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Published in:
Water Research

Link to article, DOI:
10.1016/j.watres.2024.122127

Publication date:
2024

Document Version
Publisher's PDF, also known as Version of record

Citation (APA):
Jiang, Y., Yang, X., Zeng, D., Su, Y., & Zhang, Y. (2024). Nitrate recovery from groundwater and simultaneous upcycling into single-cell protein using a novel hybrid biological-inorganic system. *Water Research*, *263*, Article 122127. https://doi.org/10.1016/j.watres.2024.122127

Water Research 263 (2024) 122127

Contents lists available at ScienceDirect

Water Research

journal homepage: www.elsevier.com/locate/watres

Nitrate recovery from groundwater and simultaneous upcycling into single-cell protein using a novel hybrid biological-inorganic system

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ARTICLE INFO

Keywords:
Nitrate
Hydrogen-oxidizing bacteria
Single-cell protein
Groundwater
Carbon capture

ABSTRACT

Nitrate pollution in groundwater is a serious problem worldwide, as its concentration in many areas exceeds the WHO-defined drinking water standard (50 mg/L). Hydrogen-oxidizing bacteria (HOB) are a group of microorganisms capable of producing single-cell protein (SCP) using hydrogen and oxygen. Furthermore, HOB can utilize various nitrogen sources, including nitrate. This study developed a novel hybrid biological-inorganic (HBI) system that coupled a new submersible water electrolysis system driven by renewable electricity with HOB fermentation for in-situ nitrate recovery from polluted groundwater and simultaneously upcycling it together with CO_2 into single-cell protein. The performance of the novel HBI system was first evaluated in terms of bacterial growth and nitrate removal efficiency. With 5 V voltage applied and the initial nitrate concentration of 100 mg/L, the nitrate removal efficiency of 85.52 % and raw of 47.71 % (with a broad amino acid spectrum) were obtained. Besides, the HBI system was affected by the applied voltages and initial nitrogen concentrations. The water electrolysis with 3 and 4 V cannot provide sufficient H_2 for HOB and the removal of nitrate was 57.12 % and 59.22 % at 180 h, while it reached 65.14 % and 65.42 % at 5 and 6 V, respectively. The nitrate removal efficiency reached 58.40 % and 50.72 % within 180 h with 200 and 300 mg/L initial nitrate concentrations, respectively. Moreover, a larger anion exchange membrane area promoted nitrate removal. The monitored of the determination of different forms of nitrogen indicated that around 60 % of the recovered nitrate was assimilated into cells, and 40 % was bio-converted to N_2. The results demonstrate a potentially sustainable method for remediating nitrate contaminant in groundwater, upcycling waste nitrogen, CO_2 sequestration and valorization of renewable electricity into food or feed.

1. Introduction

Groundwater, which is more reliable and accessible than surface water, is the preferred drinking water source globally and is used to supply more than 2 billion people (Carrard et al., 2019; Kuang et al., 2024). Nitrate contamination of groundwater has become a serious environmental issue worldwide since the 1970s, due to the use of fertilizers in agricultural activities, wastewater discharge and solid waste disposal during rapid industrialization (Fida et al., 2024; Rivett et al., 2008; Wakida and Lerner, 2005; Xin et al., 2021). The World Health Organization (WHO) has set a maximum contaminant level (MCL) concentration for drinking water to 50 mg/L of nitrate (10 mg/L nitrate-nitrogen) (WHO, 2022). High nitrate levels in drinking water pose health risks such as methemoglobinemia, gastric problems in adults and decreased functioning of the thyroid gland (Alam et al., 2023; Jiang et al., 2018; Kwon et al., 2021). Moreover, as the global population is set to rise to 9.7 billion by 2050. Several physicochemical (e.g. membrane), electrochemical (e.g. electrodialysis) and biological technologies (denitrification) have been developed to address this challenge (Abascal et al., 2022; Aliaskari and Schafer, 2021; Amoako-Nimako et al., 2021; Jensen et al., 2014; Sharma and Bhattacharya, 2016). However, most technologies focus on removal rather than recovery and upcycling, so more efficient and sustainable ways of recovering and upcycling nitrate need to be pursued.

Besides groundwater contamination with nitrate, conventional agriculture is under pressure to meet the demand for food and facing a series of environmental challenges (Sekoai et al., 2024; Zhang et al., 2022). Protein is the building block of life and an essential part of the

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https://doi.org/10.1016/j.watres.2024.122127
Received 23 April 2024; Received in revised form 13 July 2024; Accepted 20 July 2024

human diet for maintaining the body and cellular functions; consequently, population growth means an increased need for protein (Henchion et al., 2017; Kim et al., 2019; Nadathur and Wanasundara, 2017). Single-cell protein (SCP) is a product of fermentation, and as one of the more novel and alternative protein sources, it has gained interest in the past few years (Bratosin et al., 2021; Hadi and Brightwell, 2021). Among others, hydrogen-oxidizing bacteria (HOB) is a facultative autotrophs that can use hydrogen and oxygen (as the electron donor and acceptor, respectively) to fix CO_2 into protein-rich biomass (Givirovskiy et al., 2019; Mishra et al., 2020; Pous et al., 2022; Yang et al., 2022b). Thus, SCP synthesis by HOB contributes to carbon capture and utilization. Nitrogen is an essential component of dietary proteins, constituting 16 % thereof. Different forms of nitrogen, including ammonium and nitrate, can be recovered from wastewater and assimilated into SCP by HOB (Gilland, 2015; Lassaletta et al., 2016; Yang et al., 2021); thus, nitrate from groundwater could be an alternative nutrient source for SCP production. Nevertheless, a novel non-invasive approach that can recover nitrate in-situ from groundwater and simultaneously upcycle it into SCP is still missing.

This study developed a new hybrid biological-inorganic system (HBI) for in-situ nitrate recovery from groundwater and simultaneous upcycling into SCP by combining a novel submersible water electrolysis cell with HOB fermentation into one chamber. The interior of the HBI system was separated from the groundwater through an anion exchange membrane (AEM), and when it was submerged in groundwater, nitrate was first transported through the AEM into the HBI reactor, due to the concentration gradient. Next, hydrogen and oxygen generated from the water electrolysis process, driven by renewable electricity and externally dosed CO_2 were taken up by HOB to produce protein-rich biomass. First, the performance of the HBI system, in terms of HOB growth condition and nitrate removal efficiency, was evaluated. The voltage applied for water eletrolysis, and initial nitrate concentration in groundwater, determine the substrate supplied for HOB, thereby affecting the growth performance of HOB as well as nitrate removal. In addition, the AEM was identified as a key factor for the nitrate recovery rate. Furthermore, anions other than nitrate in groundwater may reduce recovery efficiency due to how they compete with nitrate over the membrane, which needs to be explored further. Finally, nitrogen transformation and the amino acid profile were analyzed. This study provides a strategy for in-situ nitrate recovery from contaminated groundwater and upcycling into a value-product, achieved simultaneously by hydrogen-oxidizing bacteria.

2. Methods and materials

2.1. HBI system setup and operation

The HBI system was a chamber electrochemical membrane reactor with an AEM at one end (170 mL working volume), making it submersible into a container simulating groundwater (working volume of 2 L) (Fig. 1). In the HBI chamber, alloy mesh coated with IrO_2 (4 × 4 cm) and titanium mesh (4 × 4 cm) were used as the anodic and cathodic electrodes, respectively. Nitrate (NO_3^-) in the groundwater passed through the AEM into the HBI reactor, driven by the concentration gradient. Electricity was applied via a power supply (30 V/5A DC, Sagitta, Sweden) to split the water into H_2 and O_2, which were then used

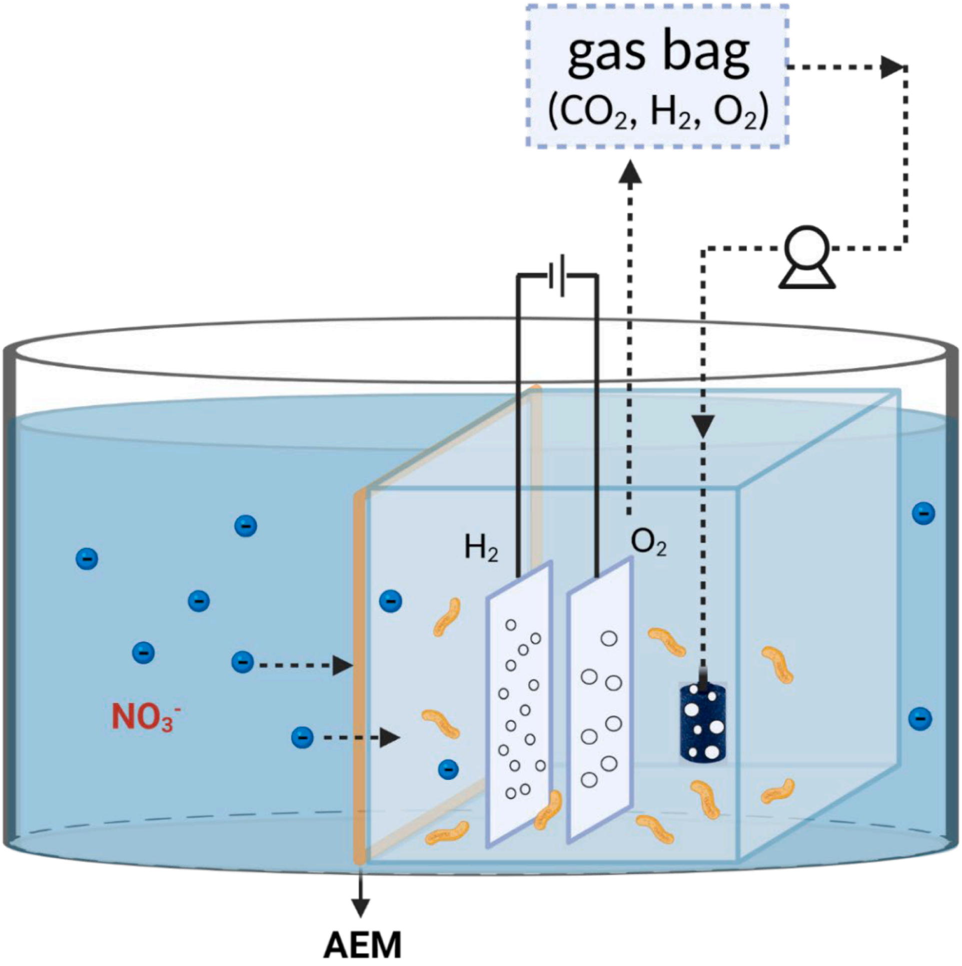

Fig. 1. The schematic illustration of the HBI system: including a groundwater container and the HBI reactor.

in-situ along with CO_2 by HOB. CO_2 filled a gas bag connected to the HBI reactor and was recirculated using a peristaltic pump (BT100-2J, LongerPump, China) at a speed of 5 mL/min. A magnetic stirrer (VS-C4, VWR International, USA) was used to promote gas-liquid mass transfer in the HBI reactor and the movement of NO_3^- in the groundwater container.

2.2. *Microbial strain, medium, and substrate*

Cupriavidus necator H16 (DSM 428) was purchased from DSMZ (Leibniz Institute DSMZ-German Collection of Microorganisms and Cell Cultures GmbH). The medium in the HBI reactor followed the recipes Medium 81 (except NH_4Cl) and Medium 27 (DSMZ_Medium 27; DSMZ_Medium 81) (Table S1). Nitrate-contaminated synthetic groundwater was prepared with tap water and $NaNO_3$.

2.3. *Experimental procedure*

The HBI reactor was submerged in synthetic groundwater containing 100 mg/L nitrate, and 5 V was applied between the anode and cathode electrodes. A semi-continuous mode (R - Batch) was adopted during the experiment. The medium for HOB growth in the HBI reactor and gas bag represented the batch charges and the synthetic groundwater continuously provided nitrate for HOB. The groundwater was replaced with new synthetic groundwater at the end of every test (including several batches), and the medium in the HBI reactor and gas in the bag were changed for every batch. A decrease in the DO value indicated the end of a batch. *C. necator* H16 was inoculated at 12 h for every batch run to ensure sufficient nitrate was transferred to the HBI reactor for bacteria growth. The conductivity of HOB medium, which is the electrolyte, was ~ 4.26 mS/cm at the beginning (no nitrogen source). In total, 1500 mL CO_2 gas was injected into the recycling gas bag at the beginning of each batch. Once the bacteria stopped growing, the OD optical density at 600 nm (OD_{600}) was no longer increasing, and the medium in the HBI reactor and the gas bag were refilled. NO_3^--N in the synthetic groundwater provided a continuous nitrogen source for the HBI reactor until the concentration dropped to an insufficient level suitable for HOB growth. Reference the previous study by Yang et al. on the recovery of ammonia nitrogen in wastewater, different supplied voltages (3, 4, 5, and 6 V) were chosen to supply for water splitting (Yang et al., 2022b). Subsequently, different initial nitrate concentrations (50, 100, 200, and 300 mg/L) in synthetic groundwater were investigated. Moreover, the nitrate removal efficiencies were compared between the HBI reactors using one and two sides of AEM. In the end, other anions, such as bicarbonate, sulfate, and chloride, were added to the synthetic groundwater to explore the competition between anions of passing AEM.

2.4. *Analytical methods and calculations*

The OD_{600} was tested with a spectrophotometer (Varian Cary 50 Bio, Varian, Australia) to evaluate the growth of *C. necator* H16. The biomass concentration collected from the remaining culture medium was expressed in dry cell weight (DCW, g/L), which also implies raw protein weight. The gas composition and amino acid profile of the SCP were analyzed according to the methods described in previous study (Jiang et al., 2022; Li et al., 2021; Yang et al., 2021). The pH was measured with a pH meter (PHM92, Radiometer analytical, Denmark), and the conductivity of the solution was measured by a meter (Hach, HQD Field case). The voltage across the resistor (10 Ω) was monitored using a multimeter (Mode 2700, Keithley Instruments, USA) every 30 min while operating the HBI reactor.

The concentrations of NO_3^-, NO_2^- and NH_4^+ were analyzed by kits (LCK 339 Nitrate, LCK 341 Nitrite, and LCK 304 Ammonium, Hach Lange GmbH), and the samples were tested by the spectrophotometer (DR 3900, Hach Lange GmbH).

3. Results and discussion

3.1. *The HBI system performance*

A proof of concept study was conducted, and the results are shown in Fig. 1. In this case, the concentration gradient was the driving force for nitrate recovery, and a pre-test was performed first to assess the ability of nitrate diffusion through the AEM, with no power supplied. Figure S1a highlights the change of nitrate concentrations in the HBI reactor and synthetic groundwater when the initial nitrate concentration in the groundwater was 100 mg/L. During the first 11 h, the nitrate recovery rate reached 0.64 mg/h, while it was only 0.11 mg/h from 11 to 48 h, and as the concertation gap decreased, the recovery rate slowed down. The nitrate concentration in the groundwater did not change greatly because the volume of the groundwater container (2 L) was almost 12 times larger than the HBI reactor (170 mL). The maximum nitrate concentration in the HBI reactor and the final nitrate concentrations in groundwater were 67.72 mg/L and 95.50 mg/L, respectively. The nitrate concentrations between AEM did not reach the same because of the hindrance from the membrane (Epsztein et al., 2019). The blank and control tests were also performed (Figure S1b).

After the pre-tests, the HBI system was operated for seven consecutive batches (361 h), with 5 V applied voltage and 100 mg/L nitrate in synthetic groundwater. The initial pH of the medium in the HBI reactor was approximately 6.9, and it was maintained within the range of 6.3~6.9 throughout the operation. The slight decrease in pH was due to the introduction of CO_2 into the medium. As HOB consumed CO_2, the pH remained within a relatively stable range. The OD_{600} value and nitrate concentrations in the HBI reactor were growing at the beginning of batches (Fig. 2a), following which they dropped while HOB continued to grow, indicating that HOB consumed the nitrate. Furthermore, HOB continued to grow even after no nitrate was detected, because nitrate fed into the HBI reactor was consumed simultaneously. The demand for nitrogen sources increased as the cell density increased. Thus, the OD_{600} decreased and then moved to the next batch. It is evident that the maximum OD value of each batch decreased due to the nitrate concentration in the groundwater constantly declining over time, as well as the nitrogen source diminishing. After seven batches, nitrate concentration in the groundwater was 14.48 mg/L, which is lower than the nitrate maximum contaminant level set by WHO. The removal efficiency of groundwater reached 85.52 %. Another HOB strain, namely TH20, has resulted in a nitrate removal efficiency of $>$ 99 % with 80 mg/L NO_3^--N, because it was inoculated into the medium containing nitrate (Chen et al., 2022), while in our study, nitrate recovery and assimilation were separated. We monitored the gas volume and content in every batch to ensure that it was sufficient in terms of supporting HOB growth (Fig. 2b). The operating cycle and HOB growth condition differed for every batch, so the gas consumption was different. The results indicate that the limiting factor here for HOB growth was the lack of the nitrogen source.

Nitrogen transformation with 100 mg/L nitrate (22.58 mg/L NO_3^--N) in groundwater was explored (Fig. 3a). The NO_3^--N concentration decreased significantly for the batch and was 3.16 mg/L after the seventh cycle. Around 60 % nitrate was assimilated to biomass, while 40 % was mitigated to N_2 gas and emitted from the biosphere into the atmosphere. Chen et al. and Zhang et al. reported that NH_4^+-N and NO_2^--N are generated or accumulated during nitrite assimilation by the HOB process (Chen et al., 2022; Zhang et al., 2020), but there was neither NH_4^+-N nor NO_2^--N detected in our study. However, it should be noted that we only tested nitrogen at the end of the batch instead of monitoring it during the batch run. In addition, it was unable to determine if the denitrification and dissimilatory to ammonium (DRNA) pathway occurred because ammonium can also be a nitrogen source for *C. necator* H16 (Chen et al., 2022; Jiang et al., 2022). Thus, ammonium could be utilized once generated. As for nitrite, it may be reduced to N_2 completely in aerobic denitrification. The metabolic process should

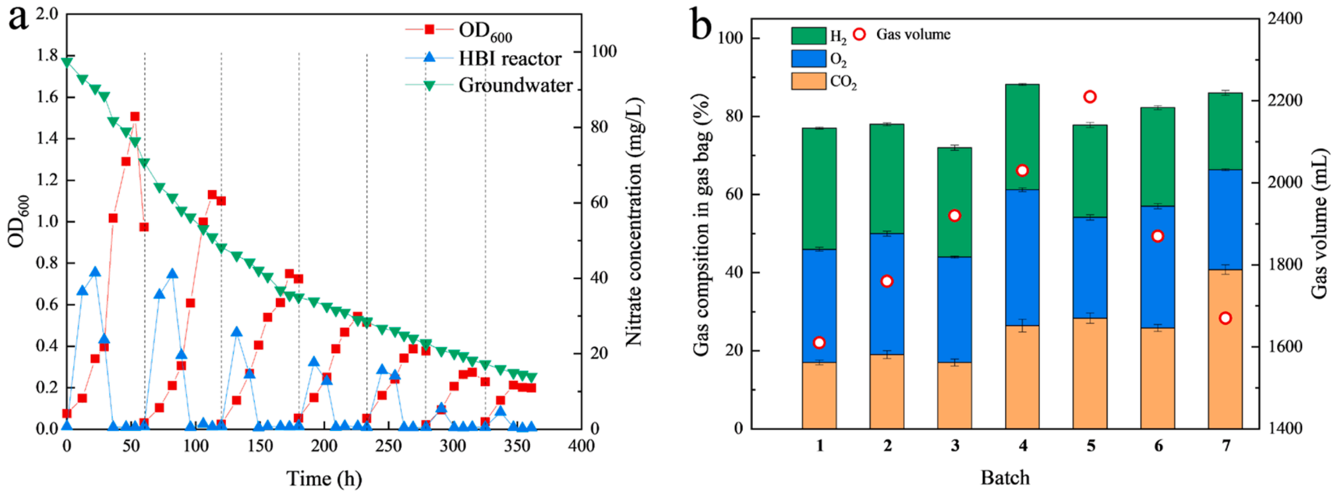

Fig. 2. The HBI system performance for seven consecutive batches with 5 V applied voltage for water splitting, and 100 mg/L initial nitrate concentration in groundwater, (a) the changes of OD value of *C. necator* H16, and nitrate concentrations of both in HBI reactor and groundwater over time, (b) gas volume and composition at the end of every batch in the gas bag connected with the HBI reactor.

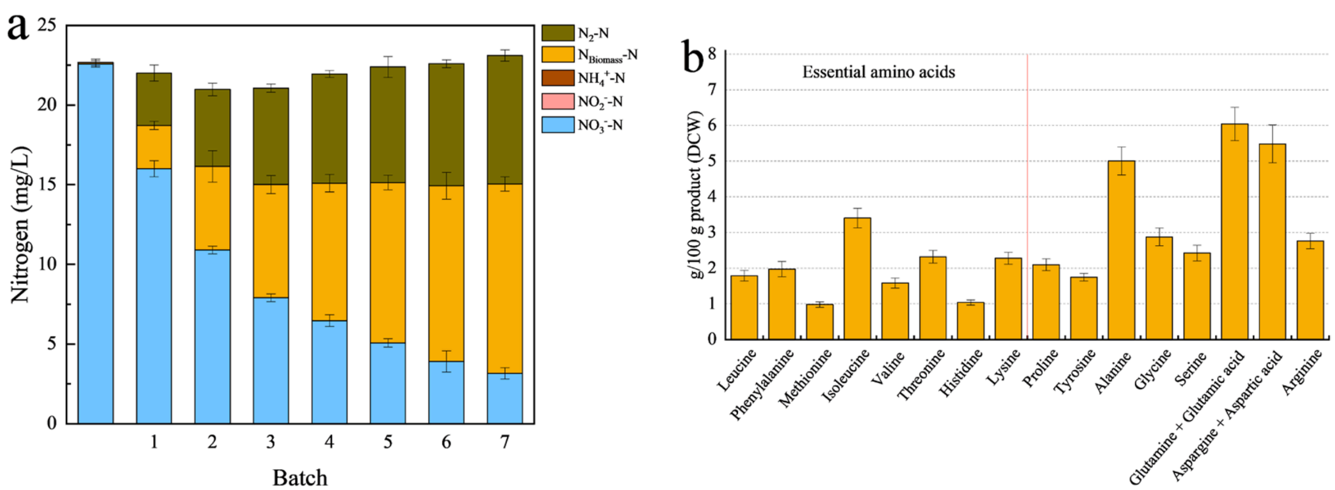

Fig. 3. (a) Various nitrogen forms at every batch end during the nitrate assimilation process by *C. necator* H16 with an initial concentration of 100 mg/L nitrate (22.58 mg/L NO_3^--N). (b) Amino acid profile of biomass produced by *C. necator* H16.

therefore be explored further. High-quality SCP was obtained from the biomass collected from the HBI reactor (Fig. 3b). Up to 16–18 amino acids were detected, accounting for 47.71 ± 2.27 % of DCW, and eight essential amino acids were detected at 35.46 % (Table S2), which is close to the content in soybean and fishmeal (Food and Agriculture Organization of the United Nations (n.d.); FoodData Central (n.d.)).

Table 1 illustrates the cost estimation of producing 1 kg SCP with an initial nitrate concentration of 100 mg/L in groundwater and the applying voltage of 5 V on the HBI reactor. The cost mainly includes chemicals in the medium, energy consumption, CO_2 supply, and the dehydration and drying of biomass. The production cost of 1 kg SCP using our HBI system is approximately 18.99 euros, with a treatment capacity of 3501 L of contaminated groundwater. The reactor was expected to operate with long-term, and the construction fee not factored into the analysis. Currently, the biomass production costs by HOB, microalgae and yeast are significantly higher than the current cost ~1.7 USD (https://www.indexmundi.com/commodities/?commodity=fish-meal, 2024) per kg for fishmeal (Hulsen et al., 2022; Zeng et al., 2023). The electricity consumption accounts for a high proportion. For our HBI system, selecting a more effective electrolysis technology or using renewable electricity can produce low-cost SCP (Khoshnevisan et al., 2022).

Table 1
Financial cost for SCP production (per kg).

Items	Cost (€)
Phosphate buffer solution (KH_2PO_4 and $Na_2HPO_4 \cdot 2H_2O$)	8.25
Inorganic salts, trace elements, and vitamins	0.00022 (Zeng et al., 2023)
Energy consumption (water splitting and reactor operation)	10.5
CO_2 supplementary	0.066
Dehydration and drying	0.17 (Li et al., 2024)
Estimated total cost	**18.99**

3.2. *The influence of operating parameters on the performance of the HBI system*

3.2.1. *Applied voltage*

H_2 and O_2 are the electron donor and acceptor, respectively, for *C. necator* H16 to grow, and their production depends on the water-splitting performance. Different voltages (3, 4, 5, and 6 V) were applied to the electrodes, and the nitrate concentration in groundwater was 100 mg/L. The currents under each voltage were stable (Figure S2) during the three consecutive cycles. As shown in Fig. 4, the maximum OD value of every batch increased with the voltage and was lower with 3 V compared with other voltages. It is known that higher voltage contributes to higher water splitting efficiency, which means there would be more soluble gases in the medium for HOB growth. With 3 V applied voltage, O_2 and CO_2 were sufficient, while generated H_2 was insufficient to support bacterial growth (Fig. 4b, c, and d). H_2 content with 4 V was relatively low compared with 5 and 6 V, leading to a slower HOB growth rate. The HOB growth performance with 5 and 6 V was similar, so the gas generated with 5 V could support HOB growth well. The nitrate removal efficiencies at the end of the 3rd cycle were 57.12 %, 59.22 %, 65.14 %, and 65.42 % with 3, 4, 5, and 6 V, respectively. Nitrate was utilized as the nitrogen source for *C. necator* H16, whose growth status also affected the nitrate recovery and assimilation. Appropriate voltage should be selected to achieve high nitrate removal efficiency while saving energy.

3.2.2. Different initial nitrate concentrations in synthetic groundwater

Nitrate contamination in groundwater varies in regions or areas, and some cases showed high nitrate concentrations of up to 300∼400 mg/L (Kurwadkar et al., 2020; Sun et al., 2021). Different nitrate concentrations ranging from 50 to 300 mg/L in synthetic groundwater were tested with an applied voltage of 5 V based on the results from different applied voltages. The maximum OD value reached around 2.78 with an initial nitrate concentration of 300 mg/L in the first cycle, while it was only 1.51 with 100 mg/L of nitrate (Fig. 5). In this case, the diffusion model follows Fick's law, indicating the substance diffusion rate across the unit area was proportional to the concentration gradient (Davis and Kenny, 2015). Fick's Law shows the mass transport of charge flux due to a concentration gradient as formula (1) (Sundén, 2019)

$$J_i = -D_i \cdot \nabla C_i \tag{1}$$

where J is the diffusion flux and C is the concentration gradient. The initial nitrate concentration in groundwater was the concentration gradient in our case. Thus, higher initial nitrate concentration indicated that more nitrate passed through the AEM from groundwater into the HBI reactor and was assimilated into cells. 50 mg/L nitrate is within WHO's acceptable level, but it could be further reduced to 36.00 mg/L after 60 h, and the OD value reached 0.73. The nitrate removal efficiencies were 65.14 %, 58.40 %, and 50.72 %. Meanwhile, the removal rates were 0.35, 0.66, and 0.86 mg/L/h after three cycles with 100, 200, and 300 mg/L initial nitrate concentrations, respectively. The results suggested the HBI system could remain stable even with high nitrate loads. If running more cycles, the nitrate concentration can continue to

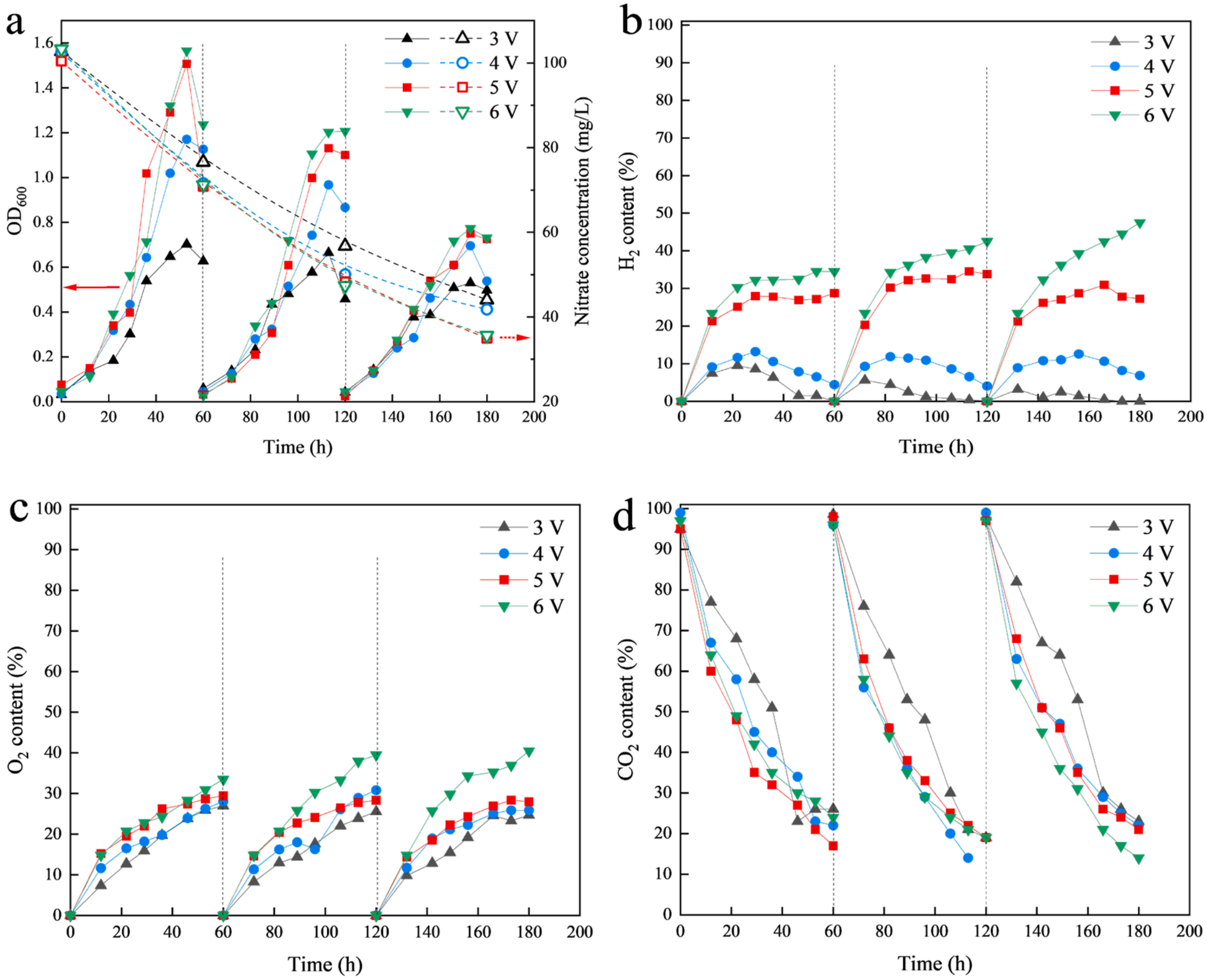

Fig. 4. The performance of the HBI system with different applied voltages under 100 mg/L initial nitrate concentration in groundwater. The changes of (a) OD_{600} and nitrate concentration in groundwater, (b) H_2, (c) O_2, and (d) CO_2 content over time.

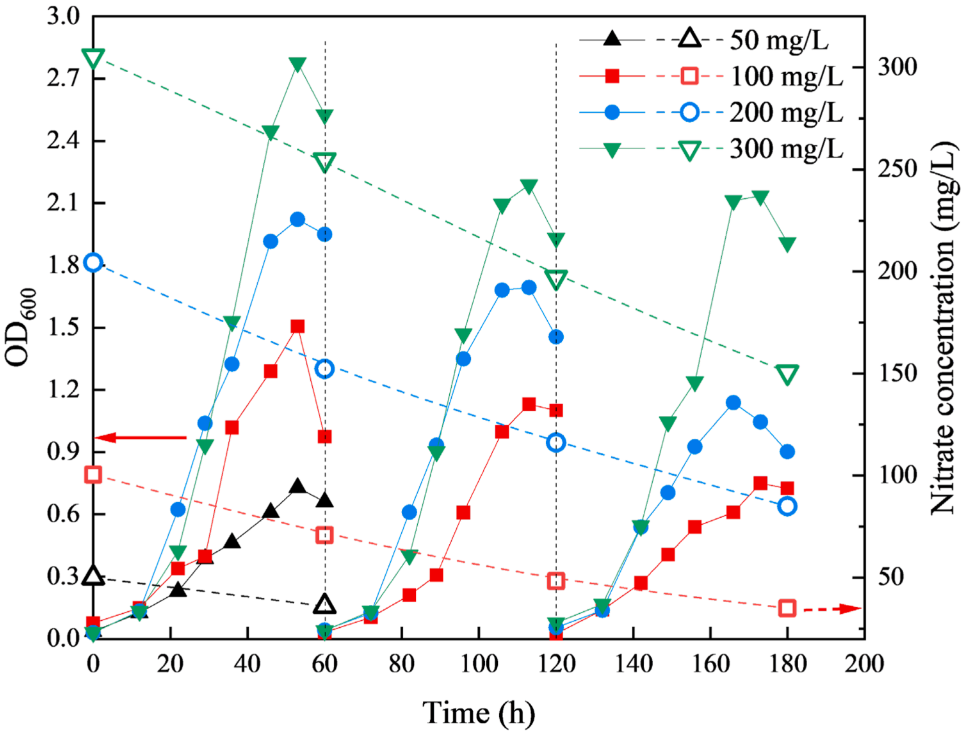

Fig. 5. The performance of the HBI system with different initial nitrate concentrations in synthetic groundwater under 5 V applied voltage.

decrease until below the maximum pollutant level.

3.2.3. AEM areas

Fick's Law of diffusion describes the time course of the transfer of a solute between two compartments that are separated by the membrane, given by (Enderle, 2012)

$$\frac{dq}{dt} = -DA\frac{dc}{dx} \tag{2}$$

where q is the quantity of solute and A is the membrane surface area, suggesting that within the unit time, the quantity of transfer solute is proportional to the area of the membrane. As shown in the schematic diagram (Fig. 1), we only installed the AEM on one side (5 cm × 5 cm). Comparatively, HBI with AEM on the two ends were explored with 5 V and 100 mg/L nitrate concentration. The OD value and nitrate concentrations were monitored within two cycles (Fig. 6). In the first cycle, the OD value could reach 2.15 with two sides AEM, almost 1.5 times that of HBI reactor with AEM on one end. While in the second cycle, the maximum OD value of two sides of AEM was lower than it of one side. At the beginning of the second cycle, the nitrate concentration in groundwater were 70.86 and 58.90 mg/L with one and two AEM, so the nitrate content was not high enough for HOB to grow. The nitrate concentration in the HBI reactor dropped to 0 quickly after HOB was cultivated. The removal efficiency reached 51.45 % and 62.20 % at 120 h. The results proved that a larger AEM area promoted nitrate recovery.

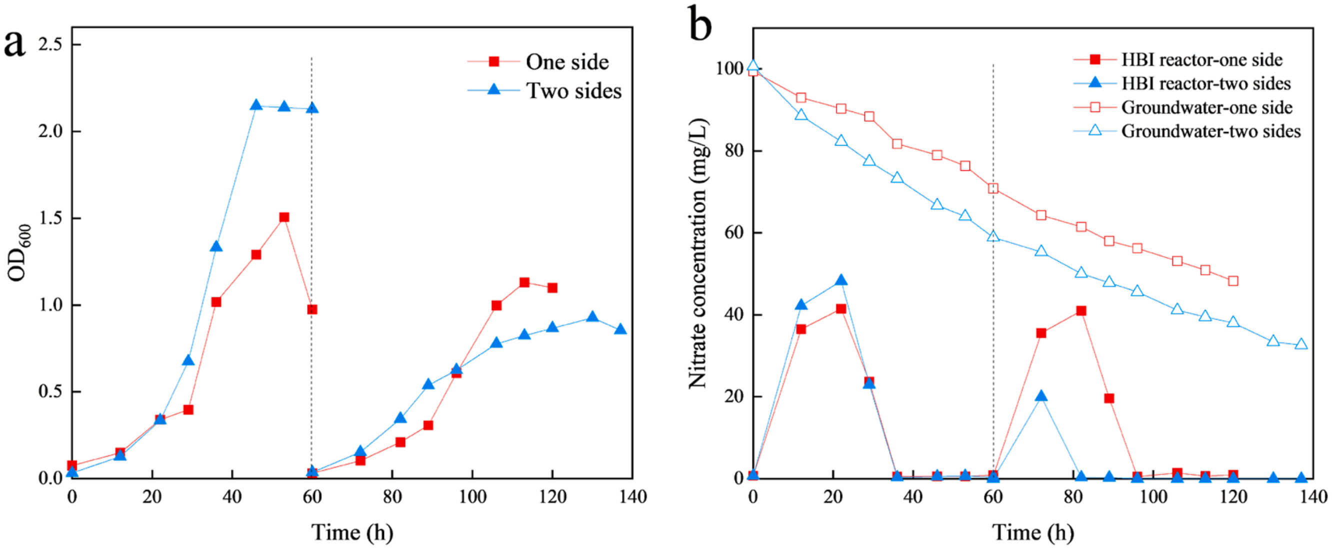

Fig. 6. The performance of the HBI system with different AEM areas. The changes of (a) OD$_{600}$, and (b) nitrate concentrations in the HBI reactor and synthetic groundwater, over time.

3.2.4. *Coexisting anions*

In actual groundwater, in addition to nitrate, other anions such as bicarbonate, sulfate, and chloride also exist, and competition may occur when anions pass through the AEM. Therefore, bicarbonate, sulfate, and chloride (each at a concentration of 100 mg/L) were added to the synthetic groundwater, in addition to nitrate, to investigate their effect on nitrate recovery. As shown in Fig. 7, regardless of the presence of anions, there was no significant difference in the HOB growth condition or the nitrate recovery efficiency. The results indicated that the anion exchange capacity was high enough to allow anions to pass in our study, and the concentration gradient was the only influencing factor.

3.3. *Significance and perspectives*

The study demonstrated how nitrate could be recovered from groundwater as an alternative nitrogen source for *C. necator* H16 to SCP production through a novel designed HBI system that integrated the wastewater treatment and hydrogenotrophic biosynthesis process. The results showed the potential of the HBI system to recover nitrate and simultaneously assimilate nitrogen into protein-rich biomass. Nitrate recovery was achieved through the concentration gradient rather than electricity-driven, a way to save energy. The HBI performance was assessed when it was submerged in 100 mg/L nitrate-contaminated synthetic groundwater and with 5 V electricity supply. The nitrate concentration after removal (14.48 mg/L) was well below the level set by WHO (50 mg/L), and a high quality of biomass containing a broad amino acid spectrum was collected. Several operating parameters were tested based on this system. The supply of gaseous substrates was the basis for efficient nitrate upcycling during the biosynthesis process. Thus, the applied voltage should be high enough to ensure sufficient H_2 and O_2 production for HOB growth. Considering the nitrate concentration in the real groundwater, a wide range from 50 to 300 mg/L was tested in the HBI system, which displayed good performance even under high nitrate loading. In the diffusion process, the area of the membrane was a key parameter. After increasing the AEM area, the nitrate recovery rate was accelerated. These explorations offer insights into developing a new reactor design which turns waste into treasure efficiently and sustainably.

Though promising, some limitation or improvement needs to be addressed. The submersible HBI reactor configuration may offer a possibility for in-situ groundwater remediation. AEM is designed to conduct anions; thus, anions in the medium could also pass through into groundwater, so further evaluation of groundwater quality after treatment is necessary. Besides, the compositions of real groundwater, and the hydrogeological and hydrogeochemical conditions are more complex. Thus, the feasibility and performance of the system in real applications should be further explored. Moreover, renewable electricity such as solar and wind energy can be applied to power the process, achieving the utilization and storage of renewable energy. Power-driven nutrient recovery can reach higher efficiency quickly (Yang et al., 2022a; Zeng et al., 2023), which can be investigated later in our system. Furthermore, HOB-based SCP has not been allowed to enter the market. The safety assessment and human acceptance are both critical issues, especially for the SCP produced from the waste stream.

4. Conclusions

This study established a novel hybrid biological-inorganic system (HBI) system, which achieved in-situ nitrate recovery from contaminated groundwater and simultaneous upcycling into microbial food by hydrogen-oxidizing bacteria. The main conclusions are shown below.

- The concentration gradient between the membrane drove nitrate recovery, and the cultivated *C. necator* H16 in the HBI reactor assimilated the recovered nitrate into protein-rich biomass. Both the quantity and quality of produced protein are appreciable.
- Around 60 % of the nitrate was assimilated to biomass, and 40 % was transformed into N_2. No ammonium and nitrite were detected in this case.
- 14.48 mg/L of nitrate (or 85.52 % nitrate removal efficiency) was obtained with the initial nitrate concentration of 100 mg/L and 5 V applied voltage.
- H_2 and O_2 produced through water electrolysis were the limiting factors for HOB growth. Appropriate voltage should be explored for the HBI system.
- The concentration gradient was the driving force for nitrate recovery, and higher nitrate concentration in groundwater enhanced its upcycling.
- Increasing the area of the anion exchange membrane can accelerate nitrate recovery.
- The coexistence of other anions did not affect the operation and performance of the HBI system.

Overall, this study realized nitrate recovery, waste nutrient

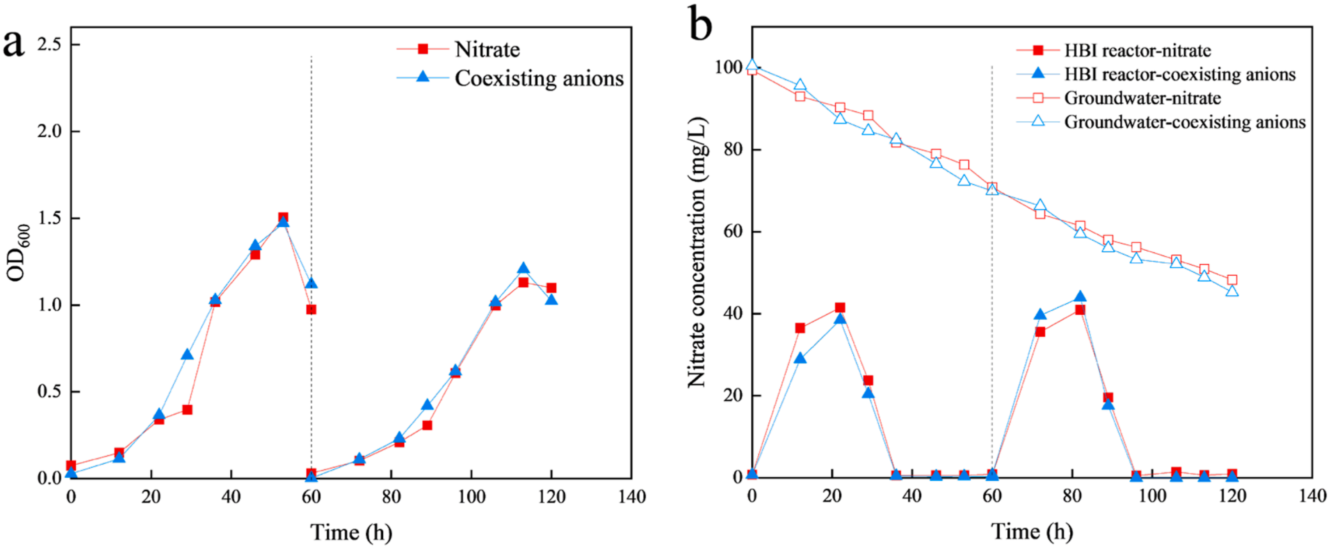

Fig. 7. The performance of the HBI system with different anions in synthetic groundwater. The changes of (a) OD_{600}, and (b) nitrate concentrations in the HBI reactor and synthetic groundwater, over time.

upcycling combined carbon capture, waste stream utilization, biosynthesis, and valued product production. The unique HBI system provides an efficient, sustainable, and energy-saving strategy for nutrient recovery and microbial food production.

CRediT authorship contribution statement

Yufeng Jiang: Writing – review & editing, Writing – original draft, Visualization, Validation, Methodology, Investigation, Data curation, Conceptualization. **Xiaoyong Yang:** Writing – review & editing, Methodology, Investigation, Formal analysis. **Danfei Zeng:** Writing – review & editing, Methodology, Formal analysis. **Yanyan Su:** Writing – review & editing, Supervision, Resources, Methodology, Conceptualization. **Yifeng Zhang:** Writing – review & editing, Supervision, Resources, Project administration, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Acknowledgements

Yufeng Jiang, Xiaoyong Yang, and Danfei Zeng would like to acknowledge the China Scholarship Council for financial support. Yifeng Zhang thanks The Carlsberg Foundation (CF18-0084), The Ministry of Foreign Affairs of Denmark (No. 21-08-DTU), the VILLUM Experiment Programme (No. 40828), the European Union under HORIZON-JU-IA programm (No. 101112345), and the Independent Research Fund Denmark (Project 1, 1032-00028B) for funding the research.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.watres.2024.122127.

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