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Isolation and Optimization of Ammonia Nitrogen (AN) Degrading Bacteria from Industrial Wastewater

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ABSTRACT

Ammonia Nitrogen (AN) is the major pollutant in water pollution. A large number of nitrogens containing compounds was discharged from industrial and agricultural production causing the problem of nitrogen pollution in water environment. As we know, ammonia nitrogen discharged into water bodies will result in water quality decline or water Eutrophication and will pollute the environment health. A total of two bacterial XY1,XY2 were isolated from the wastewater sample using enrichment culture techniques. The degradation ability of XY1, XY2 was further characterized under different growth conditions, including temperature, pH, and initial ammonium nitrogen concentration. The results showed that XY1 had the highest degradation efficiency at a temperature of 35°C , a pH of 7.0 and initial ammonia nitrogen concentration of 200 mg/l , the degradation of ammonia nitrogen in wastewater initial concentration of 131 mg/l was observed. The result of XY2 showed that degradation efficiency at temperature of 30°C a pH of 7 and initial ammonia nitrogen concentration 30 mg/l , the degradation of ammonia nitrogen in wastewater initial concentration of 131 mg/l . The consortium applied for the degradation of ammonia nitrogen , the highest amount of degradation observed at 131 mg/l concentration Finally, based on the efficiency of these isolated bacteria it can be concluded that effective ammonia removal can be achieved using these bacteria as consortium is suitable method for efficient ammonium removal from industrial wastewater and reduction of ammonia concentration.

Keywords: *Industrial Wastewater, Biodegradation, Ammonia Nitrogen Degradation, amonical nitrogen degrading strain, Ammonia nitrogen removal by consortia.*

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INTRODUCTION

In India, with major development and growth in the industrial, agriculture and petrochemical sector various chemicals are released in large amount. The majority of industry releases different types of pollutants like phenolic compounds, quinoline, ammonia nitrogen etc. These chemical compounds cause major damage to the environment, harm to human health and aquatic animals [1]. Ammonia nitrogen is a major pollutant for the environment. So, when ammonia nitrogen discharged into the water it decreases the quality of water which causes eutrophication and makes water in-habitat for aquatic life [2]. Eutrophication in water also causes decrease in dissolved oxygen (DO), reduce transparency, algal proliferation and increase turbidity [3-10]. Some of the effects of ammoniacal nitrogen are Acidification: Ammonia nitrogen can contribute to acidification of the environment. When ammonia nitrogen is converted to nitrate, it can release hydrogen ions, which can lower the pH of the soil and water [11]. Air pollution: Ammonia nitrogen can also contribute to air pollution. When it is released into the air, it can react with other pollutants to form fine particulate matter, which can cause respiratory problems in humans and animals [12]. Human health: Exposure to high levels of ammonia nitrogen can cause irritation of the skin, eyes, and respiratory system. It can also lead to headaches, nausea, and vomiting [13]. Damage to crops: Ammonia nitrogen can also damage crops by altering soil pH and nutrient availability, and by causing toxicity in plants [14]. Several detection methods have been developed for the analysis of ammonia

nitrogen in water. Physical, chemical and biological are some of the methods for treating ammonia nitrogen [15] including spectrophotometric methods and ion-selective electrodes [16]. Ion exchange method, breakpoint chlorination method, electrochemical method etc. are some of the physical and chemical methods. Biological methods are commonly used for ammonia nitrogen degradation because they are a sustainable and effective approach to removing this pollutant from industrial wastewater [17]. There are several reasons why biological methods are preferred over other methods of ammonia nitrogen removal: Environmentally friendly: Biological methods use natural processes to remove pollutants, and therefore have a lower environmental impact compared to other methods, such as chemical treatment. Cost-effective: Biological methods are typically less expensive than other methods, such as adsorption or membrane filtration, as they require less energy and fewer chemicals. High removal efficiency: Biological methods can achieve high levels of removal efficiency, especially when coupled with other treatment processes such as membrane filtration [15]. Ease of operation: Biological methods are relatively easy to operate and maintain, requiring only periodic monitoring and adjustment [18]. Overall, biological methods are a sustainable and effective approach to ammonia nitrogen removal from industrial wastewater. They offer several advantages over other methods, including environmental friendliness, cost-effectiveness, high removal efficiency, versatility, minimal waste generation, and ease of operation [19]. In this experiment we have isolated different organisms of ammonia nitrogen degrading bacteria along with we have also identified a degradation efficiency of ammonia nitrogen capacity strains from industrial wastewater. Preliminary physiological and biochemical identification was also conducted.

MATERIALS AND METHODS

Materials [1] [20]

The source of sample

The sample of wastewater sample was collected in a sterile container from industrial area of Ahmedabad.

The culture medium

a) The enriched medium: distilled water 500mL, $C_6H_{12}O_6$ 2.5 g, $(NH_4)_2SO_4$ 1.0 g, K_2HPO_4-3H $_2O$ 0.5 g, NaCl 1.0 g, $MgSO_4 \cdot 7H_2O$ 0.25 g, $FeSO_4 \cdot 7H_2O$ 0.2 g, pH 7.2 - 7.4, 121˚C stream sterilization 20 mins [1].

b) The isolation medium: add 20% agar to the enriched medium, 121˚C stream sterilization 20 mins [1].

c) The screening culture medium: 5% of $(NH_4)_2SO_4$ solution was added gradually and other components with the same as the isolation medium [1].

d) An Ammonia nitrogen removal detection medium containing 1.0 g of NaCl;0.5 g of K_2HPO_4; 0.25 g of $MgSO_4 \cdot 7H_2O$; 30mg,80mg and 200mg of ammonium sulfate (corresponding to 102,298 or 810ppm of AN), and a certain amount of glucose in 1,000 ml of filtered water was prepared, and the pH levels were adjusted to the required values (e.g., 6.0, 7.0, and 8.0) with HCl or NaOH.[20]

Methods

Culture conditions [1]

The wastewater sample was added at a ratio of 10% to enrichment culture medium which was then cultured at 30˚ C on a rotary shaker at 150 rpm. Each day 1 mL of 5% $(NH_4)_2SO_4$ solution was added in the enrichment culture medium for adaptation of organisms This step was continued about 7 days. After that, the microorganisms isolated from enrichment culture medium were inoculated into screening culture medium with a 5% inoculation quantity and 1ml of 5% $(NH_4)_2SO_4$ solution was added continuously for about 7 days. Two weeks the bacteria were cultured with NH_4^+ as a sole source of nitrogen. To preserve the isolated bacteria N-agar slant was used.

Morphological and biochemical identification of isolated bacteria

(A) Morphology:

Morphological characteristics were identified through naked eyes, which show shape, size, surface, transparency, edge, roughness, pigmentation.

(B) Biochemical Characteristics:

Different types of bacteria exhibits different biochemical activities, for identification of this different biochemical activity of bacteria various biochemical tests are done [22].Isolated organism's biochemical characteristics were tested including, Indole test, Voges-Proskaur test, Starch hydrolysis, Glucose fermentation, Sucrose fermentation, Citrate test.

- Indole test is carried out for the organism which has the ability to degrade tryptophan, an amino acid. An amino acid tryptophan undergoes deamination and hydrolysis by bacteria which releases tryptophanase enzyme [23]. These enzyme convert tryptophan into indole gas, which is detected by using Ehrlich's reagent and Kovac's reagent.

Ehrlich's reagent contains ethanol whereas Kovac's reagent contains dimethyl amino benzaldehyde in isoamyl alcohol and concentrated hydrochloric acid (HCl). The indole gas produced when react with these

reagents red color rosindole dye as a ring structure on the surface of medium will form which indicates the positive results [22].

- Voges-Proskaur test is used to identify those bacteria which produce acetoin (acetyl methyl carbinol or 3-hydroxybutanone) as an intermediate product via butanediol pathway. This intermediate product is identified by using alpha-naphthol and 40% KOH. Acetoin is oxidised to diacetyl in presence of KOH. Diacetyl react with the peptone component, guanidine which gives red color as a positive result [22] [23].
- Starch hydrolysis test is used to identify those organisms which produce the alpha amylase and oligo-1,6 glycosidase enzyme that help them to hydrolyse the starch. This test is also used to differentiate between the strains *Clostridium* and *Bacillus*. Iodine is added into the agar medium in order to check hydrolysis of starch which will turn to dark brown color indicating the positive result [22].
- Citrate test is used to identify those organisms which produces catalase enzyme. Hydrogen peroxide when kept on the slide in which the culture are introduced, if the bacteria produce catalase enzyme the enzyme will neutralize the hydrogen peroxide and bubbles will produce within 10 second which indicates the positive results [22] [24].

(C) Degradation of Ammonia nitrogen:

(i) Experiment of Ammonia nitrogen Degradation using synthesized medium:

For activating the culture, 5 ml of bacterial suspension was inoculated in Nutrient broth. Optimization activity of bacterial culture with different parameters like ammonia nitrogen concentration, pH, and temperature was checked. To perform degradation activity 30 mg/L, 80mg/L, 200 mg/L, 3000 mg/L, 8000 mg/L, 20,000 mg/L of ammonia nitrogen concentration was taken, the initial pH value was 6,7,9 and the temperature value was 25°C, 30°C, 37°C, 40°C.

(ii) Experiment of Ammonia nitrogen Degradation in wastewater:

Different wastewater was taken from chemical industries and wastewater treatment plants which contain higher amount of BOD (Biological Oxygen Demand) and COD (Chemical Oxygen Demand) value. The initial ammonia nitrogen concentration of wastewater sample was 131 mg/L, 640 mg/L. 10% inoculum size of isolated bacterial suspension was taken for ammonia nitrogen degradation. Also, simple consortia of bacterial suspension were taken to perform an ammonia nitrogen degradation experiment.

(D) Detection method of ammonia nitrogen:

Ammonia nitrogen was monitored by using pelican equipment Kelplus-Distyl EMS semi-automatic kjeldhal distillation unit **[25] [26]**.

The Kjeldahal method may be broken down into three main steps:

DIGESTION- The decomposition of Nitrogen in organic samples utilizing a concentrated Acid solution. This is accomplished by boiling a homogenous sample in concentrated sulphuric acid and digestion catalyst. The end result is an ammonium sulphate solution.

DISTILLATION- Distillation involves adding base to the acid digestion mixture to convert NH4+ to NH3.This is followed by boiling. Finally, NH3 gas is condensed and trapped in a receiving solution (boric acid).

TITRATION- Quantifying the amount of Ammonia ions in the receiving solution the percentage of Nitrogen can be calculated.

REAGENTS: In kjeldahal method we used 0.02N H_2SO_4 solution, 0.1N NaOH, phenolphthalein indicator, Boric acid solution for detection of ammoniacal nitrogen solution.

PROCEDURE: 10 ml sample was taken, into which 0.1N NaOH was added along with 2-3 drops of phenolphthalein indicator, 25 ml of Boric acid solution was placed simultaneously for ammoniacal nitrogen detection. After 3-4 min titration was carried out by using 0.02 N H_2SO_4 until the color changes from green to purple. This reading was considered as Biuret (A) reading.

RESULTS AND DISCUSSION

Identification of bacteria:

1) Colony observation

Two bacteria colonies were isolated from the wastewater, XY1 and XY2. On the basis of Gram staining method and morphological observation the XY1 bacteria was gram-positive rod-shaped bacteria whereas the XY2 bacteria were gram-negative short rod bacteria.

2) Biochemical observation

Table no 1: Results of biochemical test of the organisms "+" indicates a positive result, while "-" indicates a negative result, "++" indicates a strong positive.

Optimization activity And Ammonia nitrogen Degradation activity:

1) Effect of pH:

The pH level of cultural media affects the growth of microorganisms. The high pH value, either too high or too low, is not conducive for the cell growth [24].

Ammonia nitrogen removal was also impacted by the pH of the synthetic medium in this study. Results showed that the XY1 bacteria can only degrade ammonia nitrogen at a rate within a narrow pH range (6.0–9.0), and that higher acidic pH or alkaline pH conditions have a negative impact on bacterial activity.

Figure.1 shows the ammonia nitrogen degradation of XY1 at the different pH levels ranging from 5 to 9 and initial concentration of ammonia nitrogen was 8mg/l .The XY1bacteria at pH 5,that cannot survive, at pH 6 able to degrade 51%, at pH 7 the degradation rate increased 71% after 96 h incubation time. When the pH was 9, degradation rate was 10%. So that the optimum pH of XY1 was 7. At the pH 9 the XY1 bacterial cells loss of water due to excessive negative ionic attraction and exchange. Less degradation observed at pH 9 which indicate that the alkaline pH conditions inhibited cell growth. When the pH is too high or too low, the ammonia nitrogen degradation enzymes lose their capacity to function. Because the enzyme was denature so the reactions of ammonia nitrogen degradation was not properly working in the nitrification and dinitrification pathway.

Ammonia nitrogen removal was also affected by the pH of synthetic medium in this studies showed that the XY2 bacteria can degradation rate within narrow pH range(5.00-9.0) , and higher acidic pH,or alkaline pH conditions have negative effects on bacteria activity and also limiting their ability to remove the ammonia nitrogen

Figure.2 depicts the XY2 ammonia nitrogen degradation rate at various pH values from 5 to 9, where the original ammonia nitrogen content was 8 mg/l. After 96 hours of incubation, XY2 bacteria have a 38% degradation capacity at pH 5, a 70% degradation capacity at pH 6, and a 9.7% degradation capability at pH 9. The ideal pH for XY2 was therefore 6. The XY2 bacterial cells may experience water loss at pH 9 as a result of excessive negative ionic attraction and exchange. Less degradation was seen at pH 9 around 9.6%, At the acidic pH XY2 bacteria could degrade ammonia nitrogen because they were isolated from chemical industrial waste water and could withstand acidic pH while inhibiting cell growth in alkaline pH settings. The ammonia nitrogen degradation enzymes become inactive when the pH is too high or too low. The ammonia nitrogen degradation processes in the nitrification and dinitrification pathway were not functioning properly because the enzyme was denatured.

2) Effect of Temperature:

Temperature is an essential factor for microbial survival; it can influence microorganism development. Because enzymes are required for the absorption and utilization of growth substances, the metabolic process of bacteria changes with temperature. [27].

Figure.3 shows the effect of temperature on the ammonia nitrogen degradation using XY1. The temperature ranges from 25°c to 40°C. The initial concentration of ammonia nitrogen was 8 mg/l. The isolated XY1 bacteria at 25 °C temperature, degraded around 25% , at 30°C 46% , at 35°C around 70% degradation after 96h incubation time , and the 40°C around 20% . So that the optimum temperature of XY1 was 35°C. At the 25°C the ammonia nitrogen degradation rate was low that indicating the lower temperature inhibited the bacteria XY1, However the highest amount of ammonia nitrogen degradation rate at 35°C temperature and also higher temperature 40°C decrease the degradation rate. At 35°C the bacterial cell density and substrate biomass uptake capacity was increased. While the 40°C high temprature , that can cause the denature (lose of enzyme shape) , stop working and decrease the degradation rate.

Figure. 4 shows the effect of temperature on the ammonia nitrogen degradation using XY2. The temperature ranges from 25°c to 40°C and initial concentration of NH3-N was 8mg/l . The isolated bacteria XY2 at 25 °C 30% degradation of ammonia nitrogen , at 30°C ***78%*** degradation after 96h incubation **time** , at 35°C around 43% degradation , at 40°C 19% . Therefore, the optimum temperature of XY2 was 30°C. At the 25°C the ammonia nitrogen degradation rate was low indicating the lower temperature inhibited the bacteria XY2, However the highest amount of ammonia nitrogen degradation rate at 30°C temperature and also higher temperature 40°C decrease the degradation rate. At 35°C the bacterial cell density and substrate biomass uptake capacity was increased. While the 40°C the highest temperature, that can cause the denature (loss of enzyme shape), stop working and decrease the degradation rate. This process was irreversible, which can result in massive death of XY2 bacteria, only small number of XY2 bacteria was surviving. Thus the growth of XY2 bacteria affected by temperature.

3) Effect of ammonia nitrogen degradation (by using synthesized medium):

Figure. 5 shows ammonia nitrogen degradation, of the synthesized media having ammonia nitrogen concentration was 30, 80, 200 mg/L with 10 % of inoculums size. Low and high concentration of NH3-N was affected degradation effect. When the 30mg/l initial concentration, the degradation rate was 89% and the 80 mg/l concentration, the degradation rate was 41%, and the 200 mg/l concentration, the degradation

rate was 90% after 96h incubation time. At low concentrations, the degradation of ammonia nitrogen can be rapid and efficient, as the microbial populations can readily utilize the ammonia nitrogen as a nitrogen source for growth and metabolism. However, at higher concentrations, the degradation may be slower, as the microbial populations may become inhibited. At high concentrations of ammonia nitrogen can inhibit the growth of some microorganisms, leading to a reduction in the overall rate of degradation, and Some XY1 bacteria died, and the adsorbed NH3-N to it was desorbed, which led to a slight increase in NH3-N concentration. Ammonia nitrogen degradation using synthesized media having (**Figure. 6)** ammonia nitrogen of the 30, 80,200 mg/L with 10 % of inoculums size, the isolated XY2 the 30 mg/l concentration of NH3-N, the degradation rate was 91% and the 80 mg/l concentration, the degradation rate was 92% ,and the 200 mg/l concentration, the degradation rate was 60% after 96 h time . At low concentrations, the degradation of ammonia nitrogen can be rapid and efficient, as the microbial populations can readily utilize the ammonia nitrogen as a nitrogen source for growth and metabolism. However, at higher concentrations, the degradation may be slower, as the microbial populations may become inhibited, at high concentrations, the microbial populations may become surviving and unable to keep up with the rate of ammonia nitrogen input. This can lead to an accumulation of ammonia nitrogen in the synthetic medium environment, which can increase the concentration of ammonia nitrogen because Some XY2 bacteria died, and the adsorbed NH3-N to it was desorbed, which led to a slight increase in NH3-N concentrations.

Figure. 7 shows ammonia nitrogen degradation of wastewater sample, at the different ammonia nitrogen concentration of 3000, 8000, 20000 mg/L with 10 % of inoculums in which the degradation at 3000, 8000, 20000 mg/l concentration was 47 %, 22.26%, 28.2% by XY1 isolated bacteria after 216 h incubation time .because the very higher amount of ammonia nitrogen concentration that can affect the bacteria growth, also the long lag phase are required for the adaptation in to higher concentration. High concentrations of ammonia nitrogen can lead to changes in the pH of the synthetic medium, which can affect the growth and metabolism of the microbial populations. As the concentration of ammonia nitrogen increases, the pH of the medium

may become more basic, which can inhibit the activity of acidophilic microorganisms, the availability of other nutrients and electron acceptors can also affect the degradation of ammonia nitrogen. For example, the availability of oxygen, nitrate, or organic carbon can affect the rate and efficiency of ammonia nitrogen degradation.

Figure. 8 shows ammonia nitrogen degradation of wastewater at the different ammonia nitrogen concentration of 3000, 8000, 20000 mg/L with 10 % of inoculums in which the degradation was 42.01 %, 37.08%, 46.36% by isolated XY2 bacteria after 216 h incubation time. At the 20000 mg/l concentration of ammonia nitrogen degradation rate was higher than 3000mg/l because the At high concentrations, the microbial populations may become surviving and unable to keep up with the rate of ammonia nitrogen input. This can lead to an accumulation of ammonia nitrogen in the synthetic medium environment, which can increase the concentration of ammonia nitrogen because Some XY2 bacteria died, and the adsorbed NH3-N to it was desorbed, which led to a slight increase in NH3-N concentrations.

4) Effect of ammonia nitrogen degradation (by using wastewater):

We had inoculated the single bacteria XY1 and XY2 with 10% inoculum and its consortia into the wastewater sample which contain 131 mg/L of ammonical nitrogen concentration into it. Results shows that **(Figure. 9)** XY1 bacteria degradation rate was 93.21% in 92h time period whereas the XY2 colony degradation rate was 93.52 % in 92h time period. Consortium (mixture of XY1+XY2) 10% inoculum size degraded the 95.54 of ammonia nitrogen in wastewater sample. So that in the wastewater the consortium was degraded the maximum amount of NH3-N. Consortium of microorganisms is more effective in the degradation of ammonia nitrogen than single culture of organisms, Ammonia nitrogen is complex compund so that requires the different enzymes to the degradation. So the consortium contain mixture of bacteria that can work together and degrade the ammonia nitrogen efficiently. Consortium can may grow in wider range of optimal growth condition and thus have higher chance to better degradation of ammonia nitrogen. The presence of consortium may enhance the overall stability of the degradation process, that bacterial mixture provide mutual support and prevent the dominance of single bacteria. So the consortium of microorganisms can be more effective in the degradation of ammonia nitrogen than the single culture due to the complimentary metabolic pathways, wider optimal growth conditions and enhanced stability provided.These two bacteria (XY1, XY2) isolated from industrial wastewater, they were adapted to water with higher ammonia nitrogen concentration level and enhanced their ability to degrade NH3-N.

XY1 bacterial degradation rate was 46.18% % after 216 hours **(Figure. 10)** whereas the XY2 bacterial degradation rate was 68.5% % in 216 h time period. Consortium (mixture of XY1+XY2) of 10% inoculum size degraded the 78% of ammonia nitrogen in wastewater sample. So the consortium was degraded maximum amount of ammonia nitrogen in wastewater, because Ammonia nitrogen is complex compund

so that requires the different enzymes to the degradation. So the consortium contain mixture of bacteria that can work together and degrade the ammonia nitrogen efficiently. The presence of consortium may enhance the overall stability of the degradation process, that bacterial mixture provide mutual support and prevent the dominance of single bacteria and may prevent the development of antibiotic resistance and maintain the stability of the degradation process. So the consortium of microorganisms can be more effective in the degradation of ammonia nitrogen than the single culture due to the complimentary metabolic pathways, wider optimal growth conditions and enhanced stability provided.

Table no 1: Biochemical test

Test	XY1	XY2
Glucose fermentation	++	+
Voges-Proskaur	-	-
Indole test	-	-
Starch Hydrolysis	+	+
Catalase test	+	++
Motility	-	-

Positive: + Negative: -

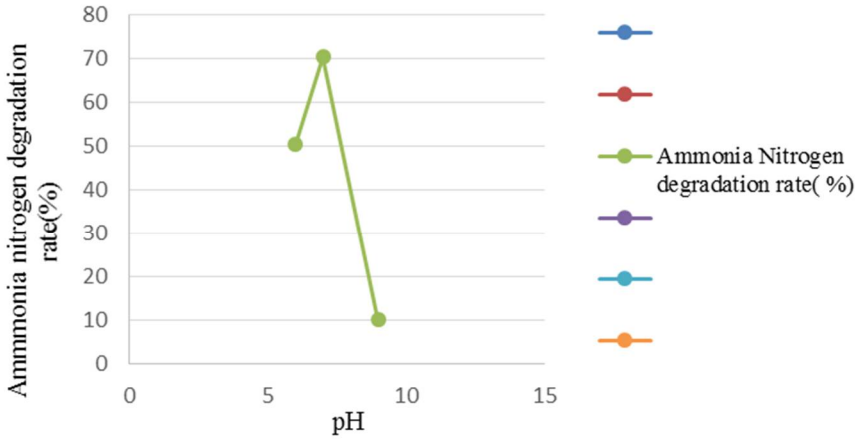

Figure 1: Effect of pH on ammonia nitrogen degradation by XY1

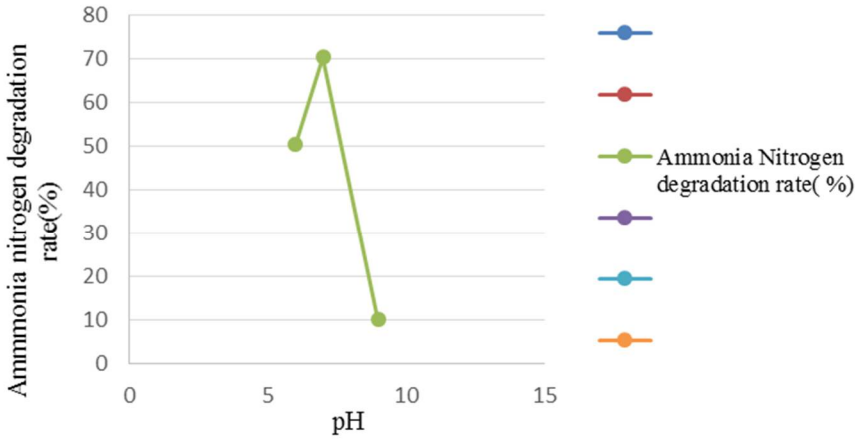

Figure 2: Effect of pH on ammonia nitrogen degradation by XY2

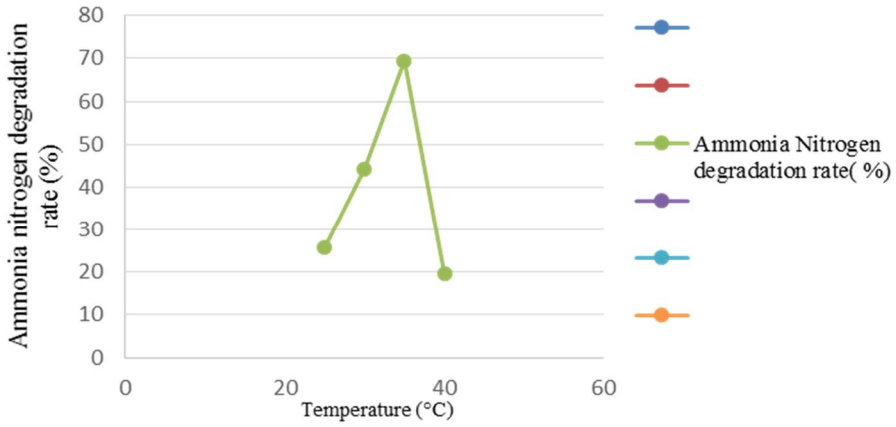

Figure 3: Effect of temperature on ammonia nitrogen degradation by XY1

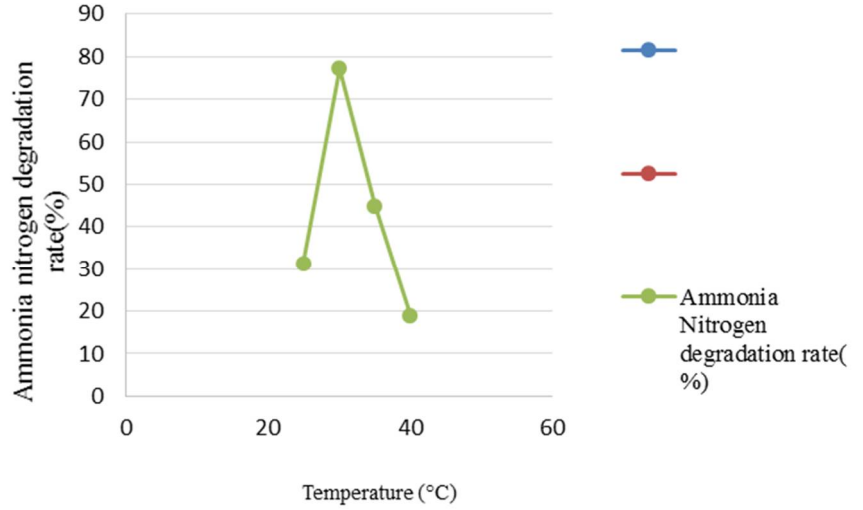

Figure 4. Effect of temperature on the ammonia nitrogen degradation using XY2.

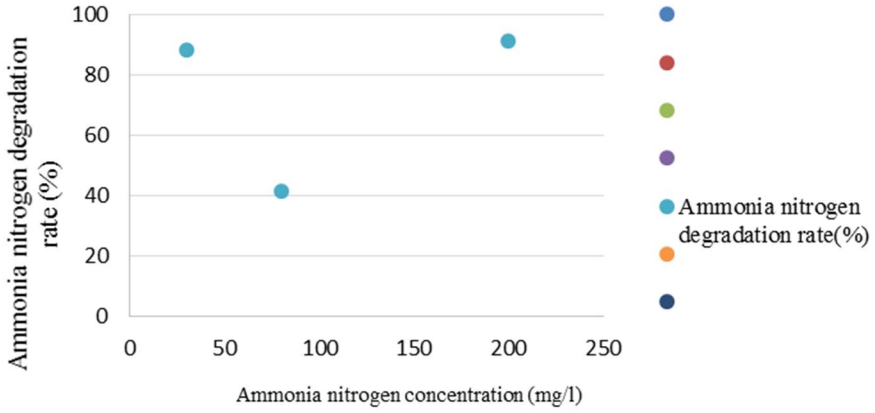

Figure 5: The Ammonia nitrogen concentration degradation by XY 1

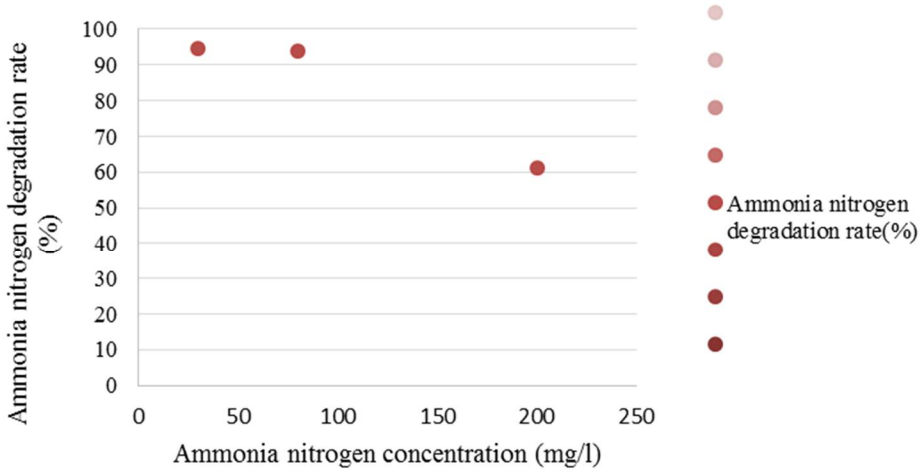

Figure 6: Effect of different amonical nitrogen concentration on degradation using XY2 in synthesized media

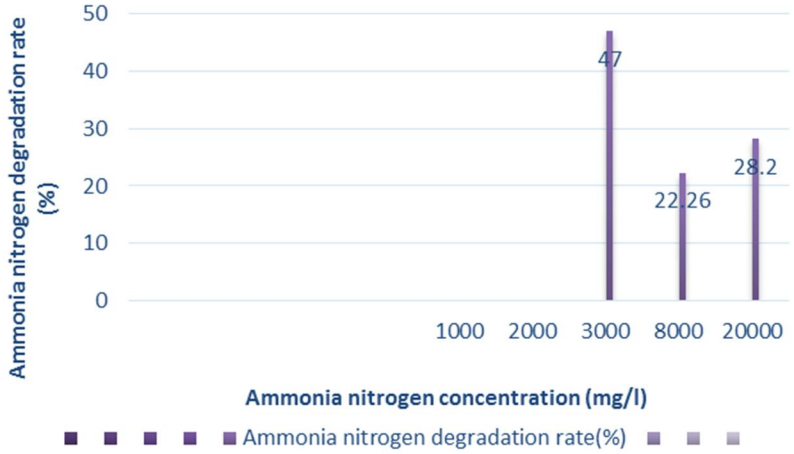

Figure 7: Ammonical nitrogen degradation rate at different ammonia nitrogen concentration with 10 % inoculum of XY1 in wastewater sample

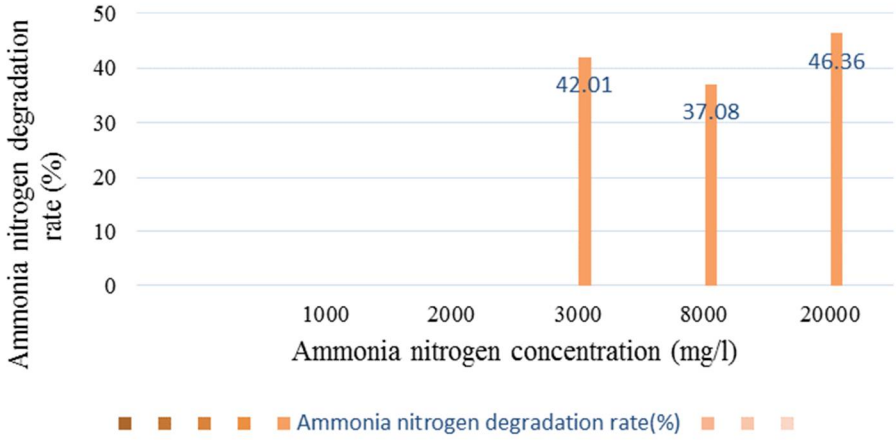

Figure 8: Ammonia nitrogen degradation rate at the different ammonia nitrogen concentration of 10% inoculums of XY2 wastewater sample.

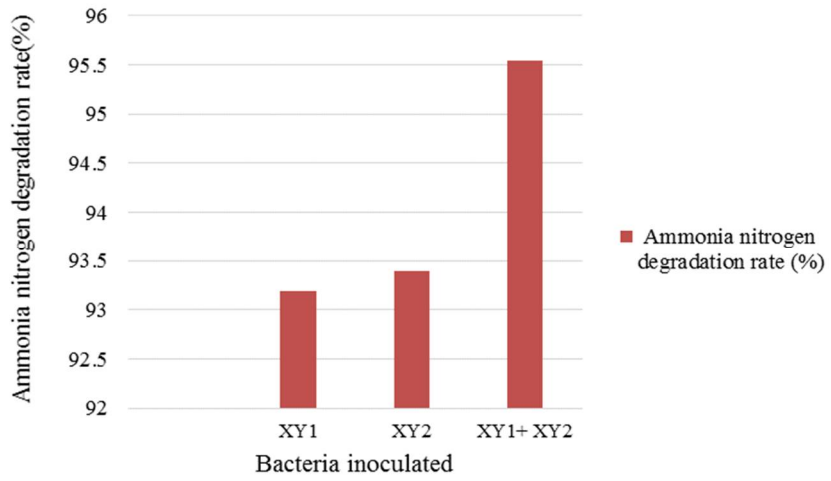

Figure 9: Amonical nitrogen degradation rate in wastewater sample by isolates and consortia at after 92 hours of incubation.

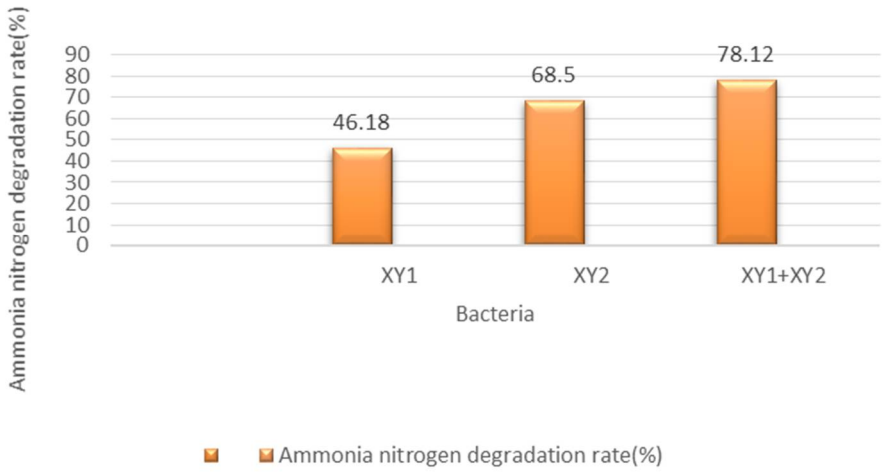

Figure 10: Amonical nitrogen degradation rate in wastewater sample by isolates and consortia at after 92 hours of incubation

CONCLUSION

By performing this experiment, the MSM medium that contains ammonium sulphate as only nitrogen source was used to isolate and screening of ammonia nitrogen degradation bacteria from chemical industry wastewater. Mainly two types of bacterial colony (XY1,XY2), which are capable of efficiently degrading ammoniacal nitrogen by the optimization activity. The XY1 degradation effect was the best at the 35°C, pH 7, and the initial concentration of ammonia nitrogen was the 200mg/l , the degradation rate was 92% after the 92h incubation time, in the waste water the 93.25% degradation rate at the 131mg/l concentration. By the comparing the best degradation effect of XY2 at the 30°C, pH 6 and the 92% degradation rate at the 80mg/l initial ammonia nitrogen concentration after 92h incubation time. In the waste water around 93.54% degradation rate at the 131mg/l concentration after 92h incubation to time. These bacteria can be potentially used in the development of effective bioremediation techniques for treating wastewater

REFERENCES

1. Yu, C. H., Wang, Y., Guo, T., Shen, W. X., & Gu, M. X. (2012). Isolation and identification of ammonia nitrogen degradation strains from industrial wastewater.
2. Zhang, X. N., Guo, Q. P., Shen, X. X., Yu, S. W., & Qiu, G. Y. (2015). Water quality, agriculture and food safety in China: Current situation, trends, interdependencies, and management. *Journal of Integrative Agriculture*, *14*(11), 2365-2379.

3. Wang, Y., Wang, J., Zhao, X., Song, X., & Gong, J. (2016). The inhibition and adaptability of four wetland plant species to high concentration of ammonia wastewater and nitrogen removal efficiency in constructed wetlands. *Bioresource technology*, *202*, 198-205.
4. Liu, Y., Shi, X. R., Cui, Y. B., & Li, M. (2013). Toxic effects of high concentrations of ammonia on Euglena gracilis. *Huan Jing ke Xue= Huanjing Kexue*, *34*(11), 4386-4391.
5. Lu, N. N., Liu, M. H., & Song, W. C. (2015). Effects of nitrogen and phosphorus on the growth of chlorella in water of the Yellow River Diversion Reservoir [J]. *Chinese Journal of Environmental Engineering*, *9*(03), 1098-1102.
6. Huang, X., Chen, W., Cai, M., & Hei, L. (2022, February). The screening of high-efficiency ammonia-nitrogen degrading bacteria and their influencing factors were studied. In *IOP Conference Series: Earth and Environmental Science* (Vol. 983, No. 1, p. 012103). IOP Publishing.
7. Zhang, L. J., Ma, Y. H., & Wang, G. L. (2010). Harm of farmland nitrogen pollution in agricultural non-point source pollution and its prevention measures [J]. *Agricultural Environment and Development*, *27*(04), 48-52.
8. Zhou B, Xing M N, Liu D, et al. 2019 Analysis of eutrophication and prevention and control measures of inland freshwater lakes and reservoirs in my country[J]. Resources Economization & Environmental Protection 0(12) 110-111
9. Dai Y R, Liang W and Wu Z B 2010 Research progress in the denitrification of low-carbon and high-nitrogen wastewater in constructed wetlands[J]. Journal of Agro-Environment Science 29(S1) 305-309.
10. hu Y X, Pei S F, Zhang H B, et al. 2019 Characteristics and research progress of nutrients and eutrophication in Laizhou Bay[J]. Marine Geological Frontiers 35(04) 1-9.
11. arak, P., Jobe, B. O., Krueger, A. R., Peterson, L. A., & Laird, D. A. (1997). Effects of long-term soil acidification due to nitrogen fertilizer inputs in Wisconsin. *Plant and soil*, *197*, 61-69.
12. Erisman, J. W., Bleeker, A., Galloway, J., & Sutton, M. S. (2007). Reduced nitrogen in ecology and the environment. *Environmental pollution*, *150*(1), 140-149.
13. Dasarathy, S., Mookerjee, R. P., Rackayova, V., Rangroo Thrane, V., Vairappan, B., Ott, P., & Rose, C. F. (2017). Ammonia toxicity: from head to toe?. *Metabolic brain disease*, *32*, 529-538.
14. Britto, D. T., Siddiqi, M. Y., Glass, A. D., & Kronzucker, H. J. (2001). Futile transmembrane NH4+ cycling: a cellular hypothesis to explain ammonium toxicity in plants. *Proceedings of the National Academy of Sciences*, *98*(7), 4255-4258.
15. Huang, X., Chen, W., Cai, M., & Hei, L. (2022, February). The screening of high-efficiency ammonia-nitrogen degrading bacteria and their influencing factors were studied. In *IOP Conference Series: Earth and Environmental Science* (Vol. 983, No. 1, p. 012103). IOP Publishing.
16. Li, D., Xu, X., Li, Z., Wang, T., & Wang, C. (2020). Detection methods of ammonia nitrogen in water: A review. *TrAC Trends in Analytical Chemistry*, *127*, 115890.
17. Li, H., Cao, Y., Liu, P., Li, Y., Zhou, A., Ye, F., & Yue, X. (2022). Ammonia-nitrogen removal from water with gC3N4-rGO-TiO2 Z-scheme system via photocatalytic nitrification-denitrification process. *Environmental research*, *205*, 112434.
18. Ronan, E., Aqeel, H., Wolfaardt, G. M., & Liss, S. N. (2021). Recent advancements in the biological treatment of high strength ammonia wastewater. *World Journal of Microbiology and Biotechnology*, *37*(9), 158.
19. Kinidi, L., Tan, I. A. W., Abdul Wahab, N. B., Tamrin, K. F. B., Hipolito, C. N., & Salleh, S. F. (2018). Recent development in ammonia stripping process for industrial wastewater treatment. *International Journal of Chemical Engineering*, *2018*.
20. Zhang, Y., Fu, C. Y., Li, X. H., Yan, P. P., Shi, T. H., Wu, J. Q., ... & Liu, X. L. (2019). Optimizing suitable conditions for the removal of ammonium nitrogen by a microbe isolated from chicken manure. *Open Chemistry*, *17*(1), 1026-1033.
21. Bergey, D. H. (1994). *Bergey's manual of determinative bacteriology*. Lippincott Williams & Wilkins.
22. Shoaib, M., Muzammil, I., Hammad, M., Bhutta, Z. A., & Yaseen, I. (2020). A mini-review on commonly used biochemical tests for identification of bacteria. *A Mini-Review on Commonly used Biochemical Tests for Identification of Bacteria*, *54*(1), 8-8.
23. MacWilliams, M. P. (2012). Indole test protocol. *American Society for Microbiology, Washington, DC*.
24. Hari, R., Talwalkar, T., & Suneetha, V. (2018). Screening and Identification of Bacteria from Vellore Polycarbonate Lenses. *Research Journal of Pharmacy and Technology*, *11*(9), 3927-3931.
25. Sáez-Plaza, P., Michałowski, T., Navas, M. J., Asuero, A. G., & Wybraniec, S. (2013). An overview of the Kjeldahl method of nitrogen determination. Part I. Early history, chemistry of the procedure, and titrimetric finish. *Critical Reviews in Analytical Chemistry*, *43*(4), 178-223.
26. Sáez-Plaza, P., Navas, M. J., Wybraniec, S., Michałowski, T., & Asuero, A. G. (2013). An overview of the Kjeldahl method of nitrogen determination. Part II. Sample preparation, working scale, instrumental finish, and quality control. *Critical Reviews in Analytical Chemistry*, *43*(4), 224-272.
27. Wang, N., Ingersoll, C. G., Hardesty, D. K., Ivey, C. D., Kunz, J. L., May, T. W., ... & Barnhart, M. C. (2007). Acute toxicity of copper, ammonia, and chlorine to glochidia and juveniles of freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry: An International Journal*, *26*(10), 2036-2047.