Magnetic field enhanced denitrification efficiency of immobilized bacterial particles

Liangang Hou, Yang Liu, Sa Fan and Jun Li

ABSTRACT

The effect of the magnetic field on denitrification process in immobilized bacteria particles was investigated in this study. The magnetic field could enhance the denitrification efficiency, especially for wastewater with low C/N ratios, and the average removal efficiencies of NO_3^--N increased by 6.58%. High-throughput sequencing analysis revealed that the magnetic field had substantial impacts on the stability of microbial community structure and relative abundance in immobilized bacteria particles, which was beneficial for the stability of denitrifying bacteria. Through the research in this paper, we suggest that magnetic field can be used to improve the denitrification performance of immobilized bacteria particles in the wastewater treatment industry.

Key words | denitrification, high-throughput sequencing, immobilized bacteria particles, magnetic field, wastewater treatment

Liangang Hou
Yang Liu
Sa Fan
Jun Li (corresponding author)
College of Architecture and Civil Engineering,
Beijing University of Technology,
Beijing 100124,
China
E-mail: *bjutlijun@sina.com*

INTRODUCTION

Microbial immobilization technology uses chemical or physical methods to immobilize free microbial cells in a defined spatial region while retaining their catalytic activities for repeated and continuous use (Saetang & Babel 2010; Barbosa *et al.* 2013; Ranjan *et al.* 2019), it has been getting more attention from experts because of its high wastewater treatment efficiency (Dong *et al.* 2017; Zhang *et al.* 2019). At present, the research about microbial immobilization technology for wastewater treatment is mainly focused on the synthesis of immobilized materials (Zhu *et al.* 2009; Chen *et al.* 2015; Sekoai *et al.* 2018) and the cultivation of bacterial strains (Zhang *et al.* 2017). In the process of wastewater treatment using microbial immobilization technology, there are few reports on the influence of environmental factors such as electric field and magnetic field.

As an environmental factor, magnetic field may affect the survival of living things (Perazzoli *et al.* 2017; Amoli-Diva *et al.* 2019; Ghanbari *et al.* 2019; Pirsaheb *et al.* 2019). According to related reports (Klyachko *et al.* 2012; Zhao *et al.* 2017; Ahmed & Siddique 2019; Sabouri *et al.* 2019; Wu *et al.* 2019), magnetic field can affect reaction heat, reaction direction, reaction rate, activation energy, entropy and many other aspects of chemical reactions (Shafiee *et al.* 2017; Hashemi *et al.* 2016), and also has an impact on biological tissue, growth process, genes, bacteria metabolism (Plonsey 1995; Wang *et al.* 2012; Shanehsazzadeh *et al.* 2015; Liu *et al.* 2019). However, there is little information available in literature about the effect of magnetic field on immobilized denitrifying bacteria particles.

To investigate the influence of magnetic field on the immobilized heterotrophic-denitrifying bacteria, a static magnetic was applied to the bioreactor which uses immobilized bacteria for denitrification in this study. The effect of immobilized heterotrophic-denitrifying bacteria by magnetic field was studied, and the changes of microbial in immobilized particles was analyzed using high-throughput sequencing analysis. This work might add some new insights to the application of microbial immobilized technology and improve the denitrification efficiency in wastewater treatment.

MATERIALS AND METHODS

Immobilization procedure

Waterborne polyurethane with carboxylate groups can be dispersed in water, and the waterborne polyurethane emulsion would be crosslinked to form a hard gel block when mixed with the suspended cells with the addition of the

doi: 10.2166/wst.2020.156

promoter and initiator (Dong *et al.* 2012; Chen *et al.* 2014). In this study, about 15 g (15%, w/v) cultured sludge (Hou *et al.* 2019) which contains denitrifying bacteria such as *Diaphorobacter* and *Paracoccus* was mixed with an equal amount of waterborne polyurethane and a suitable amount of water, added 1% (w/v) potassium persulfate solution and 0.5% (w/v) N,N,N,N-tetramethylethylenediamine solution to induce polymerization. It took approximately 10 min to become a gel at 27 °C, then was cut into 3 mm × 3 mm × 3 mm cubes, cultured in a solution containing sodium nitrate and glucose for later use.

Experimental operation

The schematic of bioreactor is shown in Figure 1. Serum bottle (500 mL) was used as the denitrification bioreactor of immobilized bacteria particles. The feeding medium used in this experiment was simulated nitrate-containing water made by adding sodium nitrate and glucose to tap water. Simulated water (405 mL) and immobilized particles (45 mL, 10% (v/V), sampled and marked S0) were added to each serum bottle. Magnet was added to the experimental group (magnetic flux density was 30 mT), and there was no magnet in the control group (0mT). The bioreactors were operated in sequential batch mode under 26–30 °C. In the start-up stage, hydraulic residence time (HRT) was set as 12 h (influent 10 min, reaction 11.5 h, effluent 20 min). The NO_3^--N was 100 mg/L and chemical oxygen demand (COD) was 400 mg/L in influent, meanwhile, pH = 7.863 and dissolved oxygen (DO) was <0.63 mg/L. In the stable operation stage, HRT was set as 8 h (influent 10 min, reaction 7.5 h, effluent 20 min). Immobilized bacteria particles were sampled after the stable operation period, and the experimental group was marked as SA and the control group as SC.

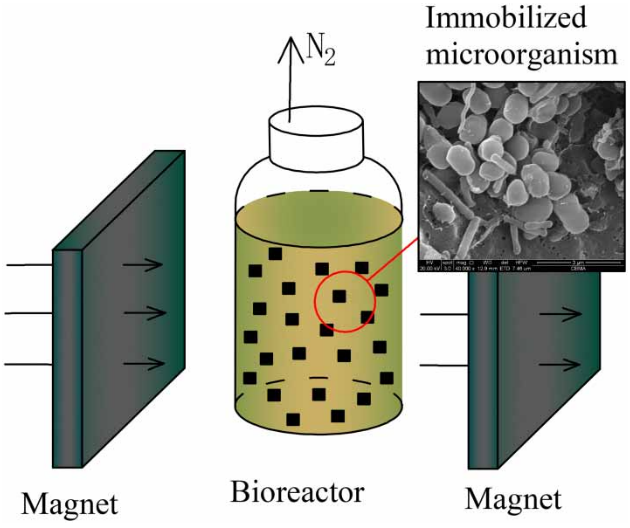

Figure 1 | Schematic diagram of the denitrification bioreactor.

Analytical methods

Samples of water quality measurements were collected and analyzed every day. The concentrations of NO_3^--N, NO_2^--N and COD in influent and effluent were analyzed according to standard methods (Rice *et al.* 2012). All tests were repeated twice and the temperature, pH and DO were measured near the midway of the bioreactor by using a WTW analyzer (Multi 3620IDS, Germany). The magnetic flux density was measured by a Tesla Meter (TD 8620, China).

Microbiological analysis

High-throughput sequencing of 16S rRNA gene fragments was used to analyze the microbial communities in immobilized particles samples from the bioreactor. Bacterial genomic DNA was extracted using an E.Z.N.A.® Soil DNA Kit (Omega Bio-tek, Inc.) after the samples (S0, SA and SC) were pretreated, following manufacturer's instructions. The PCR amplification used the universal bacteria primers (for 16S rRNA all bacteria) which incorporated MiSeq platform V3–V4 hypervariable regions. The PCR products were sequenced on the MiSeq 2 × 300 bp platform by Sangon Biotech, Shanghai, China. All sequence data have been deposited in the NCBI Sequence Read Archive database, and the BioProject ID was PRJNA578014.

RESULTS AND DISCUSSION

Performance of denitrification bioreactor

It can be seen from Figure 2 that in the start-up period (the first 15 days), the removal rates of COD (Figure 2(a)) and NO_3^--N (Figure 2(b)) were relatively low, and there was no significant difference between the two bioreactors. The removal rates showed an upward trend with time, and the highest removal efficiency of nitrate in the experimental group was 85.9%, while it was 78.36% in the control group. During 42 days operation, the average removal rates of COD and nitrate were 62.98% and 71.85%, respectively, in the experimental group, while in the control group it was 58.16% and 66.31%, so the magnetic field increased the removal rates of COD and nitrate by 4.82% and 5.54%, respectively. There was no significant difference in nitrite concentration of the

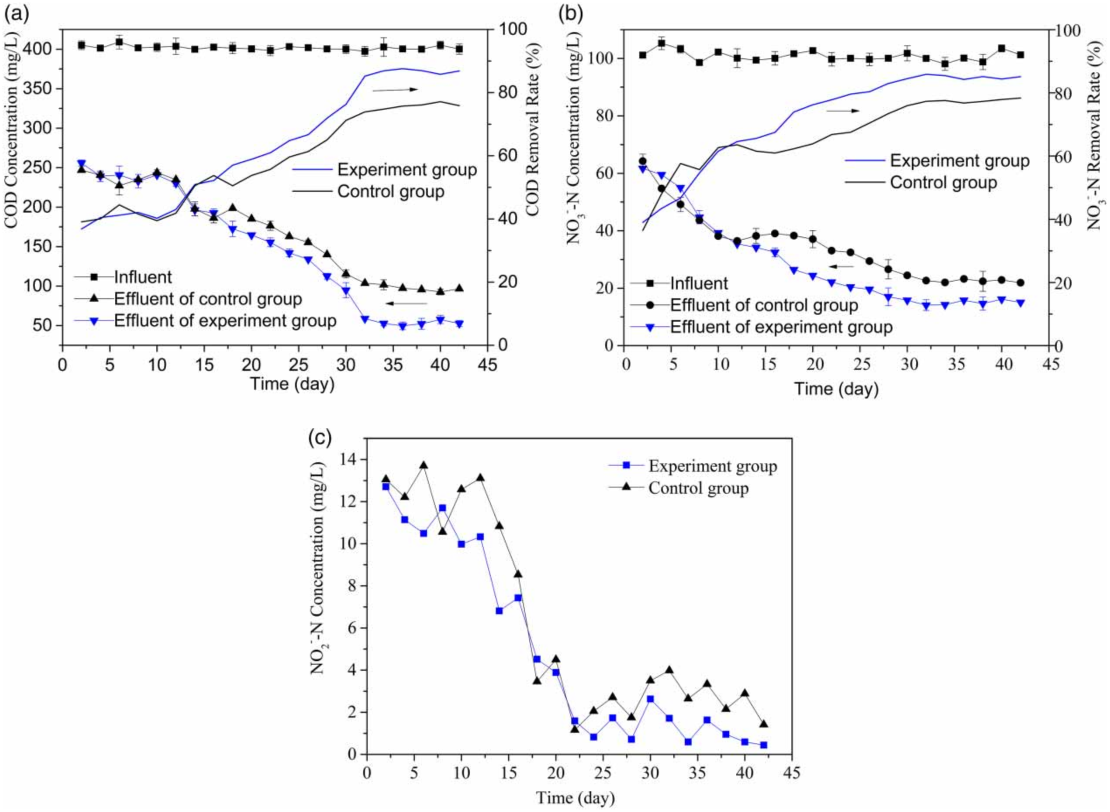

Figure 2 | Changes of concentration and removal efficiency during the start-up period of COD (a), NO_3^--N (b) and NO_2^--N (c) in the bioreactor.

two groups during the start-up process (Figure 2(c)), so the magnetic field had no detailed influence on the accumulation of nitrite in the denitrification process. Therefore, during the start-up process of denitrification bioreactor which uses immobilized bacteria particles, the magnetic field was beneficial for the COD degradation and nitrate removal. According to Figure 3, during the stable operation stage (43–75 days), the average removal rate of nitrate in the experimental group was 85.26%, significantly higher than that in the control group (78.68%). With the use of magnetic field, the nitrate removal rate increased by 6.58% on average.

Denitrification is a biochemical reaction process which converts NO_3^--N to N_2 (Kuypers *et al.* 2018) and achieves nitrogen removal, and denitrifying bacteria was the key link. The enzyme activity (Guo *et al.* 2018) of denitrifying bacteria directly affects the denitrification efficiency. Most denitrifying bacteria were sensitive to environment (Galloway *et al.* 2004; Dvorak *et al.* 2017), and were easily affected by the water quantity and the hydraulic loading (Flemming & Wuertz 2019). The research found that the physiological activity of the heterotrophic-denitrifying bacteria in the immobilized particles was affected by magnetic field, and it was beneficial to the start-up of denitrification bioreactor and can improve the denitrification efficiency. We speculated that the magnetic field enhanced the activity of some enzymes in the denitrification process, promoted the growth of heterotrophic-denitrifying bacteria in the immobilized particles, and thus improved the denitrification efficiency.

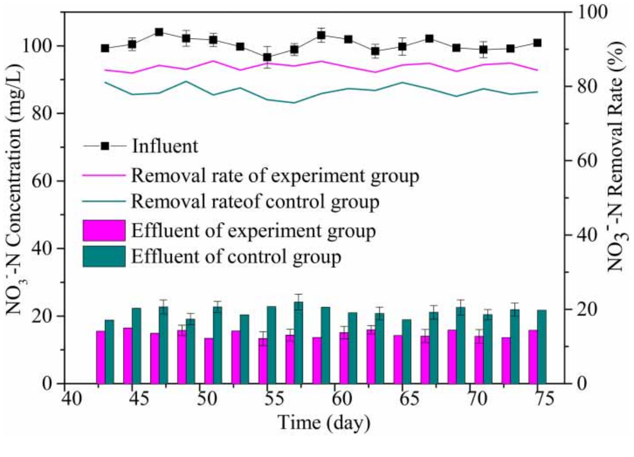

Figure 3 | Changes of concentration and removal efficiency during stable operation stage of NO_3^--N in the bioreactor.

Effect of magnetic field using batch treatment

The performance of the bioreactor in denitrification was further investigated under different carbon-to-nitrogen ratios (C/N) through a discontinuous batch test during the stable operation stage (day 65). Data in Figure 4 show the removal efficiency of NO_3^--N under different influent C/N ratios when NO_3^--N concentration in influent was 100 mg/L. NO_3^--N removal efficiency of the experimental group was 33.53%, 49.13%, 65.36%, and 85.74% when influent C/N ratios of 1.0, 2.0, 3.0, and 4.0; meanwhile, it was 26.68%, 42.94%, 59.75%, and 81.05% in the control group, respectively. The experimental group was significantly higher than that in control group by 6.85%, 6.19%, 5.61%, 4.69%, respectively. These results showed that the lower C/N ratios in influent, the better of denitrification effect under a magnetic field, and this proved that magnetic field can improve the denitrification efficiency, especially for wastewater with low C/N ratios.

The denitrification process needs carbon compounds to provide electrons (Zubrowska-Sudol 2018), and carbon is generally needed in wastewater treatment which increases the cost. In the bioreactor, the carbon source is not only utilized by the heterotrophic-denitrifying bacteria, but also consumed by other heterotrophic bacteria. In this study, the magnetic field increased denitrification efficiency, especially for low C/N ratio wastewater, and we speculated that the magnetic field inhibited the activity of heterotrophic bacteria and reduced the consumption of carbon sources, and the carbon sources were utilized by heterotrophic-denitrifying bacteria to the maximum extent under a magnetic field, which ensures the utilization rate of carbon sources in the bioreactor.

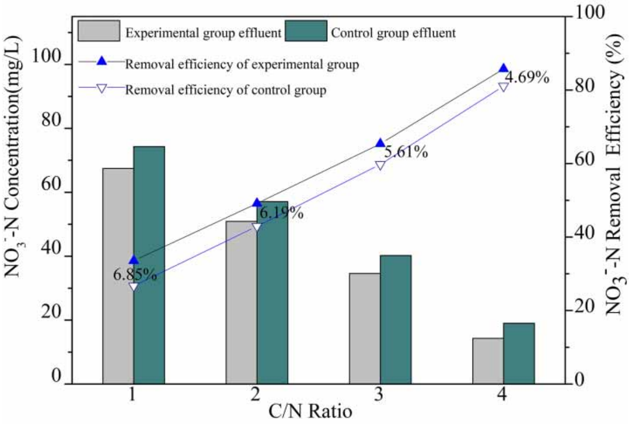

Figure 4 | Treatment efficiency of NO_3^--N under different influent C/N ratios.

Bacterial community structure analysis

To identify and compare core taxa, operational taxonomic units (OTUs) were summarized and normalized at the genus level. The number of OTUs and the richness as well as diversity of three samples was compared (Table 1). More OTUs were detected in the Samples SA compared with SC, However, both SA and SC have fewer OTUs than S0, this implies that the existence of the magnetic field can prevent microbial richness, which was also reflected by the Chao1 and Ace indexes. The low Shannon index and high Simpson index suggest low diversity in Sample SA, indicating that the microbes were highly selected and community diversity has enlarged due to the special life conditions of the magnetic field.

The microbial community of immobilized particles was characterized that based on 16S rRNA analysis. It can be seen from Figure 5 that the total identified phyla for Samples S0, SA and SC were 19, 18, and 16, respectively. Different bacteria were identified inside the immobilized particles. Sample S0 was mainly composed of *Proteobacteria* (64.09%), *Bacteroidetes* (10.02%), *Chloroflexi* (7.79%), *Ignavibacteriae* (3.64%), *Spirochaetes* (3.26%), *Acidobacteria* (1.66%), *Actinobacteria* (1.57%), and *Verrucomicrobia* (1.16%) (eight species with relative abundance >1%). Sample SA was mainly composed of *Proteobacteria* (62.58%), *Bacteroidetes* (14.78%), *Chloroflexi* (7.22%), *Spirochaetes* (5.67%), *Firmicutes* (1.8%), *Acidobacteria* (1.32%), *Ignavibacteriae* (1.22%), and *Planctomycetes* (1.14%) (eight species with relative abundance >1%). Sample SC was mainly represented by *Proteobacteria* (49.19%), *Chloroflexi*

Table 1 | Community richness and diversity indices of immobilized particles samples

Sample	OTUs number	Coverage	Chao1	Ace index	Shannon	Simpson
S0	2727	0.97	10,505.33	18,032.48	4.65	0.04
SC	1723	0.98	11,811.01	23,717.15	4.23	0.04
SA	1994	0.98	12,942.36	31,132.27	4.14	0.05

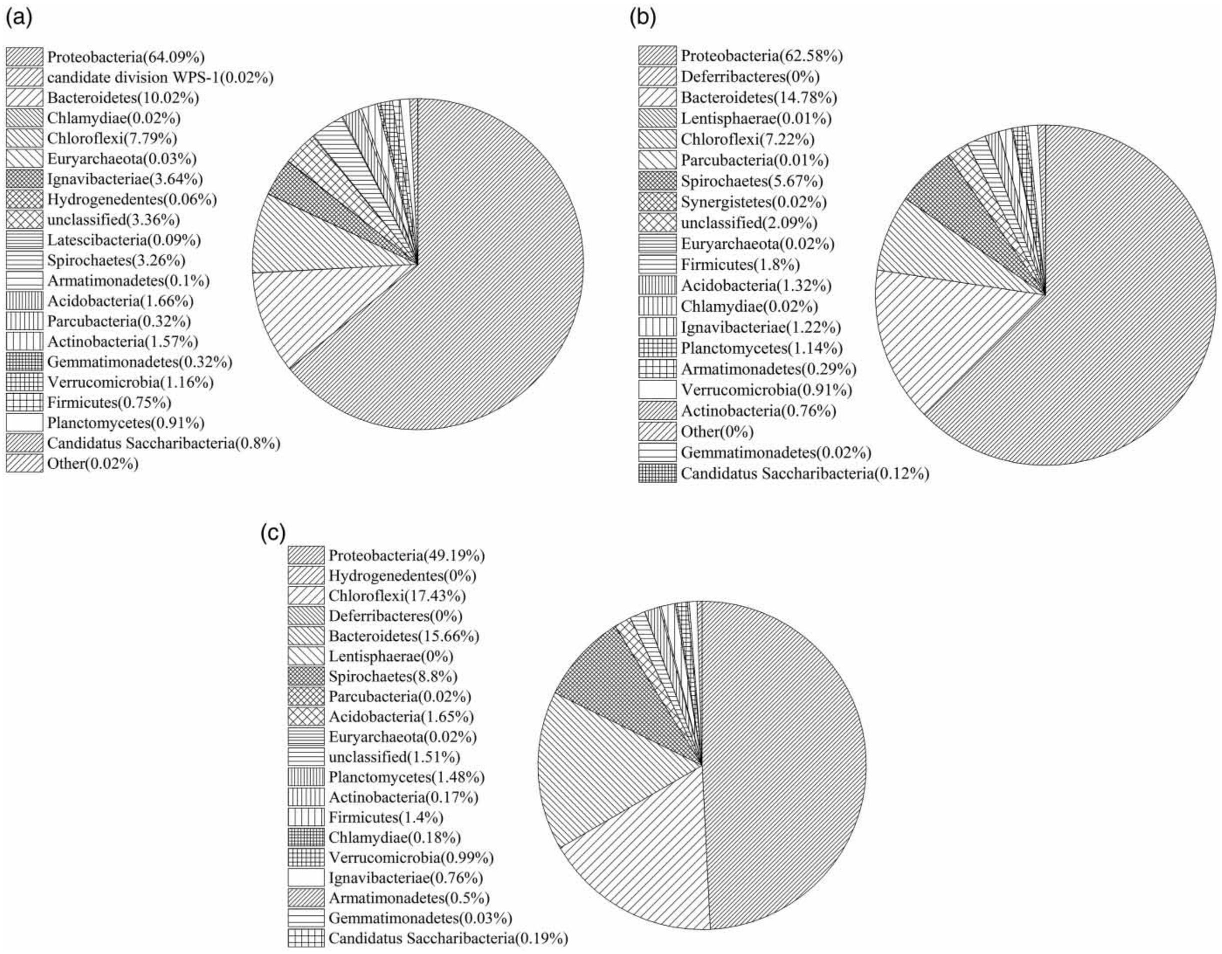

Figure 5 | 16S RNA analysis of the immobilized particles ((a) S0; (b) SA; (c) SC), phyla identified based on sequencing are shown.

(17.43%), *Bacteroidetes* (15.66%), *Spirochaetes* (8.8%), *Acidobacteria* (1.65%), *Planctomycetes* (1.48%), and *Firmicutes* (1.4%) (seven species with relative abundance >1%). It was known that the major phyla and relative abundances among the three samples were different, and the magnetic field was beneficial to maintain the stability of bacteria in the immobilized particles at the phyla level.

The use of magnetic field was conducive to maintaining the stability of *Proteobacteria* and *Chloroflexi*, and increasing the relative abundance of *Firmicutes* in immobilized particles, meanwhile, inhibition the reduce of *Actinobacteria* and *Ignavibacteriae*. These bacterial phyla were widely distributed over various wastewater treatment plants, and the influence of magnetic field on the physiological activity of these bacteria was very important to maintaining the stability of the immobilized microorganism. As can be seen from Figure 5, the magnetic field facilitated the reduction of *Candidatus* Saccharibacteria, and reduced *Acidobacteria*. Meanwhile, the magnetic field can inhibit the increase of *Spirochaetes*, *Armatimonadetes* and *Bacteroidetes* in immobilized particles.

Venn analyses were employed to evaluate the similarity of species diversity, the number of the total observed genera in the three communities was 31, with eight genera (about 25.8% of the total) shared by them (Figure 6(d)). As shown in Figure 6(d), there were both 19 genera in SA and SC, and the percent of their common genera were 61.3% (19/31). Although 17 genera coexist in SA and SC (Figure 6(d)), their relative abundance varies widely. At the genus level (Figure 6), the relative abundance with more than 2% were 9, 9, 7 in S0, SA and SC, respectively. The dominant genus in S0 was *Paracoccus* (11.99%), *Diaphorobacter* (7.95%), *Thauera* (3.46%), *Ignavibacterium* (3.16%), *Simplicispira* (2.86%), *Treponema* (2.56%), *Petrimonas* (2.29%), *Phyllobacterium* (2.23%), and *Ornatilinea* (2.22%), and in SA was *Comamonas* (14.15%), *Petrimonas*

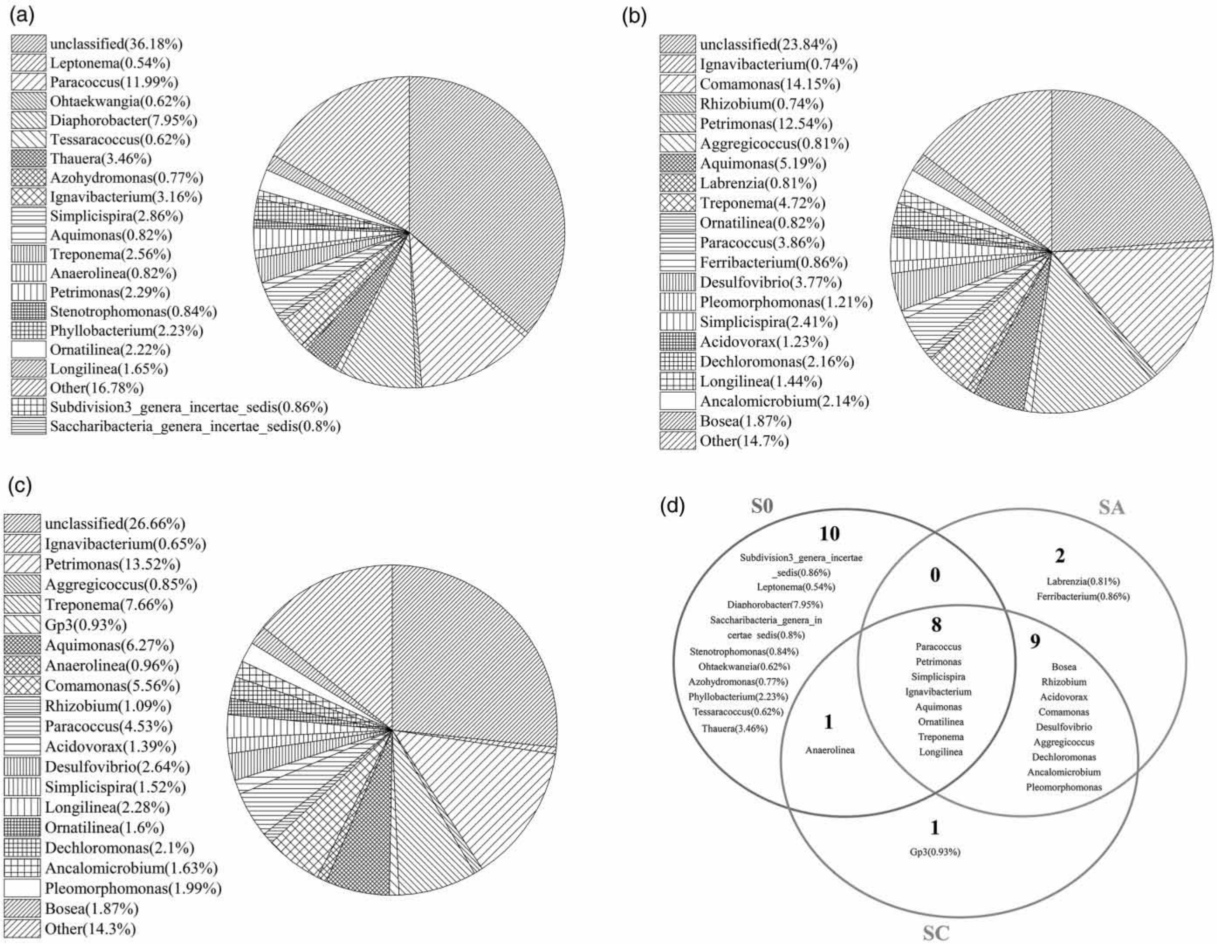

Figure 6 | 16S RNA analysis of the immobilized particles ((a) S0; (b) SA; (c) SC; (d) Venn diagram analysis of genera numbers in samples); genus identified based on sequencing are shown.

(12.54%), *Aquimonas* (5.19%), *Treponema* (4.72%), *Paracoccus* (3.86%), *Desulfovibrio* (3.77%), *Simplicispira* (2.41%), *Dechloromonas* (2.16%), and *Ancalomicrobium* (2.14%), while in SC was *Petrimonas* (13.52%), *Treponema* (7.66%), *Aquimonas* (6.27%), *Comamonas* (5.56%), *Paracoccus* (4.53%), *Desulfovibrio* (2.64%), and *Dechloromonas* (2.1%). The presence of magnetic field made the dominant genus in immobilized particles reduced, which indicated that magnetic field has selectivity effect on the evolution of microorganisms in immobilized particles, and made denitrifying bacteria become dominant bacteria. When SA and SC were compared with S0, the differences of genus in immobilized particles was obvious (Figure 6). The magnetic field inhibited the growth of *Anaerolinea*, *Aquimonas*, *Longilinea*, *Petrimonas*, *Pleomorphomonas*, *Rhizobium*, *Treponema*, etc. The magnetic field promoted the reduction of *Ornatilinea* and *Paracoccus*, meanwhile, it promoted the increase of *Comamonas*, *Ancalomicrobium*, and *Desulfovibrio*. It has been reported (Yang *et al.* 2017; Han *et al.* 2019; Zhao *et al.* 2019) that *Anaerolinea*, *Aquimonas*, *Pleomorphomonas*, *Rhizobium* and *Treponema* were competitive with denitrifying bacteria, and the presence of these bacteria was not conducive to the denitrification, this study showed that the magnetic field could inhibit its activity and promote the denitrification.

The results of high-throughput sequencing showed that under the effect of magnetic field, the composition of microbial community at phylum and genus levels of immobilized particles varied greatly. Our investigation demonstrated that the magnetic field was beneficial to the selection of dominant microorganisms in the denitrification immobilized particles, and can maintain the stability of functional bacteria during the denitrification process, so the magnetic field enhanced the denitrification efficiency.

CONCLUSIONS

By applying a magnetic field to the denitrification process of immobilized heterotrophic-denitrifying bacteria particles, the key finding of this paper is that a magnetic field can increase the denitrification efficiency of the immobilized bacteria particles, and the average removal rate of nitrate increased by 6.58%. The physiological activity of heterotrophic-denitrifying bacteria in the immobilized particles was affected by the magnetic field, which was beneficial to the selection of dominant bacteria and the stability of denitrifying bacteria. It is suggested that magnetic field can be used to increase the denitrification efficiency of immobilized particles in the wastewater treatment industry.

CONFLICTS OF INTEREST

There are no conflicts of interest to declare.

ACKNOWLEDGEMENTS

The authors are grateful for financial support from the Major Science and Technology Program for Water Pollution Control and Treatment (No. 2017ZX07103-001).

REFERENCES

Ahmed, A. & Siddique, J. I. 2019 The effect of magnetic field on flow induced-deformation in absorbing porous tissues. *Mathematical Biosciences and Engineering* **16** (2), 603–618.

Amoli-Diva, M., Anvari, A. & Sadighi-Bonabi, R. 2019 Synthesis of magneto-plasmonic Au-Ag NPs-decorated TiO_2-modified Fe_3O_4 nanocomposite with enhanced laser/solar-driven photocatalytic activity for degradation of dye pollutant in textile wastewater. *Ceramics International* **45** (14), 17837–17846.

Barbosa, O., Torres, R., Ortiz, C., Berenguer-Murcia, A., Rodrigues, R. C. & Fernandez-Lafuente, R. 2013 Heterofunctional supports in enzyme immobilization: from traditional immobilization protocols to opportunities in tuning enzyme properties. *Biomacromolecules* **14** (8), 2433–2462.

Chen, Y., Wang, L., Ma, F., Yang, J. X. & Qiu, S. 2014 Tracking composition of microbial communities for simultaneous nitrification and denitrification in polyurethane foam. *Water Science and Technology* **69** (9), 1788–1797.

Chen, G. H., Li, J., Tabassum, S. & Zhang, Z. J. 2015 Anaerobic ammonium oxidation (ANAMMOX) sludge immobilized by waterborne polyurethane and its nitrogen removal performance-a lab scale study. *RSC Advances* **5** (32), 25372–25381.

Dong, Y. M., Zhang, Z. J., Jian, Y. W., Lu, J., Cheng, X. H., Li, J., Deng, Y. Y., Feng, Y. N. & Chen, D. N. 2012 Nitrification characteristics of nitrobacteria immobilized in waterborne polyurethane in wastewater of corn-based ethanol fuel production. *Journal of Environmental Sciences* **24** (6), 999–1005.

Dong, H., Wang, W., Song, Z., Dong, H., Wang, J., Sun, S., Zhang, Z., Ke, M., Zhang, Z., Wu, W. M., Zhang, G. & Ma, J. 2017 A high-efficiency denitrification bioreactor for the treatment of acrylonitrile wastewater using waterborne polyurethane immobilized activated sludge. *Bioresource Technology* **239**, 472–481.

Dvorak, P., Nikel, P. I., Damborsky, J. & de Lorenzo, V. 2017 Bioremediation 3.0: engineering pollutant-removing bacteria in the times of systemic biology. *Biotechnology Advances* **35** (7), 845–866.

Flemming, H. C. & Wuertz, S. 2019 Bacteria and archaea on earth and their abundance in biofilms. *Nature Reviews. Microbiology* **17** (4), 247–260.

Galloway, J. N., Dentener, F. J., Capone, D. G., Boyer, E. W., Howarth, R. W., Seitzinger, S. P., Asner, G. P., Cleveland, C. C., Green, P. A., Holland, E. A., Karl, D. M., Michaels, A. F., Porter, J. H., Townsend, A. R. & Vorosmarty, C. J. 2004 Nitrogen cycles: past, present, and future. *Biogeochemistry* **70** (2), 153–226.

Ghanbari, F., Ahmadi, M. & Gohari, F. 2019 Heterogeneous activation of peroxymonosulfate via nanocomposite CeO_2-Fe_3O_4 for organic pollutants removal: the effect of UV and US irradiation and application for real wastewater. *Separation and Purification Technology* **228**, 115732–115742.

Guo, G., Wang, Y. Y., Hao, T. W., Wu, D. & Chen, G. H. 2018 Enzymatic nitrous oxide emissions from wastewater treatment. *Frontiers of Environmental Science & Engineering* **12** (1), 1001–1012.

Han, W., Li, T. M., Cheng, L. L., Liu, L., Yu, L. J. & Peng, Z. X. 2019 Effect of adding microorganism and carbon source to substrate on nitrogen removal treating the drainage of WWTP. *Water Science and Technology* **79** (10), 1947–1955.

Hashemi, E., Niayesh, K. & Mohseni, H. 2016 Effect of transverse magnetic field on low pressure argon discharge. *Turkish Journal of Electrical Engineering and Computer Sciences* **24** (6), 4957–4969.

Hou, L., Li, J., Zheng, Z., Sun, Q., Liu, Y. & Zhang, K. 2019 Cultivating river sediments into efficient denitrifying sludge for treating municipal wastewater. *Royal Society Open Science* **6** (9), 190304–190311.

Klyachko, N. L., Sokolsky-Papkov, M., Pothayee, N., Efremova, M. V., Gulin, D. A., Pothayee, N., Kuznetsov, A. A., Majouga, A. G., Riffle, J. S., Golovin, Y. I. & Kabanov, A. V. 2012 Changing the enzyme reaction rate in magnetic nanosuspensions by a non-heating magnetic field. *Angewandte Chemie-International Edition* **51** (48), 12016–12019.

Kuypers, M. M. M., Marchant, H. K. & Kartal, B. 2018 The microbial nitrogen-cycling network. *Nature Reviews Microbiology* **16** (5), 263–276.

Liu, Q., Li, H. & Lam, K. Y. 2019 Optimization of the cell microenvironment in a dual magnetic-pH-sensitive hydrogel-based scaffold by multiphysics modeling. *Bioelectrochemistry* **129**, 90–99.

Perazzoli, S., Michels, C. & Soares, H. M. 2017 Magnetite nanoparticles influence the ammonium-oxidizing bacteria activity during nitritation process. *Water Science and Technology* **75** (1), 165–172.

Pirsaheb, M., Moradi, S., Shahlaei, M., Wang, X. K. & Farhadian, N. 2019 Simultaneously implement of both weak magnetic field and aeration for ciprofloxacin removal by Fenton-like reaction. *Journal of Environmental Management* **246**, 776–784.

Plonsey, R. 1995 Magnetic field stimulation of multicellular excitable tissue approximated by bidomain. *Medical Biological Engineering Computing* **33** (3), 337–340.

Ranjan, B., Pillai, S., Permaul, K. & Singh, S. 2019 Simultaneous removal of heavy metals and cyanate in a wastewater sample using immobilized cyanate hydratase on magnetic-multiwall carbon nanotubes. *Journal of Hazardous Materials* **363**, 73–80.

Rice, E. W., Baird, R. B., Eaton, A. D. & Clesceri, L. S. 2012 *Standard Methods for the Examination of Water and Wastewater*. American Public Health Association, American Water Works Association, Water Environment Federation, Washington, DC, USA.

Sabouri, Z., Akbari, A., Hosseini, H. A., Hashemzadeh, A. & Darroudi, M. 2019 Bio-based synthesized NiO nanoparticles and evaluation of their cellular toxicity and wastewater treatment effects. *Journal of Molecular Structure* **1191**, 101–109.

Saetang, J. & Babel, S. 2010 Fungi immobilization for landfill leachate treatment. *Water Science and Technology* **62** (6), 1240–1247.

Sekoai, P. T., Awosusi, A. A., Yoro, K. O., Singo, M., Oloye, O., Ayeni, A. O., Bodunrin, M. & Daramola, M. O. 2018 Microbial cell immobilization in biohydrogen production: a short overview. *Critical Reviews in Biotechnology* **38** (2), 157–171.

Shafiee, S., McCay, M. H. & Kuravi, S. 2017 The effect of magnetic field on thermal-reaction kinetics of a paramagnetic metal hydride storage Bed. *Applied Sciences-Basel* **7** (10), 1006–1021.

Shanehsazzadeh, S., Lahooti, A., Hajipour, M. J., Ghavami, M. & Azhdarzadeh, M. 2015 External magnetic fields affect the biological impacts of superparamagnetic iron nanoparticles. *Colloids and Surfaces B-Biointerfaces* **136**, 1107–1112.

Wang, X. H., Diao, M. H., Yang, Y., Shi, Y. J., Gao, M. M. & Wang, S. G. 2012 Enhanced aerobic nitrifying granulation by static magnetic field. *Bioresource Technology* **110**, 105–110.

Wu, E. H., Li, Y. X., Huang, Q., Yang, Z. K., Wei, A. Y. & Hu, Q. 2019 Laccase immobilization on amino-functionalized magnetic metal organic framework for phenolic compound removal. *Chemosphere* **233**, 327–335.

Yang, C. Y., Wang, Q., Simon, P. N., Liu, J. Y., Liu, L. C., Dai, X. Z., Zhang, X. H., Kuang, J. L., Igarashi, Y. S., Pan, X. J. & Luo, F. 2017 Distinct network interactions in particle-associated and free-living bacterial communities during a *Microcystis aeruginosa* bloom in a plateau lake. *Frontiers in Microbiology* **8**, 1–15.

Zhang, K., Liu, Y. H., Luo, H. B., Chen, Q., Zhu, Z. Y., Chen, W., Chen, J., Ji, L. & Mo, Y. 2017 Bacterial community dynamics and enhanced degradation of di-n-octyl phthalate (DOP) by corncob-sodium alginate immobilized bacteria. *Geoderma* **305**, 264–274.

Zhang, W., Ren, X. H., He, J., Zhang, Q. R., Qiu, C. & Fan, B. M. 2019 Application of natural mixed bacteria immobilized carriers to different kinds of organic wastewater treatment and microbial community comparison. *Journal of Hazardous Materials* **377**, 113–123.

Zhao, Q., Xu, H. & Tao, L. 2017 Unsteady bioconvection squeezing flow in a horizontal channel with chemical reaction and magnetic field effects. *Mathematical Problems in Engineering* **2017 pt.1**, 1–9.

Zhao, Y. P., Jiang, B., Tang, X. & Liu, S. S. 2019 Metagenomic insights into functional traits variation and coupling effects on the anammox community during reactor start-up. *Science of the Total Environment* **687**, 50–60.

Zhu, G. L., Hu, Y. Y. & Wang, Q. R. 2009 Nitrogen removal performance of anaerobic ammonia oxidation co-culture immobilized in different gel carriers. *Water Science and Technology* **59** (12), 2379–2386.

Zubrowska-Sudol, M. 2018 Carbon source recovery from excess sludge by mechanical disintegration for biological denitrification. *Water Science and Technology* **77** (7), 1942–1950.

First received 3 December 2019; accepted in revised form 25 March 2020. Available online 6 April 2020