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Assessment of Endotoxin Removal from Reclaimed Wastewater Using Coagulation-Flocculation

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Abstract

Following biological treatment, wastewater continues to have endotoxic active materials. However, because there is a trend of potable reuse and because endotoxic active materials potentially have harmful effects on human health, their removal from water is crucial. Lipopolysaccharide endotoxin has hydrophobic groups, and their removal using a coagulation-flocculation alternative is believed to be efficient. Thus, their removal from reclaimed wastewater using the coagulation-flocculation process was assessed. Secondary effluent samples from a wastewater treatment plant located in Sapporo, Japan, were investigated. It was found that this process gave satisfactory results in removing endotoxins, with an optimum removal rate of up to 40.5%. The endotoxin removal was maximized by adjusting the pH at the low range 4 - 5.5, with an aluminum sulfate dose of 80 mg/L. Further increases of the coagulant dose did not improve the removal efficiency. DOC and turbidity removal were at their optimum at higher pH range 5.5 - 6.5. Thus coagulation and flocculation could be considered as the first barrier and should be followed by other treatments to safely reuse reclaimed wastewater.

Keywords

LPS Endotoxins, Wastewater, Coagulation-Flocculation, Potable Reuse

1. Introduction

Potable reuse of reclaimed wastewater is emerging as an alternative to alleviate stresses on conventional fresh-

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water resources and augment potable water supply. Nowadays, we count several potable reuse plants treating wastewater to a potable level. Reliance on potable reuse is expected to grow in coming years. Moreover, groundwater recharge using reclaimed wastewater has become a widespread practice around the globe [1] [2]. With the popularization of potable reuse and groundwater recharge using reclaimed wastewater, the risks associated with ingestion of treated wastewater increase. Hence, in order to ensure public safety, potable reuse plants adopt a multi-barrier approach where an integrated system of procedures, processes and tools collectively controls contaminants from passing to water supply chain. In addition, thousands of tests are performed daily to validate the treatment. Even though these colossal efforts, risks are not prevented. Indeed, potable reuse plants are not designed to treat all chemicals found in water. Lipopolysaccharide Endotoxins (LPS) are among the chemicals of concern. These are outer membrane cell wall components of most Gram-Negative Bacteria (GNB) and some cyanobacteria and they are known as pyrogens [3]-[5]. These LPS endotoxins are ubiquitous in aqueous environments and are found in large amounts in reclaimed wastewater because they are released during biological reactions and feature low biodegradability [6] [7]. LPS endotoxins are complex molecules ranging from a few kilos Daltons to hundreds of kilos Daltons in size and are composed of three main regions: lipid A, core polysaccharide, and O antigens [8]. LPS Endotoxin evokes a wide variety of pathophysiological reactions, including endotoxin shock, tissue injury, and death [9]. Indeed, if injected into the blood stream, LPS causes a broad range of negative health effects, including fever, asthma, exothermic inflammation, and hypotension that contribute to organ failure or death [10] [11]. Hence, parenteral and medical devices are strictly controlled to preserve human health [12]. Other routes of exposure include LPS endotoxin inhalation (air contaminated with endotoxins), such as in cotton fields, agricultural fields, fiberglass workshops and in wastewater treatment plants. Several studies correlate asthma with the LPS endotoxin presence in air [13]-[17]. LPS endotoxin ingestion is another potential exposure route, especially with the increasing interest in the potable reuse of the reclaimed wastewater, which is found to contain high levels of LPS endotoxins. LPS endotoxin ingestion is not yet well documented in the literature. However, few researchers reported that an amount of the ingested LPS endotoxin could be adsorbed by intestinal-epithelial cells and then detected in mice blood [18] [19]. Ghoshal 2009 estimated that up to 0.25% of the ingested endotoxin could be detected in blood. Humans are constantly exposed to LPS endotoxin found in drinking water, food, oral and intestinal bacteria and air among others. If today LPS endotoxins absorbed by the intestines do not threaten human health, it may not continue to be safe in the case of potable reuse. Indeed, it is reported that reclaimed wastewater shows extremely high concentration levels of endotoxins [6] [7] [20] [21]. It should be noticed that an amount of 1 ug/Kg in blood is enough to cause significant systemic inflammation in human [22]. From this perspective, LPS endotoxin has become an emerging contaminant of significant concern in reclaimed water. In wastewater treatment plants, LPS endotoxin is generated during a biological reaction [6]. These chemicals are uncontrollable at the source nor during treatment, and therefore post treatments are required. Moreover, LPS endotoxins can be considered to be temperature and pH stable, making their removal as one of the most difficult and challenging problems [23] [24]. Several techniques were tested for LPS endotoxin removal from reclaimed wastewater. These alternatives have been reviewed by Guizani *et al*. [25]. One common technique used for removing endotoxin contaminants uses membrane ultrafiltration taking advantage of the different sizes of the endotoxin and water [21] [26]. However, Kimura *et al*. [27] claim that severe irreversible membrane fouling is caused by polysaccharides fractions, rendering LPS endotoxin removal by ultrafilters a challenging task. Soil aquifer treatment was also tested for LPS endotoxin removal and gave satisfactory results [20]. However, its treatment efficiency was not stable over time.

Several other removal techniques such as affinity adsorbents, anionic-exchange chromatography, gel filtration chromatography, and Triton X-114 phase separation among others were employed to remove LPS endotoxin form biological preparations [28]. However, all these techniques are not cost effective in water treatment sector. Since LPS endotoxin removal from reclaimed wastewater has not yet been resolved satisfactorily, it is mandatory to investigate other methods for LPS endotoxin removal taking into consideration the properties of these chemicals. The LPS endotoxin chemicals are amphiphilic molecules containing both hydrophobic and hydrophilic groups [29]. The hydrophobic character of these chemicals is used to form large groups and aggregates to promote their settling and therefore their removal from water [30]. In addition, several researchers [31]-[33] associated the biologic activity of LPS endotoxin (e.g., toxicity) to the Lipid A. The lipid A has a net negative charge (−) and therefore the LPS endotoxins can be easily attracted to positively charged particles of coagulants and produce compact flocs suitable for easy removal by either settlement. Thus, coagulation flocculation, a common wastewater treatment alternative, is being evaluated for its efficiency in removing endotoxins from

reclaimed wastewater. The optimum conditions that insure a better removal are discussed. The ultimate objective of this study is to assess LPS endotoxin removal from reclaimed wastewater using coagulation flocculation process and find optimum conditions for their removal in terms of initial pH and aluminum sulfate dose.

2. Materials and Methods

2.1. Samples

Spot samples from the secondary effluent were collected from the settling tank (secondary treatment) of the activated sludge-operated wastewater treatment plant in Sapporo, Japan. The samples were immediately transported to the laboratory, and their characteristics were examined and recorded without delay. The assays were performed in triplicate, and average values are presented. **Table 1** summarizes the characteristics of the collected water samples from the secondary effluent of the activated sludge-operated treatment plant.

2.2. Coagulation-Flocculation test

Jar test experiments were performed to assess turbidity, DOC and LPS endotoxin removal as well as coagulation-flocculation kinetics at various pH values. The secondary effluent water samples were placed in a variable speed ZR4-6 jar test device (SuidoKikoKiasha ltd.), and their pH was adjusted from 4.5 to 8.5 (at an increment of 1) using either 0.1M sodium hydroxide (NaOH) or 0.1M hydrochloridric acid (HCl). According to Pernitsky and Edzwald (2006), favorable pH conditions for alum coagulation generally occur between a pH of 5.8 - 6.5. Then, these samples were subjected to the Coagulation-flocculation (CF) test. Aluminum sulfate, a common coagulant and a preferred reagent with several advantages, including high efficiency at low doses, low cost, low toxicity and ease of availability, was used. The test was performed in a 1-liter beaker at ambient temperature by varying the aluminum sulfate content and pH values (other parameters including rapid mixing speed and mixing time were kept constant). A change of one variable at a time was adopted in this study. First, at a given dose, a wide range of pH values was covered. From this test the optimum pH value for the best LPS endotoxin removal was obtained. The second test involved the variation of coagulant dose while setting the pH at its optimum value obtained from the previous test. As explained earlier, pH was adjusted using sodium hydroxide and hydrochloridric acid. The samples were tested using the following sequence: 60 seconds of rapid mixing at 110 rpm to enhance coagulation, followed by 30 minutes of slow mixing at 25 rpm to favor flocculation, and 30 minutes for settling. These operating conditions were selected randomly. Several coagulation test sets were conducted at different pH values, as mentioned above. The considered operating conditions are summarized in **Table 2**. For the sake of simplicity, the initial turbidity and initial endotoxin concentration effects as well as the mixing speed and time are outside the scope of this paper.

To assess the treatment efficiency, the samples were analyzed before and after the Coagulation-flocculation process. The water turbidity and DOC were measured in a HACH spectrophotometer, using a HACH kit according to the method 10173 of the HACH water analysis book (HACH, 2001) [34]. The LAL end point chromogenic assay was used to quantify the endotoxins in water samples from coagulation flocculation tests.

2.3. Endotoxin Determination Using the LAL assay

The LAL end point chromogenic assay using a general purpose colorimeter was used to quantify the endotoxins (Anonymous, 2006). A standard curve was established using a negative control of depyrogenated water (Et. and

Table 1. Characteristics of water samples collected from the secondary effluent of the wastewater treatment plant in Sapporo, Japan.

Parameters	Average value	Standard deviation
LPS Endotoxin (EU/ml)	1490	131
Dissolved Organic Carbon DOC (mg/L)	21	1.2
Turbidity (NTU)	12.9	0.6
Electric conductivity EC (µs/cm)	749	69

Table 2. Operating conditions considered in this study.

Parameters	Values
Rapid mixing (rpm)	110
Rapid mixing times (seconds)	60
aluminum sulfate doses (mg/L)	20, 40, 80 and 250
pH	4.5, 5.5, 6.5, 7.5, 8.5

beta glucan free) and CSE at endotoxin activities of 1, 0.1, 0.025 and 0.00625 EU/ml. The samples were incubated with the LAL reagent at 37˚C. A general purpose spectrophotometer was used to measure the absorbance at 405 nm. Because absorbance is related to endotoxin activity, the endotoxin activity in the unknown sample was determined by comparison to the standard curve. To validate the readings, positive controls spiked with 0.1 EU/ml of endotoxins were used to determine the recovery ratio. The spike recovery ratio for each sample must be between 50% and 200% to demonstrate a range of insignificant interference and to determine the appropriate sample dilutions. To prevent the pH interference during the LAL assay, TrisHCl buffer was used to maintain the pH at approximately 6.

The total endotoxin activities, DOC and turbidity of water samples, before and after coagulation tests, were measured in triplicates.

2.4. Reagents

An endospecy ES 24 reagent kit for the chromogenic assay of endotoxin was purchased from the SEIKAGAKU Corporation, Japan. The kit consists of a lysate of Limulus Polyphemus (LAL reagent) and a synthetic chromogenic substrate, a buffer solution to dissolve the LAL reagent and depyrogenated water (β-glucan free). The control standard endotoxin (CSE, 90 EU/vial), Pyrocolor Diazo reagent (for use with the endospecy ES 24 kit), and the LAL reagent water were purchased from the Associates of Cape Cod, Inc.

2.5. Glassware

Depyrogenated glass dilution and reaction tubes and depyrogenated pipette tips were purchased from SEIKAGAKU Corporation, Japan. Other glassware items were washed, rinsed with depyrogenated water, and finally, heat treated for 120 minutes at 250˚C or above.

3. Results

3.1. LPS Endotoxin Measurement Validation

LPS endotoxin measurements were validated using a spike test. In this test a sample with a known LPS endotoxin is spiked with a known amount of LPS endotoxin. To valid the LAL test the percentage of spike recovered should be between 50% and 200%. This validation indicates that there is no interference (enhancement or inhibition) from test samples. In this study, after the appropriate dilutions, the recovery of endotoxin-spiked samples for the LPS endotoxin detection, using the LAL assay, ranged from 50% to 200%. This validates the LAL assay for the tested samples in this work.

3.2. Dosed Water pH Adjustment

The pH value is an important parameter in determining effective coagulation. Hence, the pH of samples was adjusted from 4.5 to 8.5 (at an increment of 1) then coagulant was added. It is worth mentioning, that the solution pH value decreased in all cases as a function of the aluminum sulfate dose addition. **Figure 1** illustrates the effect of varying the coagulant dose on pH for each initial pH value. From this figure we can determine the target pH value of the solution after addition of coagulant dose.

3.3. Optimum pH for Endotoxin Removal

The effect of the initial pH on endotoxin removal is discussed in this paragraph. The coagulant dose was fixed at

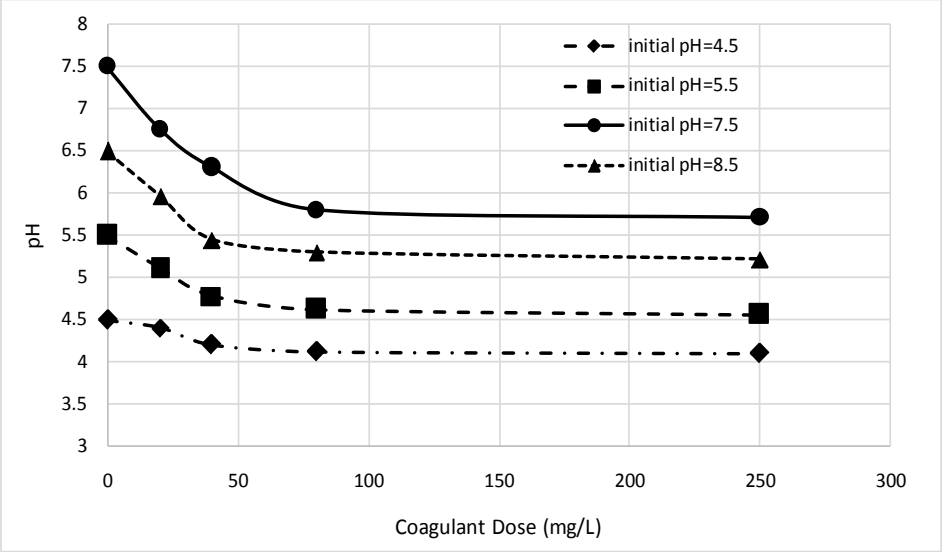

Figure 1. pH change in a solution (with a preset pH) as a function of aluminum sulfate dose.

80 mg/L. During the experiments it was observed that the LPS endotoxin, DOC concentrations and turbidity decreased during the laboratory CF test. The average DOC concentration prior to the CF test was equal to 21 mg/l (the average of triplicate measurements). The endotoxin concentration was equal to 1490 EU/ml (average). The average effluent DOC concentrations at the end of the CF test were 11.3 mg/l, 8.9 mg/l, 9.8 mg/l, 10.5 mg/l, and 11.2 mg/l for pH values stabilized at 4.13, 4.63, 5.3, 5.8 and 6.35 after coagulant addition, respectively. As shown in **Figure 2**, the corresponding average removal was 46.2%, 57.7%, 53.3%, 50% and 46.7% for pH values of 4.13, 4.63, 5.3, 5.8 and 6.35, respectively. The average effluent LPS endotoxin concentrations at the end of the CF test were 885.5 EU/ml, 887 EU/ml, 885.1 EU/ml, 912 EU/ml and 946.3 EU/ml for pH values of 4.13, 4.63, 5.3, 5.8 and 6.35, respectively. The corresponding average removal was 40.6%, 40.4%, 40.6%, 38.8% and 36.5% for pH values of 4.13, 4.63, 5.3, 5.8 and 6.35, respectively (**Figure 2**). The turbidity decreased from 12.9NTU before the test to 5.63NTU, 4.32NTU, 3.57NTU, 4.72NTU and 4.66NTU for pH values of 4.13, 4.63, 5.3, 5.8 and 6.35, respectively. The corresponding average removal was 56.4%, 66.5%, 72.4%, 63.4% and 63.9% for pH values of 4.13, 4.63, 5.3, 5.8 and 6.35 (**Figure 2**).

It is worth mentioning that the percentage of endotoxin removal was at its highest values at lower pH (4 - 5.5). The optimum LPS endotoxin removal was achieved at a pH of 5.3, when 40.6% of the endotoxin was removed (**Figure 2**). A further increase of pH did not improve the removal efficiencies. Rather, the efficiency gradually dropped at a higher pH. Generally, coagulation is achieved in different ways. At higher pH (>6), negatively charged species dominate and entrapment is the main mechanism of removal. In this case, aluminum hydroxide ($Al(OH)_2$) precipitates forming flocs that tend to capture suspended solids as it settles out of suspension. At a lower pH (<6), positively charged species start to exist in the solution and dominate at a lower pH (4 - 5) and the metal ions (Al^{3+}) directly neutralize the negatively charged LPS endotoxin. Because of the relatively high dose of aluminum used in this case (80 mg/L), the neutralization and entrapment contribute simultaneously to the removal of LPS endotoxin at low pH resulting in a relatively good removal efficiency (40.6%).

However, a different trend was observed for the DOC and turbidity removal. The optimum pH for DOC and turbidity removal is 5.8 and 6.35, respectively. This suggests that the main removal mechanism is entrapment as negatively charged species dominates in this range of pH. The turbidity removal ranged from 72.6% to 83.2%, and the DOC removal varied from 45% to 57.3%. The best pH value for the turbidity and DOC endotoxin is in the range of 5.8 to 6.35, while the best pH value for LPS endotoxin removal is rather in the low pH zone (4.3 - 5.3) (**Figure 2**). Our findings confirm the previously reported results in the literature with respect to the DOC removal in cold water (15 degree Celsius) with an approximate removal percentage of 50%. It should be noticed that that the coagulation test in this study was conducted during the cold Sapporo season and the water temperature was in the range of 14 to 17 degree Celcius.

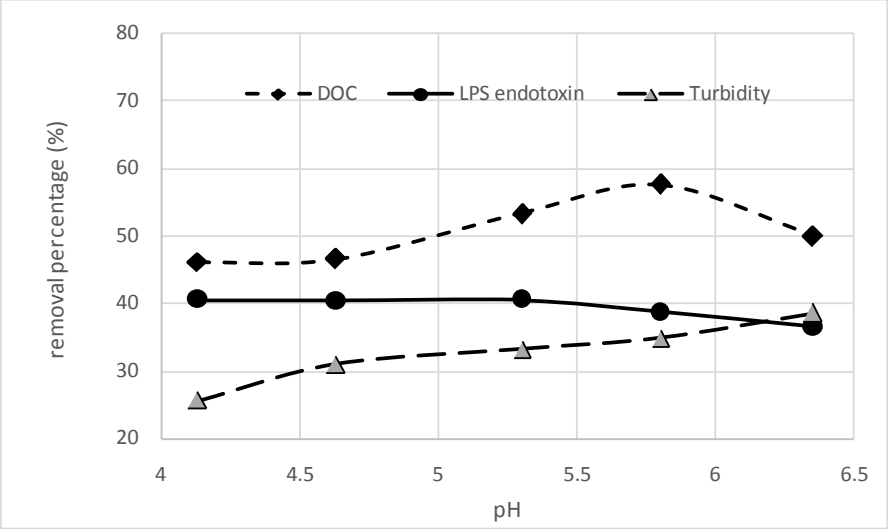

Figure 2. Removal of LPS endotoxin, DOC and turbidity vs. pH (aluminum sulfate dose: 80 mg/L).

3.4. Effect of Coagulant Dosage

The coagulant dose effect on the endotoxic active material removal is summarized in this paragraph. The samples were flocculated using 20 mg/L, 40 mg/L, 80 mg/L and 250 mg/L aluminum sulfate doses. Since the highest LPS endotoxin removal was observed in the pH range 4 - 5.5, the optimization of aluminum sulfate dosage was performed by adjusting sample pH to 5.5 while varying the alum dosage from 20 - 250 mg/L. As shown in **Figure 1**, after addition of coagulant the pH will drop but remains in the range of 4 - 5.5. In addition, for comparison, the data will be presented for the samples with their pH values adjusted to a higher value (pH = 8 before coagulant addition). During the experiment, it is observed that no or a very low number of flocks are formed at the lower coagulant doses (20 mg/L and 40 mg/L). At the highest coagulant dose (250 mg/L), a large number of flocks are observed. However, at the dose of 80 mg/L the number of flock is much higher than the case of 250 mg/L dose. **Figure 3** illustrates the well-defined trends of endotoxin removal, versus the aluminum sulfate dose. It indicates that an alum dosage of 80 mg·L^{-1} (as Al^{3+}) resulted in the lowest residual LPS endotoxin. This dosage reduced the pH from 5.5 to 4.69 which favored sweep flocculation through the formation of aluminum hydroxide precipitates [35]. Further, the isoelectric point of alum occurs at pH 8 indicating that pH values less than 8 yield positively charged precipitates, which are able to neutralize the negatively charged particles in the water sample [36]. At a pH stabilized at 4.69 after 80 mg/L coagulant addition, the LPS endotoxin decreased from 1490 EU/ml to 538 EU/ml corresponding to an average removal percentage of 40.5 At a higher pH value (pH = 8 before coagulant addition), a similar shape of the LPS endotoxin removal rate curve is observed but with a lower efficiency. The highest efficiency was observed again at 80 mg/L aluminum sulfate dosage and reached 36.5%. It is to be notice that this dosage reduces pH from 7.5 to 6.35.

Comparable shapes of removal percentage curves were observed for DOC and turbidity removal (**Figure 4** and **Figure 5**). However the best removal at 80 mg/L coagulant dosage were observed for a pH of 6.35 (after coagulant addition), validating the findings related to optimum pH discussed earlier.

It is to be noticed that increasing the aluminum sulfate dosage is not in favor of turbidity removal. In some cases we found that at higher doses of aluminum sulfate (250 mg/L) were often observed to add turbidity to the water (**Figure 4**). Turbidity removal is considered to be an important parameter because turbid matter may provide good protection for bacteria. Furthermore, in the presence of trace amount of organic matter the bacteria regrowth will lead to an increase of the LPS endotoxin after the CF process. Thus, the lower residual water turbidity is the target to better control endotoxins.

4. Discussion

The process of coagulation/flocculation produced satisfactory results as an alternative for removing endotoxins.

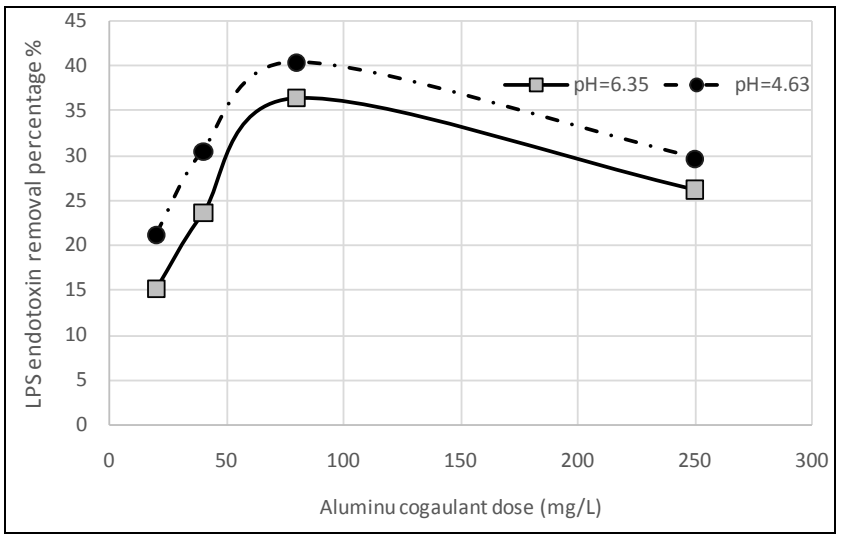

Figure 3. Removal of LPS endotoxin vs. aluminum sulfate dose (pH: 4.63 and 6.35).

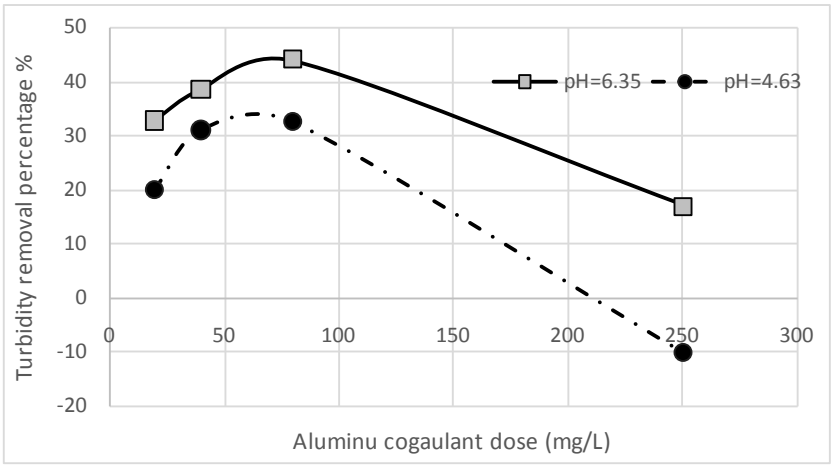

Figure 4. Turbidity vs. the aluminum sulfate dose (pH: 4.63 and 6.35).

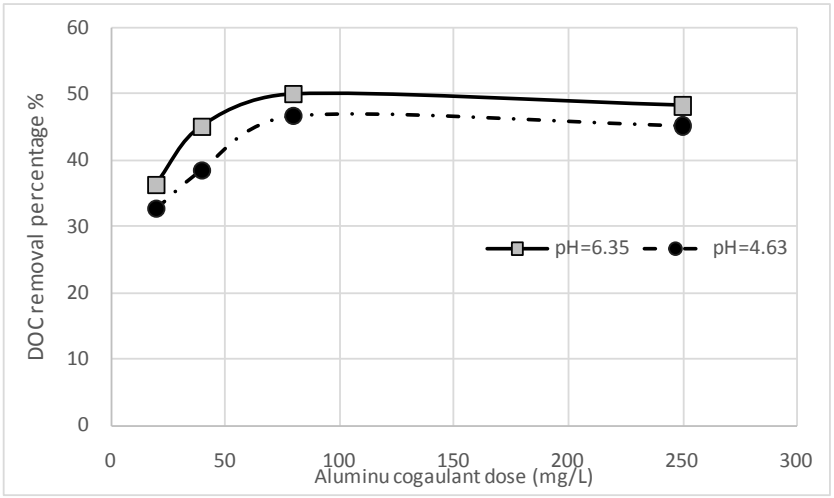

Figure 5. DOC removal vs. aluminum sulfate dose (pH: 4.63 and 6.35).

The LPS endotoxin removal using the coagulation flocculation process achieved satisfactory but not excellent results, with up to 40.5% LPS endotoxin removal at its highest efficiency. The LPS endotoxin concentration in the supernatant of the coagulation flocculation process was found to be significantly higher than the values reported for tap water and groundwater (Anderson *et al*., 2002). Without standards, further treatments are required to meet the current LPS endotoxin levels in tap water and groundwater. Hence, CF is an effective first barrier to reduce endotoxins in the reclaimed wastewater.

In comparison with other treatment alternatives (e.g., sand filtration, membrane filtration, oxidation and UV treatment), coagulation flocculation is less efficient but is still an attractive cheap and affordable method. Indeed, the soil filtration approach reported by Guizani *et al*. in 2011 exhibited a good removal efficiency (75.6%), but with high instability in time. However, the process is not recommended for the shallow aquifer application. Indeed, long soil columns are required for better efficiency (90 cm). The nano-filtration and reverse osmosis methods have a removal efficiency of more than 90% [21]. However, these alternatives are expensive. The oxidation and UV treatments were tested only for water that was contaminated with endotoxins, other than wastewater and secondary-treated effluents. These methods are not appealing because their efficiency did not exceed 40%, although the relatively low endotxicity initially present in these waters [37]. Coagulation-flocculation is a cheap and interesting alternative for reducing endotoxicity in the treated wastewater. It can be combined with other treatment options to achieve the best removal efficiency. However, with the common trend of potable reuse, the higher coagulant dose is of concern. In addition, as optimum turbidity and LPs endotoxin could not be achieved under the same conditions. The low turbidity removal at the optimum pH for LPS endotoxin removal, may lead to the bacterial regrowth and LPS endotoxin regeneration, which is a concern. Moreover, it is worth mentioning that this study, which is based on one variable variation at a time, provided us with optimum settings for pH and coagulant dose for better endotoxin removal. Rapid mixing speed and mixing time were set randomly. However, these two parameters may affect significantly the efficiency of coagulation flocculation process as they provide the interaction speed and time between molecules and particles in the water and a coagulant [38]-[40]. Hence, the influence of rapid mixing velocity and rapid mixing time will be investigated in future studies. In addition, the optimum conditions can better estimate if all possible cases are tested, which is, practically, impossible. Thus, an optimization technique that uses statistical methods, such as response surface methodology, is of interest [41].

5. Conclusion

Potable reuse of reclaimed wastewater is becoming a worldwide common trend. Hence efficient removal of potential contaminants found in reclaimed water is of great concern. LPS endotoxins are among the emerging contaminants of concern. In this study, taking advantage of their net negative charge and hydrophobic character, the removal of LPS endotoxin from reclaimed wastewater has been assessed using coagulation flocculation test with the use of aluminum sulfate as coagulant. The coagulation and flocculation process provided satisfactory results in reducing LPS endotoxins in water as a first barrier. The highest endotoxin removal was achieved at an aluminum sulfate dose of 80 mg/L at a pH of 4.69. However, the optimum pH for DOC and turbidity removal was 6.35. The residual LPS endotoxin concentration was significantly higher than the reported value in tap and groundwater. This suggests a need for further treatments for safe potable reuse. Furthermore, even though there is a significant reduction of DOC and turbidity, the remaining turbid matter presents a risk of LPS endotoxin release following the bacteria regrowth. To prevent bacteria regrowth, further treatments are essential. Briefly, the CF process is a satisfactory first barrier for LPS endotoxin removal treatment.

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