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AMMONIACAL NITROGEN REMOVAL IN SYNTHETIC WASTEWATER USING SEAWEED-BASED IONIC LIQUID AND *BACILLUS SPP*.

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Abstract

Ammoniacal nitrogen (AN) is a hazardous component and a pollutant in rivers that is challenging to be treated using conventional chemical treatment. The purpose of the project was to remove the AN in synthetic wastewater using a seaweed-based ionic liquid and *Bacillus spp.* Bacterium, which is required to achieve the Department of Environment (DOE) environmental quality (industrial effluent) requirement 2009. It is set at 20 ppm AN for Standard B area. The Standard B is a water quality indexed set by the DOE to ensure the effluent discharge from industry. A denitrification and ion exchange process occurs in the bioreactor that remove the ammonia ions. The process variables used to evaluate the removal efficiency are pH and temperature while taking into account the COD, BOD and sludge of the effluent. The pH used was between 6 to 8 and a temperature that varies between 30°C to 40°C. Furthermore, to evaluate the effect of pH and temperature during the biological treatment process of ammonia removal. The optimum pH and temperature values set as design parameters are 7 and 37°C, respectively. The result was then used as a constant to study the optimum condition for the bacteria either in aerobic and anaerobic condition. It was found that the aerobic condition was the best condition. The ammonia removal rate was 94% with a final concentration of ammonia at 5.8 ppm treated using 12.0 g of bacteria for 24 hour in a bioreactor.

Keywords: Ammoniacal nitrogen, Bacteria, Ionic liquid, Denitrification process, Wastewater.

1. Introduction

The demand for clean water has increased throughout the years, which have revolutionized science and engineering in wastewater treatment to meet the demands of the society. Water demand is increasing every year due to the increasing population, rapid industrial development and climate change. Butler and Memon reported that the demand and usage of clean water increased in the past 31 years and has been forecasted to continuously increase in year 2025 to fulfil a population of 4800 million people but there is a decrease in water availability of 13,300 km^3/yr compared to year 1985 that only have a population of 2930 million people with water availability of 13,700 km^3/yr [1]. This data has shown how with an increasing population will require higher demand of clean water however the availability is drastically decreasing over the years.

With a continuous need of clean water, a better solution is required to meet the future demands. Wastewater has played a large contribution in providing water to the population and has a high potential to be further developed in providing further access to clean water in Malaysia [2]. Wastewater known as liquid wastes and wastes from households, commercial establishment, industries, storm water and other surface runoff water. Based on research, 43% of the water supply were from Malaysia rivers that originates from industrial wastewater [2]. The challenge is to provide access to clean water using wastewater to meet the Department of Environment (DOE) regulation based on the Environmental Quality Act 1974, which is the Environmental Quality (Industrial Effluent) Regulations 2009 as seen in Table 1.

Table 1. Water quality standard in Malaysia [4].

Parameters	Unit	Standard A	Standard B
pH	-	6.0-9.0	5.5-9.0
BOD5 at 20℃	mg/L	20	50
COD	mg/L	50	100
Ammoniacal nitrogen (AN)	mg/L	10	20

Referring to the DOE environmental report 2013, 72% of 473 rivers in Malaysia was found polluted and 25% were classified as highly polluted [3]. Furthermore based on the DOE environmental quality report 2014-2015, there was a decrease of ammoniacal nitrogen (AN) in polluted rivers from 151 in 2014 to 136 in 2015 [4]. The water pollution is classified in the form of Biochemical Oxygen Demand (BOD), AN and suspended solids (SS). The AN is a form of nitrogen in contact with water and is a challenged to be removed in wastewater based on the DOE environmental report. This shows a high potential threat to the environment in terms of water quality and water pollution. Thus, further research is required to overcome this issue.

Wastewater is treated before consumption. Based on the current wastewater treatment technology, ammoniacal nitrogen removal is not sufficient to meet the water quality index based on the environmental quality report 2015 [4]. The two types of treatment available for ammonia removal are biological treatment or chemical treatment but research has found that the biological has a better opportunity in terms of environmental and economical aspect [5]. Biological treatment involves utilizing the bacteria either aerobes or anaerobes to remove the ammonia through a denitrification or nitrification process. Aerobic bacteria require the presence of oxygen while an anaerobic bacterium does not require oxygen to break down ammonia. Chan et al. [5] reported on the advantage of aerobic removal efficiency but has a larger sludge disposal compared to anaerobic treatment. It was suggested to use anaerobic-aerobic bioreactors to overcome the limitations but has only been tested on a small scale compared to a large-scale wastewater treatment.

Referring to the properties, the aerobic *bacillus spp*. bacteria and is a denitrifying bacteria. Denitrification is the reduction of ammonium nitrogen to nitrogen gas. Foglar et al. [6] stated that denitrification is environmentally friendly and cost effective compared to nitrification which supports Soares assumption [7] that it may be the most economical strategy for polluted water. Zhou et al. [8] and Ergas and Rheinheimer [9] stated that denitrification has an advantage due to the nitrogen gas as the end product based on Van Der Hoek et al. research [10]. Also, Yang et al. [11] and Kim et al. [12] stated about the *Bacillus spp.* strain shows potential as a biological treatment bacteria to treat ammonia removal in soil and water. Although ionic liquid and zeolite have been studied to remove metal ions from wastewater but there has not been a research on the removal of AN. Thus, using ionic liquid to remove AN in wastewater is rather scarce.

2. Methodology

A two-step process applied in removing of AN. Firstly, biological treatment using the *Bacillus spp*. through denitrification process with an initial concentration of 100 ppm of ammonia solution in the bioreactor that was run for 24 hours. Secondly, 100 mL was extracted from the bioreactor into 4 sample sets that was used to improve the AN removal using the ionic liquid. Other than that, pH and temperature were set as a manipulated parameter to investigate the effect of the parameters to AN removal.

2.1. Material preparation

100 ppm ammonia solution added into the bioreactor for an autoclaving process. 1500 ppm NaOH and 36000 ppm NaCl was prepared and diluted using distilled water to 2000 ppm at 2L. Then, the solutions added into the bioreactor pH beaker.

2.2. Bioreactor setup

The bioreactor was cleaned, followed by filling with ammonia solution. The bioreactor was changed from an open system to a closed system by closing all the openings with either a clip and was covered with an aluminium foil to avoid damage to the equipment .Furthermore, the bioreactor was added into an autoclave at 121℃ and 0.1 atm for 15 min based on ISO standard 17655 [13]. The purpose was to sterilise the bioreactor internally and externally to ensure no biohazard contamination occurs.

After sterilizing, the bioreactor was set up with the set parameters to operate for 24 hr as a batch process. The run was tested using 4.0 g, 8.0 g and 12.0 g of bacteria at a constant condition of 37ºC, pH 7, 100 rpm and an aerator at 7.0 L/s. Urea was added into the bioreactor as a nutrient source to the bacteria with a ratio of 1.0 g of bacteria to 7.0 g of urea. Between the range of 4.0 to 8.0 g of urea is able to grow in a sustainable environment [14].

2.3. Improvement AN removal using the ionic liquid

After the denitrification process, 4 samples extracted from the bioreactor after 24 hr using a syringe attached to the bioreactor. Each of the samples collected consist of 50 mL and placed into a beaker. After obtaining the 4 samples, 1.0 to 4.0 mL of the ionic liquid added into the samples with an interval of 1.0 mL. Each sample covered using an aluminium foil and left for 5 min. After 5 min, the samples moved into a clean cuticle using a pipette before measuring the ammonia concentration using the spectrophotometer.

2.4. Experimental analysis and test

There were a total of 4 testing methods, which were AN reading using the American Standard Test Method (ASTM) using an UV/VIS spectrophotometer (Spectroquant® Prove 700, Merck). The jar test (Floc Tester, ET 750, Lovibond) was conducted and sludge volume index (SVI) was calculated.

2.4.1. Ammoniacal nitrogen (AN)

The AN nitrogen analysis methodology was adapted from ASTM D1426-08 [15]. A sample of 0.0, 10.0, 20.0, 30.0, 40.0 and 50.0 mL of the AN standard was prepared using deionised water. Then, the sample was mixed with Zinc Sulphate (ZnSO4) at 0.5 mL/L, which was then left to settle for 5 min. The sample was filtered using a filter paper to remove any sediment before adding 1.0 mL of Nessler reagent to avoid error in the photometer reading. After 30 min, each samples were added into a cuvette, which were placed in the photometer (Spectroquant® Prove 700, Merck).The absorbance measurement was set at 425 mm based on the standard and was tested using a blank (Nesslerized ammonia-free water) followed by each sample. A calibration curve was generated to calculate the value.

2.4.2. Chemical oxygen demand (COD) and Biological oxygen demand (BOD).

The COD and BOD value was analysed using the spectrophotometer. The COD test kit has a range of 15-300 mg/L while the BOD has a range of 0.5-3000 mg/L. Each test kit has a specific method of testing based on the ASTM standard. For the COD test, 2.0 mL of the sample was added to the Merck sample tube and shaken at the cap until its well mixed and then heated in a thermos-reactor for 2 hour at 150℃ [16].After 2 hour, the sample tube was cooled for 30 min on a rack and tested using the photometer. The test method for BOD was modified based on the winkler dissolved oxygen method [17]. There were 3 reagents, which were SP 1, SP 2 and SP 3. The sample was poured into an oxygenated bottle until its full and bubble free. 5 drops of SP1 and 10 drops of SP2 were added and slowly stirred for 10 sec and was closed and set aside for 1 min to react. 10 drops of SP 3 was added and stirred for another 10 sec. The sample left for 5 min before taking the sample from the bottle and tested in the spectrophotometer.

2.4.3. Jar test

The purpose of jar test was to know the amount of coagulant concentration needed for optimum sludge settlement, which was based on the ASTM jar test of water [18]. This jar test was highly related to sludge volume index to determine the amount of sludge in the mixture. This is because the amount of sludge determine the condition of the bacteria and consider a physical treatment before obtaining the final ammonia concentration. Lime was used as the coagulant component. 1000 mL of six samples were prepared after AN removal. The jars were placed on a stirrer (Lovibond ET 750, Taylors University) with paddles positioned perpendicularly to each beaker. At 10-40 rpm with an interval of 10, the liquid was mixed for 30 sec. The first beaker left as a blank while the other 5 beakers added with lime at different concentration. The samples then stirred for 60 sec at 75-300 rpm with 75 rpm interval. It reduced to 40 rpm for 160 sec and settled for 15 min. The appearance and time of settlement for each of the samples were recorded. Zinc sulphate used to reduce the turbidity in the water. The steps repeated at the intervals and the results compared to obtain the amount of coagulant concentration needed for the sludge to settle.

2.4.4. Sludge volume index (SVI)

The SVI was used to determine and compare the mixed solution to know the settleability in the liquid which were based on the Wastewater and Water standard of America [19]. This was to determine the amount of sludge used before and after the denitrification process and the relation between denitrification process and sludge volume. The volume includes mixed liquor solid settleability (MLSS) concentration and the formula can be seen in equation (1). The volume was in mL occupied by 1.0 g of activated sludge that has settled within 30 min as was related to MLSS concentration in g/L. The MLSS concentration was approximately same with the amount of organism in the bioreactor. A good SVI is when it was in between 80-100 [16].

$$SVI = \frac{Mixed\ Liquar\ solid\ settled\ in\ 30\ min}{\frac{\left(MLSS\ concentration\,\frac{mg}{L}\right)}{1000}} \tag{1}$$

3. Results and Discussion

3.1. Ammonia removal based on aerobic and anaerobic conditions

Two type of condition was applied to the bacteria in terms of aerobic condition or anaerobic condition and each conditions provides different in the sustainability of the bacteria that can result in a diverse difference in term of ammonia removal rate.

The two aerobic and anaerobic conditions inhibited different growth rate of the bacteria, which were highly co-related to the ammonia removal rate. By comparing the results, aerobic bacteria has a higher removal rate of 56 to 94 % while anaerobic was only able to remove 37 to 46%. The aerobic condition has a higher efficiency in AN removal compared to anaerobic bacteria. This could be highly due to the characteristic of AN that were readily biodegradable and has a high oxygen demand. Oxygen shows to be a limiting factor in bacterial growth [20]. Referring to the Table 2, AN removal rate are higher at the aerobic condition. The aerobic bacteria showed a better result and the anaerobic able to remove a certain amount of ammonia only. Thus, the growth of the bacteria has an ability to survive in both the condition.

Based on the study conducted by Sun et al. [21], the results of *Bacillus spp.* shows a similar growth trend in which the bacteria were able to grow in both aerobic and anaerobic conditions however it shows a higher growth rate in aerobic conditions. Two factors that affect the AN removal rate were the bacteria growth in different conditions and amount of bacteria added to the treatment. Thus, based on the results, 12.0 grams of bacteria in the aerobic condition has the highest ammonia removal rate of 94% in the ammonia biodegrading process.

Table 2. Ammonia removal rate based on aerobic or anaerobic conditions in 24 hour.

Condition	Bacteria (g)	Initial concentration, ppm	Final concentration, (ppm)	Ammonia removal rate (%)
Aerobic	4	100	44.0	56.0
	8	100	20.0	80.1
	12	100	5.83	94.2
Anaerobic				
	4	100	63.1	36.9
	8	100	59.2	40.8
	12	100	53.5	46.5

The COD, BOD and sludge weight are showed in Table 3. The aerobic condition shows a BOD of 3.2 mg/L and COD of 12.0 mg/L with an SVI of 91. The BOD and COD were within the range for the DOE requirement standard B of 10 mg/L and 100 mg/L, respectively. However, the initial BOD was 8.9 mg/L and dropped to 3.2 mg/L, which has only achieved a removal of 66%. Corsino et al. [22] able to achieve 90% removal of BOD that was higher than the resulted outcome. This could be because of the bacteria and wastewater composition used in the research. Furthermore, COD has an initial reading of 1261.0 mg/L and dropped to 12.0 mg/L that archived a 99% removal rate, which was an excellent result compared to Corsino et al. [22], that was only able to remove 90% of the COD. The BOD removal efficiency using a pre-treatment method or a filtering method recommended by Al-Jlil [23] were activated carbon and sand filtration. Based on the SVI at 91.000, there were still remaining organics suspended within the solution because it was above the range of 80 and states that there was still an amount of suspended organic that hasn't been removed yet [24]. This can be further improved by increasing the floc size of the sludge either by changing the coagulant or adding treatment beforehand as suggested by Rosman et al. [25].

Table 3. COD, BOD and sludge weight in based on 12 grams of bacteria.

Condition	Initial BOD (mg/L)	Final BOD (mg/L)	Initial COD (mg/L)	Final COD (mg/L)	SVI
Aerobic	9.6	3.2	1261	12	91

3.2. Improving the AN removal using the ionic liquid

Ammonia removal rate was further improved using ionic liquid after the denitrification process in the bioreactor. The ionic liquid was based on the ion composition of 40:60 of negative to positive ions based on the ionic liquid characteristic of 4 samples from the reactor were tested.

Table 4 shows the relationship of ionic liquid based on two conditions with different bacteria weight. For aerobic condition, 100 mL samples were tested using 1.0 -4.0 mL of ionic liquid with 1.0 mL interval which was set as a basis. 4.0 g of bacteria plus 3.0 ml of ionic liquid decreased the AN concentration to 6.8 ppm. It increased to 11.0 ppm with 4.0 ml of ionic liquid. 8.0 g of bacteria resulted decreased the AN to 19.5 mg/L using 2.0 mL of ionic liquid. A 12.0 g of bacteria decreased the AN to 11.1 mg/L using 2.0 mL of ionic liquid. It was found that the aerobic condition, certain amount of ionic liquid added would slightly increase the AN concentrations. After removing excess ammonia ions using the ionic liquid there no ions left to be bound and resulted in the excess ionic liquid to be in the solution. However, Fiset and Riveros [26], was studying on the removal of metal ions in wastewater but shows similar reasoning based on her results that there was an excess of ions due to ionic liquid unable to bind with the metal ions.

Table 4. AN concentrations (ppm).

Ionic liquid (mL)	AC (4.0 g)	AC (8.0 g)	AC (12.0 g)	ANC (4.0 g)	ANC (8.0 g)	ANC (12.0 g)
1.0	35.1	20.3	16.6	52.4	50.8	42.9
2.0	6.2	19.5	11.1	37.0	38.8	20.6
3.0	6.8	20.0	24.1	22.7	21.67	18.9
4.0	11.1	19.7	24.8	20.3	15.5	29.9

For anaerobic conditions, with 4.0 g of bacteria the concentration of ammonia decreases until 20.3 mg/L with the increase of ionic liquid added. For 8.0 grams of bacteria, AN concentration can be reduced to 15.5 mg/L and for 12.0 g of bacteria the ammonia concentration was until 18.9 mg/L then increased slightly to 29.9 mg/L. Based on the decrease of ammonia concentration in each of the samples, there were more un-bound ions compared to aerobic, which was due to the poor biodegrading of AN in anaerobic condition, which was similar to Rodríguez et al. [27] and Foresti et al. findings [28]. Since the initial bacteria denitrification stage produces poor results, ionic liquid was able to remove the excess ions that the bacteria. This results in a higher removal rate during the second removal process using the ionic liquid.

3.3. Effect of pH on AN removal rate

In the aerobic condition with 12.0 g of bacteria has the highest ammonia removal rate was selected to study the effects of pH on Ammoniacal Nitrogen removal ranged from pH 6 to 7 (Table 5). It was found that the highest ammonia removal was at pH 7 with a final concentration of 5.83 ppm with an ammonia removal of 94.2%, while the lowest was at pH 6 with a final concentration of 50.3 ppm with an ammonia removal of 49.7%. There was a slightly higher ammonia removal rate at pH 8 of 74.8% compared to pH 6, which states that there was a higher biodegrading process of AN in an alkaline environment compared to the acidic environment. This can be highly due to the characteristic of the bacteria to either be able to withstand an alkaline or acidic environment for the bacteria to fully function in a stressful growth environment.

Cheba et al. [29] found that the *Bacillus sp.* were highly stable between pH 7 and 8 and this condition was able to sustain its growth. In addition, Sabumon [30], found that the ammonia removal process using a batch process with pH less than 7 ammonia removal rate was 44%. A stressful growth environment can induce the bacteria into homeostasis mode in which the bacteria stopped multiplying and be in a standstill state to survive the harsh environment [31]. Thus, there was no biodegration of AN due to the inactivity of the bacteria. A bacillus spp. bacterium optimally grows at a pH of 7 and was slightly tolerant to an alkaline environment.

Table 5. Ammonia removal based on pH.

pH	Concentration (ppm)	Ammonia removal rate (%)
6	50.3	49.7
7	5.83	94.2
8	25.2	74.8

3.4. Effect of temperature on ammonia removal rate

The 12.0 g of bacteria with highest rate of removal of AN also selected to investigate the effects of temperature on AN removal. Temperature ranged from 30℃, 35℃, 37℃ and 40℃ were used in this study. Table 6 shows that the AN removal rate was around 50%, which slowly increased to 72% at 35℃. However, the temperature of 37℃ was initially tested and found that the removal rate was at 94% and was then decreases at 40℃ with a removal rate of 48%. The temperature then drops as it slowly increases to 40℃. *Bacillus spp*. has always been known as a thermophilic bacterium and a mesophilic bacterium depending on the genus [32]. For this experiment, the *Bacillus spp*. used was a mesophilic bacteria based on the bacteria characteristic. With this environment, the bacteria was not able to thrive beyond 40℃ and under 30℃ and has an optimum growth at 35-37℃ [32]. Lopex-Vaxquez et al. [33] also tested the removal of ammonia in industrial wastewater treatment using various bacteria and found that all failed to show bacterial activity above 40℃ which results in poor ammonia removal rate. Sudarno et al. [34], also tested until a temperature of 40℃ in a similar test and found that it was optimal between 37ºC. This shows that ammonia removal rate was highly dependent on the growth or condition of the bacteria.

Table 6. Ammonia removal based on the temperature.

Temperature (℃)	Concentration (ppm)	Ammonia removal rate (%)
30	50.341	49.659
35	27.730	72.270
37	94.173	94.173
40	51.712	48.288

4. Conclusion

Ammonia removal was conducted through biological treatment using bacteria with similar traits to *Bacillus spp*. and further improvement of ammonia removal using ionic liquid. Based on the two growth conditions which were aerobic and anaerobic, aerobic conditions provides the most suitable environment for denitrification process to take place. The amount of bacteria used in the aerobic conditions also was an important factor in which 12 grams of bacteria was able to remove 94%. Further ammonia removal was also highly dependent on the amount of ammonia ions that was inside the solution. In addition, the pH and temperature was an up most important parameter to ensure smooth ammonia removal and bacteria growth. Thus, the optimum pH was 7 and temperature of 37ºC

Based on the research objective, 5.8 ppm can be achieved under the aerobic conditions with 12.0 g bacteria and 20 ppm with 8.0 g of bacteria that was able to meet standard B set by the DOE. Thus, anywhere between the range of 8.0 to 12.0 g of bacteria in the aerobic condition for 24 hour was able to achieve the DOE standard B also taking into account the BOD, COD and SVI at 3.2 mg/L, 12.0 mg/L and 91.0. In conclusion, AN proposed treatment able to meet the DOE requirement with a removal rate of up to 94.2%.

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Abbreviations

AC	Aerobic condition
ANC	Anaerobic condition
AN	Ammoniacal Nitrogen
ASTM	American Standard Test Method
BOD	Biological Oxygen Demand
COD	Chemical Oxygen Demand
DOE	Department of Environment
NAOH	Sodium Hydroxide
NACL	Natrium Chlorida
SVI	Sludge Volume Index

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