Journal of Advances in Environmental Health Research

Ammonium removal in granular activated carbon up-flow submerged reactors containing native bacterial consortium

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Original Article

Abstract

Free ammonium in industries wastewater could be one of the worst toxic contaminants of aquatic life if diluted in water. Biological nitrogen removal (BNR) is the most common method for removing ammonium and nitrate from wastewater. Attached growth and suspended growth are the main BNR systems. The aim of the present work was to study the treatment of petrochemical wastewater (ammonium and nitrate removal) using native bacterial consortium isolated from Kermanshah Wastewater Treatment Plant, Iran, in two laboratory-scale high performance, granular activated carbon up-flow submerged reactors (GAC-USRs). The average maximum removal efficiency of NH_4-N and NO_3-N was 97.46% and 97.58% at the nitrification rate (NR) and denitrification rate (DR) of 2.44 kg NH_4-N/m^3.day and 2.31 kg NO_3-N/m^3.day, respectively. It was confirmed that the immobilized native bacterium on GAC could achieve a high ammonium and nitrate removal efficiency. The results of this study showed that the Bio-GAC-USRs can be an efficient method for complete ammonium and nitrate removal from wastewater.

KEYWORDS: Ammonium, Denitrification, Native Bacterial Consortium, Petrochemical Wastewater, Up-flow Submerged Reactors, Granular Activated Carbon

Date of submission: 15 Sep 2015, ***Date of acceptance:*** 23 Dec 2015

Citation: Khademikia S, Godini H. **Ammonium removal in granular activated carbon up-flow submerged reactors containing native bacterial consortium.** J Adv Environ Health Res 2016; 4(1): 25-33.

Introduction

Nitrogen enters water in numerous forms, including both inorganic (ammonia/ammonium, nitrate, and nitrite) and organic forms (proteins, amino acids, urea, and living or dead organisms).[1] Ammonium is one of several forms of nitrogen that have direct and indirect toxic effects on aquatic environments; direct effects via municipal and industrial effluent discharges and the excretion of nitrogenous wastes from animals, and indirect effects via nitrogen fixation, air deposition, and runoff from agricultural lands.[1,2] Natural levels of ammonium range from 0.2 mg/l in groundwaters to 12 mg/l in surface waters. Higher natural contents (up to 3 mg/l) are found in strata rich in humic substances.[3] The free ammonium in the wastewater of petrochemical, pharmaceutical, fertilizer, and food industries can be one of the worst toxic contaminators of aquatic life if diluted in water.[1-3] The presence of ammonium at higher than geogenic levels is an important indicator of faecal pollution (toxic to fish and other aquatic organisms). Environmental and health concerns regarding nitrogen can be grouped as human health, aquatic life toxicity, eutrophication (resulting in oxygen-deprived or hypoxic waters), and nitrogen gasses and atmospheric concerns.[3-5]

In dealing with nitrogen-related problems

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in aquatic environments, there are important options for achieving safe ammonia and nitrate levels. Biological nitrogen removal (BNR) and physical-chemical systems are the treatment processes used for removing ammonium and nitrate from wastewater.[6] However, the BNR could be the most common removal method because of its efficacy. Nitrification and denitrification are the key parameters in the design of a BNR.[7] Nitrification is formally a two-step process; in the first step, ammonia is oxidized to nitrite (NO_2-N) by Nitrososphaera, Nitrosomonas and Nitrosococcus, and in the second step, nitrite is oxidized to nitrate (NO_3-N) by bacteria of the genus Nitrobacter and Nitrospira. The denitrification process occurs in anoxic conditions, and converts nitrate to nitrogen gas (N_2). Denitrification is performed by heterotrophic or autotrophic bacteria (Pseudomonas, Bacillus, Sprylyum, Acinetobacter, Rhizobium, Alcaligenes, Thiobacillus, and Corynebacterium).[6-9]

Attached growth and suspended growth are the main BNR systems. Biological filtration system (BFS), as an emerged BNR, is a relatively new method in the attached growth systems for ammonium and nitrate removal from wastewater.[10-12]

Today, a large amount of research is devoted to BFSs. These can have very high concentrations of biological mass because of their wide and porous surface.[13] The increase in BFSs use in today's industrial world is largely due to the inherent advantages of the technology such as; chemical and physical inactivity, high performance, long lifetime, media geometries, filter bed material, and reduced number of channeling issues.[14-18]

Some of the most frequently used inert solid material include perlite, porous ceramics, activated carbon, porous lava, and polyamide and polypropylene beads.[6,10,19]

In previous studies, activated carbon beads showed significantly higher attachment capacity for the nitrifying and denitrifying consortium.[10,19]

Therefore, in this study, the performance of two laboratory-scale submerged columns with granular activated carbon substrate with native nitrifying and denitrifying bacteria on the nature of the fixation was evaluated in terms of suitability for nitrogen removal.

Materials and Methods

Isolation and Inoculation of Nitrifier and Denitrifier (ND) Bacterium

The ammonium and nitrate-degrading bacterial consortium was previously isolated from the biological activated sludge process of a petrochemical wastewater treatment plant in Kermanshah, Iran, and tested in nitrification/denitrification experiments with a two stage biofiltration column.[20] The inoculated solid media were incubated at 30 °C for 72 hours to obtain the pure single colonies shown in figure 1.[15]

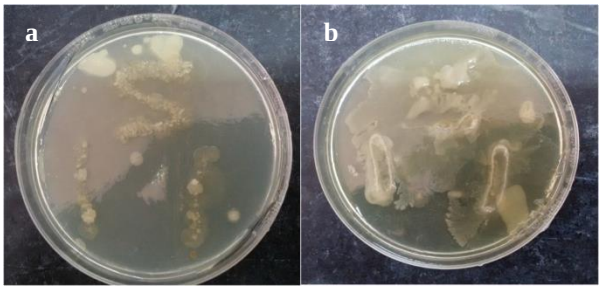

Figure 1. Pure single colonies of (a) denitrifiers and (b) nitrifiers

The mixed culture of acclimatized nitrifying and denitrifying (ND) bacteria were inoculated into the two granular activated carbon submerged up-flow reactors (GAC-USRs) after 48 hours of re-acclimatization (on a rotary shaker with 140 rpm) to grow on the GAC.[21-24] Before the injection of bio-GAC inside the reactors, the following preparation of bio-GAC was performed.

Pretreatment of bio-GAC

GAC was used as a packing material in the experimental USRs. Hydrochloric acid (1N) was used for the GAC pretreatment. The GAC was soaked several times in deionized water, and then, dried at 105 °C for 5 hours and used further as a carrier for the

attachment of the ND consortium.[25]

The GAC was transferred into USRs, and the re-acclimatized consortium was pumped onto the GAC columns. The volumetric ratio of GAC to total reactor volume was 0.5. The growth and re-acclimatization of biomass on the GAC was maintained for 2 months by controlling the environmental and operational parameters; pH of 7 ± 0.2, temperature of 29 ± 1 °C, NH_4–N of 1000 mg/l, and NO_3-N of 1000 mg/l.[16] Oxidation–reduction potential (ORP) and pH were monitored using a pH/ORP meter (WTW pH Meter, Model 330i, Wissenschaftlich-Technische Werksta tten GmbH, Weilheim, Germany). Dissolved oxygen (DO) and water temperatures were measured with a portable DO meter (YSI Model 52, Yellow Springs Instruments, YellowSprings, OH).

Reactor Set-up

Two GAC-USRs were operated to eliminate the high concentrations of nitrogen through simultaneous nitrification/denitrification - filtration process. The GAC-USRs were used to investigate the nitrification/denitrification of wastewater with specific emphasis on the effect of the nitrogen loading rate. The solid substrate material in the USRs is typically supported by porous plates. The fluid is then forced through the distributor and up through the solid material. In this type of reactor, a fluid is passed through a granular solid catalyst material to suspend the solid and cause it to behave as though it were a fluid. A multiport peristaltic pump set (Masterflex L/S drive, 8-roller pump head, small cartridge model no. 07519–85, Cole-Parmer Instrument Company, Vernon Hills, IL, USA) provided the fluid flow to the bioreactors. The pump head rotational speed (RPM) was adjusted every other day to ensure that the flow rates were as close to 15 ml/minute as possible.[15,16] The GAC-USRs were built from Plexiglas tubes as shown in figure 2.

Figure 2. The granular activated carbon submerged up-flow reactors, (a) nitrification column and (b) denitrification column

Each Plexiglas column was 42 cm in height and had an inner diameter of 10 cm and a screen layer of 2 mm diameter was installed at the bottom of each column. The reactors were made watertight at the bottom and top using O-ring flanges to prevent the transfer of atmospheric gases to the anaerobic zone. GAC (500 g) with a size of 2–4 mm was packed into both columns with a height of 30 cm as bio carrier. The total volume of the reactors was 1.5 l. The effluent ports were located 20 cm above the plate and the influent ports 20 cm below it in the nitrification and denitrification columns. All experiments were operated at 30 °C.[16]

To inoculate the biofilter media with bacteria, each bioreactor was filled with synthetic media and inoculated with 3×10^8 colony-forming units (CFU)/ml ND bacterium. After the static periods, wastewater was circulated through the reactors in a closed loop. This recirculation was continued until there was a substantial decline in the nitrate and ammonium concentration of wastewater. During this acclimation period, wastewater in the nitrification column was amended with the addition of ammonium to improve bacterial growth and wastewater in the denitrification column was amended with the addition of nitrate and methanol. The ammonium and nitrate eliminated by the GAC-USRs were calculated using the following equations:[15,16]

Nitrification rate: $\frac{[NH4]_{in}-[NH4]_{out}\times R}{V}$ (1)

Denitrification rate: $\frac{[NO3]_{in}-[NO3]_{out}\times R}{V}$ (2)

Where R is the wastewater flow rate, V is the reactor volume, [NH_{4in}] is the influent and [NH_{4out}] the effluent NH_4 concentrations (g NH_4-L), and [NO_{3in}] is the influent and [NO_{3out}] the effluent NO_3 concentrations (g NO_3-L). Statistical analyses were carried out on data from each loading rate at pseudo-steady-state conditions.

Results and Discussion

ND bacteria

The ND bacterium isolated from the petrochemical wastewater by culture method was the best bacterium with a high ammonium and nitrate removal rate at a low retention time. The nitrifying and denitrifying reactors of the present study were inoculated with the same microbial population, and then, developed towards specialized biomass inoculums (owing to the specific experimental conditions). Microbial fixation and biofilm formation on the support surface are two of the most important factors because they affect the levels of elimination of every pollutant.[16]

Formation of tiny, irregularly shaped, and well settling granules was apparent within two weeks of the operation. The morphology of granules, which evolved in the reactors during high strength nitrification and denitrification, is shown in figure 3. The sludge predominantly consisted of granules as is evident from visual observations, microscopy, and settling characteristics.[15] Long rod-shaped microorganisms were evident on the surface of granules (Figure 3).

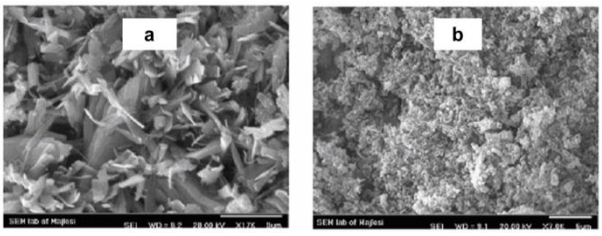

Figure 3. Scanning electron microscope image of (a) granules and (b) the morphology of the microorganisms on the surface of granules

Start-up period

The start-up period of the GAC-USRs lasted about 2 months of operations, until the biomass concentration reached an optimal level. During the first days of the start-up period, NH_4-N and NO_3-N removal efficiencies were low (10-20%). Later, the removal efficiencies increased reaching a stable high level in approximately 3 weeks.

Nitrification

The nitrification process realization was evaluated by NH_4-N measurements in the effluent of the nitrification GAC-USR (NGAC-USR). Steady state operation was achieved throughout most of the start-up period with complete NH_4-N removal through regulation of recycled wastewater flow and biomass development onto the GAC. The ammonium removal cycle is affected by many factors such as the initial ammonium concentration, hydraulic conditions, temperature ranges, and suspended solids.[2] During the experiments, the agreement of pH with the theoretical values acceptable for the design of the nitrification systems was assessed. A value of 7.5 was chosen for pH (the optimal pH for the nitrification process is in the range of 7.5–8.5).[26] Under aerobic conditions, it is energetically more favorable for bacteria to utilize molecular oxygen in the presence of organic electron donors. An aquarium water pump (2 l/minute) was used to create a current in the water (column A).

Figure 4 shows the concentrations of ammonium in the influent and the effluent of the nitrifying system and the ammonium removal percentage during the 120 days of operation (4 runs). Figure 5 shows the effect of applied nitrification rate on the NH_4-N accumulations in the NGAC-USR effluents. Near complete NH_4-N removal was obtained at 1.1 kg/m.day of nitrification rate (NR) and 98.75% NH_4-N removal was achieved with an effluent NH_4-N concentration of lower than 5 mg/l. As shown in figure 5, it was evident that the system was able to provide an

ammonium removal rate of more than 4.44 kg NH_4-N/m^3/day. NO_2-N and NO_3-N were detected as the main nitrification products in the effluent of the NGAC-USR through the experimental study.

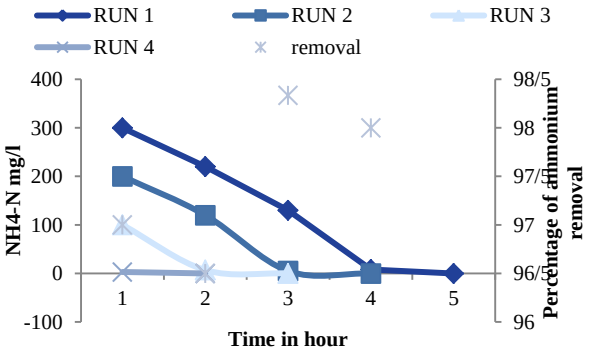

Figure 4. Ammonium concentration in the influent and effluent of the nitrification granular activated carbon up-flow submerged reactors (NGAC-USR) and the percentage of ammonium removal throughout the study

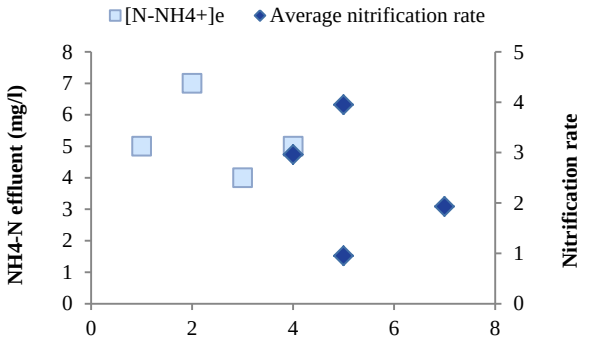

Figure 5. Effect of applied nitrification rate on the NH_4-N accumulations in the nitrification granular activated carbon up-flow submerged reactors (NGAC-USR) effluents

Table 1 shows the operational parameters of the runs. The average nitrification rate was very high in all runs (2.44 ± 1.27 kg N-NH_4^+/m^3/day). The maximum ammonium removal percentage was also very high throughout the study (97.46%). These results reject the concept of ammonia removal by stripping or other biological methods.[27]

Table 1 shows that the nitrification rate was high. The increase in ammonium removal from the system could be explained by the cyclic growth and attachment of the biofilm in the submerged biofilter.[10,12] Nitrifires grow faster than the denitrifires at elevated temperatures (> 20 °C). In this study, the temperature in the laboratory was kept constant at 29 ± 1 °C which was the standard optimum value of 25–30 °C for nitrifiers.[28] Van Benthum et al. reported a nitrification rate of 0.23 kg N-NH_4^+/m^3/day using a nitrifying biofilm growth airlift suspension reactor coupled with a chemostat.[29] A review on literatures showed that the nitrification rates for various systems treating wastewater at 30 °C were 0.6–1.3 kg NH_4-N/m^3/day for nitrifying sludge, 0.8–1.0 kg NH_4-N/m^3/day for nitrifying biofilm SBR, and 0.47–1.6 kg NH_4-N/m^3/day for partial nitrification using a down-flow hanging sponge reactor.[30,31]

Denitrification

Methanol was used as the external carbon source in the denitrification GAC-USR (DGAC-USR). The DGAC-USR was operated in a start-up mode by feeding NO_3-N to acclimate microorganisms in the synthetic wastewater. After this time (sludge formation and steady state), the effluent of the NGAC-USR was recirculated to the DGAC-USR. The effluent was treated in the DGAC, using methanol as external carbon source, during different periods. In the long term operation test, the synthetic wastewater was fed applied with the following conditions: 50-500 mg NO_3-N/l, methanol as the carbon source, and an adjusted pH of 7.2. The pH of the effluent (7.58) never exceeded the acceptable maximum (8.5).

Table 1. Operational parameters for the nitrification granular activated carbon up-flow submerged reactors (NGAC-USR) throughout this study

Runs	Period (days)	$[N\text{-}NH_4^+]_{in}$ (mg N/l)	$[N\text{-}NH_4^+]_e$ (mg/Nl)	HRT (hours)	Average nitrification rate (kg N-NH_4^+/m^3/day)
4	120	250 ± 129.09	5.75 ± 2.21	1-4	2.44 ± 1.27

HRT: Hydraulic retention time

Table 2. Operational parameters for the DGAC-USR throughout this study

Runs	Period (days)	$[N\text{-}NO_3^+]_{in}$ (mg/NL)	$[N\text{-}NO_3^+]_e$ (mg/NL)	HRT (hours)	Average denitrification rate (kg $N\text{-}NO_3^+/m^3$/day)
4	120	237.5 ± 205.65	6.25 ± 6.29	1-4	2.31 ± 1.99

Figure 6 and table 2 show the operational parameters, the influent and effluent nitrate concentrations, and the nitrate removal percentage during the 120 days of operation (4 runs). Figure 7 shows the effect of applied denitrification rate on the NO_3-N accumulations in the DGAC-USR effluents. During this period, NO_x-N effluent concentration never exceeded 15 mg/l. In this experiment, the temperature was maintained at 30 °C and the external carbon source flow was adjusted to produce an optimal influent COD/N ratio of an acceptable value for the design of the denitrification systems, thus, ensuring that the system was not limited by the organic matter. The 120 days of the external carbon source study include 4 runs. In the present experiments, maximum nitrate removal was evaluated after the first 24 hours. The denitrification rate increased by operating the reactor at this temperature and the optimum operation. ANOVA showed no significant difference among media types ($P > 0.05$). This could be attributed to the possibility that the denitrifying biomass had already developed with GAC media performance. The denitrification ability of the DGAC at the optimum carbon source (3:1 ratio) proved stable under relatively low hydraulic retention time (HRT) (1-4 hours) and at an average denitrification rate of 2.31 kg NO_3-N/m^3/day at 30 °C. The maximum nitrate removal percentage was also very high throughout the study (97.58%). The concentration of ammonia in the simulated wastewater was very low and resulting effluent concentrations remained very low. The ORP readings of the effluent were always greater than 145 mV, relatively higher than the 200 mV limit for possible excessive nitrite and sulfide formation. ORP also decreased with nitrate removal from 20 mV in the influent. Effluent pH readings were between 8.32 and 9.47, confirming alkalinity production.

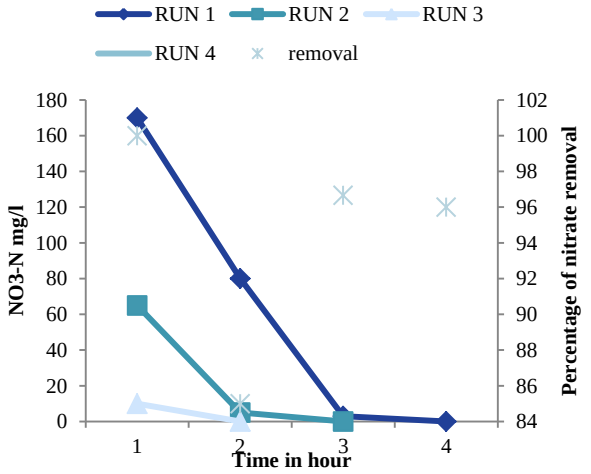

Figure 6. Nitrate concentration in the influent and effluent of the nitrification granular activated carbon up-flow submerged reactors (NGAC-USR) and the percentage of nitrate removal throughout the study

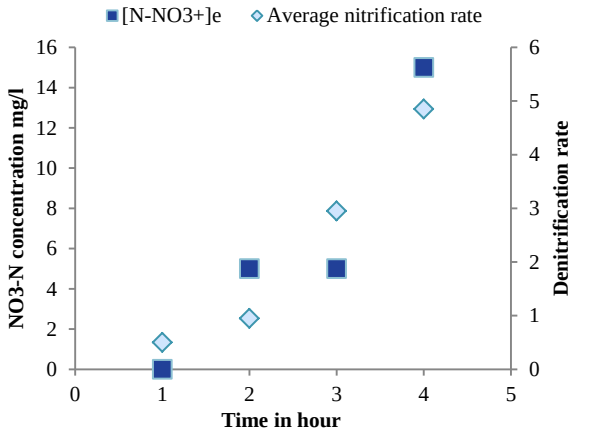

Figure 7. Effect of applied denitrification rate on the NO_3-N accumulations in the denitrification granular activated carbon up-flow submerged reactors (NGAC-USR) effluents

Saliling et al. reported a denitrification rate of 1.36 kg NO_3-N/m^3/day using a wood chips/wheat straw anoxic biofilter.[32] They evaluated wood chips and wheat straw as

inexpensive and readily available alternatives to more expensive plastic media for denitrification processes in treating aquaculture wastewater. The results showed that both wood chips and wheat straw produced comparable denitrification rates to the Kaldnes plastic media. However, one has to consider the abundance, cost, and expected life of the media before deciding which to use.[32] For a packed bed with polyethylene media and using methanol as a carbon source, the value reported for a study by Suzuki et al. was 43 g.N/(m^3/day).[33] The range of volumetric denitrification rates of 36–400 g.N/(m^3/day) for various denitrifying reactors were reported by Van Rijn et al.[34] A maximum biological denitrification rate of 0.88 kg NO_3-N/(m.day) with HRT of 3 hours for 60 mg/l nitrate was reported by Kesseru et al. in a continuous flow pilot bioreactor containing immobilized pseudomonas butanovoracells.[23]

The volumetric denitrification rate reported by Foglar et al. using zeolite particles with bacterial culture was 39.2–51.28 mg NO_3–N/Lh.[35] In the continuous denitrification process, complete denitrification was achieved at 25 °C with the nitrate and methanol loading rate of 4.35 mg NO_3-N = 1 hour and 23 mg O_2/1 hour, respectively. The denitrification rates reported in a previous study were 0.36 kg NO_x-N/m^3/day in a submerged biofilter, 0.2–0.38 kg NO_2-N/m^3/day in a packed film reactor, and 3.23–18.70 kg NO_2-N/(m^3.day).[36] It can be said that because of low influent NH_4-N and NO_x-N concentrations in the nitrification and denitrification reactors and the performance of the operation at higher temperatures than other studies, high removal efficiency was obtained by GAC-USRs in present work. At present, application of denitrification in commercial recirculating systems is conducted at a limited scale. It seems that full scale implementation of denitrification is feasible. However, the lack of studies on large-scale recirculating systems has limited the commercial application of denitrification in recirculating systems.

Conclusion

This study developed two comprehensive GAC-USRs as a promising system to eliminate nitrogen components. The mixed culture of acclimatized ND bacteria (originating from Kermanshah petrochemical wastewater treatment plant after acclimation) were inoculated into the two GAC-USRs. The nitrification and denitrification proceeded under oxic and anoxic conditions with the mixed bacterial culture, respectively. Based on the experimental results of this study, it can be concluded that native ND immobilized on GAC can be used effectively for nitrate and ammonium removal. Isolated native bacteria had a high ability for nitrification and denitrification processes. Therefore, the ammonia in the effluent can be removed with high efficiency using such bacteria. The reactors performed better ammonium removal at elevated temperatures (> 20 °C). The results showed a complete removal of ammonium and nitrate at HRT of 1-4 hours. Experimentally, it was confirmed that the immobilized bacterium on GAC could achieve a maximum ammonium and nitrate removal efficiency of 97.46% and 97.58%, respectively. These bioreactors containing immobilized native ND cells proved an efficient denitrification system with a relatively low retention time. The results obtained in this laboratory-based study could be incorporated in a larger-scale test of a biofiltration column using real wastewater. This process has the potential to be applied to ammonium removal at contaminated sites.

Conflict of Interests

Authors have no conflict of interests.

Acknowledgements

The authors are grateful for the financial support of this project by the School of Health of Lorestan University of Medical Sciences.

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