



Effect of pre-treatment with a tannin-based coagulant and flocculant on a biofilm bacterial community and the nitrification process in a municipal wastewater biofilm treatment unit



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ABSTRACT

Tanfloc, a natural tannin-based coagulant and flocculant, was used in this study as a pre-treatment agent for a biofilm unit that was used to treat municipal wastewater. The point of interest in this study was the effect of the extended use of Tanfloc (around 2 months) on the biofilm bacterial community. Two identical bench-scale aeration tanks were run in parallel, one of them treating settled wastewater, and the other pre-treated (flocculated/settled) wastewater using Tanfloc. The results showing the effect of Tanfloc was very apparent on the characteristics of the wastewater. At a hydraulic retention time of 4 h, the relatively short retention time was not enough to achieve any noticeable removal of ammonia without Tanfloc. In contrast, the 4 h retention time was sufficient to achieve around 70 % removal of ammonia when Tanfloc was used. This improvement in the nitrification process was attributed to the bacterial community of the biofilm as a consequence of Tanfloc use. A bacterial 16S rRNA analysis of the biofilm showed a significant increase in the percentage of ammonia-oxidizing bacteria (3.33 %) and nitrite-oxidizing bacteria (7.8 %) in the experiment using Tanfloc, compared to only 0.073 % and 0.19 % respectively, in the experiment without Tanfloc.

1. Introduction

Coagulation and flocculation have been used extensively in the treatment of water [1] and wastewater [2]. Different materials have been used as coagulants and flocculants, with the conventional materials being metal ions such as Al^{3+} and Fe^{3+} [3]. In addition to the suspected effect on public health, these inorganic materials produce large amounts of inorganic sludge, which act as an additional burden to the environment. For that reason, many researchers pay special attention to the use of natural resources in the hope of finding environment-friendly alternatives for conventional coagulants. This emerging trend has resulted in two main categories of natural coagulants and flocculants, depending on the exploited natural resources. The first category are the extracellular bio-polymeric substances secreted by certain species of microorganism [4], while the second category are the materials that are extracted from other natural resources such as polymeric plant extracts [5].

The material investigated in this study was a tannin-based coagulant

and flocculant. Generally, tannin is a polyphenolic compound with a low molecular weight and high solubility in water. Raw tannin is extracted from trees such as the *Acacia mearnsii* De Wild. In order to improve the characteristics of raw tannin as a coagulant, it should undergo a cationization process (granting a cationic character to the tannin molecule) to enable the modified tannin to obtain a charge neutralization mechanism [6].

A commercial tannin-based coagulant and flocculant has been produced under the name of Tanfloc. The *Acacia mearnsii* De Wild tree is the source of the tannin that is used in Tanfloc. This tannin is polymerized by the addition of formaldehyde, quaternary nitrogen (NH_4Cl) and hydrochloric acid. A mixture of these three chemicals is stirred and heated. Then, the tannin extract is added. This process takes several hours until a viscous mixture containing 40 % solids is produced. Evaporation is the last step in the production of Tanfloc in powder form [7].

Tanfloc, being a new material, has attracted the attention of researchers. Bongiovani et al. [1] used Tanfloc to remove natural organic

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matter in surface water in order to reduce the potential formation of trihalomethane. Singh et al. [8] investigated the effect of Tanfloc on the removal efficiency of pollutants from domestic wastewater using a jar test. The effect of Tanfloc on the harvesting of algae from seawater was investigated by Roselet et al. [9]. The outcomes of these studies revealed a promising performance of Tanfloc as a treatment agent. To the best of our knowledge, the effect of Tanfloc as a pre-treatment agent on a biofilm bacterial community has not been investigated yet.

Tannin has an inhibitory effect on a wide spectrum of microorganisms; the suggested inhibitory mechanisms are inhibition of microbial enzymes, deprivation of substrate and metal ions required for the growth or direct effects on bacterial membranes. However, high concentrations of tannin compounds (hundreds of milligrams per liter) are required to show a serious inhibition of microbial growth [10]

In conventional biological wastewater treatment, ammonia-nitrogen ($\text{NH}_3\text{-N}$) is one of the pollutants of concern. In nitrification process, $\text{NH}_3\text{-N}$ is oxidised to nitrite ($\text{NO}_2\text{-N}$) as the first step and $\text{NO}_2\text{-N}$ is oxidised to nitrate ($\text{NO}_3\text{-N}$) as the second step commonly undertaken by the autotrophic bacteria *Nitrosomonas* and *Nitrobacter* respectively [11]. In biofilm processes, most of the biochemical oxygen demand (BOD) must be removed before the nitrifying organism can be established. In fact, the heterotrophic bacteria have a higher biomass yield and thus can dominate the surface of the biofilm carrier over nitrifying bacteria [12]. In other words, retaining a significant percentage of nitrifying bacteria within the biofilm community in biological units is difficult to be achieved because of their low growth rate. That is why its percentage in treatment processes is very minor and could be less than 1 % [13]. However, in some treatment processes with a favourable environment, nitrifying bacteria can thrive to present in a high percentage of 15 % of the total bacterial community [14].

In our previous work [15], the effect of Tanfloc as a pre-treatment for biofilm was studied in a big-scale pilot plant. However, the application of Tanfloc in the previous work was limited to only a few hours due to technical difficulties; as a result, the effect of Tanfloc on the bacterial community was not highlighted. In this work, the effect of the extended use of Tanfloc on the bacterial community in a biofilm unit was investigated in a bench-scale aeration tank.

2. Materials and methods

2.1. Materials

2.1.1. Tanfloc

Tanfloc SG was purchased in powder form and used in the experiment in solution form with a concentration of 1 g/L. This solution was prepared daily by dissolving 20 g of Tanfloc in 20 L tap water.

2.1.2. Municipal wastewater

A real municipal wastewater produced from a student hostel of Faculty of Engineering/ Universiti Putra Malaysia (which accommodates for 336 students) was used in the experiment. The main characteristics of this wastewater are listed in Table 1.

2.1.3. Biofilm carrier

Cosmoballs (a trademark of Pakar Management Technology/Malaysia) were used as biofilm carrier, they are hollow spherical polyethylene media with eight holes, each hole is 1 cm diameter, specific gravity of 0.9, the average diameter of 8 cm, and specific surface area of $160 \text{ m}^2/\text{m}^3$.

2.1.4. Bench scale aeration tank

In this study, the wastewater was pretreated in a big scale pilot plant explained in details in Hameed et al. [16], then the wastewater was pumped to a set of two units of 600 L storage tanks as illustrated in Fig. 1. In order to prevent the settlement of solids in the storage tank during the experiment, a submersible pump (controlled by a timer) was

Table 1
Raw wastewater characteristics.

Parameter	Unit	Average \pm Standard Deviation
Turbidity	NTU	58 \pm 9.7
Total suspended solids (TSS)	mg/L	78 \pm 14.5
Total dissolved solids (TDS)	mg/L	214 \pm 22
BOD ₅	mg/L	87 \pm 18
Chemical oxygen demand (COD)	mg/L	192 \pm 32
Conductivity	$\mu \text{ s/cm}$	386 \pm 34
Nitrate ($\text{NO}_3\text{-N}$)	mg/L as N	0.5 \pm 0.1
Nitrite ($\text{NO}_2\text{-N}$)	mg/L as N	0
Ammonia - nitrogen ($\text{NH}_3\text{-N}$)	mg/L as N	22 \pm 3.5
Total phosphate	mg/L as P	5.6 \pm 1.3
Temperature	C	28 \pm 2
pH		7.3 \pm 0.3

used in each storage tank to completely mix the water for 15 min in every hour.

Two identical peristaltic pumps (Heidolph PD 5206, Germany) were used to convey wastewater from each storage tank to subsequent aeration tank with the same flow for both parallel experiments. The effluent hose of the peristaltic pump was immersed to the bottom level of the aeration tank to ensure homogenization with the aid of air bubbles.

The two aeration tanks, each made of transparent PVC, 25 L with length 25 cm, width 23 cm, depth 50 cm, were used as two parallel aeration tanks. Each was filled with 20 pieces of Cosmoballs, which were confined between two horizontal perforated plates, one at the bottom (12 cm from the base of the tank) and one at the top (36 cm from the base of the tank). Each plate has thirty 2.5 cm diameter holes. Two identical air diffuser aquarium stones of 15 cm length and 2 cm width were glued to the bottom of each tank symmetrically. The four stones were connected to four aquarium flexible hoses of the same length and connected to four nozzles of an aquarium air pump. The intended symmetry of the aeration system ensures even distribution of the blown air to the two aeration tanks.

The wastewater was discharged from the aeration tank to a holding tank and directly discharged to the sewer. No secondary clarifier was used in this experiment; however, as a substitution for a secondary clarifier, the collected water from the aeration tank was allowed to settle for one hour in a 1 L beaker and the supernatant was considered as secondary settled wastewater (referred as effluent), this was done to maintain the same sedimentation condition spite of the increasing flow, to be able to distinguish the performance of aeration tank which is targeted in this paper.

2.2. Methods

2.2.1. Pre-treatment of the wastewater

The pretreatment process was not the same for the two identical aeration tanks. Wastewater underwent flocculation using Tanfloc in addition to sedimentation in one of the two experiments (with Tanfloc), while wastewater underwent only sedimentation process in the other experiment (without Tanfloc). Refilling of storage tanks by the pretreated wastewater was done regularly to supply enough water for the continuous flow experiment. Refilling process was usually done at midday to get almost the same quality of domestic wastewater, to reduce the effect of fluctuation to the minimum.

The flocculation process was conducted in a big-scale pilot plant as mentioned earlier. Tanfloc was made as a solution with a concentration of 1 g/L using tap water and was added by a dosing pump according to the optimum dose previously determined in the lab (35 mg/L) after adjusting the dosing pump. At the beginning, wastewater pump was off and Tanfloc was added manually to the flocculation tank, slow mixing in flocculation tank was allowed for 30 min (the design retention time), the flocs were formed clearly, finally, the continuous flow was run

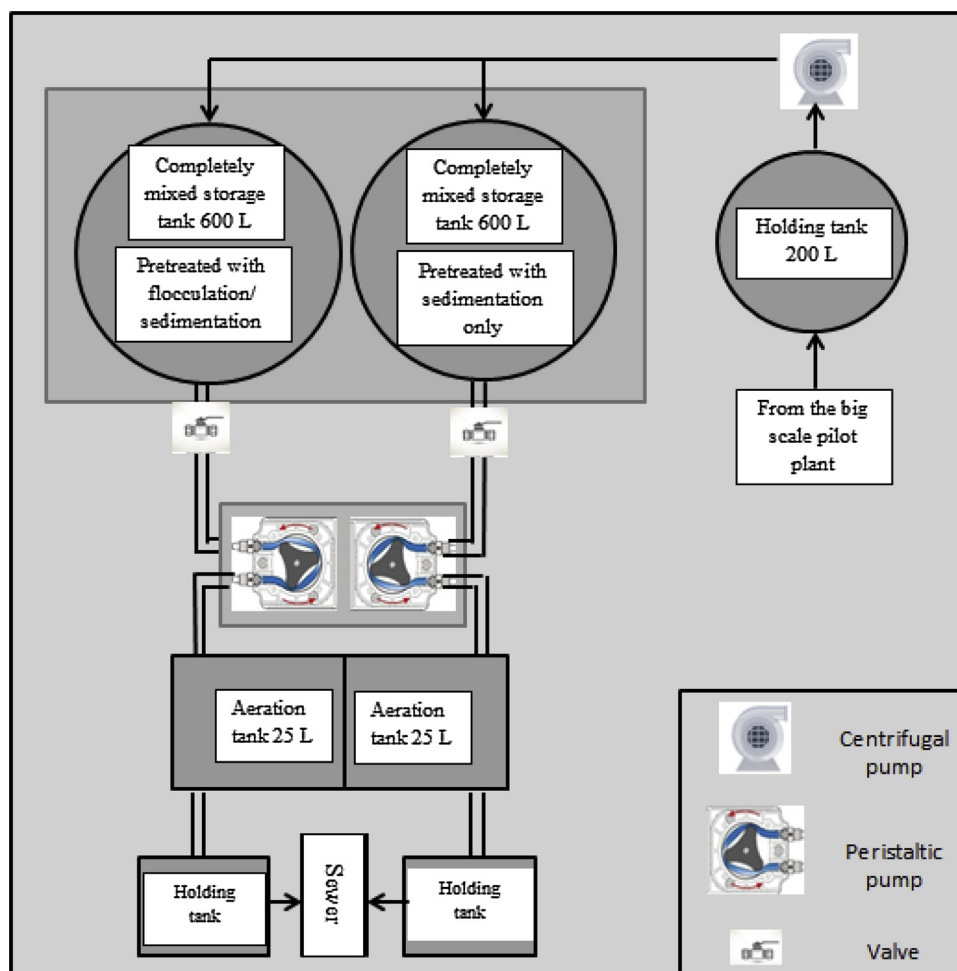


Fig. 1. Illustration of the bench scale treatment process.

simultaneously with the running of the dosing pump.

2.2.2. Sampling points and frequency

Five flows were investigated in this experiment, starting from 52 mL/min to 416 mL/min, to cover the hydraulic retention time (HRT) range of 8 to 1 h (as shown in Table 2). The evaluation began after the biofilm unit had been stabilized. Since real wastewater with fluctuating characteristics was used, it was not expected to maintain fixed removal efficiency. Consequently, when no significant improvement in performance was noticed (after about 20 days), it was considered that a steady state had been reached and the evaluation was started.

The evaluation was achieved by analyzing one sample from the storage tank (which represented the influent), and another sample from the supernatant of the settled water in the 1 L beaker mentioned in section 2.1.4 (which represented the effluent).

Each flow was evaluated in duplicate on two different days and was then left without any evaluation for one week to tolerate the change in performance after each flow increment. All the samples were tested for BOD, COD, TSS, NO_3 , NH_3 and pH.

2.2.3. Biofilm characterization (16S rRNA analysis)

Samples of the biofilm culture were taken from the pilot plant at the

Table 2

Flow rates and retention times investigated in the experiment.

Flow (mL/min)	52	69	104	208	416
Retention time (h)	8	6	4	2	1

end of the experiments with retention time of 4 and 2 h and sent for a characterization analysis. Samples of the detached biofilm taken from the outer surface of a few Cosmoballs, mixed and put in a 1.5-ml Eppendorf tube that was filled halfway with ethanol at -20°C , before being sent to a specialist lab for characterization analysis.

This test was used to identify the species of bacteria present and their percentage composition among the biofilm bacterial community. It offers an end-to-end sequencing analysis, including cluster generation, amplification, sequencing, and data analysis in a single instrument. To detect the bacteria (all the species) in the biofilm microbial community, a specific region (V3-V4) in the (16S) gene was amplified. The forward and reverse primers used in PCR analysis were 338 F (5'-ACTCCTACGGGAGGCAGCA - 3') and 806R (5' - GGACTACHVGGGT-WTCTAAT - 3').

The procedure comprised four main steps; the first one was DNA extraction (using MoBio Power Biofilm DNA Extraction Kit) according to the manufacturer's protocols. The next step was PCR amplification of the marker region of bacteria. The amplification reactions were performed in triplicate, a mixture of 20 μL (containing 4 μL of $5 \times$ Fast Pfu Buffer, 2 μL of 2.5 mM dNTPs, 0.8 μL of each primer (5 μM), 0.4 μL of Fast Pfu Polymerase, and 10 ng of template DNA) underwent certain reaction conditions (95 $^\circ\text{C}$ for 2 min, followed by 25 cycles at 95 $^\circ\text{C}$ for 30 s, 55 $^\circ\text{C}$ for 30 s, and 72 $^\circ\text{C}$ for 30 s and a final extension at 72 $^\circ\text{C}$ for 5 min). Amplicons were extracted from 2 % agarose gels and purified using the AxyPrep DNA Gel Extraction Kit (Axygen Biosciences, Union City, CA, U.S.) according to the manufacturer's instructions and quantified using QuantiFluor™. In the third step, analyzing by Illumina sequencing was conducted according to the standard protocols. Fourth

step was bioinformatics analysis using Quantitative Insights Into Microbial Ecology (QIIME version 1.9.1). The results of this test are shown as tables (alphabetically listed bacteria names with their percentages among the biofilm bacterial community) in phylum, class, order, family and genus level.

2.2.4. Analytical methods

All samples were analyzed for pH and BOD₅ in the same day but kept at 4 °C to be analyzed for ammonia on the next day. All analytical methods followed the American Public Health Association Standard Methods [17]. EUTECH instruments were used to measure Dissolved oxygen (DO) and pH.

3. Results and discussion

To evaluate the changes in the biofilm community, the nutrients concentrations, pH and DO should be highlighted due to their direct relationship to the bacterial community. Moreover, it is worth mentioning that regardless of the influent raw water temperature, it was almost stable at 30 °C in the aeration tank, and indeed, the large amount of air bubbles caused the stability.

3.1. Treatment efficiency

Tanfloc has a clear effect on treatment efficiency. The most significant effect of Tanfloc was the improvement in the nitrification process as shown in Table 3. There was no removal of ammonia at 4-h retention time unless Tanfloc was used.

Furthermore, the removal efficiency with Tanfloc was slightly lower as compared to the experiment without Tanfloc at a retention time of 8 and 6 h. This minor deterioration might be justified by the inhibitory effect of tannin on the nitrifiers [12]. In fact, high concentrations of tannin compounds (hundreds of milligrams per liter) are required to show a serious inhibition of microbial growth [10]. In this study, Tanfloc dose during the pretreatment process was only 35 mg/L, moreover, Tanfloc as a flocculant was removed with the settled flocs in the clarifier and the left concentration that entered the aeration tank was very low. Nevertheless, the inhibiting effect was overcome by the positive effect of Tanfloc on the ammonia removal process at a retention time of 4 h.

With an extremely short retention time of 2 h and 1 h, the removal of ammonia did not occur. However, this is normal because NH₃-N removal process is relatively slow and as such, the short retention time was not adequate to demonstrate any removal.

Nitrification process depends significantly on pH value. In fact, ammonia removal is an alkalinity-consuming process. The measurements of pH are depicted in Fig. 2, a noticeable drop in pH value coincided with efficient nitrification process (8-h, 6-h, and with Tanfloc 4-h experiments).

With regard to BOD concentrations, it is clear from Fig. 3 that the concentrations in the influent flow in the experiment with Tanfloc were far lower than those of their counterparts in the experiment without Tanfloc. In fact, it was obvious that the pre-treatment with Tanfloc reduced BOD concentrations by the enhancement of colloids removal,

keeping in mind that a certain portion of those removed colloids was organic.

Regarding the removal efficiencies for BOD, it was noted that during the short retention time (1 and 2 h) the process was unable to treat the high organic load and the BOD concentrations in the experiment without Tanfloc were significantly higher than their counterparts in the experiment with Tanfloc. In the experiment with Tanfloc, the influent organic load was low, and it was possible to achieve a high level of treatment despite the extremely short retention time.

3.2. DO level

DO decreased dramatically as retention time was decreased (flow was increased) as shown in Table 4. According to United States Environmental Protection Agency [18], the oxygen demand in the aeration tank depends mainly on the organic load. In fact, the more organic load in aeration tank the more heterotrophic bacterial activity and the more oxygen demand. The increment of flow leads to organic load increment, consequently, more oxygen consumption and less DO concentration.

At each flow rate, the DO level was higher when Tanfloc was used because a high percentage of organic materials (represented by BOD) had been removed during the pre-treatment as mentioned earlier, consequently, resulting in less heterotrophic bacteria activity and less oxygen consumption.

3.3. Biofilm bacterial community

The characterisation of the biofilm reveals a broad spectrum of bacteria genera for the biofilm samples that were cultured in the experiments with and without Tanfloc. However, the differences in the characteristics of the raw and flocculated water resulted in differences in the biofilm community. In other words, the characteristics of raw water may be suitable for certain species of bacteria to thrive, while other species can thrive in flocculated water. Considering the significant improvement to remove the ammonia in the experiments using Tanfloc, the bacteria of concern in this study, are centred on ammonia oxidising bacteria (AOB) and nitrite oxidising bacteria (NOB). Fig. 4 compares the relative abundance of AOB and NOB in the biofilm samples in the experiments both with and without Tanfloc, at a hydraulic retention time of 4 and 2 h.

3.4. The detected genera of AOB and NOB

The nitrification process consists of two steps. The first step is the oxidation of ammonia to nitrite by AOB, and the second step, is oxidising nitrite rapidly to nitrate by NOB. The genera recognised in the literature of AOB, includes; *Nitrosomonas*, *Nitrosospira*, *Nitrosovibrio*, *Nitrosolobus* and *Nitrosococcus*, while genera identified in the literature of NOB includes; *Nitrococcus*, *Nitrobacter*, *Nitrospira*, *Nitrospina* and *Nitrotyphis* [19,20]. The presence and domination of any of these genera will depend on the process and water characteristics.

By searching the genera list revealed by the 16S rRNA analysis, only *Nitrosomonas* was detected as AOB, and only *Nitrospira* was detected as

Table 3
Removal efficiencies of ammonia – nitrogen (mg/L) in the aeration tank.

Retention time (h)	Without Tanfloc			With Tanfloc		
	Influent to the aeration tank	Effluent of the aeration tank	% removal	Influent to the aeration tank	Effluent of the aeration tank	% removal
8	21.5 ± 4.9	4.5 ± 2.12	79.5 ± 4.95	23 ± 4.2	6.5 ± 2.1	72 ± 4.2
6	20.5 ± 0.7	6.5 ± 0.7	68 ± 4.24	23 ± 1.4	8 ± 1.4	65.5 ± 3.5
4	17.5 ± 0.7	17.5 ± 0.7	0	17 ± 0	5.5 ± 0.7	68 ± 4.2
2	23 ± 1.4	23 ± 1.4	0	22.5 ± 2.1	22 ± 2.8	2.5 ± 3.5
1	21 ± 1.4	21 ± 1.4	0	21 ± 1.4	21.5 ± 2.1	0

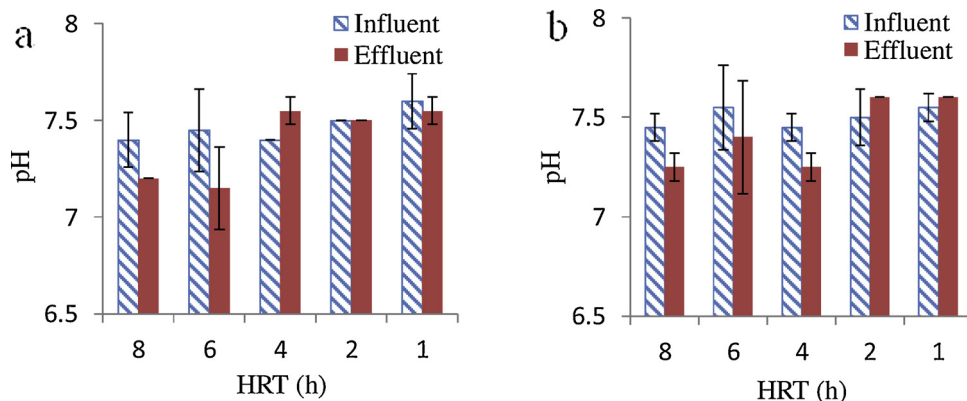


Fig. 2. pH values during ammonia oxidizing process a) Without Tanfloc b) With Tanfloc.

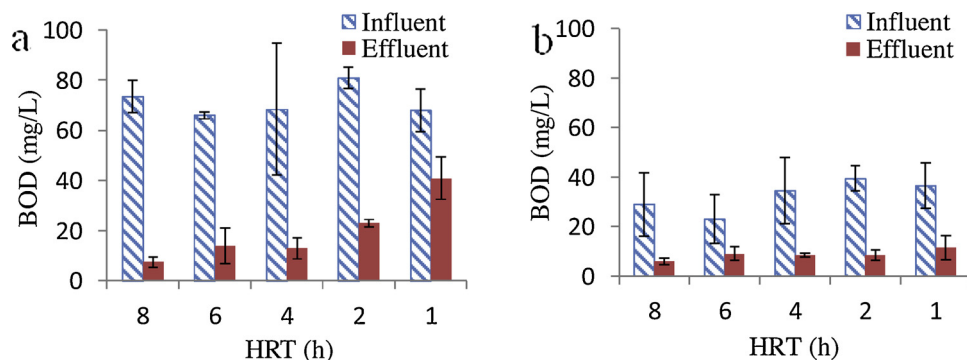


Fig. 3. BOD concentrations (mg/L) in the experiments a) Without Tanfloc b) With Tanfloc.

Table 4

DO level (mg/L) versus organic load (g/d).

	HRT									
	8 h		6 h		4 h		2 h		1 h	
	Organic load	DO	Organic load	DO	Organic load	DO	Organic load	DO	Organic load	DO
Without Tanfloc	5.17	6.6	6.5	4.6	7.5	3.5	23.4	1.25	37	1.1
With Tanfloc	1.5	7.3	1.6	6.2	3.75	5.1	10.8	3.85	18	2.9
Increasing %		10.5		35		46		200		163

Increasing = (DO with Tanfloc – DO without Tanfloc) / DO without Tanfloc.

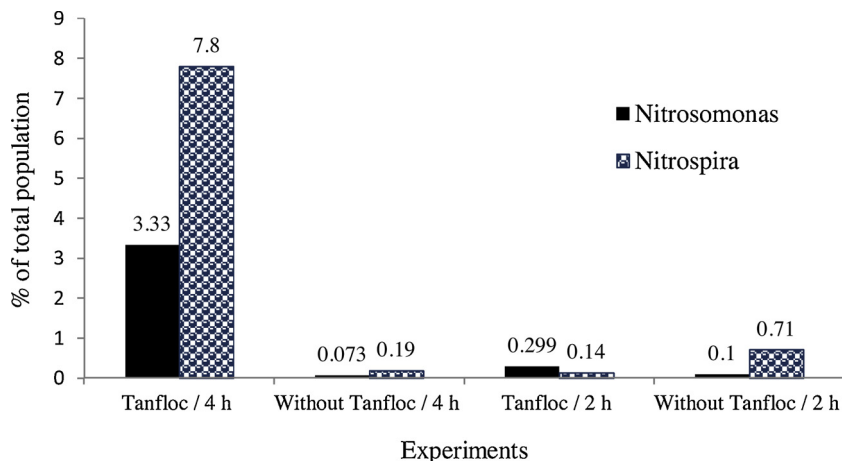


Fig. 4. Comparison between the percentage of Nitrosomonas and Nitrospira.

Table 5
Percentage of AOB and NOB in other experiments.

AOB %	NOB %	System	Reference
0.02 to 0.2	—	Biological aerated filter	[13]
3.1 to 5.4	2.7 to 4.6	Compact suspended carrier biofilm reactor	[24]
5	1	MLSS	[14]
18	5	Biofilm Carrier	
7	2	MLSS	[23]
15	3	Biofilm carrier	
7	1	MLSS	[23]
15	5	Biofilm carrier	
0.05	—	Moving Bed Biofilm Reactor	[22]
1	2	Membrane bioreactor	[23]
13 - 38	—	Sequencing batch reactor	[25]
3.33	7.8	Biofilm with Tanfloc	This study
0.073	0.19	Biofilm without Tanfloc	

MLSS: Mixed Liquor Suspended Solids.

NOB in both experiments with and without Tanfloc. In fact, both the two detected genera are the most common and important genera of municipal wastewater treatment plants amongst other genera [14,21]. These are also identified within the bacteria community in other studies [22,23].

Fig. 4 shows the percentage of *Nitrosomonas* and *Nitrobacter* among the total bacterial community. At a 4-h retention time, there was an extremely low concentration of both *Nitrospira* and *Nitrosomonas* observed in the experiment without Tanfloc, while Tanfloc showed a significant effect on the increment of these two species (3.3 % and 7.8 %). At a retention time of 2 h, the extremely low concentration of these two genera was observed in both experiments, with and without Tanfloc. These observations agreed with the removal efficiency of ammonia in these two experiments (refer to Table 3).

The relative abundance of the two species varies as reported in the literature depending on several factors in the experiments, including pH, C/N, hydraulic retention time, sludge age, DO level and other factors governing bacteria growth. Table 5 tabulates the percentages of AOB and NOB as reported by other studies.

The NOB was higher than AOB in the experiment with Tanfloc at the retention time of 4 h, and this phenomenon was also detected in other studies [23,26]. In fact, the biomass yield of NOB is far lower than that of AOB and the theoretical NOB/AOB ratio is about 0.5. However, there is a possibility to diverge from this trend if the metabolism of NOB is changed in such a way that their biomass yield increases. This could take place if the growth of NOB does not only depend on the nitrite, but also on other nutrients such as organic compounds which suggests a mixotrophic metabolism of NOB [27]. Another plausible explanation of this phenomenon was revealed by [28] that suggests a complete nitrification process by *Nitrospira* bacteria, in other words, *Nitrospira* possess the ability to oxidize not only nitrite but also ammonia, resulting in an increment in biomass yield.

3.5. The role of Tanfloc in creating a suitable environment for AOB

Dissolved oxygen (DO): the high concentration level is of immense importance for both AOB and NOB. The required oxygen level relies on many factors, with the thickness of biofilm being one such factor, where the nitrifying bacteria are distributed within the biofilm matrix. The bacteria located deep within the biofilm are exposed to lower DO concentrations, and therefore, a higher DO level is required to ensure sufficient diffusion of oxygen at the interior level of the biofilm. Although there is no specific minimum concentration of DO needed to achieve an efficient nitrification process, Tchobanoglous et al. [12] suggested that 2–3 mg/L of oxygen concentration is satisfactory for most of the suspended aerobic growth processes. For a normal nitrification process in biofilm, a much higher DO concentration may be required. It was evident from the measurements of DO in Table 4 that

Tanfloc caused an apparent jump in the DO level (as a consequence of reducing the organic load) thereby stimulating the ammonia removal process.

BOD concentration and load: high BOD concentration causes the inhibition of nitrifiers [29]. The C/N ratio of 0.3 led to the competition between heterotrophs and autotrophs with a detrimental effect resulting over the latter [30], in municipal wastewater C/N ratio is usually not less than 3 [31]. In the light of these C/N values, the opportunities for the autotrophs to dominate in wastewater are less. It is also anticipated, that the transport of ammonia from the bulk water phase to the ammonia oxidiser cell would be hindered by the presence of crowded cells of heterotrophs which consume the oxygen before it reaches the nitrifiers. Xia et al. [24] and Fatihah and Donnelly [13] mentioned that heterotrophs dominate the surface of the biofilm in the case of a high C/N ratio. Consequently, the nitrifiers will be buried in the lower layer of the biofilm causing difficulties in ammonia diffusion. Tanfloc significantly reduced the organic load in the aeration tank as shown in Fig. 3, consequently enable a more suitable environment for AOB.

4. Conclusion

The use of Tanfloc as a pre-treatment agent for municipal wastewater treatment showed a significant improvement in the nitrification process at HRT of 4 h while there was no removal of ammonia in the experiment without Tanfloc at 4 h of retention time, the removal efficiency was around 70 % when Tanfloc was used. This enhanced nitrification process promises the potential use of smaller aeration tanks in treatment plants.

The improvement in the nitrification process when Tanfloc was used reflected the significant increase in the percentage of AOB and NOB bacterial community as a consequence of the favourable environment due to the application of Tanfloc. At a hydraulic retention time of 4 h, the AOB and NOB percentages were 3.33 and 7.8 %, respectively in the experiment with Tanfloc. In contrast, there were only 0.073 % of AOB and 0.19 % of NOB in the experiment without Tanfloc.

CRedit authorship contribution statement

Yasir Talib Hameed: Methodology, Investigation, Formal analysis. **Azni Idris:** Conceptualization, Supervision, Funding acquisition. **Siti Aslina Hussain:** Resources, Writing - original draft. **Norhafizah Abdullah:** Methodology, Visualization. **Hasfalina Che Man:** Data curation, Writing - review & editing.

Declaration of Competing Interest

All authors have approved the manuscript and they have no conflicts of interest to declare.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.jece.2020.103679>.

References

- [1] M.C. Bongiovani, F.P. Camacho, P.F. Coldebella, K.C. Valverde, L. Nishi, R. Bergamasco, Removal of natural organic matter and trihalomethane minimization by coagulation/flocculation/filtration using a natural tannin, *Water*

- Treat. (2015) 1–10.
- [2] S. Ansari, J. Alavi, Z.M. Yaseen, Performance of full-scale coagulation-flocculation/DAF as a pre-treatment technology for biodegradability enhancement of high strength wastewater-recycling wastewater, *Environ. Sci. Pollut. Res.* 25 (2018) 33978–33991.
- [3] Z. Zhang, J. Wang, D. Liu, J. Li, X. Wang, B. Song, B. Yue, K. Zhao, Y. Song, Hydrolysis of polyaluminum chloride prior to coagulation: effects on coagulation behavior and implications for improving coagulation performance, *J. Environ. Sci. China (China)* 57 (2017) 162–169.
- [4] A.H.R. Aljuboori, A. Idris, N. Abdullah, R. Mohamad, Production and characterization of a bioflocculant produced by *Aspergillus flavus*, *Bioresour. Technol.* 127 (2013) 489–493.
- [5] N.A. Oladoja, Headway on natural polymeric coagulants in water and wastewater treatment operations, *J. Water Process Eng.* 6 (2015) 174–192.
- [6] J. Beltrán-Heredia, J. Sánchez-Martín, M. Gómez-Muñoz, New coagulant agents from tannin extracts: preliminary optimisation studies, *Chem. Eng. J.* 162 (2010) 1019–1025.
- [7] J. Sánchez-Martín, M. González-Velasco, J. Beltrán-Heredia, Acacia mearnsii de wild tannin-based flocculant in surface water treatment, *J. Wood Chem. Technol.* 29 (2009) 119–135.
- [8] R. Singh, S. Kumar, M. Garg, Domestic Wastewater Treatment Using Tanfloc: A Tannin Based Coagulant, *Geostatistical and Geospatial Approaches for the Characterization of Natural Resources in the Environment*, Springer, 2016, pp. 349–354.
- [9] F. Roselet, J. Burkert, P.C. Abreu, Flocculation of *Nannochloropsis oculata* using a tannin-based polymer: bench scale optimization and pilot scale reproducibility, *Biomass Bioenergy* 87 (2016) 55–60.
- [10] G.G. Gross, R.W. Hemingway, T. Yoshida, *Plant Polyphenols 2: Chemistry, Biology, Pharmacology, Ecology*, Springer Science & Business Media, 2012.
- [11] J. Yuan, W. Dong, F. Sun, K. Zhao, Low temperature effects on nitrification and nitrifier community structure in V-ASP for decentralized wastewater treatment and its improvement by bio-augmentation, *Environ. Sci. Pollut. Res.* 25 (2018) 6584–6595.
- [12] G. Tchobanoglous, F.L. Burton, Eddy Metcalf, H.D. Stensel, *Wastewater Engineering: Treatment and Reuse*, McGraw-Hill, 2003.
- [13] S. Fatihah, T. Donnelly, Spatial distribution of ammonia-oxidizing bacteria in the biofilm and suspended growth biomass of the full-and partial-bed biological aerated filters, *Can. J. Civ. Eng.* 36 (2009) 1859–1866.
- [14] J. Leyva-Díaz, A. González-Martínez, M. Muño, J. Poyatos, Two-step nitrification in a pure moving bed biofilm reactor-membrane bioreactor for wastewater treatment: nitrifying and denitrifying microbial populations and kinetic modeling, *Appl. Microbiol. Biotechnol.* 99 (2015) 10333–10343.
- [15] Y.T. Hameed, A. Idris, S.A. Hussain, N. Abdullah, H.C. Man, F. Suja, A tannin-based agent for coagulation and flocculation of municipal wastewater as a pretreatment for biofilm process, *J. Clean. Prod.* 182 (2018) 198–205.
- [16] Y.T. Hameed, A. Idris, S.A. Hussain, N. Abdullah, A tannin-based agent for coagulation and flocculation of municipal wastewater: Chemical composition, performance assessment compared to Polyaluminum chloride, and application in a pilot plant, *J. Environ. Manage.* 184 (2016) 494–503.
- [17] A.D. Eaton, M.A.H. Franson, Association, A.P.H. Association, A.W.W. Federation, W.E. Standard Methods for the Examination of Water & Wastewater, American Public Health Association, 2005.
- [18] United States Environmental Protection Agency, Evaluation of Energy Conservation Measures for Wastewater Treatment Facilities, [cited 2019 24 December]. Available from: (2010) <https://nepis.epa.gov/Exe/ZyNET.exe/P1008SBM.txt?ZyActionD=ZyDocument&Client=EPA&Index=2006%20Thru%202010&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&UseQField=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5CZYFILES%5CINDEX%20DATA%5C06THRU10%5CTXT%5C00000021%5CP1008SBM.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=20&ZyEntry=1&slide>.
- [19] G. Azziz, T. Trasante, J. Monza, P. Irisarri, The effect of soil type, rice cultivar and water management on ammonia-oxidizing archaea and bacteria populations, *Appl. Soil Ecol.* 100 (2016) 8–17.
- [20] J. Niu, I. Kasuga, F. Kurisu, H. Furumai, T. Shigeeda, K. Takahashi, Abundance and diversity of ammonia-oxidizing archaea and bacteria on granular activated carbon and their fates during drinking water purification process, *Appl. Microbiol. Biotechnol.* 100 (2016) 729–742.
- [21] A. Cydzik-Kwiatkowska, M. Zielińska, Bacterial communities in full-scale wastewater treatment systems, *World J. Microbiol. Biotechnol.* 32 (2016) 1–8.
- [22] K. Biswas, M.W. Taylor, S.J. Turner, Successional development of biofilms in moving bed biofilm reactor (MBBR) systems treating municipal wastewater, *Appl. Microbiol. Biotechnol.* 98 (2014) 1429–1440.
- [23] J. Leyva-Díaz, A. González-Martínez, J. González-López, M. Muño, J. Poyatos, Kinetic modeling and microbiological study of two-step nitrification in a membrane bioreactor and hybrid moving bed biofilm reactor-membrane bioreactor for wastewater treatment, *Chem. Eng. J.* 259 (2015) 692–702.
- [24] S. Xia, J. Li, R. Wang, Nitrogen removal performance and microbial community structure dynamics response to carbon nitrogen ratio in a compact suspended carrier biofilm reactor, *Ecol. Eng.* 32 (2008) 256–262.
- [25] J. Miao, Q. Yin, T. Hori, T. Aoyagi, H. Habe, G. Wu, Nitrifiers activity and community characteristics under stress conditions in partial nitrification systems treating ammonium-rich wastewater, *J. Environ. Sci.* (2018).
- [26] A. Benakova, J. Wanner, Application of fluorescence in situ hybridization for the study and characterization of nitrifying bacteria in nitrifying/denitrifying wastewater treatment plants, *Environ. Technol.* 34 (2013) 2415–2422.
- [27] M.K. Winkler, J.P. Bassin, R. Kleerebezem, D.Y. Sorokin, M.C. van Loosdrecht, Unravelling the reasons for disproportion in the ratio of AOB and NOB in aerobic granular sludge, *Appl. Microbiol. Biotechnol.* 94 (2012) 1657–1666.
- [28] H. Daims, E.V. Lebedeva, P. Pjevac, P. Han, C. Herbold, M. Albertsen, N. Jehmlich, M. Palatinszky, J. Vierheilig, A. Bulaev, Complete nitrification by *Nitrospira* bacteria, *Nature* 528 (2015) 504–509.
- [29] G. Zhu, Y. Peng, B. Li, J. Guo, Q. Yang, S. Wang, Biological removal of nitrogen from wastewater, *Rev. Environ. Contam. Toxicol. Springer* (2008) 159–195.
- [30] S. Ge, S. Wang, X. Yang, S. Qiu, B. Li, Y. Peng, Detection of nitrifiers and evaluation of partial nitrification for wastewater treatment: a review, *Chemosphere* 140 (2015) 85–98.
- [31] A. Khursheed, R.Z. Gaur, M.K. Sharma, V.K. Tyagi, A.A. Khan, A. Kazmi, Dependence of enhanced biological nitrogen removal on carbon to nitrogen and rCOD to sCOD ratios during sewage treatment in sequencing batch reactor, *J. Clean. Prod.* 171 (2018) 1244–1254.