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Biological denitrification using microbial electrochemical technology: a perspective of materials, the arrangement of electrodes and energy consumption

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ABSTRACT

This research aimed to study the performance of electroactive denitrifying bacteria on biological nitrate reduction using carbon cloth plate and cylindrical stainless steel mesh electrodes in a microbial electrochemical system (MES). The carbon to nitrogen (C/N) ratio, energy consumption, microbial population, nitrate coulomb-reduction rate, and hydrogen production were considered during the experiments. The optimum condition for nitrate removal was obtained at C/N = 2, applied current = 2 mA, and reaction time = 6 h. Nitrate coulomb-reduction rate and hydrogen gas generation were 3.33 mg C^{-1} and 2.2×10^{-4} moles, respectively. The consumption of electricity and power were computed 0.0104–0.096 kWh m^{-3} and 9.9×10^{-5}, respectively. The analysis of microbial community relying on 16S ribosomal ribonucleic acid (rRNA) genes demonstrated that the denitrifying bacteria mainly belonged to *Bacillus* spp., and *Pseudomonas* spp. This integrated MES makes it possible to both NO_3^- and NO_2^- removed effectively. This system could achieve a high denitrification performance with low nitrate and ammonia accumulation due to providing suitable and larger surface area for bacterial adhesion, uniform distribution and better electron exchange in redox reactions.

Keywords: Denitrification; Energy consumption; Microbial electrochemical system; Steel mesh; Carbon cloth

1. Introduction

Industrialization, urbanization and agricultural practices can cause various pollutants to enter into the environment. Compounds containing nitrogen are among those pollutants, which can lead to serious problems when discharged into the environment [1,2]. Water quality deterioration and potential risk to human or animal health are examples of these problems [3]. During the last 40 years, water has influenced by high concentrations of nitrate in various regions. It has posed as an environmental issue with global concerns [4]. Suggested control methods for nitrate reduction have some difficulties. Recently, microbial electrochemical systems (MESs) have been presented as an applicable technology for the denitrification process, electrical energy production, and generation of renewable hydrogen gas. In these systems, reduction and oxidation reactions are electrochemically catalyzed by bio-electrode interaction [5]. In MES, electrical stimulation can enhance pollutant removal due to microbial metabolism improving [6]. Applying electrical current enhances the ion migration rate, promoting the reactions occurring on the electrode surface. The reactions in the bio-electrodes can be varied and intensified [1]. Applying wastewater as a substrate containing a low carbon footprint is interesting because of the growing demand for the treatment of low-grade streams in the environment [7].

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Factors controlling denitrification by a bio-cathode and removal of organic matter at bio-anodes include electrode material and surface area, electrode spacing, organic matter concentration, electric current, and performance of bioelectrodes [5]. Hence, the electrode material acts as one important parameter in the bioreactor performance. The improved effective surface area of electrodes can promote process efficiency [8]. Among electrode materials, carbon materials have a qualified mechanical strength and a sufficiently rough surface, which is ideal for biofilm formation [5]. Carbon-based materials such as carbon cloth, are the most promising materials due to cheaper process costs and their stability, while microorganisms are attached and grown on them [9]. Stainless steel mesh can also be appropriate owing to its manageability, common strength, and suitable electrokinetic properties. It can be one of the materials achieving desirable results in organic species elimination. The bacteria with the ability to catalyze the organic matter oxidation and direct electron transfer, grow easily on these electrode surfaces and can conduct electrons to the electrode [5]. Several modifications such as coating with active polymers, electron mediators, polyaniline, and quinone groups have been suggested and investigated to promote the efficiency of electrode substances with great ohmic resistance. Some modification materials like copper, have been indicated to be improper as anode electrode because of their solubility characteristics which are toxic to the bacterial community [5]. Carbon cloth and stainless steel can be applied using with and/or without partial modification [1]. Providing larger surface areas and subsequently, more space for microbial attachment results in increased electron transfer rates. These show that power output and electricity generation is dependent on the adhesion of biofilm to the electrode surface. More electrodes conductivity can decrease the required electric current and energy consumption. So, oxidation and reduction rate in bioanode and biocathode can be improved respectively [5]. According to studies, various substances were surveyed as the electrode, but carbon cloth and steel mesh which can be good nominations for bio-electrochemical denitrification was not considered together as electroactive biofilm electrode in the studied configuration and system, especially in respect of bioelectrode formation and energy utilization. In the present work, a new integrated bio-electrochemical single-chambered system applying carbon cloth sheet and steel mesh (as the base of electrodes with different design and arrangement) was used to enhance bioelectrode formation, energy conservation, and denitrification process. The obtained results can be applied as reference data to improve the anoxic bio-electrochemical technique for the denitrification process.

2. Material and methods

2.1. Materials

The materials used in this work were sodium acetate ($C_2H_3NaO_2$), glucose ($C_6H_{12}O_6$), sodium bicarbonate ($NaHCO_3$), methanol (CH_3OH), potassium nitrate (KNO_3), potassium dihydrogen phosphate (KH_2PO_4), sodium hydrogen phosphate (Na_2HPO_4), magnesium sulfate ($MgSO_4$), sodium chloride (NaCl), hydrogen chloride (HCl), sodium hydroxide (NaOH), and sulfuric acid (H_2SO_4). All reagents and chemicals were high purity and provided from Merck, Germany.

2.2. Bio-electrochemical reactor setup

Fig. 1 schematically shows the batch experimental bioreactor. The bioreactor unit was cylindrical plexiglass (diameter 100 mm and height 250 mm) with the net volume of 2 L. The electrodes were immersed in the wastewater 20 mm spacing between each other. To obtain a correct mixture of the wastewater, a magnetic stirrer equipped with a timer was applied. The stirrer was set at 300 rpm. The direct current source was used to supply power to the system (Atten, China).

2.3. Experimental procedure

The bioreactor was inoculated by return activated sludge from a sewage treatment plant, Tehran, Iran. The initial concentration of suspended solids in mixed liquor was about 5,000 mg L^{-1}. The input wastewater was contained 0.163 g L^{-1} KNO_3, 0.5 g L^{-1} $NaHCO_3$, 0.15 g L^{-1} KH_2PO_4, 0.45 g L^{-1} Na_2HPO_4, 0.1 g L^{-1} $MgSO_4$, and 0.2 g L^{-1} NaCl. Sodium acetate as one organic carbon source was used to adjust the carbon to nitrogen ratio. The denitrification process was operated under anoxic conditions at the pH 6.5–8.5 range and room temperature (25°C ± 2°C). After bioelectrode formation, the denitrifying bacteria located on the electrode surface were adapted to electric current. Effective factors consist of carbon to nitrogen (C/N) ratios (0.5–4), carbon sources (sodium acetate, glucose, and methanol), and current intensity (1–10 mA), were evaluated. The electrical energy usage, hydrogen production, pH variation, electrical conductivity (EC), and oxidation–reduction potential (ORP) were investigated during the bio-electrochemical nitrate reduction. The concentrations of nitrate (NO_3^-), intermediate products (NO_2^- and NH_4^+), and chemical oxygen demand (COD) were measured in the effluent. The designing experiments were

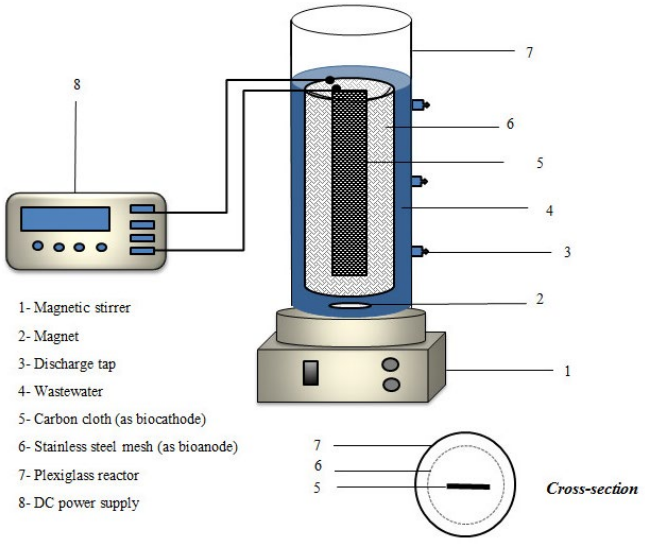

Fig. 1. Schematic of the integrated electrochemical bioreactor.

set based on the one-factor-at-a-time method. All runs were repeated with similar results so that the standard deviation of all data points was <5%. To determine the contribution of biological and electrochemical nitrate reduction in the hybrid bioreactor, two control systems were conducted with similar design and operation. The biological reactor was working on open-circuit mode, while the electrochemical system was run without any biomass.

2.4. Analytical methods

Effluent monitoring indexes such as nitrate (NO_3^-), nitrite (NO_2^-), and ammonium (NH_4^+) ions were examined using UV–visible spectrophotometer (Ray Leigh UV-9200, China) in accordance with the Standard Methods for the Examination of Water and Wastewater as follows: NO_3^- (spectrophotometric methods), NO_2^- (N-(1-naphthyl)ethylenediamine dihydrochloride), and NH_4^+ (Nesslerization method). The determination of COD was done in the optimum operating conditions using closed reflux and colorimetric method. 0.45 μm membrane (Whatman filter) was used to clarify samples before analysis. The pH and redox potential were monitored by a portable pH meter (Sension 378, HACH, USA)and ORP meters (ORPTestr 10-Eutech, USA).

2.5. Biofilm identification and bacterial population

To identify the main bacteria present in the bioreactor, the biofilm sampling was carried out by wiping the electrode surface. Then, it was cultured on a solid culture medium using a sterile loop. The multiple plates were prepared and incubated for 3 d at 37°C. Until reaching the separate colonies on the agar plates, subculturing was continued. The sample deoxyribonucleic acids were extracted using the boiling method, and the 16S ribosomal ribonucleic acid (rRNA) gene of bacteria was amplified by forward primer 27F (5′-AGAGTTTGATCATGGC-3′) and reverse primer 1492R (5′-TACCTTGTTACGACTT-3′) [10]. The conditions of polymerase chain reaction were as follows: amplification at 95°C (5 min), denaturation at 95°C (30 s), annealing at 50°C (30 s), and extension at 72°C (100 s). The number of 30 cycles was performed in a thermocycler (TPN-25, Padideh Nojen Pars, Mashhad, Iran). Finally, one extension step (10 min and 72°C) was run. The fragments sequencing was investigated by the sequencer (Genfanavaran; Macrogen, Seoul, Korea). The closest sequences were identified using the BLAST software. Studying the bacterial phylogeny was done using the 16S rRNA gene sequences. Phylograms were created using a computational phylogenetic method known as the maximum likelihood method with 500 replications applying MEGA software [11].

3. Results and discussion

3.1. Electrode materials and their configuration

In this work, carbon cloth sheet and cylindrical steel mesh were applied as bio-cathode and bio-anode, respectively. Using this fixed bed electrochemical bioreactor, the NO_3^- and NO_2^- concentrations were achieved 3.95 and 0.036 mg L^{-1}, respectively, during 6 h reaction time (Fig. 2). Simultaneously, the NH_4^+ concentration was reached to 0.7 mg L^{-1}. Fig. 3 shows the role of bioanode as an external electron acceptor for oxidation of substrates. In the proposed MES, the configuration of cylindrical/plate bioelectrodes increased the denitrification rate due to providing suitable and more surface area for bacterial adhesion; subsequently, the redox reaction area developed resulting in increased electron transfer rates. The bacteria are able to exchange electrons (donate or accept)

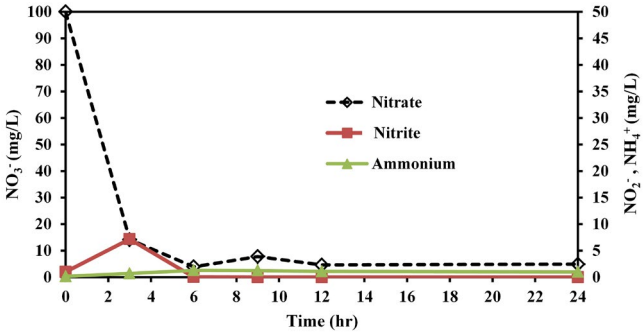

Fig. 2. NO_3^-, NO_2^-, and NH_4^+ concentrations in the microbial electrostimulation system. NO_3^- = 100 mg L^{-1}, I = 2 mA, and C/N ratio = 2.

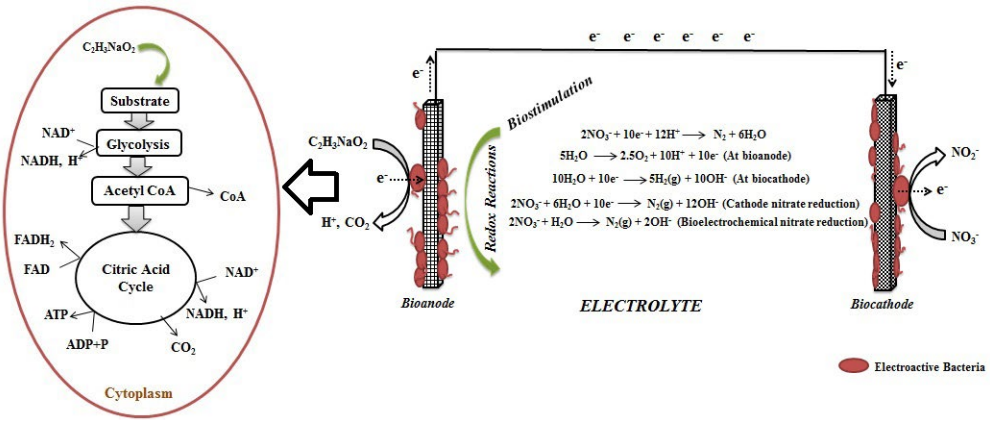

Fig. 3. Mechanism of electron exchanges in bioelectrodes; (bio-anode: citric acid cycle, NAD^+ and FAD reduction).

with solid electrodes easily adhere to electrode surfaces and transfer electrons to stimulate microbial metabolism [6,8]. The electron carriers shuttle the electrons from bacterial cytoplasm to the cell membrane and then transferred to the electrode by mediated or direct electron transfer mechanisms (MET or DET) [5]. Fig. 3 illustrates the occurrences of transferring electrons in the bioelectrodes. To enhance the efficiency of carbon type electrodes, some researchers have studied electrode modification using electron mediators, polyaniline, active polymers, and quinone groups [5,12]. Pre-treatment methods were suggested to improve electron transfer and bioelectrode performance. The generation of carboxyl functional groups could enhance the biofilm microbial composition and electron exchange [5]. In our study, some pre-treatments were examined on carbon cloth electrode including oxidation in sulfuric acid, and nitric acid. The conductivity of the electrode was enhanced by about 1 mA. The characteristics of electrode-like conductivity and ohmic resistance were not considerably improved by surface modification.

3.2. Biofilm assessment

To determine the biofilm growth on carbon cloth and stainless steel mesh electrodes, 1 cm^2 of biofilms, formed on the biocathode and bioanode, were taken and transferred on glass fiber filters. It was assumed that biofilm thickness was uniform in all parts of bioelectrodes. The filters used were dried in an oven (105°C within 2 h) and weighed before and after sampling. By subtracting two measurements, the biofilm mass was achieved. The mass per unit area in the studied fixed biofilm electrochemical reactor was specified to be 17.2 and 34.3 mg cm^{-2} over a 6 months operation in biocathode and bioanode, respectively. The results demonstrated that the steel mesh electrode had better performance in bacterial adhesion and subsequently electrons do exchanges in the electrochemical biosystem. It may be due to more conductivity and surface area of the steel mesh electrode in comparison with the carbon cloth electrode. It was found that the electrode type and conductivity are important factors in biofilm formation and its thickness. The mesh building of the stainless steel electrode creates a more and porous surface area that is favorable, as most bacteria can be captured in media [13]. The total amount of biomass on the graphite fiber brush in a microbial electrolysis cell was reported to be 126.7 ± 8.7 mg volatile solids for 3 months [13]. Some reports expressed that anodic biomass usually found to be thin compared with other biological processes [14]. In another study on biodegradation of phenolic wastewater using a biofilm reactor, biofilm density was reported to be 10.64 mg VSS L^{-1} [15]. According to 16S rRNA genes, the sequence analysis using NCBI BLAST software carried out on the microbial community present in the biofilm. The most bacteria which distinguished in the bioreactor were *Bacillus* spp. and *Pseudomonas* spp. Phylogenetic tree, relying on 16S rRNA gene sequences illustrates the phylogenetic situation of *Pseudomonas* spp. and *Bacillus* spp. (Fig. 4). *Pseudomonas* spp. strains are known as usual as denitrifying bacteria. They are gammaproteobacteria, gram-negative, rod-shaped, with species of polarly flagellated [1]. *Bacillus* spp. strains are also a member of the phylum *Firmicutes* with the ability of the denitrification under anoxic and aerobic operating conditions [16]. The ability of *Bacillus* as an effectual denitrifier has been stated by other researchers [17].

3.3. Electron donor type and C/N ratios

We studied three carbon sources; sodium acetate, glucose, and methanol, from the aspect of nitrate reduction rate (Fig. 5). Comparable maximum NO_3^- removal and NO_2^- production were determined according to the carbon sources. Sodium acetate showed a maximum denitrification rate relative to others. To determine the influence of applied carbon to nitrogen ratios on denitrification efficiency, various carbon substrate concentrations were chosen to gain desired ratios. Based on the results, C: N = 2 showed the best efficacy in the electrochemical biosystem using 2 mA applied electric current (Fig. 6). The surplus amounts of electron donors can be decreased both NO_3^- and NO_2^-, resulting in N_2 gas production. Higher C: N ratio would possibly lead to excess organic matters in the aquatic environment [18]. External organic carbon source as an electron donor is one of the affecting factors in most denitrification processes. The highest denitrification rate is achievable by the most readily biodegradable carbon sources; the slowly biodegradable COD provides a

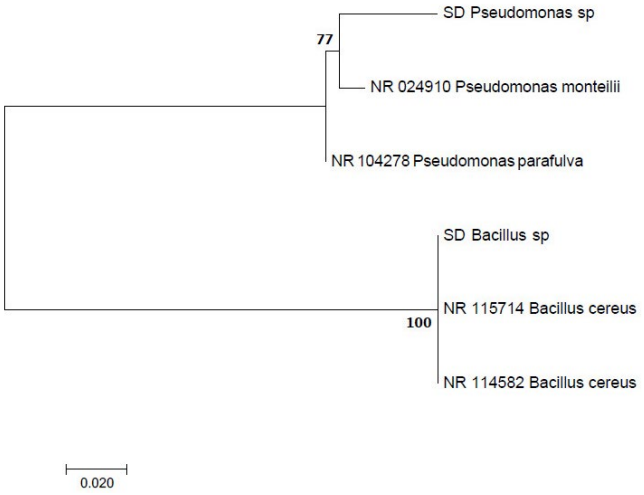

Fig. 4. Phylogenetic tree of denitrifiers in the bio-electrochemical reactor studied (scale bar = 0.02 change per sequence position).

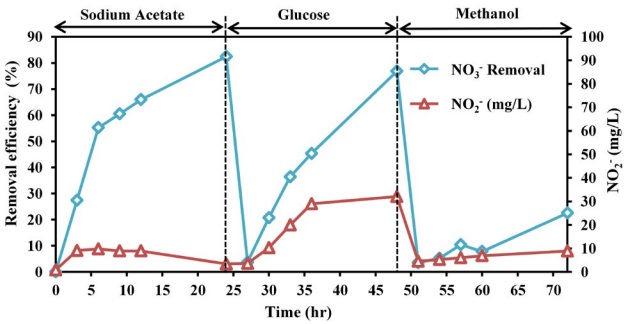

Fig. 5. NO_3^- removal (condition; NO_3^- = 100 mg L^{-1}, I = 2 mA, and C/N: 1).

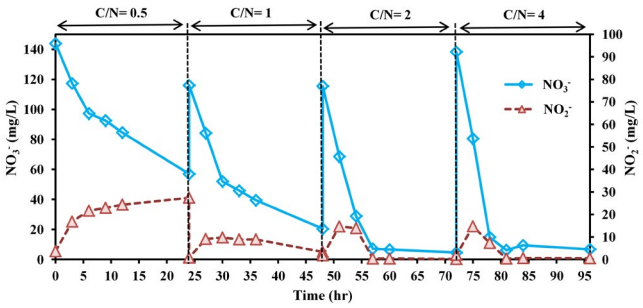

Fig. 6. Carbon to nitrogen ratio and denitrification efficiency.

slower denitrification rate because it requires hydrolyzed before the denitrification process [19]. Comparing carbon sources in commercial-scale up-flow denitrification biological filters, showed that all used carbon sources, including methanol, acetic acid, molasses, and cerelose, were able to effectively reduce NO_3^- to near-zero concentrations (influent concentrations from 11 to 57 mg L^{-1} NO_3^-). Although NO_2^- generation was not a problem once the reactors attained a constant effluent NO_3^-, ammonia production was the main problem for reactor-fed molasses [20]. A significant factor representing the influence of NO_3^- and NO_2^- on the microbial growth rate that should be considered is the yield coefficient. The biomass growth varies considerably per carbon source. The main difficulty of using sugar-rich carbon sources is the high amount of the yield coefficient, leading to more sludge production and discharge requirements. Thus, the management costs of sludge increase [19]. It is found that the acetate-fed process obtained stable and high nitrate removal at 0%–10% NaCl, while methanol was the advantageous electron donor in conditions with less than 3% of NaCl [21]. According to reports, the most nitrate reduction rate for molasses and methanol as carbon sources determined 4,094.8 ± 254.4 and 4,531.3 ± 186.1 mg N m^{-2} d^{-1}, respectively. The methanol was chosen as a suitable carbon source due to the lower risk of ammonia accumulation and H_2S generation. Assessment of COD: NO_3^-–N (3:1 to 6:1) indicated that a higher denitrification rate was achieved at a higher amount of methanol so that the maximum denitrification rate improved from 2,334 to 7,529 mg N m^{-2} d^{-1} [18]. The results of the control experiment showed that under optimum denitrification condition the contribution of each biological and electrochemical processes were 68.4% and 5.85%, respectively. It was shown that the bio-electrochemical reactor using electroactive bioelectrodes could achieve a larger capacity of treatment and higher denitrification performance.

3.4. Hydrogen production assay

Hydrogen gas was calculated according to Faraday's as Eq. (1):

$$n = \frac{It}{zF} \tag{1}$$

where n is H_2 generation amount (mol); I is electrical current intensity (A); t is the total time to which constant current was applied (s); z is the valency number of the substance ions (electrons transferred per ion) and F is the Faraday constant (96,485 C mol^{-1}) [22]. In this study, the H_2 production was 2.2×10^{-4} mol under optimum denitrification conditions. Hydrogen can be produced in MES, based on bio-electrohydrogenesis. Single chamber MES has been lately attractive to treat the waste and simultaneously obtain hydrogen gas. It was found that by immobilizing denitrifying bacteria on the cathode surface, hydrogen gas produced from the water electrolysis can be utilized. Cathodes can act as electron donors within microbial denitrification. Biocathodes are more interesting and cost-effective than abiotic cathodes due to cheaper catalysts and mediators [5]. The reactions of denitrification employing H_2 produced from water electrolysis in the process are as Eqs. (2)–(5) [5]:

$$2H_2O + 2e^- \rightarrow H_2 + 2OH^- \tag{2}$$

$$NO_3^- + H_2 \rightarrow NO_2^- + H_2O \tag{3}$$

$$2NO_2^- + 3H_2 + 2H^+ \rightarrow N_2 + 4H_2O \tag{4}$$

Overall reaction:

$$2NO_3^- + 5H_2 + 2H^+ \rightarrow N_2 + 6H_2O \tag{5}$$

In this study, the H_2 generation was obtained less than some other similar researches [23,24] because of lower electrical current used as a notable parameter influencing the hydrogen formation rate. So, it is unlikely that H_2 was a major electron donor at integrated bio-electrochemical nitrate removal. It demonstrates that the heterotrophic denitrifiers had a significant contribution to the denitrification process against autotrophic bacteria [5]. It has also reported that hydrogen production was directly proportional to the applied voltage and power consumption. When the current intensity is higher, H_2 gas generation via electrolysis increases. It is also stated that in a membrane-less microbial electrochemical cell (MEC), acetate accumulation and lower hydrogen production were observed at 0.4 and 0.55 V. By increasing the voltage, the enhancement of hydrogen generation was obtained. In membrane-based and membrane-less MES, the trend of hydrogen production was similar [5].

3.5. Electrical energy and power consumption

To assess operational costs of the integrated bio-electrical system, electrical energy and power consumption were computed by mathematical equations as follows [25,26]:

$$E = \frac{UIt}{V} \tag{6}$$

where E is electrical energy consumption (kWh m^{-3}), U is applied voltage (V), I is current intensity (A), t is reaction time (h) and V is the volume of effluent (L). In the present research, energy usage was determined 0.0104, 0.024, 0.0408, 0.0672, and 0.096 kWh m^{-3} for current intensities of 2–10 mA (1.3–2.4 V), respectively. Since the increasing applied electrical

Table 1
A summary of results obtained by some previous studies on nitrate removal, applying bio-electrochemical systems

Type of reactor	Type of wastewater	Electrode material	Culture	HRT (h)	Current intensity	NO_3–N (mg L^{-1})	Removal efficiency (%)	References
Separate reactors for electrolysis and denitrification	Aquaria water	Two plain perforated nickel electrodes	Mixed, hydrogenotrophic	12	0.3 and 0.5 A at a minimum potential of 1.5 V	20	36 mg N L^{-1} d^{-1} (90%)	[29]
Biofilm-electrode reactor	Synthetic wastewater	*Anode*: NA *Cathode*: stainless steel (plane)	Pure, heterotrophic (*Pseudomonas denitrificans*)	NA	NA	50–300 (mg L^{-1} NO_3^-)	Under 10 mgL^{-1}	[30]
Biofilm-electrode reactor (continuous)	Feed solution	*Anode*: stainless steel *Cathode*: graphite or stainless steel	Mixed, heterotrophic	NA	12 mA	20	23.8–68.3	[31]
Biofilm-electrode reactor (continuous)	Synthetic wastewater	*Anode*: one carbon rod *Cathode*: 12 carbon rods	Mixed, heterotrophic	7–36	0.06–0.09 mA cm^{-2}	200	NA	[32]
Divided electrolysis cell with graphite granules and cation exchange membrane	Synthetic wastewater	Anodic and cathodic frames were filled with graphite granules	Mixed, heterotrophic	NA	Current density: 23.4 mA cm^{-2}	0.02 and 0.11 g NO_3^-–N L^{-1}	74%	[33]
Divided electrolysis cell with proton exchange membrane	Synthetic wastewater	*Anode*: dimensionally stable (DSA) *Cathode*: Cu (plate)	Mixed, hydrogenotrophic	3.5	50 mA	22.13	98.5%	[34]
Batch bio-electrolysis cell divided by cation-selective membrane	Synthetic wastewater	*Anode*: dimensionally stable (DSA) *Cathode*: graphite felt	Mixed, hydrogenotrophic	~4	200 mA	70	~93%	[35]
Batch anoxic bio-electrochemical reactor	Synthetic wastewater	*Bioanode*: stainless steel mesh (cylindrical) *Biocathode*: carbon cloth (plate)	Mixed, heterotrophic (*Pseudomonas* spp. and *Bacillus* spp.)	6	2 mA	100 (mgL^{-1} NO_3^-)	≥95%	Present study

NA: not available.

current had no remarkable impact on nitrate removal (Fig. 7), the I = 2 mA was chosen as the best economical electric current. Table 1 shows the summarized characteristics of the present hybrid microbial electrochemical reactor in comparison with some previous researches on nitrate removal using bio-electrochemical systems. As observed in Table 1, great denitrification efficiency can be attained at lower applied electric current by the bioreactor developed in this study. Under this operation, energy usage was decreased. The power consumption calculated by Eq. (7):

$$\mathrm{EC} = \frac{VIt}{\left(\Delta\mathrm{COD} \times V\right)} \tag{7}$$

where EC is the power usage (kWh kg COD^{-1} m^{-3}), V is applied voltage (V), I is current intensity (A), t is reaction time (h) and V is the volume of the treated wastewater (L) and ΔCOD is COD_{in}–COD_{out}. In this regard, the consuming power achieved 9.9 × 10^{-5} in the present study. The average power consumption was around 4.44 Wh L^{-1} in a membrane-based MES. It was higher than a membrane-less system (2.34 Wh L^{-1}). This can be related to the additional ohmic resistance created by the membrane. Hence, a membrane-less configuration seems to be more favorable and beneficial [5]. The MEC can significantly improve the removal of pollutants by applying low energy supply. The power used for enhanced removal of Acid Orange 7 was reported about 0.012 kWh mol^{-1}. Similarly, the nitrobenzene removal occurred using a power of 0.05 kWh mol^{-1}. The aniline formation rate during nitrobenzene removal was enhanced to 8.6 and 6.7 mol m^{-3} total cathodic compartment d^{-1}, at an energy consumption of 17.1 W m^{-3} total cathodic compartment [27]. Kłodowska et al. [28] studied the effect of carbon source on denitrification efficiency in bio-electrochemical sequencing batch biofilm reactors. It was observed that organic complex substrates have more influence on the process by supplying electrons and lessening electricity consumption. Given that the power used can be considered as the major cost in the bio-electrochemical denitrification process, the nitrate coulomb-reduction rate studied using Eq. (8) [22].

$$u = \frac{CV\eta}{IT} \tag{8}$$

where u is the nitrogen coulomb-reduction rate (mg C^{-1}); C is initial nitrate concentration in influent (mg L^{-1}); V is the amount of effluent (L), η is the nitrate removal efficiency; I is electric current (A), and T is the hydraulic retention time (HRT) (s). In this study, u was obtained 3.33 mg C^{-1}. In a multi-electrode system, u was reported 0.028 mg C^{-1} which is less than the calculated value in our study. A similar result (0.019 mg C^{-1}) was also stated in a bio-electrochemical reactor developed [24]. The higher the required current intensity and lower nitrate reduction efficiency in autotrophic denitrification processes caused the mentioned results. The experimental data demonstrated that optimizing the reactor configuration for the efficient use of electric energy could be the most significant point for large-scale applications in the future.

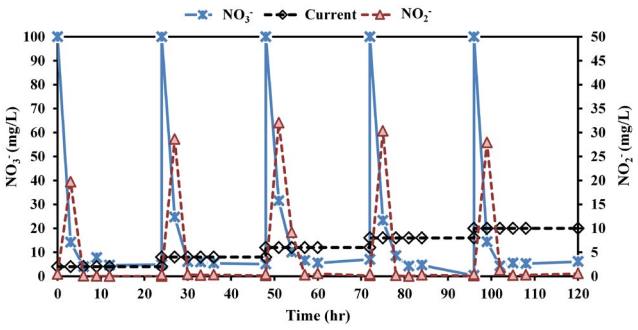

Fig. 7. Efficacy of applied current on NO_3^- removal (condition: NO_3^- = 100 mg L^{-1}, I = 2–10 mA, and C/N ratio = 2).

4. Conclusions

This study demonstrated that nitrate and nitrite were efficiently reduced in the proposed MES. The concentrations of NO_3^-, NO_2^-, and NH_4^+ reached to 3.95, 0.036, and 0.7 mg L^{-1} during 6 h reaction time. Using simple, high conductive bioelectrodes of stainless steel mesh as bio-anode and carbon cloth as bio-cathode in the unique form of cylindrical/sheet could increase the denitrification rate of more than 95%. The applied current intensity and energy consumption were reduced. The biomass per unit area in the studied fixed bed electrochemical bioreactor was determined 17.2 and 34.3 mg cm^{-2} over a 6-month operation in biocathode and bioanode, respectively. Sodium acetate showed a maximum denitrification rate compared with other carbon sources. The control experiment showed that the contribution of each biological and electrochemical process was 68.4% and 5.85%, respectively. The proposed bio-electrostimulation reactor using electroactive bioelectrodes provided higher denitrification performance at a similar operation. Accordingly, this system can be raised as a powerful and efficient approach for nitrate removal from polluted aquatic solutions.

Abbreviations

C/N	–	Carbon to nitrogen
COD	–	Chemical oxygen demand
DC	–	Direct current
EC	–	Electrical conductivity
DNA	–	Deoxyribonucleic acid
MES	–	Microbial electrochemical system
MILSS	–	Suspended solids in mixed liquor
OFAT	–	One-factor-at-a-time
ORP	–	Oxidation-reduction potential
rRNA	–	Ribosomal ribonucleic acid
SBBR	–	Sequencing batch biofilm reactors

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