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Ammonia nitrification of a petrochemical wastewater by the eight generation of microbial consortium of autotrophic bacteria

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Abstract

If present in drinking water, a nitrate concentration above the standard level may reduce to nitrite in the human digestive tract. It then converts hemoglobin to methemoglobin with a significant loss in oxygen-carrying capacity, which is responsible for developing methemoglobinemia. The nitrite may also react with amines forming carcinogenic nitrosamines. The wastewater of a petrochemical was initially treated with microorganism of the municipality of Shahrek-e-Ghareb located in Tehran. The prolonged, poor, and irreproducible results were perhaps due to the inorganic nitrogen in the wastewater and the higher ratio of heterotrophic bacteria present. The present study's results further indicated that autotrophic organisms are required to synthesize nitrification of wastewater containing inorganic nitrogen compounds. The autotrophic bacteria from wastewater of depot 93 of the Shiraz petrochemical plant was partially purified by the dilution method for 13 generations. The experimental results showed that the 8th generation microbial consortium was an effective source of bacteria for nitrification in the above wastewater. Hydrochloric acid was used to reduce the original pH of the wastewater, which was 10.5, to about the neutral range. The medium pH and alkalinity were increased to pH: 7.8 ± 0.1 by using 2 V/W % sodium carbonate, which resulted in an 80% ammonia oxidation of the wastewater. After examining the effects of parameters such as feed concentration, inoculum size, initial pH, shaker's speed, and temperature on nitrification, it was observed that using the 8th generation of a microbial consortium with a temperature maintained at about 29 ˚C, a shaker speed of 2.5 s-1, pH: 7.8, and inoculum size of 10 % V/V, resulted in approximately 85% ammonia oxidation when used in a Watson medium, and 80 % ammonia oxidation when applied to petrochemical wastewater. The data was fit into a Monod type equation, and an agreement of 99.68 % was obtained.

1. Introduction

The growth and activities of the urban population worldwide have resulted in a growing demand for fresh water to meet domestic, commercial, industrial, and agricultural needs, coupled with the depletion of its limited resources. Therefore, adequate treatment of fresh water turned to wastewater is required. However, challenges in this process, such as nitrogenous waste streams, must be solved. The volume of this type of wastewater is substantial and remains a current problem in many industries, i.e., in

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petrochemical, steel manufacturing, fertilizers, slaughterhouses, ammunitions, and farm runoff, that needs to be solved to meet the specified standards of each nation. Particularly increasing stringent environmental regulations are forcing companies to reduce the nitrate in wastewaters to the appropriate level. The influence of such compounds on global pollution is documented in the literature (Maere *et al*., 2016). The theoretical study of nitrification was initiated in 1940, and the first research on the removal of a nitrogenous compound was performed in southern California in 1969 Metcalf (Tchobanoglous, 2014). This microbial technique holds promise in providing lower-cost alternatives for nitrogen reduction than traditional energy-intensive treatment methods like ion exchange. The advantage of the biodegradation process over physico-chemical separation lies in the two-step conversion of nitrate into nitrogen gas (Capodaglio *et al*., 2015; Chawley *et al*., 2020). A nitrate concentration higher than the standard level enhances the corrosion rate due to the production of nitric acid and is toxic to fish and other vertebrates. The oxidation of [NH_4^+] to [NO_2^-] and then [NO_3^-] is an energy-yielding process, and certain microorganisms use the generated energy to assimilate CO_2. Nitrification is enhanced by the presence of enough oxygen, carbon type and concentration, and alkalinity. Carbon types play a major role in nitrification in certain processes. The nutritional requirements of the nitrifying autotrophs include ammonia, nitrite, carbon dioxide, carbonate, biocarbonate, dissolved oxygen, phosphates, sodium, calcium, magnesium, and iron.

Due to freshwater scarcity in many parts of the world, there are instances where the demand exceeds the available resources, and additional sources are not in reach even at a reasonably higher cost. In those cases, lower quality water, like treated wastewater, could be substituted to serve non-potable purposes. Therefore, the treatment of wastewater into fresh water that could be used in many medium and large-scale processes is an asset.

Literature has addressed the nitrification of municipal wastewater in many ways, i.e., using the suspended growth technique (Falås et al., 2013; Machineni et al., 2019). Further studies have shown that nitrifying bacteria have a proclivity to attach to surfaces and grow in tight clusters of cells or cysts (Picioreanu et al., 2016; Matsumoto et al., 2010; De Boer et al., 1991). In the seventies, many biological processes were equipped and treated with familiar and economical biofilm for nitrification and denitrification (Todt and Dörsch, 2016; Coelhoso et al., 1992; Van Benthum et al., 1998). The effect of initial N-NH_3 on ammonia oxidation was reported in several studies (McGhee, 1975; Zayed et al., 1986; Princic et al., 1998). A brief explanation of the mechanism is given below:

1.1. Nitrification

Lithoautotrophic nitrification: The sequential transformation of NH_4^+ via NO_2^- to NO_3^- is typically catalyzed by two phylogenetically distinct groups of bacteria, i.e., the ammonia and nitrite–oxidizing bacteria, respectively. The two main strains of these groups are Nitrosomonas and *Nitrobacter* spp. Other commonly used strains are, Nitrosobilis, Nitrospira, and Nitrovibrio (Attested *et al*., 1994; Koops, 2005). The growth rate of autotrophic ammonia-oxidizing bacteria is slow (Wijffels & Tramper 1995; Kits *et al*., 2017) and primarily controlled by substrate concentration, temperature, pH, alkalinity, light, agitation, and oxygen strength. Further, heavy metals, toxic chemicals, and pesticides have an adverse effect on nitrification, particularly Nitrosomonas. The rate-limiting step in nitrification is the conversion of ammonia to nitrite because the growth rate of Nitrosomonas is slower than Nitrobacter (Blackburne *et al*., 2007). In general, the nitrifying bacteria grows well in a temperature range of 20-35 ˚C, incubated in the dark. The autotrophic and hetretrophic microorganisms uptake and assimilate NH_4^+ and NO_3^-, convert the compounds into proteins, and grow unless the nitrogen source is depleted. The mechanism of nitrification has been summarized by (Moo-Young, 1985, Rezvani et al., 2019):

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Step 1 occurs in Nitrosomonas:

Ammonia (NH_3^+) + oxygen (O_2) + Alkalinity + Nitrosomonas →Nitrite (NO_2^-) $55\ NH_4^+ + 76\ O_2 + 109\ HCO_3^- \rightarrow C_5H_7O_2N + 54\ NO_2^- + 57\ H_2O + 10H_3CO_3$ (1)

Nitrosomonas converts NH_3 to NO_2 by the successive action of ammonia monoxygenase (AMO) and hydroxylamine oxidoreductase (HAO).

Step 2 occurs in Nitrobacter:

Nitrite (NO_2^-) + Oxygen (O_2) +Alkalinity + Nitrobacter → Nitrate (NO_3^-) (2)

$400\ NO_2^- + NH_4^+ + 4H_2CO_3 + HCO_3^- + 195\ O_2 \rightarrow C_5H_7O_2N + 3H_2O + 400NO_3^-$ (3)

The oxidation of nitrite is two electron steps; electrons are released from the enzyme-bound substrate. Moreover, it is not yet clear whether the actual substrate is a nitrite ion, undissociated nitrous acid, or a hydrated form of either one. However, nitrite oxidoreductase is repressed in the absence of nitrate. Nitrobacter consists of three proteins and cytochrome c, an undefined quinone, and NADH dehydrogenase are the constituents of the enzyme system (Sundermeyer-Klinger *et al*., 1984). Furthermore, the oxidoreductase enzyme containing iron, molybdenum, and a high of sulfur, may be present in the membrane that carries out the stoichiometric reaction as follows:

$NO_2^- + H_2O \Leftrightarrow NO_3^- + 2H^+ + e$ (4)

$2H^+ + 2e^- + 0.5O_2 \rightarrow H_2O$ (5)

Reaction (4) is reversible in an anaerobic environment; however, the significance of the reverse reaction to their survival is unclear since nitrite and nitrate are rapidly reduced. Further, ammonia and nitrite oxidizers are aerobic organisms that require a reasonably high amount of oxygen and an approximately 1 unit higher pH than neutral for growth and sound reaction.

However, there is a scant research work available in the published literature addressing nitrification of cumbersome petrochemical wastewater using fresh water from a river. However, was decided a schedule to overcome the problem and accomplish the gap realized. This study attempted to obtain an effective microbial consortium, which is a key parameter in nitrification. Additionally, the effect of parameters such as microbial inoculum and count, pH, carbon type and concentration, shaker's speed, and medium temperature on the nitrification of the above wastewater was experimentally studied. Biofilm formation on a solid particle of higher and lower density than water was developed to obtain packed stationery and fluidized beds, respectively. Furthermore, the data of such a process would help pave the way for a procedure in platforming for a larger-scale investigation.

2. Materials and methods

2.1. Microorganism, medium and analysis

The isolation schedule was performed by using wastewater samples from depot 93 of the Shiraz Petrochemicals plant. The microbial consortium was partially purified by the dilution method employing the Watson medium and phenol red as the indicator. The 8th generation was used as the main source of autotrophic nitrifiers organisms. In addition, environmental factors, such as microbial inoculum and count, pH, carbon type and concentration, shaker's speed, and medium temperature, on nitrification, have been investigated.

Wastewater from depot 93 of the Shiraz Petrochemical plant was used to partially purify the microbial consortium using the dilution Method. A series of dilutions from 10^{-1} to 10^{-4} in sequence in Erlenmeyer flasks containing 100 mL of Watson's medium and added phenol red as an indicator was used to isolate effective autrophic nitrifiers (Watson *et al*., 1981). In addition to monitoring the medium color changes from red to yellow, macroscopic observations were also checked. The dark covered shaker was gyratory at

2.5 s^{-1}, and its temperature was maintained at 29˚C with an initial pH of 7.8. The color of the medium was initially red and gradually changed from deep pink to yellow, and as the reaction progressed, the color change occurred more rapidly. The medium pH was adjusted by adding a few drops of sterilized 2% W/V sodium carbonate.

As a result, the 8th generation partially purified consortium was found to be comparatively more effective and therefore selected for further studies. The 8th microbial consortium was gradually adapted with sterilized wastewater, and then the concentration of the wastewater was increased from 10 to 100%. The wastewater was first autoclaved for 10 minutes. The composition of the initial wastewater from depot 93 is reported in Table 1.

Table 1. Analysis of wastewater of depot 93

No.	Medium	Initial ammonia (ppm)	Initial Nitrate (ppm)	Initial phosphor (ppm)	TDS (ppm)	BOD (ppm)
D	Depot 93	700-1500	400-1000	10	1200	90

A few media were initially selected from Bergey's manual (1989) and Prokaryotes handbook (Starr *et al*., 2013), and the Watson medium was chosen for this study as represented in Table 2.

Table 2. Different medium used

No.	Medium	Initial Nitrate (ppm)	Initial ammonia (ppm)	Initial pH	Reference
A	ATCC 2265	---------	1125	8.5	www.atcc.org
B	Watson et al.	----------	499		Koops et. al 2005
C	Synthetic	135	50	8	Nakos and Wolcott 1972
D	Depot 93	400-1000	700-1500		Present study

During the treatment, the pH of the medium gradually dropped, which indicates that the reaction is progressing on the proper track and acid carbonic is being produced, which turns the red colored medium (due to the phenol red indicator) to yellow (Watson *et al*., 1981; Starr *et al*., 2013; Moo-Young, 1985). The nitrification was performed for [NH_4^+], [NO_2^-], and [NO_3^-], and the samples were analyzed using a Unicam 8620 UV/VIS spectrophotometer (Fresenius *et al*., 1988). The reagent from the Macherey-Nagel (MN) Company (Germany) was used.

The MN has used an ammonia analysis procedure developed by Boltter, Bushman, and Tidwell (1961), which is a sensitive photometric determination of ammonia and is based on the reaction with phenol and hypochlorite to give an indophenol that is intensely blue in an alkaline medium and is read at 625 mμ against a blank reagent by spectrophotometric measurements. Analysis of the nitrate was performed using the MN reagents, which developed a yellow color in wastewater and had a spectrophotometer measurement of 430 nm. The analysis of nitrite (NO_2^-) was carried out as explained in Clescerl et al.'s Standard Methods for the Examination of Water and Wastewater (1999). The principle of the method is based on spectrophotometric measurements of NO_2^- through the formation of a reddish-purple azo dye produced at pH 2.0 to 2.5 by coupling diazotized sulfanilamide with N- (1-naphthyl)-ethylenediamine dihydrochloride (NED dihydrochloride) and finely powdered zinc (the reagent contains the compositions) and then measuring the color at 540 nm wavelength.

Results and discussion

3.1. Effect of microbial source

The effect of two different sources of microorganisms on ammonia oxidation was studied using a synthetic Watson medium and the wastewater from depot 93. Initially, the synthetic medium and wastewater of depot 93 were inoculated with 10% V/V Shahrak-e-Gharb municipality wastewater as the microbial source. Sampling and analysis were continuously performed for 30 days without achieving effective and reproducible nitrification results.

Furthermore, it appears that the heterotrophic bacteria initially inoculated needed a proper carbon type and concentration. The mixed culture from depot 93 resulted in complex behavior with improper effect on lengthy rate of ammonia reduction. The results presented in Figure 1 illustrate that the mixed culture containing a larger amount of heterotrophic microorganisms was unable to oxidize the level of nitrogenous compound present in the wastewater in the desired time span.

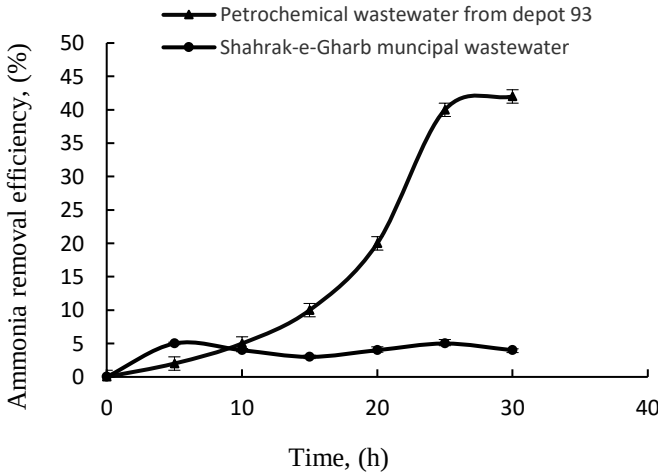

Figure 1. Wastewater source from Shahrak-e-Gharb and petrochemical wastewater

The poor results achieved in the Shiraz Petrochemical plant's wastewater from depot 93 were perhaps due to the presence of a higher ratio of inorganic nitrogenous compounds and a shortage of the proper type and percent of carbon material. Hereafter, a schedule to acquire a partially purified autotrophic bacterial consortium from the wastewater of depot 93 was designed based on the dilution method. The appropriately sterilized Watson medium with phenol red as an indicator (added separately) turned yellow with the pH reduction during the progress of the reaction.

The dilution method started with a serial 10^{-1} to 10^{-4} inoculation of 10 % V/V of wastewater from depot 93. The experiment was continued until the 10^{-4} diluted content of Erlenmeyer turned yellow. Then, the medium pH was adjusted by adding 2 % w/v sterilized sodium carbonate drop wise in the solution. The content of the Erlenmeyer flask (10^{-4} diluted) of 10% V / V was used for the following dilution preparation. The microscopically observed yeast and impurities present in the fifth generation of dilution were substantially less. The second shaker of wastewater was used to examine the effect of the consortium on the ammonia reduction through 13 generations. Experimental results showed that the 8th generation of the partially purified bacterial consortium was more effective than 9, 10, 11, 12, and 13th, and consequently, it was used for further studies. The objective to enhance the nitrification rate was achieved from date to about hours. The effect of different microbial sources, carbon types at defined molar ratios, temperature, initial pH, and shakers speed on nitrification was studied. A kinetic model based on the Monod type was developed.

3.2. Effect of carbon source

The influence of the two different carbon sources on the ammonia oxidation and initial pH variations is illustrated in Figure 2 using the 8th generation of autotrophic consortium. The carbon sources were added to the Watson medium, and the treatment of the wastewater from depot 93 was similar. The carbon sources were used to maintain the pH and provide the alkalinity of the medium. The observed results presented in Figure 2 show that the sodium carbonate in the solution has a stronger effecting edge than many carbon-containing compounds used in the present study. So, the effect of the molar ratio of carbon to nitrogenous compounds (CN^{-1} = 5) added to the medium was examined. In addition, the effects of Na_2CO_3, $CaCO_3$, NH_4 HCO_3, K_2CO_3, and $NaHCO_3$ of (CN^{-1}) 5 were also examined. Results showed that the ammonia oxidation increased as the oxygen accessibility and uptake increased in the medium. As the reaction progressed, nitric acid and carbonic acid production increased, the medium pH decreased, and nitrification ceased. It was also found that the type of inorganic carbon source and agitation speed effectively maintaining the alkalinity of the medium and provided the required CO_2. Calcium carbonate has low

solubility in water and results in an unfavorable pH of about 9; carbonate of potash is similar for the same reason. Ammonium bicarbonate has limited solubility in water and is temperature-dependent, generating a harsh pH environment. Adding sodium carbonate in the tap water to make up 2% w/v resulted in effective ammonia oxidation, which may be due to the availability of moderate alkalinity environments and enough CO_2 in the medium.

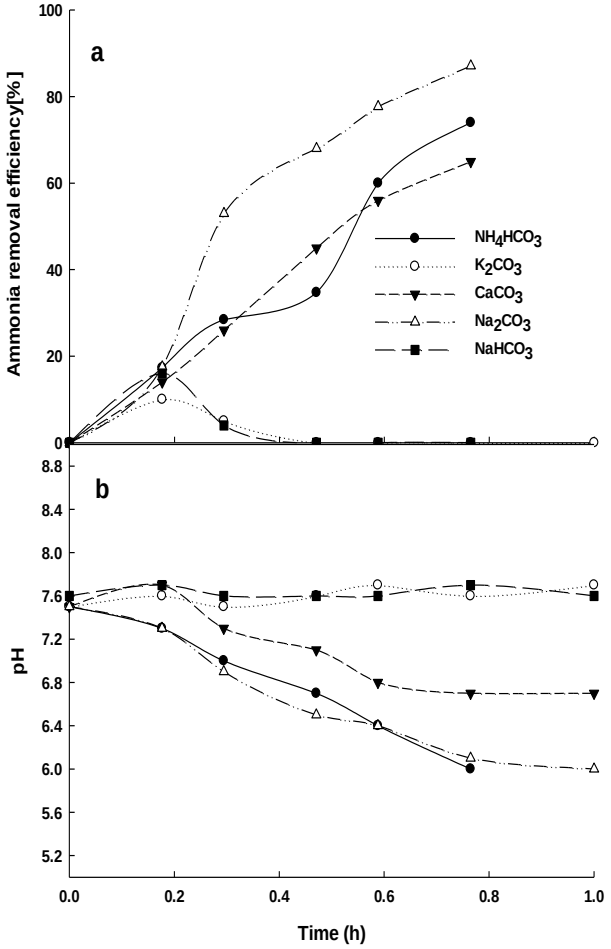

Figure 2. Effect of various carbon sources on nitrification of wastewater of depot 93 of the Shiraz Petrochemical plant.

3.3. Effect of medium temperature

The nitrification process can often be limited by temperature variation. In addition, variables such as heavy metals, pH, and ammonia concentration also strongly influence the process rate. Figure 3 shows that as the process temperature increased above 20 ºC, ammonia removal increased. Also, as the medium temperature rose to about 29 ± 0.5ºC, ammonia oxidation increased by about 75%. However, as the process temperature increased above 29 ºC, say about 32 ºC, the ammonia removal was reduced to 60 %, with the defined conditions. Therefore, a medium operating temperature of 29 ± 0.5 ºC was used in the following experiments in the present work. It was observed that a degree centigrade (ºC) variation in the medium temperature had a significant effect on ammonia oxidation, which is similar to the findings of Attested et al. (1994).

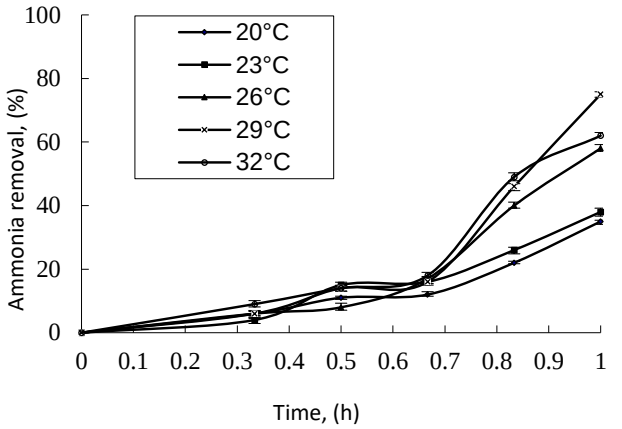

Figure 3. Effect of temperature on the ammonia removal using the wastewater of depot 93 of the Shiraz Petrochemical plant.

3.4 Effect of medium pH

The effect of a wide range of initial pH variations (from 7 to 9) on ammonia oxidation was studied. The initial pH of the wastewater of depot 93 of the Shiraz Petrochemical plant was 10.5. By adding a predefined concentration of hydrochloric acid (HCl), it was reduced to about 7. Next, a 2 % w/v sodium carbonate solution was added to adjust the medium pH to about 7.8 ± 0.1. These results were obtained during process verification, while the carbon and alkalinity level was maintained constant. Nitrification does not usually occur in extreme environments because pure cultures of nitrifiers, such as Nitrosomonas and Nitrobacter, have an optimal pH of approximately 7.5 to 8 (Bock *et al*., 1986; Ydstebo, 1991). The ammonia oxidation employing the synthetic medium and the petrochemical wastewater are depicted in Figures 4 and 5, respectively. As can be observed, adding

specific amounts of carbon species increased the initial pH of the medium from 7 to 7.8, which enhanced the ammonia oxidation.

Moreover, as the initial pH of the medium increased to about 8 ± 0.1, ammonia oxidation ceased. However, we realized that the initial pH of 7.8 ± 0.1 was more effective for ammonia oxidation than a pH of 8 using the wastewater. As illustrated in Figures 4 and 5, the ammonia oxidation achieved was 80 and 85% using the petrochemical wastewater and Watson medium, respectively.

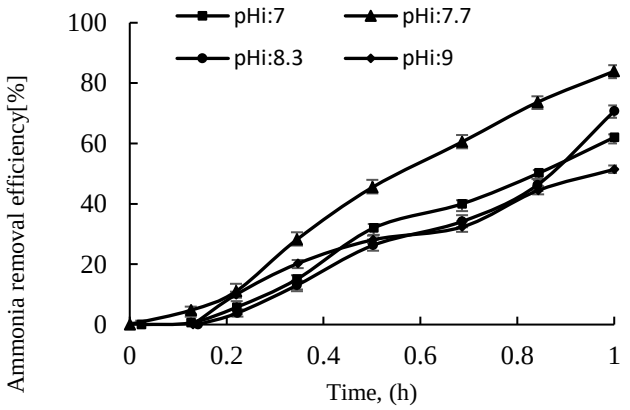

Figure 4. Effect of pH on ammonia removal using Watson medium.

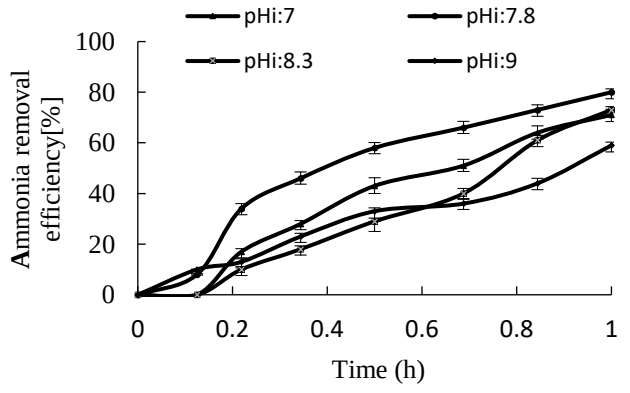

Figure 5. Effect of pH on ammonia removal using wastewater from depot 93 of the Shiraz Petrochemical plant.

Alkalinity, pH, and carbon dioxide are interrelated and exist in equilibrium. However, the alkalinity drops due to ammonia oxidation by nitrifiers; therefore, proper optimization of the process was required to keep it stabilized. As the reaction progressed, nitric acid and carbonic acid concentration in the medium increased. This accumulation of acids decreased the environment pH to less than 6, dropping it to as low as 5.6. In order to achieve sound nitrification, sufficient alkalinity should be present in the medium to balance the acidity and required CO_2. However, if buffering capacity of the medium can no longer be maintained using chemicals, the reduction in medium pH can be stopped by aerating the environment to reduce CO_2 in the medium and supply oxygen. Ydstebo suggested that all or part of the pH gradient was usually governed by the proton–yielding nitrite oxidation (Ydstebo, 1991).

3.5. Effect of shaker's speed

The effect of a wide range of shaker speed, from 0.84 to 3 s^{-1}, on ammonia oxidation was investigated. Original wastewater from depot 93 of the Shiraz Petrochemical plant was used for the ammonia removal studies. The shaker was covered, maintained at 29 ˚C, with a medium with an initial pH of 7.8 ± 0.1 (adjusted first by hydrochloric acid and then with sodium carbonate in the solution), and inoculated with 10% V/V of the 8^{th} generation partially purified bacterial consortium. Figure 6 shows that the ammonia reduction increased when the shaker speed increased from 0.84 to 1.67 s^{-1}. About 80% ammonia reduction was achieved using the wastewater by further increasing the shaker speed to 2.5 s^{-1}. It should be noted that increasing the shaker speed higher than 3 s^{-1} had shown no notable effect on ammonia reduction. Therefore, a shaker speed of about 2.5 s^{-1} was used for further experimental investigation.

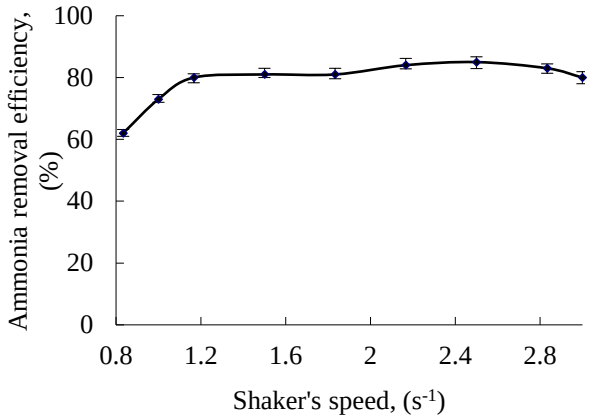

Figure 6. Effect of shaker speed on ammonia reduction of the wastewater of depot 93 of Shiraz Petrochemical plant.

Reproducibility

The experiments was performed in duplicate and the average values are reported. Values plotted are Mean ± SD of two replicates.

3.5. Kinetic verification of the process

An overall reaction for the partially purified autotrophic microbial consortium(*Nitrosomonas* and *Nitrobacter*) was presented using a Monod type of equation to obtain μ:

$$\mu = \mu^* \frac{S}{K_S + S} \tag{6}$$

The ammonia reduction could be related to the nitrifier growth rate according to:

$$-r_A = \frac{\mu}{Y_A} = -\frac{dC_A}{dt} = \frac{r_A^* C_A}{K_A + C_A} \tag{7}$$

Where r_A is the nitrification rate, and Y_A and C_A are the nitrifiers yield coefficient and ammonia concentration, respectively. Arranging and integrating equation (7) to obtain:

Rearranging equation (8) to linearise:

$$\frac{r_A^*}{K_A} t = \ln \frac{C_{A_0}}{C_A} + \frac{1}{K_A}(C_{A_0} - C_A) \tag{8}$$

$$\frac{C_{A_0} - C_A}{\ln\left(\frac{C_{A_0}}{C_A}\right)} = -K_A + r_A^* \left(\frac{t}{\ln\left(\frac{C_{A_0}}{C_A}\right)} \right) \tag{9}$$

Equation (9) fits the experimental data well with R^2 = 99.68%, as presented in Figure 7.

$$\frac{C_{A_0} - C_A}{\ln\left(\frac{C_{A_0}}{C_A}\right)} = -80 + 976.65 \left(\frac{t}{\ln\left(\frac{C_{A_0}}{C_A}\right)} \right) \tag{10}$$

Furthermore, μ was calculated using K_A = 27.459 and r_A^*= 0.2534, considering the ammonia concentration varied from 1 to 1000 mg/L. Consequently, the specific growth rate also increased value from μ = 0.0089 to 0.246 s^{-1}. For example, the specific growth rate μ = 0.174 s^{-1} was obtained for the ammonia concentration of 60 mg/L. Further improvement of the other parameters resulted in an 85 % ammonia reduction using the Watson medium. Under the specified conditions, an 80% ammonia reduction in the petochemical wastewater was reached in less than 40 to 45 minutes.

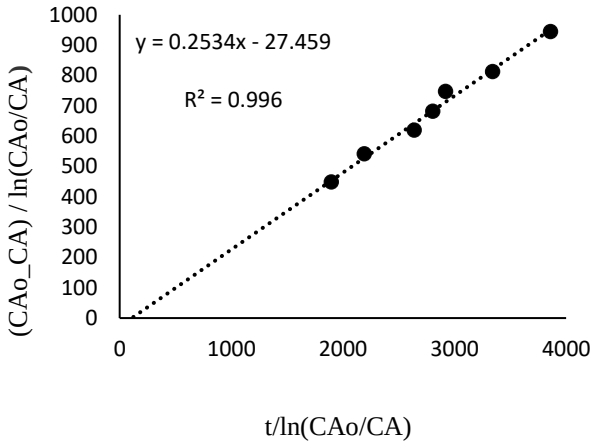

Figure 7. Fitting experimental data to equation (9).

The original medium (wastewater from depot 93) took 14 days to oxidize using partially Shahrek-e-Ghareb municipality wastewater as the inoculant; this was perhaps due to a higher ratio of heterotrophic to autotrophic bacteria, the inorganic nitrogen compound, and a harsh medium. The Watson medium containing phenol red as an indicator was used to isolate autotrophic bacteria from depot 93 of the Shiraz Petrochemical plant through 13 generations by applying the dilution method. By experimentation, it was found that the 8^{th} generation of the isolate was more effective than the others for ammonia oxidation. A carbon to nitrogen (CN^{-1}) molar ratio of 5 was examined using Na_2CO_3, $CaCO_3$, NH_4HCO_3, K_2CO_3, and $NaHCO_3$, and it was found that sodium carbonate in a solution of 2% (W/V) resulted in effective ammonia oxidation. The volume percent of petrochemical wastewater was increased to act as a nitrogen source in the experiments.

The 8^{th} microbial consortiums generation obtained from serial dilution was adapted and finally examined with a 10 V/V% inoculum of depot 93 wastewater, which resulted in an effective autotrophic microbial consortium for the removal of ammonia from the wastewater. However, when the initial pH of the wastewater was first decreased to neutral by hydrochloric acid and then increased to pH 8, an adverse effect on ammonia oxidation occurred. Eventually, a solution of 2 % w/v of sodium carbonate (Na_2CO_3) caused in a desirable carbon source to maintain the alkalinity and pH of the medium at about 7.8 ± 0.1, which resulted in the effective oxidation of ammonia. The dark covered shaker with a gyratory speed maintained at 2.5 s^{-1}, a temperature of about 29 ± 0.5ºC, and an initial pH of 7.8 ± 0.1 was found to be the optimum operating parameters. Finally, incorporating these optimized parameters, 80% removal of ammonia from the wastewater of depot 93 was achieved. The data of the present work fit kinetic equation 9 well, with an agreement of $R^2 = 99.68\%$.

Conflict of interest

The authors declare that there is no conflict of interest.

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Ethical approval

This manuscript does not contain any studies with human participants or animals performed by any of the authors.

Nomenclature

C_A: Ammonia concentration (mgL^{-1})
C_{A0}: Initial ammonia concentration (mgL^{-1})
C/N Molar ratio of carbon to nitrogen
K_A: Ammonia concentration at which $\mu = 0.5\mu^*$ (mgL^{-1})
K_S Kinetic constant (mgL^{-1})
ppm: Part per million
r_A: Nitrification rate ($mgL^{-1}\ s^{-1}$)
r_A^*: Maximum nitrification rate ($mgL^{-1}\ s^{-1}$)
S: Consumption substrate concentration for growth microorganism (mgL^{-1})
Y_A: Nitrifier yield coefficient

Greek letters

μ: Growth rate of the biomass (s^{-1})
μ^*: Maximum growth rate of the biomass (s^{-1})

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