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Use of Natural Coagulants in Removing Organic Matter, Turbidity and Fecal Bacteria from Hospital Wastewater by Coagulation-Flocculation Process

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Abstract

Hospital wastewater represents an infectious and toxic risk to human health and the environment due to its contents. Most hospitals in developing countries, including Benin, do not have a wastewater treatment plant. In this study, the wastewater from two hospitals in northern Benin was characterized and then treated with *Azadirachta indica* leaves, *Moringa oleifera* and L*uffa cylindrica* seeds by coagulation/flocculation process. The wastewater characteristics showed that the collected samples are greatly polluted by organic matter and fecal bacteria such as *Escherichia coli*, *Enterococcus* fecal and Total coliforms. Jar-test results revealed that 95.74%, 78%, 49.19% of turbidity, 51.35%, 38.32%, 22.19% of COD, 93.16%, 85.26%, 83.30% against *Escherichia coli*, 92.11%, 90.93%, 94.60% against total Coliforms and 99.37%, 91%, 99%, 55.07% against *Enterococcus* were removed from hospital wastewater using *Moringa oleifera*, *Luffa cylindrica* seed and *Azadirachta indica* leaves respectively at dose of 100 mg/L. The results highlighted that the natural coagulants could be successfully used for the removal of turbidity and fecal bacteria from hospital wastewater.

Keywords

Hospital Wastewater, Fecal Bacteria, Organic Matter, Natural Coagulants, Removal

1. Introduction

The demographic explosion has led to an increase in water needs, which satisfaction exercises great pressure on available water resources [1]. In order to limit the pressure available water reserve, there is the necessity to recycle wastewater for rational management of the existing through the implementation of processes with effective action on the physico-chemical and bacteriological pollution [2]. The challenges related to wastewater management are therefore becoming an increasingly important concern in all international communities [3]. Among the different categories of wastewater, those generated by hospitals constitute a potential threat to human health and the environment [4] [5].

In most developing countries including Benin, hospital wastewater was often released in septic tanks without any prior treatment [6]. The septic tanks used to store this wastewater have a low capacity to eliminate pathogenic bacteria and the infiltration wells can pollute the shallow groundwater used to supply the surrounding wells, a source of drinking water for many households [7]. The presence of bacteria and pathogenic parasites in drinking water could cause waterborne diseases such as diarrhea, typhoid fever and cholera [8] [9].

Faced with all these public health problems that can be caused by hospital wastewater, it is, therefore, urgent to propose a purification technology that is less expensive, easy to access and does not require any particular qualification based on the use of local products and having the effectiveness of action on physico-chemical and bacteriological pollution of hospital wastewater. Numerous processes such as coagulation-flocculation [6], adsorption [10], ozonation [11], membrane filtration processes [12], photocatalytic oxidation [13] are used to purify wastewater. Among these methods, the coagulation process is preferentially applied to remove pollutants from water because of its high effectiveness and easiness of application on a wide range of water types [14]. The implementation of the coagulation-flocculation process in the treatment of wastewater requires the use of chemical or natural coagulants. Chemical coagulants such as aluminum sulfate and ferric chloride are most commonly used in wastewater treatment. However, the use of these chemical coagulants affects the water's pH and generates significant amounts of non-biodegradable sludge and metallic residues in the treated water, sources of disease [15]. In order to reduce the use of chemical coagulants, current research is directed toward the use of natural coagulants in water treatment. Thus, the natural coagulants extracted from the seeds of *Moringa oleifera* [6], *Cicer arietinum* [16], *Jatropha curcas* [17], *Cocos nucifera* [18], *Luffa cylindrica* [19] or leaves of *Azadiracha indica* [20] were used for pathogenic bacteria [6] [21] [22] and dyes [23] [24] removal from wastewater with satisfactory results. This work investigates turbidity, COD, UV_{254}, *Escherichia coli*, *Enterococcus* and total coliforms removal from hospital wastewater by *Moringa oleifera*, *Luffa cylindrica* seeds and *Azadiracha indica* leaves.

2. Materials and Methods

2.1. Study Areas and Sample Collection

Wastewater samples were collected from Bakita (10˚18'15.22'' North, 1˚24'15.21''

East) and Zone (10˚18'39.56'' North, 1˚22'42.08'' East) hospitals in Natitingou. In both hospitals, the wastewater from each service is drained by PVC pipes to a storage tank. In this study, five wastewater sampling points labeled PE1/B, PE2/B, PE3/B, PE1/HZ, PE2/HZ are all located upstream from four followed wastewater storage tanks FS1/B, FS2/ B, FS1/HZ and FS2/HZ, as well as the tanks, were sampled from August 2021 to October 2021. The water samples were collected in 500 mL borosilicate bottles previously sterilized by Trade raypa steam sterilizer brand autoclave at 121˚C for 1 h, and then stored at 4˚C in a cooler and brought to the laboratory for analysis. Sampling collected points and storage tanks are shown in Figure 1.

2.2. Physico-Chemical Analysis

In our study, physic-chemical parameters such as pH, temperature, conductivity, TDS, dissolved oxygen and turbidity of hospital wastewater samples were carried out in-situ using a HANNA HI 991001 pH-meter, HANNA HI 99300 multiparameter conductivity meter, WTW OXI 3205 oximeter and HANNA HI 93703 turbidimeter respectively. The concentrations of nitrates and nitrites were performed by spectrophotometric analysis on UV/Visible spectrophotometer VWR 1600 PC using salicylate sodium at 416 nm and sulfanilic acid at 435 nm. Chemical Oxygen Demand was determined by hot oxidation with potassium dichromate. The dissolved organic matter was measured by spectrophotometry at 254 nm on the filtrates obtained by filtration of the sample on a membrane with a porosity of 0.45 µm.

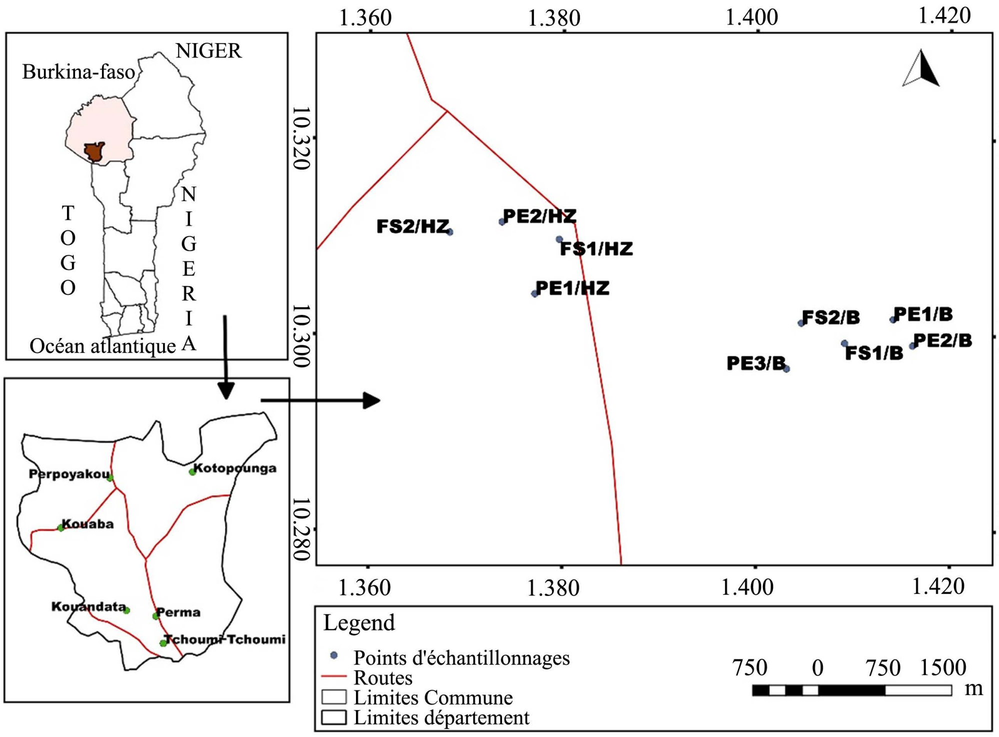

Figure 1. Located of wastewater sampling points.

2.3. Bacteriological Analysis

Bacteriological analyses were performed according to the protocol described by Nonfodji *et al.* [10]. The isolation of total coliforms, *Escherichia coli* and *Enterococcus* were carried out at 37˚C using Chromogenic Coliform Agar and Bile-Esculin-Azide agar respectively, while that of Fecal coliforms at 44˚C on Chromogenic Coliform Agar agar.

2.4. Preparation of the Coagulants

2.4.1. *Moringa oleifera* Seeds (MOs)

Moringa oleifera seeds used in this study were harvested in Natitingou. The seeds were shelled, dried at room temperature for 72 h then ground in an electric laboratory Moulinex and sieved to obtain fine particles. 2 g of residues were dispersed in 100 mL of NaCl 1 M solution, stirred for 30 min then left to settle for 15 min; afterward, the supernatant is filtered on Whatman1 filter paper.

2.4.2. *Luffa cylindrica* Seeds (LCs)

The dried and mature fruits of *Luffa cylindrica* were harvested in Natitingou; the seeds were collected, dried at room temperature for 72 h then ground using a ball mill and sieved to obtain fine particles. 2 g of the powder was dispersed in 100 mL of NaCl 1 M solution, stirred for 30 min then allowed to settle for 15 min; afterward, the supernatant is filtered on Whatman1 filter paper.

2.4.3. *Azadirachta indica* Leaves (AZl)

Azadirachta indica leaves used in this study were collected in Natitingou. The leaves were washed with distilled water, dried at room temperature for 21 days then crushed and sieved to obtain fine particles. 2 g of *Azadirachta indica* leaves powder were dispersed in 100 mL of ethanol 95%, then stirred for 48 hours on a magnetic stirrer. Afterward, the supernatant is filtered on Whatman1 filter paper.

2.5. Jar-Test Procedures

The jar-test assays were carried out on a STUART SW6 laboratory six-paddle stirrer flocculator. Increasing doses of coagulants varying from 0 to 200 mg/L were injected into 1 L of hospital wastewater sample. The mixtures were stirred for 5 minutes at 150 rpm then at 60 rpm for 30 minutes. After 2 h of settling, water sample was collected at 20 mm below top clarified water using sterile syringe for analysis Turbidity, COD, UV_{254}, pH, total coliforms, *Escherichia coli* and *Enterococcus*. The removal efficiency (R %) of each parameter was calculated by the following expression:

$$R(\%) = \frac{N_0 - N}{N_0} * 100$$

3. Results and Discussion

3.1. Physico-Chemical and Bacteriological Characteristics

Physico-chemical and bacteriological characteristics of the Wastewater samples are in Table 1 and Table 2. According to the results presented in Table 1, the collected samples points PE1/B, PE2/B, PE3/B, PE1/HZ, PE2/HZ, FS1/B, FS2/B, and FS1/HZ were moderately mineralized, unlike FS2/HZ which is highly mineralized. The pH of wastewater sampled tends towards neutrality. The temperature of the samples varies between 27.50˚C ± 1.22˚C and 29.30˚C ± 0.90˚C and would be related to the ambient temperature of the study area. Temperatures above 15˚C favor the development of microorganisms [10]. The average turbidity values measured vary between 8.16 ± 0.15 NTU to 96.00 ± 1.41 NTU, which shows that the wastewater samples do not have the same origins. All wastewater samples had low dissolved oxygen ranging between 0.14 ± 0.01 mg/L to 0.93 ± 0.14 mg/L with elevated mean values of both COD and nitrate varying to 220.70 ± 17.93 mg/L-O_2 up to 386.30 ± 2.12 mg/L-O_2 mg/L-O_2 and from 0.27 ± 0.11 mg/L up to 11.63 ± 4.95 mg/L respectively. These measured values confirm the

Table 1. Physico-chemical parameters.

Parameters	Wastewater samples									Norm*
	PE1/B	PE2/B	PE3/B	FS1/B	FS2/B	PE1/HZ	PE2/HZ	FS1/HZ	FS2/HZ	
Temperature (˚C)	28.66 ± 0.86	28.10 ± 1.26	28.53 ± 1.10	28.03 ± 1.37	29.30 ± 0.90	29.36 ± 1.30	27.50 ± 1.22	28.93 ± 1.36	28.90 ± 1.32	<30
pH	7.13 ± 0.30	7.34 ± 0.12	7.09 ± 0.17	7.45 ± 0.01	7.45 ± 0.17	6.34 ± 0.10	6.67 ± 0.19	6.99 ± 0.31	6.77 ± 0.40	5.50 - 8.50
Disolved Oxygen (mg/L)	0.48 ± 0.09	0.39 ± 0.18	0.65 ± 0.39	0.28 ± 0.08	0.93 ± 0.14	0.77 ± 0.16	0.74 ± 0.02	0.37 ± 0.12	0.14 ± 0.01	≥5
Conductivity (µS/cm)	676.00 ± 94.75	297.33 ± 29.02	314.00 ± 38.31	567.00 ± 26.96	581.00 ± 65.05	100.50 ± 14.84	88.00 ± 25.45	536.50 ± 7.77	3966.00 ± 57.15	<2700
TDS (mg/L)	342.50 ± 48.79	159.23 ± 32.14	166.90 ± 30.84	283.46 ± 12.97	289.50 ± 33.23	51.00 ± 7.07	45.00 ± 14.14	265.50 ± 3.53	2000 ± 0.00	
Turbidity(NTU)	61.00 ± 2.82	18.94 ± 2.83	24.27 ± 3.12	96.00 ± 1.41	92.00 ± 9.84	16.15 ± 0.91	8.16 ± 0.15	13.26 ± 4.35	84.33 ± 14.50	-
Nitrates (mg/L)	0.69 ± 0.33	0.27 ± 0.11	1.78 ± 0.04	11.63 ± 4.95	5.21 ± 0.70	2.35 ± 0.05	5.16 ± 0.15	0.49 ± 0.14	5.52 ± 1.97	<1
Nitrites (mg/L)	0.16 ± 0.05	0.05 ± 0.007	0.75 ± 0.13	0.25 ± 0.03	0.32 ± 0.14	0.56 ± 0.07	0.24 ± 0.04	0.23 ± 0.06	0.84 ± 0.11	<1
DCO (mg/L-O_2)	220.70 ± 17.93	239.20 ± 14.70	270.40 ± 14.71	386.30 ± 2.12	228.80 ± 44.12	270.50 ± 8.21	244.40 ± 80.89	239.80 ± 12.44	339.20 ± 11.33	<90

Table 2. Bacteriological parameters.

Bacteria	Wastewater samples								
	PE1/B	PE2/B	PE3/B	**FS1/B**	FS2/B	PE1/HZ	PE2/HZ	FS1/HZ	FS2/HZ
Total Coliforms (UFC/100mL)	$1.61 \times 10^6 \pm 0.82 \times 10^6$	$1.89 \times 10^6 \pm 0.74 \times 10^6$	$1.80 \times 10^6 \pm 0.86 \times 10^6$	$3.17 \times 10^6 \pm 0.86 \times 10^6$	$1.33 \times 10^6 \pm 0.28 \times 10^6$	$1.59 \times 10^6 \pm 0.08 \times 10^6$	$1.66 \times 10^6 \pm 0.52 \times 10^6$	$1.60 \times 10^6 \pm 0.89 \times 10^6$	$1.84 \times 10^6 \pm 0.89 \times 10^6$
Faecal Coliforms (UFC/100mL)	$4.72 \times 10^5 \pm 0.74 \times 10^5$	$2.73 \times 10^5 \pm 0.51 \times 10^5$	$1.54 \times 10^5 \pm 0.50 \times 10^5$	$4.29 \times 10^5 \pm 0.36 \times 10^5$	$2.07 \times 10^5 \pm 0.19 \times 10^5$	$2.77 \times 10^5 \pm 0.18 \times 10^5$	$1.16 \times 10^3 \pm 0.18 \times 10^3$	$1.39 \times 10^5 \pm 0.21 \times 10^5$	$2.49 \times 10^5 \pm 0.40 \times 10^5$
Escherichia coli (UFC/100mL)	$1.44 \times 10^6 \pm 0.12 \times 10^6$	$5.13 \times 10^5 \pm 0.38 \times 10^5$	$4.85 \times 10^5 \pm 1.76 \times 10^5$	$1.65 \times 10^6 \pm 0.74 \times 10^6$	$1.29 \times 10^5 \pm 0.28 \times 10^5$	$8.45 \times 10^5 \pm 0.21 \times 10^5$	$1.43 \times 10^4 \pm 0.33 \times 10^4$	$1.22 \times 10^6 \pm 0.79 \times 10^6$	$4.88 \times 10^5 \pm 0.46 \times 10^5$
Enterococcus (UFC/100mL)	$2.91 \times 10^6 \pm 0.08 \times 10^6$	$3.38 \times 10^4 \pm 0.73 \times 10^4$	$1.61 \times 10^5 \pm 0.55 \times 10^5$	$1.97 \times 10^6 \pm 0.76 \times 10^6$	$1.89 \times 10^4 \pm 0.58 \times 10^4$	$2.50 \times 10^4 \pm 0.14 \times 10^4$	$1.88 \times 10^4 \pm 0.66 \times 10^4$	$2.45 \times 10^4 \pm 0.49 \times 10^4$	$7.41 \times 10^4 \pm 0.28 \times 10^4$

pollution of hospital wastewater samples by bacteria that use dissolved oxygen to degrade organic matter and reduce nitrates to nitrites [25]. The bacteriological parameters of the hospital wastewater samples collected shown in **Table 2**, revealed the pollution of wastewater samples by Fecal coliforms, total coliforms, *Escherichia coli* and *Enterococcus*. Most of these bacteria isolated from hospital wastewater samples are multi-resistant to commonly prescribed antibiotics in human medicine [10]. The discharge of this wastewater into the environment without any prior treatment could cause contamination of diseases requiring antibiotic treatment in the surrounding populations.

3.2. Effects of Natural Coagulants on Turbidity, COD and UV_{254} Removal

The effectiveness of MOs, LCs and AZl was evaluated on the removal of Turbidity, COD and UV254 from hospital wastewater samples. The results obtained are shown in **Figures 2-4** respectively. These results highlighted that the removal efficiency increased with increase in the coagulant dosage. Turbidity removal of 95.74%, 78% and 49.19% were obtained with MOs, LCs and AZl respectively for a dose of 100 mg/L (**Figure 2**). MOs appears more effective in removing turbidity followed by LCs. This efficiency of coagulants in the elimination of turbidity would be linked to a charge neutralization mechanism. Indeed, previous work has reported the presence of a cationic protein in *Moringa oleifera* [10] [26], *Luffa cylindrica* [19] [24] seeds and *Azadirachta indica l*eaves [27] [28] which is active on the polluting particles of wastewater. The positive charges of this protein interact with the negative charges of the pollutant molecules present in the wastewater leading to the aggregation of the pollutant particles and their flocculation followed by their sedimentation. The gradual increase in the turbidity removal with the dose of coagulant reflects the increase in active sites of the cationic protein which interact with an increasingly increasing quantity of

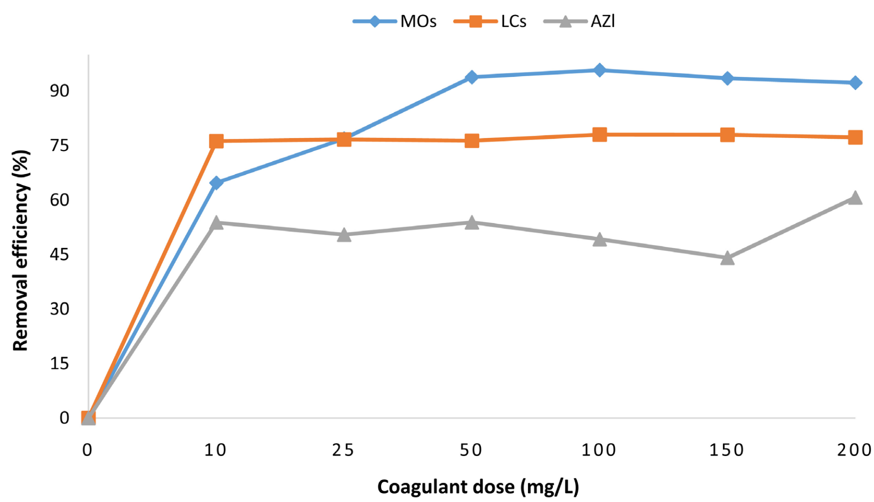

Figure 2. Turbidity removal.

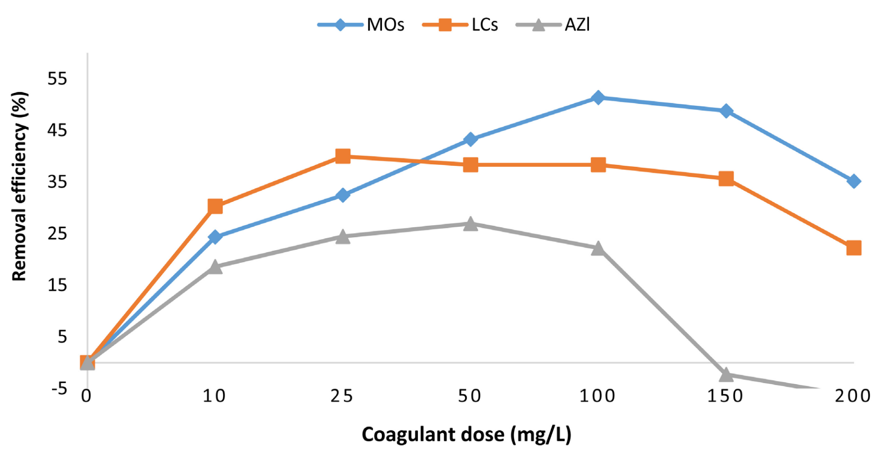

Figure 3. COD removal.

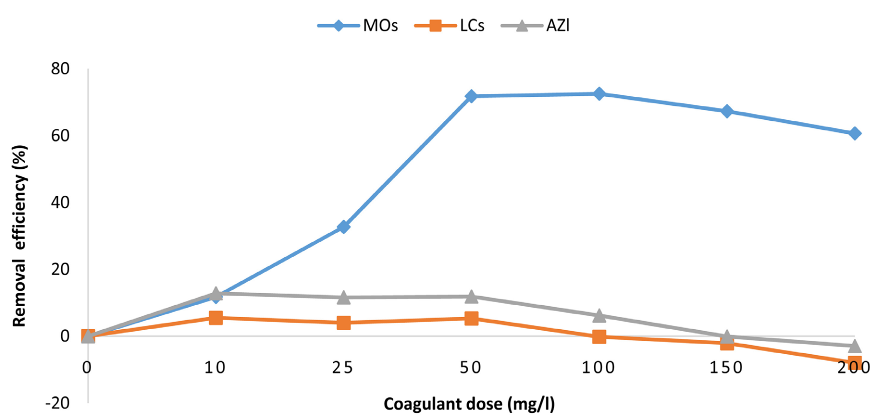

Figure 4. UV_{254} Removal.

polluting particles in hospital wastewater. COD reduction rates of 51.35%, 38.32% and 22.19% were observed with MOs, LCs and AZl respectively for a dose of 100 mg/L (**Figure 3**). For the same dose, it was noted UV_{254} removal of

72.54% with MOs compared to LCs and AZl which gave a very low reduction rate (**Figure 4**). This suggests that MOs appears effective on COD and aromatic organic matter in hospital wastewater. From these results, it appears that the crude extracts of *Moringa oleifera* seeds are effective in the elimination of turbidity, COD and aromatic organic matter from hospital wastewater.

3.3. Effects of Natural Coagulants on Fecal Bacteria Removal

The antibacterial efficiency of MOs, LCs and AZl coagulants were tested against *Escherichia coli*, total coliforms and *Enterococcus*. The results shown in **Figures 5-7** revealed that the removal percentage of bacteria increases with increasing in the coagulant dosage. So, it was also noted removal percentage passing from 69.63%, 77.86%, 70% to 95.28%, 87.32%, 90% against *Escherichia coli* (**Figure 5**), 67.88%, 82.39%, 87.22% to 94.51%, 92.54%, 95.37% against total coliforms (**Figure 6**) and 94.83%, 89.64%, 2.90% to 99.65%, 94.18%, 78.50% against *Enterococcus* with MOs, LCs and AZl respectively when increasing of the coagulant dosage from 10 mg/L to 200 mg/L. Similar observations have been made by several authors. Indeed, Mo [29] reported a reduced rate of 87% against *Escherichia*

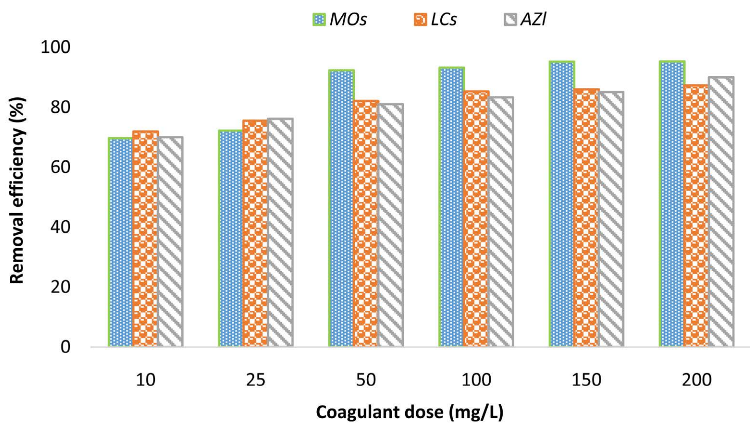

Figure 5. *Escherichia coli* removal.

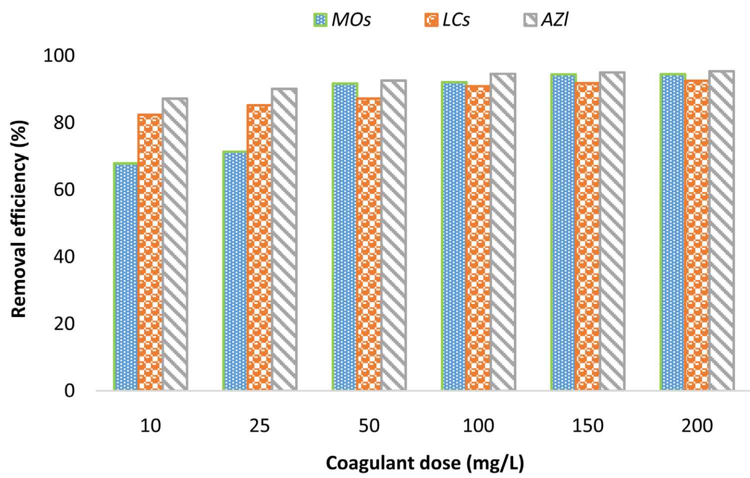

Figure 6. Total coliforms removal.

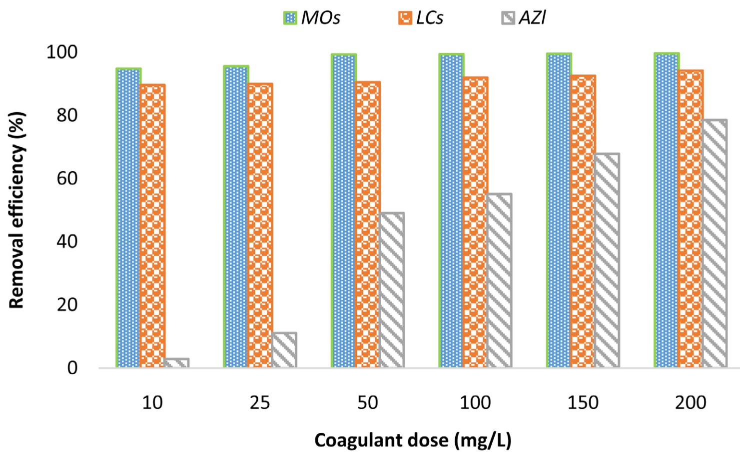

Figure 7. *Enterococcus* removal.

coli with *Moringa oleifera* seeds at a dose of 600 mg/L [21], while Shaheed obtained a removal percentage of 60% against total Coliforms with *Luffa cylindrica* seeds at a dose of 200 g/L. The efficiency of bacteria inactivation by MOs, LCs and AZl may be explained on the one hand by the coagulation process which can remove bacteria associated with the turbidity of wastewater samples by flocculation and sedimentation. On the other hand, authors have reported the presence of antibacterial compounds in *M. oleifera* [30], *L. cylindrical* seeds [31] [32] and *A. indica* leaves [22] which are responsible for the destruction of the internal and external membranes of bacterial cells.

4. Conclusion

Wastewater samples from Bakita and Zone hospitals in northern Benin were collected characterized and treated with *Moringa oleifera* (MOs), *Luffa cylindrica* (LCs) seeds and of *Azadirachta indica* leaves (AZl) extracts. The wastewater characteristics revealed the presence of organic matter and fecal bacteria such as *Escherichia coli*, *Enterococcus*, fecal and total coliforms. Jar-test results revealed that 95%, 75%, 60.30% of turbidity, 70%, 8%, 5% of UV_{254}, 53%, 40%, 25.21% of COD, 95%, 81.75%, 88% against *Escherichia coli*, 97%, 89.40%, 93% against total Coliforms and 96%, 90.98%, 75% against *Enterococcus* were obtained with MOs, LCs and AZl, respectively. The results also revealed that *Moringa oleifera* has a high affinity for hospital wastewater pollutants compared to *Luffa cylindrica* and *Azadirachta indica*.

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Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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