L	12100 – 21367	16233.5
Chemical oxygen demand	COD	mg/L	21900 – 28800	25350.0
Turbidity	–	NTU	2733 – 3250	2991.5
Moisture content	–	%	68.8 – 72.2	72.0

Inadequate wastewater treatment systems in the developing countries can be solved by introducing the more cost-effective point-of-use (POU) methods such as coagulation [6]. Coagulation and flocculation are one of the chemical unit processes which are used to destabilize colloidal particles in the wastewater, which leads to the agglomeration and aggregation of particles. The coagulation process neutralises particles that are charged, while flocculation causes the small flocs to combine and form larger flocs that precipitate. The removal of particles is done by particle separation subsequent to the gravity sedimentation and filtration process [7]. In the conventional treatment method, chemical-based coagulants such as alum ($AlCl_3$) and ferric chloride ($FeCl_3$) are added into the POME for treatment purpose. While the treatment performance of alum and ferric chloride are highly efficient, the drawbacks related to the usage of these chemical coagulants should not be neglected. The futile attempts to apply them in cold solutions, their high chemical cost, irreversible side effect on the human body, large volumes of sludge produced and the high pH in operating conditions are some of the limitations for the application of chemical coagulants. Therefore, the use of natural coagulants, such as plant-based coagulants, have been suggested to replace chemical coagulants [8].

The main advantages of natural coagulants (bio-coagulants) are that they are cost effective and eco-friendly [9]. Moreover, they do not generate much sludge or contaminants in the end of the process. Many types of plants have been used as bio-coagulants [4,9]. However, bio-coagulants need to be studied and tested whether it is suitable for POME treatment. Most bio-coagulants are polysaccharides or proteins, which are in polymer form. Polymers will interact with the solvent in the solution due to the presence of the hydroxyl (–OH) functional group, which is partially charged along its chain. Thus, natural polyelectrolytes may involve adsorption and interparticle bridging mechanism for the removal of contaminants from POME [10].

For this study, an unconventional bio-coagulant, which is *Opuntia* cactus, was selected and investigated for its performance in POME treatment. Previously, *Opuntia* has been used in other wastewater treatment systems with high efficiencies, but thus far it has not been tested for POME treatment. *Opuntia* is a plant that has potential to be used as a natural bio-coagulant because it possesses all the desired qualities of a coagulant and it is a cheap and abundant source that may be grown very easily in Malaysia. The inner and outer parts of *Opuntia* may be extracted for wastewater treatment purpose. It was reported that the high coagulation capability of *Opuntia* is most likely

attributed to the presence of mucilage which is viscous and the presence of complex carbohydrate within the cactus inner and outer pads that has great water retention capacity [8]. Carbohydrates such as L-arabinose, D-galactose, L-rhamnose, D-xylose, and galacturonic acid are identified based on the previous research [10]. Some researchers have reported that galacturonic acid is the possible active ingredient that supports the coagulation capability of *Opuntia*. At the same time, it has been reported that *Opuntia* operates predominantly through bridging-coagulation mechanism where solution particulates do not directly contact with each other but are bound to a polymer-like material [12]. In another study, presence of viscous natural polyelectrolytes bearing negative surface charges under acidic conditions have some coagulation activity shown by most of the parts of *Opuntia* [13]. The plant therefore can act as a cheap and natural coagulant for wastewater treatment.

Upon coagulation, flocculation is needed for the treatment process as it helps to bring together the small flocs formed and sediment them. Various types of bio-flocculants have been used for wastewater treatment [4,9]. Okra (*Abelmoschus esculentus*), is among a plant species that has the common name "lady's finger" or "Gumbo Okra". It is a crop that has a fast-vegetative cycle and grow relatively easily, with high yield. The mucilage in okra has been studied for wastewater treatment purpose by several researchers [9]. The mucilage which contains polymer molecules that may enhance the flocculating activity through chemical reaction and end up as complex product formation [14].

Although the utilisation of these natural coagulants has been reported in literature, there is lack of understanding of the true mechanism of the process and systematic optimization of the operating parameters specially for the treatment of POME. Therefore, in this study, a comprehensive analysis of cactus *Opuntia* and okra was carried out to study the effects of essential process parameters such as pH, coagulant dosage flocculant dosage and suspended solids settling time and the possible mechanisms involved. The present study also compared the performance of coagulant alone with the combination of coagulant and flocculant in POME treatment.

2. Materials and Methods

2.1 *Materials*

2.1.1 *Characterisation of POME*

POME was collected from Seri Ulu Langat Palm Oil Mill, located in Dengkil, Selangor. Samples were collected one day before the experiment, as it was allowed to clarify first to remove larger particles that settle due to gravity. Raw POME was collected at the first aerobic pond, right after the anaerobic ponds. The reason is because coagulation and flocculation works well with water that has been partly clarified, so the best point to study this would be at the aerobic ponds. POME samples were homogenised by inverting the container a few times. In order to reduce the biodegradation of POME, it was stored in the refrigerator at temperature 4℃. The required sample volume was withdrawn and regulated at room temperature before the experiments were carried out. The quality of POME may vary according to time, thus different samples of POME were tested and measured to obtain the average parameter values. The characteristics of the collected POME from the aerobic pond is given in Table 2.

Table 2 Characteristics of POME from the aerobic pond

Parameter	Unit	Value
pH	-	6.5
Total suspended solids (TSS)	mg/L	16233.50
Turbidity	FNU	2991.50
Chemical oxygen demand (COD)	mg/L	25350

2.1.2 *Coagulant preparation*

Fresh *Opuntia* cactus pads were obtained from a plantation in Mantin, an area in Negeri Sembilan, Malaysia. The *Opuntia* pads were first rinsed with distilled water and then the thorns and the outer layer of cactus were peeled off manually by using a cutting knife. The flesh of the *Opuntia* was weighed using an electronic balance and the initial mass reading was recorded. The flesh was cut as thin as possible in strip shapes and dried in an oven at 60 ℃ for 24 hours. After the drying process, the dried *Opuntia* flesh strips were weighed and the reading was recorded for the calculation of moisture content in the *Opuntia* samples. The dried *Opuntia* flesh strips were subsequently ground into fine powder by using a blender. The powder was then sieved by using a Retsch AS 200 vibratory sieve shakers to obtain powder with the particle size range of $200\sim500\mu m$ in diameter. The sieved *Opuntia* powder was then filled and stored in a bottle and kept in a desiccator. In the next processing phase, extraction of the active agents in *Opuntia* was done by using 1 M sodium chloride (NaCl) standard solution. 1 g of *Opuntia* powder was dissolved and stirred in the 1 L NaCl standard solution bottle to prepare 1 g/L of *Opuntia* coagulant solution [10].

2.1.3 *Flocculant preparation*

Old and unwanted okra was obtained and collected from a local farm. The okra's outer skin was rinsed with tap water to remove impurities. The okra was cut into small pieces and unwanted seeds were removed. For mucilage obtaining, approximately one part of inner okra gum was immersed in one part of distilled water in beaker (ratio of 1:1). The okra immersed in water was left for 24 hours. Okra gums were removed from the solution when the mucilage extraction process was completed and the sticky liquid with mucilage was stored in a bottle at room temperature for further use [9].

2.2 *Coagulation-flocculation process*

Batch coagulation tests were performed by using the Lovibond™ ET 750 flocculator (jar test apparatus). Six 1000 mL beakers were prepared and filled with 400 mL of raw POME sample for each test run. For each jar test, the centre of beaker was aligned with the impeller shafts to enable uniform mixing. The stirring was started by adjusting the speed of the impeller to 200 rpm for rapid mixing during coagulation. The impeller stirring speed was then reduced to 40 rpm after 3 min of rapid mixing. The slow mixing process was continued for 30 min for flocculation. A stopwatch was used to record the total reaction time. Upon completion of the coagulation and flocculation experiments, the beakers were left undisturbed for the clarification process to take place. The sample of the supernatant was extracted by using a syringe for further analysis to determine the treated POME characteristics. For the jar test experiments, coagulant dosages used were 2, 4, 6, 8, 10 and 12 g/L and flocculant dosages used were 50, 100, 150, 200, 250 and 300 mL. For the settling time experiment, extracted supernatant was measured and the readings were collected with intervals of 60 min until the sample was fully settled. All experiments were carried out at room temperature of 27°C, and each test run was performed 3 times to get the average value for repeatability [2,15].

2.3 *Analytical methods*

The obtained supernatant was tested for its COD, TSS and turbidity removal efficiencies. The supernatant was diluted 10 times for the purpose of analysis. The turbidity measurement was performed by using MARTINI Mi 415 turbidity meter. Turbidity removal efficiency was calculated using Eq. (1) [5]:

$$\text{Turbidity removal (\%)} = \frac{\text{Turbidity}_{\text{Raw POME}} - \text{Turbidity}_{\text{Treated POME}}}{\text{Turbidity}_{\text{Raw POME}}} \times 100 \quad (1)$$

The TSS concentration in the supernatant was determined by using a HACH DR 3900 spectrophotometer at 620 nm with programme 630 suspended solids within the range of $0 - 750\ mg/L$ TSS. TSS removal efficiency was calculated using Eq. (2) [5]:

$$\text{TSS removal (\%)} = \frac{\text{TSS}_{\text{Raw POME}} - \text{TSS}_{\text{Treated POME}}}{\text{TSS}_{\text{Raw POME}}} \times 100 \quad (2)$$

For COD determination, 2 mL of supernatant was extracted to mix with reagent, while a blank was prepared using 2 mL distilled water mixing with reagent in the COD vial. The sample was mixed well and immediately heated up by using COD reactor (HACH DRB 200) for 2 hours at temperature 150℃. The samples were allowed to cool down to room temperature before measurements were taken by using HACH DR 3900 spectrophotometer at 435 nm under programme 435 COD HR within the range of $0 - 1500\ mg/L$ COD. The reduction in COD was calculated using Eq. (3) [5]:

$$\text{COD reduction (\%)} = \frac{\text{COD}_{\text{Raw POME}} - \text{COD}_{\text{Treated POME}}}{\text{COD}_{\text{Raw POME}}} \times 100 \quad (3)$$

Qualitative analysis on the main functional groups in *Opuntia* was performed by using the Fourier-Transform Infrared Spectroscopy (FTIR) analysis. The main functional groups were identified by obtaining IR spectrum of *Opuntia* through the Perkin Elmer FTIR spectrum instrument in solid phase. For the FTIR study, *Opuntia* powder was mixed with KBr powder in the ratio of 1:200, which was later encapsulated by KBr powder to obtain a homogeneous and transparent thin film for sample testing. The sample was scanned from the range 4000 cm^{-1} until 400 cm^{-1} with 48 number of scans [16,17].

The zeta potential test was performed to optimize the coagulant dosage in POME treatment. The cactus coagulant solution was extracted and sonicated by using an ultrasonic bath and then inserted into a cuvette. The cuvette was placed in the Malvern Zetasizer and the average zeta potential value of the sample was obtained after three scans. The experiments were repeated by using POME sample, okra solution and cactus coagulant solutions.

2.4 *Optimization study*

Four stages of experiments were conducted to identify the optimum conditions for applying cactus *Opuntia* with okra solution for POME treatment. Optimization of coagulant dosage was first conducted, followed by that of the flocculant dosage, and then the effects of pH and finally the settling time were studied.

2.4.1 *Cactus Opuntia dosage optimization*

A total of six different *Opuntia* dosage concentrations were tested. *Opuntia* coagulants in NaCl solution were prepared at dosages of 2, 4, 6, 8, 10 and 12 g/L respectively. 100 mL of the prepared solutions was added to the jars (beakers) in the flocculator. For each experiment, supernatants were extracted from each beaker and allowed to sediment with the settling time of 24 hours for the analysis of turbidity, TSS and COD values.

2.4.2 *Okra solution dosage optimization*

A total of six different okra solution dosages were tested to improve the performance of *Opuntia* coagulants for POME treatment. Okra solutions which contain mucilage were prepared at amounts of 50, 100, 150, 200, 250 and 300 mL and added to the flocculation beakers. Some researchers have suggested that adding acetone to precipitate the gum and subsequently making the okra solution into dried into powder form is much better [18]. But in this study, it was found that the direct application of okra solution gave better results. In each experiment, supernatants were extracted from each beaker and allowed to sediment with the settling time of 24 hours for the analysis of COD, TSS and turbidity values.

2.4.3 *Effect of pH*

The concentration of hydrogen ion is an important parameter that will affect the treatment efficiency of natural coagulants. The flocculator was operated at five pH conditions (3, 5, 7, 9 and 11). The pH was varied by adding droplets of 1 M of NaOH solution and 1 M of HCl solution gradually. pH readings were measured and checked by using a pH meter. *Opuntia* coagulant and okra flocculant were maintained at optimum conditions obtained at *Section 2.4.2* and supernatants were extracted from each beaker and allowed to sediment with the settling time of 24 hours for the analysis of COD, TSS and turbidity [5].

2.4.4 *Effect of settling time*

The flocculated suspensions were left undisturbed for 60 min to set up settling mechanism. The supernatant was formed when sedimentation process occurred and it was extracted and measured for COD, TSS and turbidity in the given time interval of 60 min at optimal experimental conditions. The minimum applicable settling time of *Opuntia* with okra solution combination was determined when the results of COD, TSS and turbidity become constant and stable.

2.5 *Comparison of Opuntia with flocculant and without flocculant*

The flocculator test was also repeated by using *Opuntia* coagulant alone for comparison purpose with combination of *Opuntia* coagulant and okra flocculant. The removal efficiencies of both sets were investigated by performing the experiments at optimum treatment conditions obtained in *Section 2.4.4*. The final COD, TSS and turbidity values were recorded and compared.

3. Results and Discussion

Opuntia powder with NaCl solution extraction was used as the bio-coagulant in treating POME based on the preliminary studies, as it has the ability to neutralize particles that do not readily settle. NaCl was used as an extracting agent because it was found to be more efficient than water, because it speeds up the splitting of protein interactions and solubility of proteins due to the increase in ionic strength. In other words, using NaCl increases what is called the ‘salting-in’ mechanism [19]. The performance of the coagulation-flocculation process was tested by using *Opuntia* alone as well as combined with okra solution as flocculant in order to increase the treatment efficiency of POME by investigation on parameters such as COD, TSS and turbidity. In the end, the treatment efficiency of *Opuntia* with okra solution was compared with cactus *Opuntia* alone.

3.1 *Fourier transform infrared (FTIR) spectroscopy*

As mentioned in Section 2.3, background scan was done to obtain IR-active atmospheric components. Based on the analysis graph obtained, a strong doublet was detected around 2300 cm^{-1} and spiky peaks denoting the presence of carbon dioxide and OH- functional groups for water vapour in atmosphere were observed at 3800 *and* 1600 cm^{-1}.The subsequent infrared spectrum of cactus *Opuntia* powder was obtained and shown in Fig. 1. According to the spectrum, there are eight main features as listed in Table 3 [20, 21].

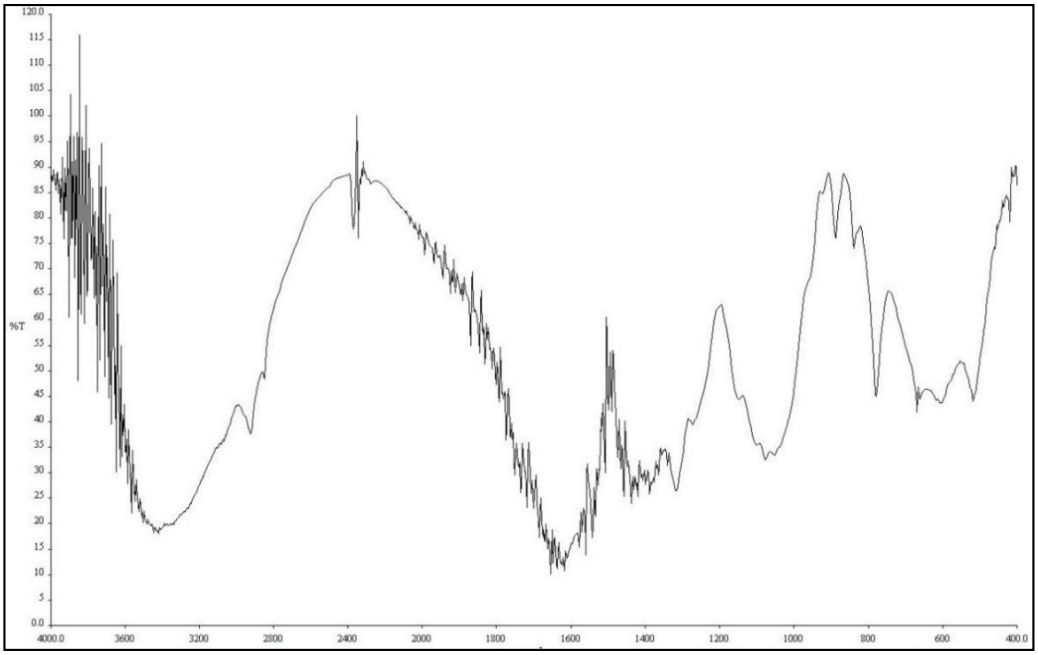

Fig. 1. FTIR spectra of *Opuntia* powder

Table 3 FTIR spectral characteristics of *Opuntia* powder

No.	Wave range/peaks (cm^{-1})	Assignment
1	4000-2400	Bonded hydroxyl group (OH)
2	2925	C-H stretching
3	2371-2345	Carbon dioxide gas
4	2000-1500	C=O, C=C double bonds
5	1632, 1400	Antisymmetric and symmetric COO- stretching
6	1740-1710	C-O stretching of COOH
7	1390-1340	Antisymmetric and symmetric COO-
8	1077	C-O absorption

Opuntia is one of the family members in cactus species. It shares some common characteristics with plant fibres, such as cellulose structure, pectin that forms cell wall of green plants. Thus, carboxylic, hydroxylic and carbonyl groups are expected to be identified in the FTIR spectroscopy process [22]. Based on Fig. 1 which displays the FTIR spectra of *Opuntia*, it can be seen the most significant absorption peak in this spectrum was found at 3422 cm^{-1}. The peak is located in the broadband with

the range of $2400 - 4000\ cm^{-1}$, which corresponds to the OH stretching of alcohol and carboxylic acids, which have -OH groups that are involved in intermolecular hydrogen bonding of polymeric compounds (macromolecular associations), such as alcohols, phenols and carboxylic acids, as in pectin, cellulose and lignin [22, 23].

Two wide bands at 1632 cm^{-1} and 1400 cm^{-1} were observed. The bands correspond to the antisymmetric and symmetric COO- stretch characteristic of carboxylic acid salts, which is the deprotonated carboxylate functional group of cellulose. The stretching vibration of C-O bonds due to non-ionic carboxyl groups (-COOH, $-COOCH_3$) was identified in the range of (1740-1710 cm^{-1}) [22, 23]. The changes in peaks that are observed in the bands 1390-1340 cm^{-1} are the indication of the presence of antisymmetric and symmetric carboxylic groups (COOH-) of pectin. This is explained by the presence of uronic acid in pectin molecules [16]. Uronic acid is the oxidised form of galacturonic acid which has the carboxylic groups at C1 and C6 [24]. Besides that, the additional peaks in the spectrum in the longer wavelength of 1077.71-400 cm^{-1} region, represents the fingerprint region of *Opuntia* [25].

The presence of uronic acid and the identification of aldehyde (C-OH) and carboxylic (COO-) functional groups have proven the presence of galacturonic acid in *Opuntia*. These results agree well with some researchers who identified the presence of galacturonic acid cactus *Opuntia* [26]. Galacturonic acid is the main component of pectin which exists in polymeric form (polygalacturonic acid). It has the anionic nature since carboxylic functional group is 'proton donor' when it is present in aqueous solutions. The existence of -OH groups also suggests the possible intra-molecular interactions which may distort the relative linearity of chain. Therefore, it is suggested that future works such as physicochemical characterization can be done on *Opuntia* by using X-ray and morphology analysis to provide ion exchange mechanism for colloid adsorption and to get an idea of the microscopic structure of the polymer adsorbent's surface, respectively [10].

3.2 *Optimum coagulant (Opuntia) dosage*

Fig. 2 shows the effect of *Opuntia* powder dosage on the removal of COD, TSS and turbidity. As the *Opuntia* powder dosage gradually increases from 2 to 8 g/L, the solution appeared clear on the top part of suspension with the presence of small colloidal particles inside the suspension. At higher dosage concentration which above 8 g/L, the POME turned into a darker colour.

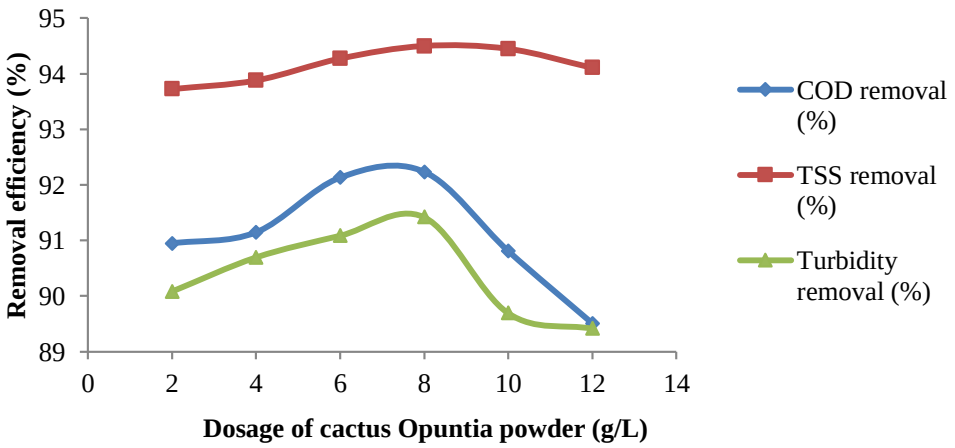

Fig. 2. Effect of cactus *Opuntia* powder dosage on final COD, TSS and turbidity of POME

Based on results shown in Fig. 2, COD, TSS and turbidity share the similar trend of removal efficiency. The percentages of COD, TSS and turbidity removal increased with the increase of *Opuntia* powder dosage to a maximum level, which is 8 g/L *Opuntia* powder that provided the best treatment performance in reduction of COD, TSS and turbidity as much as 92.2 %, 94.5% and 91.4%, respectively. It subsequently declined with further increase of *Opuntia* powder. The mean value was calculated by three determinations taken over per test when the supernatant extraction was obtained. It was observed that *Opuntia* powder dosage has negligible effects on the TSS removal efficiencies compared with COD and turbidity removal efficiencies. In the end of experiment, the results proved and identified that 8 g/L cactus *Opuntia* powder is the optimum dosage for POME treatment.

The main mechanism of the coagulation with *Opuntia* appears to be adsorption and interparticle of the colloidal charges [27]. *Opuntia* is a long chain high molecular weight and high charge density compound. The coagulation activity is due to the polymer bridge formation mechanism. During the coagulation process, this anionic polymer provides number of available active sites for the attachment of surface of particles that are found in POME solution. Due to the particle collision promoted by the agitation process in flocculator, bridged particles will interact with another and forms three dimensional larger particles with intertwined structure. This results in rapid sedimentation, since denser large particle structure will settle down easily by gravitational pulling effect. The particle-polymer bridge has leaded to the formation of sediment in the bottom part of the jars, as observed in *Section 3.2* [7].

Adequate coagulants must be used in coagulation-flocculation process to aid the sedimentation process. Hence *Opuntia* powder dosage was studied in the given range (2 – 12 g/L), in order to attain the optimum coagulant dosage for POME treatment. COD, TSS and turbidity removal efficiencies share the same trend as shown in Fig. 2 in the optimum coagulant (*Opuntia*) dosage experiments. The maximum level (8 g/L *Opuntia* powder) implies that the improvement of treatment efficiency of three parameters is limited within a certain range. The increasing treatment efficiency suggests the increment of coagulant dosage does enhance the coagulation-flocculation process, as it provides enough adsorption sites for the adsorption of polygalacturonic chains onto the POME colloidal particles to form polymer bridges [7].

However, with further increase in *Opuntia* powder dosage to 12 g/L, the performance becomes poor. This happened due to the increase of organic loading in the POME sample with extra *Opuntia* powder which mainly contains organic constituents. Polygalacturonic acid and other polymeric organic substances, which are the main constituents in *Opuntia* has led to the increase in number of organic metabolites which are harder to digest [10]. In the end, the oxygen demand for organism to break down the extra organic waste has been increased and it subsequently increases the COD loading and reduces the COD removal efficiency [15].

The same conditions are applicable to the TSS and turbidity removal. The increase of non-biodegradable organic load origin from extra *Opuntia* powder contributed to the high concentration of suspended solids, which resulted in higher TSS loading and water turbidity, and consequently the reduction in TSS and turbidity removal efficiency. It should be noted that turbidity is not a measurement for suspended solids content, it is the amount of light that is scattered by particles [28]. Thus both removal performances do share the same trend, yet there are the differences that exist in terms of removal efficiency and the affected removal efficiency range by adjusting of coagulant dosage concentration.

Chemical precipitation can achieve higher removal percentage of TSS including some colloidal particles compared to COD [7]. The chemical precipitation was performed in primary treatment stage where most of the TSS and COD were removed. According to the graph studies in this research, *Opuntia* coagulant does share the similarities with chemical precipitation in industrial practice where TSS removal efficiency (94.5 %) is higher than COD and turbidity removal efficiencies (92.2 % and 91.4 %).

As an overall, the results have shown that the optimum coagulant (*Opuntia*) dosage fall in 8 g/L in NaCl solution, which is capable to remove 92.2 %, 94.5 % and 91.4 % COD, TSS and turbidity, respectively.

3.3 *Zeta potential test*

The zeta potential for various *Opuntia* powder dosages in NaCl solution is shown in Fig. 3, with the corresponding final turbidity value of treated POME in experiment performed in *Section 3.2*. The magnitude of the negative zeta potential was decreased by increasing the dosage of 2 to 4 g/L and it reached the maximum level (-22.57 mV) at the background salt solution of 1 M NaCl. The zeta potential value was more positive when the *Opuntia* powder dosage increased to 12 g/L, though fluctuation was observed at 10 g/L powder dosage. The zeta potential failed to reach isoelectric point (0 mV) at the optimum dosage of 8 g/L where the zeta potential and turbidity were recorded as -25.33 mV and 257 NTU respectively [29].

Zeta potential test was also performed on *Opuntia* powder dissolved in distilled water, followed by okra solution and raw POME sample. The measured values were -5.98 mV, -22.3 mV and -12.77 mV respectively, which were the mean readings for three repeating measured data.

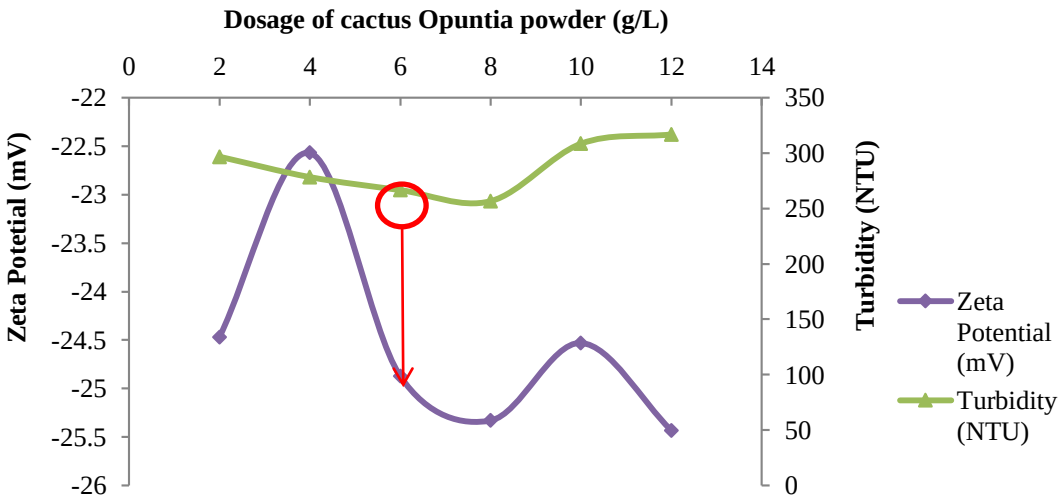

Fig. 3. Zeta potential control of cactus powder dosage

Zeta potential test is an important tool that is used to optimize the *Opuntia* dosage in POME treatment. In order to promote the coagulation process in POME treatment, reduction of their zeta potential with coagulants is needed for colloid removal by using cationic polymer coagulants [29].

From the FTIR investigation, *Opuntia* powder is categorized as an anionic polymer coagulant, thus expected to have the highest zeta potential value in this case. The results of the zeta potential test in *Section 3.3* for POME and *Opuntia* powder dissolved in distilled water have shown that the zeta potential increased from -5.98 mV to an average of -24.53 mV [29]. Based on the experiments, the existing repulsive forces have no effect on the performance of POME treatment using 8 g/L of *Opuntia* powder at this highest zeta potential condition (-25.33 mV). This has proven that the bridging mechanism occurs in POME treatment by *Opuntia* coagulants. The long polymer chains can adsorb on the colloidal particles' surface despite the presence of repelling electrostatic forces [7].

The established zeta potential results have provided an alternative way to control the coagulant dosage. If the measured value of POME is more negative than target zeta potential value, then the

increase of *Opuntia* powder dosage is needed, and vice versa. In this case a zeta potential of -25.33 mV corresponds to the lowest filtered water turbidity (257 NTU) and would be used as the targeted zeta potential value [29].

3.4 *Optimum flocculant (Okra solution) dosage*

Fig. 4 illustrates the COD, TSS and turbidity removal efficiencies by applying different flocculant (okra solution) dosages. The trends of COD, TSS and turbidity removal efficiencies varied when the okra solution dosage increased from 50-300 mL. The removal of COD achieved stability when the okra solution dosage reached 200 mL, with 92.8 % removal efficiency. At the same time, TSS removal efficiency reached maximal value (93.0 %) by adding 200 mL okra solution while maximal turbidity removal efficiency (94.4 %) was obtained at 300 mL okra solution. It was observed that TSS removal efficiency is the most affected parameters by adjusting the dosage of okra solution, with the affected range (88.1-93.0 %) as compared with COD and turbidity removal efficiencies.

In the comparison of the parameters in Fig. 4, 200 mL okra solution was chosen as the optimum okra solution dosage for subsequent analysis. The ratio of okra solution to 400 mL POME sample was recorded as 1:2. At 200 mL okra solution condition, removal percentages of COD, TSS and turbidity were achieved at 92.8 %, 93 % and 92.8 %, respectively.

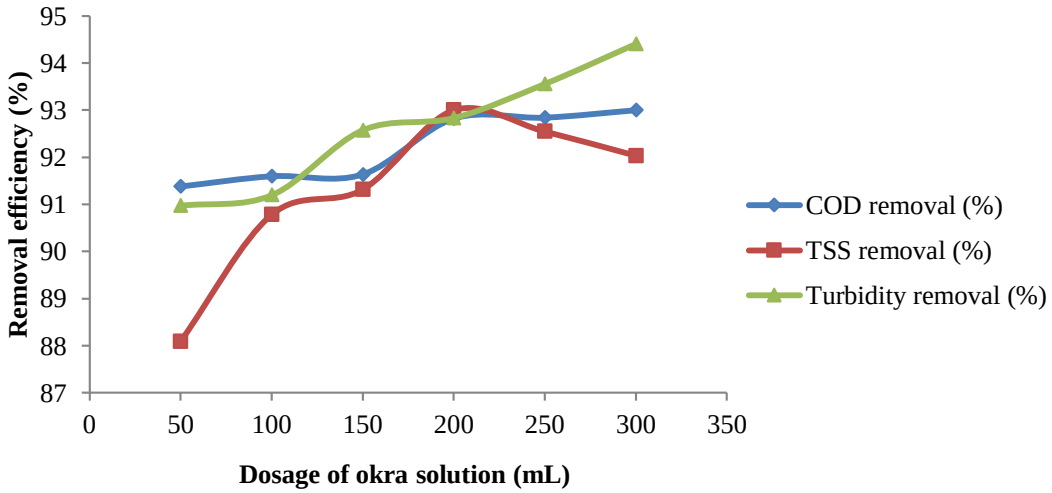

Fig. 4. Effect of okra solution dosage on final COD, TSS and turbidity of POME

The main function of the okra solution is as a flocculant to aid the coagulation-flocculation process in POME treatment. Okra solution contains mucilage, which is extracted by distilled water, and is sticky in nature. It contains polymer molecules which act as flocculants to enhance the flocculation process. The amount of okra solution required for the flocculation process needs to be quantified so that the ratio of bio-flocculant to POME can be determined. As observed in Fig. 4, the removal percentages of COD, TSS and turbidity increases with okra solution dosage but slows down beyond a dosage of 200 mL. This is because there is an addition of organic loading into the solution beyond this point [15].

3.5 *Effects of pH*

The effect of pH was investigated for pH among 3 to 11 and the results were presented in Fig. 5. It was noticeable that the removal efficiency trends of COD, TSS and turbidity were similar of that in *Section 3.2*, where these trend lines gradually increased from pH 3-5 and reached maximum level at

pH 9 and declined steeply after it exceeded pH 9. At the maximum level, which is pH 9, *Opuntia* achieved the best removal performance, with COD, TSS and turbidity removal recorded as 92.2 %, 95.8 % and 93.3%, respectively. It was observed that the pH environment of POME has less impact on TSS removal performance compared with COD and turbidity, which the recorded range (94.3-95.8 %). Yet the TSS removal performance is better than the duo with the recorded highest percentage (95.8 %) at pH 9. At the end of experiment, pH range of 7-9 gave identical and better performance in aiding coagulant-flocculant process. pH 9 was chosen as the optimum pH for POME treatment though close performance gap between pH 7 and 9 was observed in the treatment process.

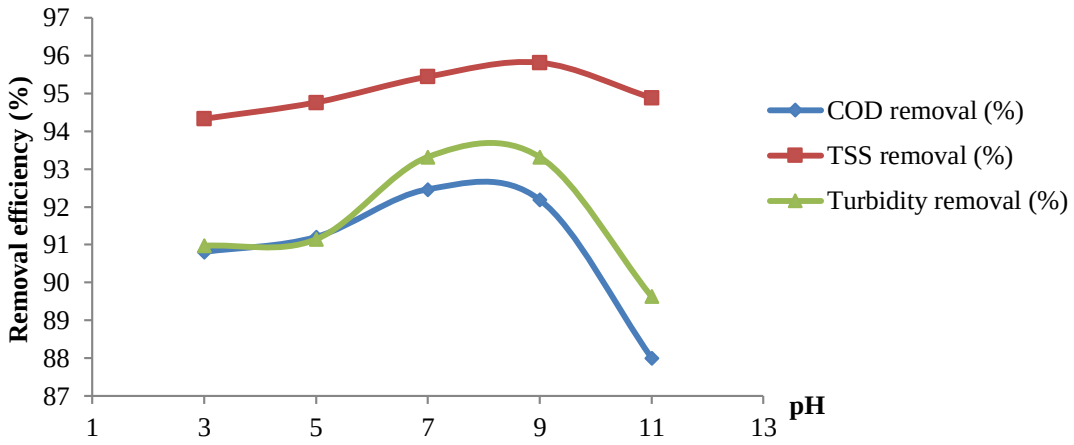

Fig. 5. Effect of pH on final COD, TSS and turbidity of POME

In the POME treatment process, pH adjustment is needed to prevent severe impact on the treatment performance of coagulation-flocculation process due to the extreme environment (highly acidic and alkaline) [5]. In the case of *Opuntia* with okra solution, the optimum pH is slightly basic since the identified optimum pH at about 9. It has been suggested that galacturonic acid (main component found in pectin) plays a key role in removal of COD, TSS and turbidity. Since galacturonic acid has negatively charged functional groups present in the long polymer chains, the following reactions (Eq. 4 and 5) take place during the treatment [6]:

$$R\text{-}COOH \leftrightarrow R\text{-}COO^{-} + H^{+} \quad (4)$$

$$R\text{-}COO^{-} + H_2O \leftrightarrow R\text{-}COOH + OH^{-} \quad (5)$$

For the reaction in Eq. (4), the carboxyl group partially dissolves and generates the chemical adsorption sites for the attachment of colloidal particles. Chemical equilibrium occurs on polygalacturonic acid for the reaction in Eq. (5). The quantity of available adsorption sites in polygalacturonic acid is affected by the concentration of OH^{-} present in the solution. 1 M NaOH and 1 M HNO_3 were added to adjust the pH environment (between 3-11) and the pH value was measured by using pH meter [10]. In this study, reversible reaction was observed, which proved that the amount of active sites in polymeric galacturonic chain was affected by the pH environment.

Based on the graph in Fig. 5, COD, TSS and turbidity share the same trend in terms of removal efficiencies. Charge balance is the main variable in this case, where it is associated with the balance of H^{+} and OH^{-} ions present in the POME samples at different pH conditions. When the pH value increases above 3, the solution becomes more basic with the increase in OH^{-} concentration. According to Le Chatelier's Principle, the position of equilibrium will shift to the left, which enables the carboxyl group to form water molecules and exposes more COO- available active sites for the adsorption of polygalacturonic chain onto the POME colloidal particles.

For the present study, the removal efficiencies of COD, TSS and turbidity reached maximum level (92.2 %, 95.8 % and 93.3 %) at pH 9 [30]. However, with further increase in pH value to 11, a drop in the COD removal performance is observed. This was because further increase in the concentration of OH^- ions, caused competition with organic molecules from POME for adsorption process. Besides that, the stronger influence of electric double layer repulsion which beyond the bridge aggregation contributes to the number of organic residues in POME, subsequently increasing POME's organic loading, which as a result reduces the COD removal efficiency [5].

TSS and turbidity removal had the similar trend. At pH higher than 9, POME sample colloidal particles were predominantly negative charges, which caused the repulsion effect between anionic polyelectrolytes and the colloidal particles. The suspended particles failed to attach to the polygalacturonic chain in this case, and the polymer will eventually fold back on itself. In this case, it is not possible to form polymer bridges, thus the TSS and turbidity removal efficiencies will decrease [29].

The close performance of coagulation-flocculation process among pH 7 and pH 9 has suggested that *Opuntia* with okra solution works well within the range of pH 7-9. If operating costs are concerned, pH 7, which slightly higher than the natural pH condition (6.5) for the POME sample can be considered for the POME treatment process, which little additional pH adjustment material is required in the process. However, if higher pollutant removal is desired, then pH 9 must chosen as the optimum pH for treating POME. For the present study, pH 9 was chosen as the optimal pH based on its better treatment efficiencies.

3.6 *Settling time determination*

Fig. 6 represents the TSS and turbidity removal efficiencies of *Opuntia* with okra and *Opuntia* alone at various settling times. It was obvious that TSS and turbidity removal increase with settling time. The increment of settling time from 0-300 min allowed the *Opuntia* with okra combination to achieve 96.1 % TSS removal and 93.6 % turbidity removal. The experiment was stopped when TSS and turbidity removal remained constant along the progress of settling time experiment. Based on the graph, it can be concluded that at 240 min, settling time is the limiting factor for the coagulation-flocculation process.

The removal efficiencies of *Opuntia* with okra solution and *Opuntia* alone were compared in this case. From the observed trends in Fig. 6, the turbidity removal efficiency of the treatment process experienced greater increment (6.2 % for *Opuntia* + okra, 7.1 % for *Opuntia* alone) throughout the experiment compared with TSS removal efficiency (2.3 % for *Opuntia* + okra, 3.5 % for *Opuntia* alone). COD removal efficiency was determined at the end of the test where *Opuntia* + okra recorded 93.9 % while *Opuntia* alone recorded 91.2 %.

The separation of suspended solids in the coagulation-flocculation process is essential for producing clean and clarified effluent. Therefore, the settling mechanism is involved in the experiment where adequate time will be needed to ensure gravity settling of particles and form supernatant on the top part of the suspension. During the settling of particles, longer settling time results in clearer solution obtained in the end of experiment. However, the total cycle time will be delayed thus a reduction in POME treatment capacity [15].

Therefore, enough settling time for coagulation-flocculation process is important in treating POME in order to achieve high removal efficiencies with minimal treatment cost. The settling time was investigated by measuring the COD, TSS and turbidity data in given 60 min time interval. The operating conditions such as coagulant (*Opuntia*) dosage, flocculant (okra solution) dosage, and pH were maintained at 8 g/L, 200 mL, pH 9, respectively, which are the optimum values that determined in the previous experiments. The settling time experiment was performed by *Opuntia* with okra solution and *Opuntia* alone.

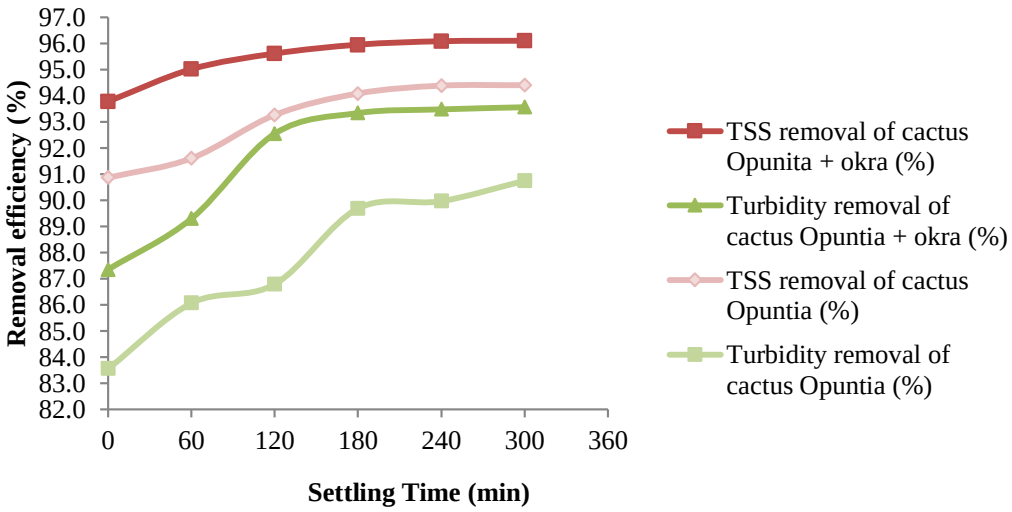

Fig. 6. Determination of settling time of treatment process

As inferred from Fig. 6, settling time at 240 min was identified as the limiting factor since the removal efficiencies of TSS and turbidity by using *Opuntia* with okra solution reached a maximum plateau value at this time. The great improvement of turbidity removal efficiency from 87.4 % to 93.6 % indicated that the particles in POME samples were able to form sediments in bottom part of beaker if adequate settling time is provided, thus producing best supernatant clarity with lower turbidity value. The same situation has been applied to *Opuntia* alone, with greater increment (from 83.6 % to 90.7 %) in terms of turbidity removal efficiency.

The settling time experiments did not work well for COD removal due to the time-consuming steps involved in testing and collecting data. The long digestion period for COD reagent vial (2 h) is the main obstacle and constraint since the experiment's data collected in 60 min time interval. It has been suggested to increase the time interval for the experiment to determine COD value or prepare numbers of COD reactor to compensate the instrument cycle time. Nevertheless, the final COD value was tested at the settling time 240 min, and the overall results have proved that *Opuntia* with okra achieved better treatment performance compare to *Opuntia* alone.

4. Conclusion

Based on the results obtained in this research, cactus *Opuntia* with okra solution with an optimal 8 g/L *Opuntia* dosage, 1:2 ratio of okra solution to POME, pH 9 and settling time of 240 min gave the best results for POME clarification. The average clarification percentages achieved were about 93.9% for COD and turbidity removal, and 96.1% for TSS removal. A summary of the results obtained in this study is given in Table 4.

From the present study, it can be concluded that *Opuntia* has great potential in being used as a cheap source of bio-coagulant for the treatment of POME. The addition of okra as a bio-flocculant seemed to improve the removal percentages of COD, TSS and turbidity, but slightly only (Table 4). Thus, this step may be omitted in the process, as using *Opuntia* alone seemed to give reasonably good results. Thus, further research is needed to see if *Opuntia* can be developed to be used both as a bio-coagulant and bio-flocculant. There is much potential in this area, and it is worth exploring.

Table 4 Optimum performance comparison in POME treatment

		Cactus *Opuntia*		Cactus *Opuntia* + Okra		
Parameters (mg/L)	**Influent (mg/L)**	**Treated effluent (mg/L)**	**Removal efficiency (%)**	**Treated effluent (mg/L)**	**Removal efficiency (%)**	**Change (%)**
COD	25350.0	2225.0	91.2	1555.0	93.9	2.7
TSS	16233.5	909.0	94.4	634.0	96.1	1.7
Turbidity	2991.5	277.0	90.7	192.0	93.6	2.9

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References

[1] Chong, M.F., Modeling, Simulation and Design of Membrane Based Palm Oil Mill Effluent (POME) Treatment Plant from Pilot Plant Studies. LAP Lambert Acad, (2009), pp.1-3.
[2] V. Kalaidevi and S. S, Preetha, Study on traditional plants utilized for the treatment of effluent with special reference to palm oil mill industry. Universal Journal of Environmental Research and Technology, 3(4), (2013), pp. 490-496.
[3] Montgomery, M. and Elimelech, M., Water and Sanitation in Developing Countries: Including Health in the Equation. Environmental Science & Technology, 41(1), (2007), pp.17-24. DOI: 10.1021/es072435t
[4] Brian Lee Choong Lek, Angela Paul Peter, Kevin Hwang Qi Chong, Pavithran Ragu, Vasanthi Sethu, Anurita Selvarajoo and Senthil Kumar Arumugasamy, Treatment of palm oil mill effluent (POME) using chickpea (*Cicer arientinum*) as a natural coagulant and flocculant: Evaluation, process optimization and characterization of chickpea powder, Journal of Environmental Chemical Engineering 6 (2018), pp. 6243-6255. DOI: 10.1016/j.jece.2018.09.038
[5] Bhatia, Subhash, Zalina Othman, and Abdul Latif Ahmad, Pretreatment of Palm Oil Mill Effluent (POME) using *Moringa oleifera* seeds as natural coagulant, Journal of Hazardous Materials, 145 (1-2), (2007), pp. 120-126. DOI: 10.1016/j.jhazmat.2006.11.003
[6] Miller, S., Fugate, E., Craver, V., Smith, J. and Zimmerman, J., Toward understanding the efficacy and mechanism of *Opuntia spp*. as a natural coagulant for potential application in water treatment, Environmental Science & Technology, 42(12), (2008), pp. 4274-4279. DOI: 10.1021/es7025054
[7] Tchobanoglous, G., Wastewater engineering: treatment and resource recovery, (2014). Boston: McGraw-Hill.
[8] K. Edzwald, J., Drinking Water Treatment: Past, Present, and Future. 1st ed. [ebook] New York City: Columbia University, (2015), pp. 1-27. Available online at: http://www.columbia.edu/~kc2288/Edzwald.pdf
[9] Chung Chee Yap, Anurita Selvarajoo, Vasanthi Sethu, Apurav Krishna Koyande, Arvin Arputhan, Lim Zhi Chien, Treatment of Palm Oil Mill Effluent (POME) using coagulation-flocculation process: Evaluation and optimization of peanut–okra and wheat germ–okra, Clean Technologies and Environmental Policy, 20 (2018), 1951-1970. DOI: 10.1007/s10098-018-1619-y
[10] Yin, Chun-Yang, Emerging usage of plant-based coagulants for water and wastewater treatment, Process Biochemistry, 45 (9), (2010): 1437-1444. DOI: 10.1016/j.procbio.2010.05.030
[11] Vijayaraghavan, G., Sivakumar, T., Vimal Kumar, A., Application of plant-based coagulants for wastewater treatment, International Journal of Advanced Engineering Research and Studies, 1 (1), (2011), pp. 88-92
[12] Sáenz, Carmen, Elena Sepúlveda, and Betty Matsuhiro, *Opuntia Spp* Mucilage's: A functional component with Industrial Perspectives, Journal of Arid Environments, 57(3), (2004), pp. 275-290.
[13] Bustillos LGT, Carpinteyro-Urban S, Departamento, Carlos Orozco, Production and characterization of *Opuntia ficus indica* mucilage and its use as coagulant–flocculant aid for industrial wastewaters, International Journal Biotechnology Research, 1 (3), (2013), pp. 38–45.

[14] Renukha A. Binayke and M.V. Jadhav, Application of natural coagulants in water purification, International Journal of Advanced Technology in Civil Engineering, 2(1), (2013), pp. 118-123.
[15] Chan Yi Jing, Mei Fong Chong and Chung Lim Law, Optimization on thermophilic aerobic treatment of anaerobically digested Palm Oil Mill Effluent (POME), Biochemical Engineering Journal, 55 (3), (2011), pp. 193-198. DOI: 10.1016/j.bej.2011.04.007
[16] Introducing the NEW Perkin Elmer Spectrum 100 FT-IR and FT-NIR Spectrometers. 1st ed. USA: PerkinElmer Life and Analytical Sciences, (2005), Available online at: https://www.perkinelmer.com/lab-solutions/resources/docs/BRO_Spectrum100FTIR.pdf
[17] Sample Preparation for FT-IR - NIU - Chemistry Analytical Lab (2019), Available online at: https://www.niu.edu/chembio/research/analytical-lab/ftir/sample-preparation.shtml
[18] Zaharuddin, N.D., Noordin, M.I. and Kadivar, A., The use of *Hibiscus esculentus* (Okra) gum in sustaining the release of propranolol hydrochloride in a solid oral dosage form, BioMed Research International (2014), pp.1-8. DOI: 10.1155/2014/735891
[19] Tetsuji, O., Aloysius UBaes, Wataru Nishijima, Mitsumasa Okada, Isolation and characterization of coagulant extracted from *Moringa oleifera* seed by salt solution, Water Research 35 (2), (2001), pp. 405-410, DOI: 10.1016/S0043-1354(00)00290-6
[20] Infrared Spectroscopy (IR), Available online at: https://chem.libretexts.org/Bookshelves/Physical_and_Theoretical_Chemistry_Textbook_Maps/Supplemental_Modules_(Physical_and_Theoretical_Chemistry)/Spectroscopy/Vibrational_Spectroscopy/Infrared_Spectroscopy
[21] Infrared Spectroscopy'. N.p., 2015. Available online at: https://www2.chemistry.msu.edu/faculty/reusch/VirtTxtJml/Spectrpy/InfraRed/infrared.htm
[22] Mohamed Ali Wahab, Hatem Boubakri, Salah Jellali, Naceur Jedidi, Characterization of ammonium retention processes onto cactus leaves fibers using FTIR, EDX and SEM Analysis, Journal of Hazardous Materials, 241-242 (2012), pp.101-109. DOI: 10.1016/j.jhazmat.2012.09.018
[23] Fox, Dawn I, Thomas Pichler, Daniel H. Yeh, Norma A. Alcantar, Removing heavy metals in water: The interaction of cactus mucilage and arsenate, (As (V)), Environmental Science & Technology 46 (8), (2012), pp. 4553-4559. DOI: 10.1021/es2021999
[24] D-Galactopyranuronic Acid, Available online at: https://pubchem.ncbi.nlm.nih.gov/compound/D-Galacturonic-Acid
[25] Four Main Regions of IR Spectrum, Available online at: https://chem.libretexts.org/Bookshelves/Organic_Chemistry/Book%3A_Organic_Chemistry_with_a_Biological_Emphasis_(Soderberg)/Chapter_04%3A_Structure_Determination_I/4.3%3A__Infrared_spectroscopy
[26] Zhang, J., Zhang, F., Luo, Y., Yang, H., A preliminary study on cactus as a coagulant in water treatment, Process Biochemistry, 41 (3), (2006), pp. 730 – 733. DOI: 10.1016/j.procbio.2005.08.016
[27] Manunza, B., Salvatore D., Marco P., Carlo, G., Molecular dynamics study of polygalacturonic acid chains in aqueous solution, Carbohydrate Research 300 (1), (1997), pp. 85-88, DOI: 10.1016/S0008-6215(97)00027-X
[28] Turbidity, Accessed online at: https://archive.epa.gov/water/archive/web/html/vms55.html
[29] Zeta Potential: A Complete Course in 5 Minutes, Available online at: http://www.zeta-meter.com/5min.pdf
[30] David Bevan, Chemistry Study Guide for A-Level, (2011), Hodder Education, London.