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A state-of-the-art review and guidelines for enhancing nitrate removal in bio-electrochemical systems (BES)

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ABSTRACT

Excess unmanaged nitrogen pollutes the environment. A sustainable wastewater treatment system must achieve better pollutant removal efficiency at a lower cost, and the feasibility of integrating biological nitrogen removal into bio-electrochemical systems (BES) has been reported as a tool in green technology. Nitrogen in the form of nitrate (NO_3) is a common pollutant in both surface and ground waters, and a high level of NO_3 makes water unsuitable for drinking water. This analysis and review of BES for treating NO_3 polluted water investigate BES's components and operational factors and their importance on the NO_3 removal efficiency to design more powerful but economic systems. The NO_3 removal efficiencies were analyzed by the influence of electrode materials, working mode, number of chambers, type of inoculum, capacity, and microbial community structure. Overall, the electrode materials, significantly influence the NO_3 removal rate. The operational parameters, such as working mode, the number of chambers, inoculum type and the systems' capacity, were deemed important and have significantly influenced the NO_3 removal efficiencies when analyzed by the random forest classification algorithm. *Proteobacteria* and *Firmicute* were the prominent phyla observed in BES treating NO_3 polluted water. Besides the denitrification (abundance of *narG*, *nirS*, *nirK*, *nosZI*, and *nosZII* genes) process in BES, there is evidence of electrochemical support for anaerobic ammonium oxidation (ANAMMOX) (abundance of *hzsB* or ANAMMOX-specific 16S rRNA genes) and dissimilatory NO_3 reduction to ammonium (DNRA) (abundance of *nrfA* genes) processes. Our analysis suggest that BES, as a continuous two-chamber system with cathode and anode materials as granular carbon and carbon paper, respectively, with denitrifying microbes as inoculum type, would contribute to optimum NO_3 removal efficiencies.

1. Introduction

Nitrogen (N) has been fixed artificially for food production in vast amounts since the advent of the Haber-Bosch process. N fertilizers usually are administered as nitrates (NO_3), ammonia (NH_3) and urea (CH_4N_2O), which oxidize easily to NO_3. NO_3 leaches into groundwater, and improper management of the agricultural runoff causes excessive eutrophication of water bodies, threatening the aquatic flora and fauna, and rendering groundwater hazardous for consumption, causing methemoglobinemia (blue baby syndrome) and linked to cancer and various other health consequences [1]. The threshold stated by the United States environmental protection agency is 44.3 mg NO_3/L, and by the European Union (EU) and World Health Organization (WHO) regulations, the upper limit for nitrates is 50 mg NO_3/L. Many regions in Asia, Europe, and Africa have observed higher NO_3 concentrations in groundwater compared to WHO recommendations [2], although an average of 60 % of rural regions in Asia and Africa rely on groundwater for drinking [3].

N has a complex cycle consisting of many different NO_3 and nitrite transforming processes (e.g., denitrification, anaerobic ammonium oxidation (ANAMMOX), dissimilatory NO_3 reduction to ammonium (DNRA)), which usually require anaerobic conditions. NO_3 is the most stable form of oxidized N and, therefore, majorly studied while understanding N removal [4]. The anaerobic process of reduction of soluble NO_3 or nitrite (NO_2) to dinitrogen gas (N_2) is complete denitrification. Incomplete denitrification results in nitrous oxide (N_2O) gas, missing the last step of complete denitrification [5]. Bacteria and archaea can both carry out denitrification, but the extensive denitrification by archaea is understudied, denitrifying microbes can be autotrophs, heterotrophs, or mixotrophs on the modes they gain their energy from inorganic or organic compounds. They can use a wide range of inorganic and organic substrates for autotrophic and heterotrophic denitrification,

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respectively [6]. Depending on the environmental conditions, DNRA is happening in which organic carbon is used to reduce NO_3 to ammonium, or higher NO_3 concentrations may increase N_2O yield relative to ammonia [7]. ANAMMOX is mediated by an anaerobic bacteria that oxidize ammonia using nitrite as the terminal electron acceptor and generates N_2 [8].

NO_3 has been acknowledged as a major nutrient pollutant [9]. Various physiochemical treatments (e.g., electro-dialysis, nano-filtration, ion exchange, and reverse osmosis) have been explored for the removal of NO_3, but they consume a lot of energy and generate byproducts, e.g., sludge [10]. In addition to the physiochemical methods, the biological treatment for NO_3 removal is also widely applied. Incomplete denitrification was observed in a micro-aerobic sequence batch reactor which employed biological NO_3 removal, ANAMMOX process was also supported by the reactor [11]. Biological denitrification was observed to be enhanced using reduced graphene oxide as a catalyst, which enhanced denitrification via donating electrons [12]. Solid phase denitrification is largely utilized for biological denitrification, which consists of biodegradable polymers which provide surface area for microbes and also act as carbon sources, but they have displayed limitations due to carbon availability and N_2O accumulation [13].

Various sustainable mitigation methods have been recognized in the last few decades [9]. Sustainable ways such as constructed wetlands (CW) have been readily utilized for the treatment of wastewater, but their utilization for NO_3 removal remains limited [14]. Incomplete denitrification in CW has shown evidence of N_2O gas emissions, which questions the system's sustainability for NO_3 removal [15].

Electrochemistry has also been recently applied for the N treatment [16], and the treatment of various pollutants, microbial action, and electrochemical manipulation can be explored in BES for enhancement of pollutant removal. BES has displayed potency in NO_3 removal from contaminated waters. The exploration of BES has been attractive because it provides a contained space for microbial communities as well as enhances the reduction of N oxides by the generation of electrons. Autotrophic and heterotrophic denitrification can both be exploited in BES [17]. The microbial communities largely influence the efficiency of the systems, and the co-existence of chemolithotrophic and chemoheterotrophs were found to co-exist in the biofilms [18]. BES can be successfully utilized for treating wastewater with low carbon content as well, by enhancement of autotrophic bacteria [19,20]. The advent of exoelectrogens, which help in the transport of electrons outside the cells, helps in the enhancement of the reduction process. Exoelectrogens display interspecies electron transfer and form a network via their extracellular polymeric substances (EPS) for aggregates formation; during NO_3 stress, a mutualistic interaction is formed between anaerobic microbes aiding NO_3 reduction [21]. The external electric supply has been observed to enhance the reductive efficiency of the system [22].

BES can be largely classified as Microbial Fuel Cell (MFC), Microbial Electrochemical Snorkel (MES), and Constructed Wetland conjugated BES (CW-BES). MFC redirects electrons generated by microbial oxidation of substrates to generate electricity, this microbial activity can be employed for NO_3 reduction [23–25]. Microbial electrolytic cells (MEC), transfer electrons to the cathode from microbes thus, accelerate the intracellular reduction metabolism. When electrons are transferred from anode to cathode in an MFC, the substrate is reduced, whereas in MECs, bacteria and electron shuttles dictate the potential perched on the cathode since they have various potential differences [26]. MES is the most recent advancement in the field of BES, they utilize concentrated electrons from sediment, generated by microbial activity to reduce overlying NO_3-polluted water [22]. CW-BES consists of electrodes embedded in CW creating a system for employing microbial communities for intensive NO_3 removal.

The removal of NO_3 is an electron conducive process ($2\,NO_3^- + 10e^- + 12H^+ \rightarrow N_2 + 6\,H_2O$), and the lack of electron donors could hinder the reduction of NO_3 in the absence of an organic carbon source [27]. With the advent of electrogenic bacteria such as *Geobacter*, with the aid of electrodes, these communities act as an electric network to reduce NO_3 [28]. The extracellular, interspecies and intracellular electron chains enhanced the removal of NO_3 [29]. The energy consumption for the treatment of NO_3 by electrodialysis is around 4.3 kWh kg NO_3 [30], whereas BES systems can not only be employed for energy production in case of wastewater with high organic loads such as MFC or utilize minimal energy for igniting the electrogens in case of MES. BES can be employed for wastewater of wider characteristics. BES can be more conveniently operated and does not involve huge infrastructure requirements like traditional wastewater treatment plants. Biological removal using single stage nitration-ANAMMOX processes coupled with desalination in BES has been observed to impart a synergistic approach to nitrogen removal [31]. BES have been recognized as a green energy source for the electrochemical treatment of wastewater [32].

Engineering efficient BES systems and conjugation of BES with natural ecosystems or treatment wetlands can enhance the removal of NO_3 polluted water, and managing highly contaminated agricultural and industrial runoff and help in restoring the ecosystem and prevent the repercussions of NO_3 pollution.

Though this field of research is making strides toward the successful management of NO_3 pollution, there are no systemic ways in which all research articles published are comparable due to the need for more standardization in reporting the removal rates. The complete understanding of the effect of varying parameters, which make up these systems, on the NO_3 removal rate remains to be determined. In this review, we discuss the state of the art of knowledge and research on enhancing NO_3 removal in BES and provide guidelines for designing efficient systems for NO_3 removal in BES based on a comparison of parameters such as working modes, electrode materials, systems' capacities, inoculum type, number of chambers, microbial community structure, and design of BES, and their significance on the NO_3 removal rate.

2. Material and methods

2.1. Data collection

To analyze the articles that investigate BES for NO_3 removal, data was collected using the Google Scholar search engine and NCBI database (data was accessed between March 2022 and March 2023); the terms 'bioelectrochemical system', 'biocathode', 'denitrification', 'microbial community', 'nitrate removal', 'DNRA' and 'ANAMMOX' were used alone or in combinations and in singular or plural. To comprehensively cover all available papers, we examined all references cited in the papers collected. The papers included in the review are till March 2023. A total of 75 individual observations were included in our analysis. The articles were chosen by the following criteria: a) BES which were specifically used for NO_3 removal were included in the study; b) Different types of BES were included to increase the scope of the study i.e. microbial fuel cells (MFC), microbial electrochemical snorkels (MESs), and constructed wetlands conjugated with bio-electrochemical systems (CW-BES); c) Articles specifying the rates of removal of NO_3 and the percentages of NO_3 removal; d) Articles possessing relevant information for calculating the NO_3 removal rates and percentages were chosen for the study. The different types of BES and NO_3 transformation processes are schematically represented in Fig. 1.

2.2. Data classification for the analysis

The operating parameters and components of the systems were classified systemically for analysis, and two data sets were created (Tables 1, 2). The working mode of the systems was classified as a) batch mode, where no extra feed is utilized through the process, b) fed-batch mode, where feeding of a substrate can continue like carbon source or nutrients and c) continuous mode, where the feed rate and the harvest rate are in equilibrium to maintain continuous functioning of the

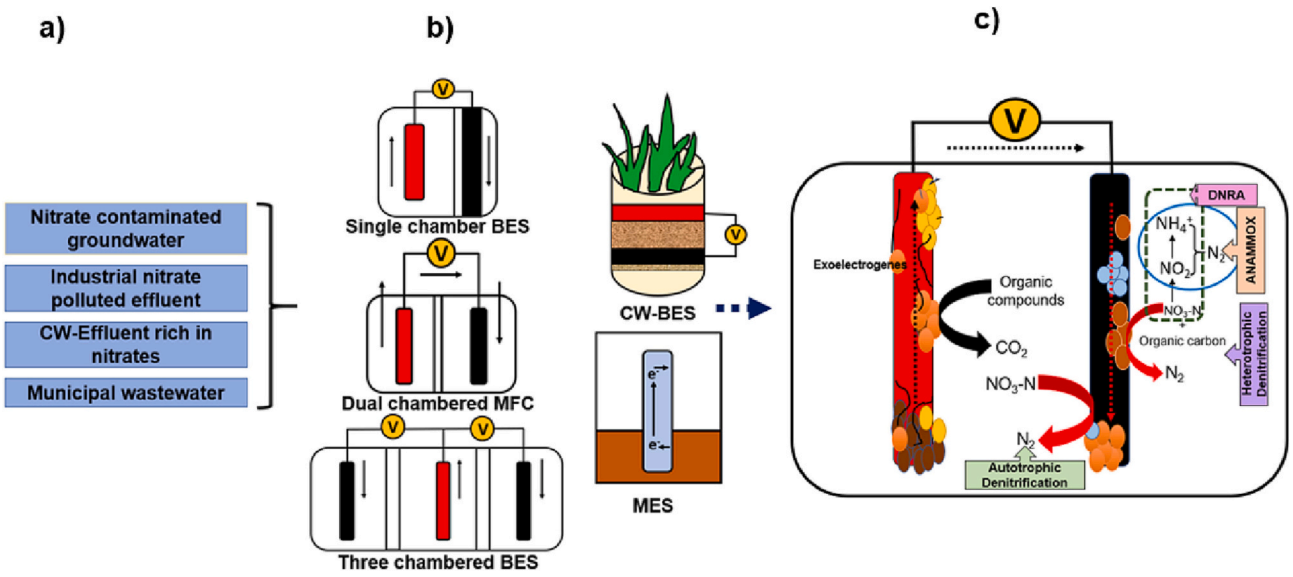

Fig. 1. a) Sources of nitrate polluted water, b) Types of Bio-electrochemical systems (BES), c) Schematic diagram of the processes of nitrate transformation in BES. (red electrode represents-anode, black electrode represents-cathode).

system.

The inoculum type largely influenced the microbial structure in the systems and was largely grouped as i) Activated sludge, sludge used as inoculum from the wastewater treatment plant; ii) Sediment, which included sediments collected from water bodies, agricultural fields (Paddy fields, bamboo park), and constructed wetlands; iii) Denitrifying bacteria, inoculum from a denitrifying reactor and directly inoculated denitrifying bacteria such as *Thiobacillus denitrificans* and *Pseudomonas stutzeri* and iv) Uninoculated, the system without any specific microbial community.

The electrode materials were classified as various elements utilized as electrodes such as i) stainless steel; ii) Graphite was simplified to carbon for a more concise and coherent understanding. Carbon electrodes with different configurations were further classified as cloth, felt, fiber, granules, paper, and rod as the surface area of the electrode would largely influence the efficiency of the system; iii) Combination electrodes included all the electrodes which utilized combined elements such as Carbon felt electrodes with stainless steel mesh, and immobilized elements on Carbon felt or stainless steel electrode, they were all classified as combination or combined electrodes.

The chambers were classified as a) number of chambers (one, two, three), b) 3D-BES when the cell is made of multiple electrodes, and c) CW-BES when electrodes were embedded in constructed wetlands. The capacities of the reactors were grouped into three groups: I (a volume of less than a liter (<L)), II (volumes 1–3 l (1−3 L)), and III (volume >3 l (>3 L)), the scales were distinguished for the microcosms, mesocosms, and pilot scales respectively.

The N-transforming genes and the microbial community structure were only studied in some of the collected papers (Table 3), and we also made an overview of them to understand their influence on the NO_3 removal capacities of the systems.

2.3. Calculations

Different studies represent the rate of NO_3 removal in various units such as 'mg m^{-2} d^{-1}', 'm^{-3} NCV d^{-1} (NCV-Net cathodic volume)', 'g m^{-3} d^{-1}', 'kg m^{-3} d^{-1}', '%', 'mg cm^{-2} d^{-1}', 'g m^{-2} d^{-1}', 'g m^{-3} h^{-1}'. It is difficult to compare data from various studies without normalization, therefore, all the units were normalized to a comparable unit of NO_3 removal 'mg liter^{-1} day^{-1}'. It specifically quantifies how much NO_3 is removed in a day per volume of the system. In contrast, percentages give just the amount removed from the BES without specifying any duration of time consumed for the removal.

Nitrate removal rate (mg liter^{-1} day^{-1}):

$$Inlet\ nitrate(mg) - Outlet\ nitrate(mg) \div Liter \times Day$$

Nitrate removal percentage (%):

$$Inlet\ nitrate(mg) - Outlet\ nitrate(mg) \div Inlet\ nitrate(mg) \times 100$$

The removal rate of 849 mg liter^{-1} day^{-1} [40] (the majority of articles included in the analysis have removal rates between 0 and 300 mg liter^{-1} day^{-1}) was excluded from our analysis. CW-BES and 3D-BES (i.e. number of chambers parameter) were also excluded while representing graphically because they consist of just 4 viable ($n = 4$) observations respectively.

2.4. Statistical analysis

The software R version 4.2.1 was used to analyze and visualize the data. The feature selection was carried out by using the package Boruta version 8.0 [74]. The random forest classification algorithm is a feature selection tool that helps in understanding which parameters are crucial to be considered when selecting to build models. It creates randomness in the data set by shuffling copies of all features called Shadow Features, then it trains a random forest classifier which measures mean decrease accuracy to evaluate the importance of each feature. It compares the features along with the Shadow minimum (ShadowMin), mean (ShadowMean), and maximum (ShadowMax), attributes determined by the algorithm. We utilized this feature to determine the relevance of the parameters utilized for building and running the BES. Boruta determines the importance of the parameter and whether or not they might be statistically significant. We used the tentative rough fix on tentative parameters to assess their importance.

ANOVA was carried out to determine the statistical significance among the varying groups of the parameters. Inorder to determine differences between various groups (pairwise) in particular factor, post hoc test i.e. Tukey's HSD (honestly significant difference) was performed. ANOVA and Tukey's HSD was carried out for the logarithmic values of the rate of NO_3 removal in order to quantifiy the relative change and to be insensitive to the skewness.

Table 1
Parameters classified for analysis and removal of nitrate rate normalized to a common unit (mg liter^{-1} day^{-1}). The letters after the removal of nitrate rate are showing the differences between studies if other parameters were the same in the table (a – anode condition (abiotic, biotic), circuit connection; b – substrate (NO_3, $NO_2 + NO_3$); c – proportion of cathodes embedded in simulated aquifier (0 %, 100 %); d – cathode potential (V); e – cathodic nitrate loading rate; f – hydraulic retention time (HRT), potential; g – carbon sources; h – chronoamperometry; i – circuit connection; j – C/N ratio; k – polarization (periodic, continuous); l – C/N ratio, HRT; m – inversion times (12, 24 and 48 h)). *Capacity (<1 L = I, 1-3 L = II, >3 L = III).

Study	Working mode	Type of inoculum	Cathode material	Anode material	Number of chambers	Capacity*	Volume (L)	Removal nitrate rate (mg L^{-1} day^{-1})
[33]	Continuous	Sediment	Combination	Combination	one	I	0.17	23.52
[17]	Batch	Activated sludge	Carbon felt	Carbon felt	two	I	0.7	0.63 a
[17]	Batch	Activated sludge	Carbon felt	Carbon felt	two	I	0.7	1.53 a
[17]	Batch	Activated sludge	Carbon felt	Carbon felt	two	I	0.7	1.56 a
[17]	Batch	Activated sludge	Carbon felt	Carbon felt	two	I	0.7	2.39 a
[17]	Batch	Activated sludge	Carbon felt	Carbon felt	two	I	0.7	1.7 a
[17]	Batch	Activated sludge	Carbon felt	Carbon felt	two	I	0.7	1.4 a
[17]	Batch	Activated sludge	Carbon felt	Carbon felt	two	I	0.7	1.51 a
[34]	Fed-Batch	Activated sludge	Carbon paper	Carbon paper	two	I	0.4	204 b
[34]	Fed-Batch	Activated sludge	Carbon paper	Carbon paper	two	I	0.4	188 b
[35]	Fed-Batch	Activated sludge	Carbon rod	Carbon rod	two	II	1	0.345
[36]	Batch	Activated sludge	Carbon felt	Carbon felt	two	I	0.7	0.831 c
[36]	Batch	Activated sludge	Carbon felt	Carbon felt	two	I	0.7	0.322 c
[37]	Fed-Batch	Uninoculated	Carbon granules	Carbon granules	one	II	1.05	65.279 d
[37]	Fed-Batch	Uninoculated	Carbon granules	Carbon granules	one	II	1.05	113.75 d
[38]	Continuous	Activated sludge	Combination	Combination	three	II	1.8	24.59 e
[38]	Continuous	Activated sludge	Combination	Combination	three	II	1.8	50.28 e
[38]	Continuous	Activated sludge	Combination	Combination	three	II	1.8	76.8 e
[38]	Continuous	Activated sludge	Combination	Combination	three	II	1.8	100.21 e
[38]	Continuous	Activated sludge	Combination	Combination	three	II	1.8	117.32 e
[38]	Continuous	Activated sludge	Combination	Combination	three	II	1.8	136.17 e
[38]	Continuous	Activated sludge	Combination	Combination	three	II	1.8	150.1 e
[38]	Continuous	Activated sludge	Combination	Combination	three	II	1.8	141.4 e
[38]	Continuous	Activated sludge	Combination	Combination	three	II	1.8	122 e
[39]	Continuous	Activated sludge	Carbon fiber	Carbon fiber	two	I	0.5	26 f
[39]	Continuous	Activated sludge	Carbon fiber	Carbon fiber	two	I	0.5	50 f
[39]	Continuous	Activated sludge	Carbon fiber	Carbon fiber	two	I	0.5	64 f
[39]	Continuous	Activated sludge	Carbon fiber	Carbon fiber	two	I	0.5	96 f
[39]	Continuous	Activated sludge	Carbon fiber	Carbon fiber	two	I	0.5	56 f
[39]	Continuous	Activated sludge	Carbon fiber	Carbon fiber	two	I	0.5	78 f
[39]	Continuous	Activated sludge	Carbon fiber	Carbon fiber	two	I	0.5	114 f
[40]	Fed-Batch	Denitrifying microbes	Combination	Combination	two	II	2.5	849
[41]	Batch	Activated sludge	Carbon rod	Carbon rod	one	I	0.45	10.56 g
[41]	Batch	Activated sludge	Carbon rod	Carbon rod	one	I	0.45	22.8 g
[41]	Batch	Activated sludge	Carbon rod	Carbon rod	one	I	0.45	24 g
[41]	Batch	Activated sludge	Carbon rod	Carbon rod	one	I	0.45	26.16 g
[42]	Continuous	Activated sludge	Combination	Carbon fiber	two	I	0.25	208.2
[43]	Fed-Batch	Denitrifying microbes	Carbon rod	Carbon rod	one	I	0.018	2.64 h
[43]	Fed-Batch	Denitrifying microbes	Carbon rod	Carbon rod	one	I	0.018	1.62 h
[43]	Fed-Batch	Denitrifying microbes	Carbon rod	Carbon rod	one	I	0.018	2.78 h
[44]	Batch	CW Sediment	Stainless Steel	Stainless Steel	CW-BES	II	1.575	0.5 i
[44]	Batch	CW Sediment	Stainless Steel	Stainless Steel	CW-BES	II	1.575	2.97 i
[45]	Batch	Activated sludge	Carbon rod	Carbon rod	one	I	0.45	16.56 j
[45]	Batch	Activated sludge	Carbon rod	Carbon rod	one	I	0.45	26.16 j
[46]	Batch	Activated sludge	Carbon cloth	Stainless Steel	two	III	5.86	1.4
[20]	Batch	Denitrifying microbes	Carbon cloth	Carbon felt	one	III	5.8	3.81
[47]	Continuous	Activated sludge	Combination	Stainless Steel	two	II	1.37	62.15
[48]	Continuous	Activated sludge	Combination	Carbon rod	two	I	0.716	146
[49]	Batch	Activated sludge	Carbon granules	Carbon rod	two	I	0.364	345
[50]	Batch	Activated sludge	Carbon granules	Carbon rod	two	I	0.672	175
[51]	Batch	Denitrifying microbes	Carbon cloth	Carbon cloth	one	II	2.43	186
[52]	Batch	Activated sludge	Carbon paper	Carbon paper	two	I	0.4	204
[53]	Continuous	Denitrifying microbes	Carbon granules	Combination	two	I	0.35	233 k
[53]	Continuous	Denitrifying microbes	Carbon granules	Combination	two	I	0.35	205 k
[54]	Continuous	Activated sludge	Carbon felt	Carbon rod	one	I	0.45	39.6
[55]	Batch	Sediment	Carbon rod	Carbon rod	two	I	0.5	19.92
[56]	Batch	Activated sludge	Carbon felt	Carbon mesh	two	II	1	170
[57]	Batch	Activated sludge	Carbon fiber	Combination	3D BES	II	3	0.85 l
[57]	Batch	Activated sludge	Carbon fiber	Combination	3D BES	II	3	1.08 l

(*continued on next page*)

Table 1 (*continued*)

Study	Working mode	Type of inoculum	Cathode material	Anode material	Number of chambers	Capacity*	Volume (L)	Removal nitrate rate (mg L^{-1} day^{-1})
[57]	Batch	Activated sludge	Carbon fiber	Combination	3D BES	II	3	1.07 l
[57]	Batch	Activated sludge	Carbon fiber	Combination	3D BES	II	3	0.98 l
[58]	Fed-Batch	Denitrifying microbes	Carbon cloth	Carbon fiber	one	I	0.028	50 e
[58]	Fed-Batch	Denitrifying microbes	Carbon cloth	Carbon fiber	one	I	0.028	100 e
[58]	Fed-Batch	Denitrifying microbes	Carbon cloth	Carbon fiber	one	I	0.028	75 e
[59]	Batch	Activated sludge	Carbon paper	Stainless Steel	two	I	0.1	1.5 m
[59]	Batch	Activated sludge	Carbon paper	Stainless Steel	two	I	0.1	1.1 m
[59]	Batch	Activated sludge	Carbon paper	Stainless Steel	two	I	0.1	0.44 m
[60]	Batch	Activated sludge	Carbon fiber	Carbon fiber	two	I	0.43	48
[61]	Fed-Batch	Denitrifying microbes	Carbon rod	Carbon felt	two	I	0.1	48.4
[62]	Continuous	Activated sludge	Carbon felt	Carbon granules	CW-BES	III	25	3.53 j
[62]	Continuous	Activated sludge	Carbon felt	Carbon granules	CW-BES	III	25	5.47 j
[63]	Batch	Activated sludge	Stainless Steel	Stainless Steel	one	III	3.57	0.27
[64]	Continuous	Activated sludge	Carbon fiber	Combination	three	II	1.07	2.83

3. Results

3.1. Cathode and anode materials

We investigated the effect of the cathode and anode materials on the NO_3 removal rate. Different materials utilized as cathodes in our data set are stainless steel, combination electrodes, and carbon utilized in different configurations such as cloth, felt, fiber, granules, paper, and rod. We observed that the most commonly used material is carbon, specifically graphite felt (Tables 1, 2). Significant differences were observed between varying cathode electrode materials for their NO_3 removal efficiencies ($p < 0.01$) (Fig. 2a). According to Tukey's HSD, significant differences were observed among six pairs, i.e. carbon granules-carbon felt, combination-carbon felt, carbon rod-carbon granules, stainless steel-carbon granules, combination-carbon rod, stainless steel-combination ($p < 0.01$).

The carbon granules followed by carbon paper have displayed higher NO_3 removal rates. There is high variability observed in the removal rates by carbon granules and carbon paper compared to other cathode materials, which show lower variability among the removal efficiencies.

Different materials studied for anode material are stainless steel, combination electrodes, and carbon utilized in different configurations such as cloth, felt, mesh, fiber, granules, paper, and rod. Carbon paper has displayed the highest removal efficiency compared to other anode materials, the material also shows low variability among its removal rates (Fig. 2b). Significant differences were observed in the removal rates between the varying anode type ($p < 0.05$) (Fig. 2b). According to Tukey's HSD, significant differences were observed among eight pairs, i.e. carbon fiber-carbon felt, carbon paper-carbon felt, carbon rod-carbon felt, combination-carbon felt, stainless steel-carbon fiber, stainless steel-carbon paper, stainless steel-carbon rod, stainless steel-combination ($p < 0.05$).

3.2. Operational parameters

The Batch mode is practical when dealing with BES as one can monitor the substrates in the system more easily. Nevertheless, due to functional and commercial reasons, there are also run in a fed-batch or continuously. The most studied operating mode of BES is continuous, followed by batch and fed-batch (Tables 1, 2). The three modes display significant differences in efficiency for their NO_3 removal rates ($p < 0.01$) (Fig. 3a). According to Tukey's HSD, there are significant differences observed among continuous-batch mode of operation ($p < 0.01$), fed batch-batch ($p < 0.05$), although continuous mode showed on average highest NO_3 removal rates. The continuous mode has shown higher NO_3 removal rates, followed by fed-batch and batch.

The importance of the volume of the reactors on NO_3 removal was also analyzed, the majority of the systems studied had volumes between 1 and 3 l (Tables 1, 2). There were no statistically significant differences observed among BES with different scales on their NO_3 removal efficiencies ($p > 0.05$) (Fig. 3b).

The inoculum acts as the starter culture that aids in establishing the microbial structure of the system along with the NO_3-polluted water; if not inoculated, the system builds its community by the presence of microbes in the water that is treated. Most of the research articles have utilized sludge from wastewater treatment plants as inoculum (Tables 1, 2), as it consists of a high heterogeneous microbial load. Though studies specifically utilize denitrifying microbes to enhance denitrification. There were no statistically significant differences observed among the type of inoculums for their removal rates ($p > 0.05$) (Fig. 3c). Still, the highest removal rates are observed in systems inoculated with denitrifying microbes. High variability was observed between the removal rates with BES inoculated with activated sludge and denitrifying microbes compared to uninoculated and BES inoculated with sediment.

Multiple chambers are hypothesized to aid in enhancing NO_3 removal. Two-chambered BES are the most commonly studied, and single-chambered snorkel systems are also studied in recent years (Tables 1, 2). The maximum rate of removal was observed in two-chambered systems. There were no statistically significant differences observed among the number of chambers for their NO_3 removal rates ($p > 0.05$) (Fig. 3d).

3.3. Microbial community structure in BES for NO_3 removal

The microbial community structure in NO_3-removing BES was observed to possess *Proteobacteria* with the highest phyla proportion, followed by *Firmicutes* and *Bacteriodetes* (Fig. 4, Table 3). The majority of the N-transforming bacterial communities belong to the *Proteobacteria* phylum. Species specifically observed populating the biocathode are *Thiobacillus, Nitratireductor, Shinella, Dyella, Paracoccus, Simplicispira, Geobacter, Thauera, Thermomonas, Azoarcus, Ottowia Nitrospira, Denitratisoma, Dechloromonas,* and *Candidatus Competibacter* (Table 3). Bioanodes were largely populated with *Pseudomonas, Curtobacterium,* and *Aeromonas* species.

The presence and abundance of denitrification genes such as *narG, napAB, norAC, nirS, nirK, nosZI, and nosZII* were observed to be enhanced in BES treating NO_3-rich water [66,72,75]. *nrfA* gene responsible for the DNRA process is significantly enhanced in BES systems [20,72]. The

Table 2
Parameters classified for analysis with NO_3 removal percentage (%). The letters after the removal of nitrate rate are showing the differences between studies if other parameters were the same in the table (a – pH; b – anode condition (abiotic, biotic), circuit connection; c – substrate (NO_3, $NO_2 + NO_3$); d – facilitated cathode; e – HRT, cathode potential (V); f – substrate concentration; g – HRT, C/N ratio; h – HRT; i – facilitated cathode; j – size of cathode; k – pH; l – nitrate loading; m – inversion times (12, 24 and 48 h)). *Capacity (<1 L = I, 1-3 L = II, >3 L = III).

Study	Working mode	Type of inoculum	Cathode material	Anode material	Number of chambers	Capacity*	Volume (L)	Removal of nitrate (%)
[65]	Continuous	Activated sludge	Carbon fiber	Carbon rod	one	II	2.5	83.71 a
[65]	Continuous	Activated sludge	Carbon fiber	Carbon rod	one	II	2.5	97.21 a
[65]	Continuous	Activated sludge	Carbon fiber	Carbon rod	one	II	2.5	93 a
[33]	Continuous	Sediment	Combination	Carbon rod	one	I	0.17	51.5
[17]	Batch	Activated sludge	Carbon felt	Carbon felt	two	I	0.7	50 b
[17]	Batch	Activated sludge	Carbon felt	Carbon felt	two	I	0.7	43 b
[17]	Batch	Activated sludge	Carbon felt	Carbon felt	two	I	0.7	43 b
[17]	Batch	Activated sludge	Carbon felt	Carbon felt	two	I	0.7	28 b
[34]	Fed-Batch	Activated sludge	Carbon paper	Carbon paper	two	I	0.4	87.9 c
[34]	Fed-Batch	Activated sludge	Carbon paper	Carbon paper	two	I	0.4	85.4 c
[34]	Fed-Batch	Activated sludge	Carbon paper	Carbon paper	two	I	0.4	84.7 c
[66]	Batch	Denitrifying microbes	Carbon fiber	Carbon fiber	two	II	1.1	59 d
[66]	Batch	Denitrifying microbes	Carbon fiber	Carbon fiber	two	II	1.1	95 d
[67]	Continuous	Activated sludge	Carbon rod	Stainless Steel	two	II	1.43	90
[39]	Continuous	Activated sludge	Carbon fiber	Carbon fiber	two	I	0.5	94 e
[39]	Continuous	Activated sludge	Carbon fiber	Carbon fiber	two	I	0.5	85 e
[39]	Continuous	Activated sludge	Carbon fiber	Carbon fiber	two	I	0.5	74 e
[39]	Continuous	Activated sludge	Carbon fiber	Carbon fiber	two	I	0.5	65 e
[39]	Continuous	Activated sludge	Carbon fiber	Carbon fiber	two	I	0.5	96 e
[39]	Continuous	Activated sludge	Carbon fiber	Carbon fiber	two	I	0.5	91 e
[39]	Continuous	Activated sludge	Carbon fiber	Carbon fiber	two	I	0.5	81 e
[68]	Batch	Sediment	Carbon rod	Carbon rod	two	I	0.06	64 f
[68]	Batch	Sediment	Carbon rod	Carbon rod	two	I	0.06	84 f
[68]	Batch	Sediment	Carbon rod	Carbon rod	two	I	0.06	84 f
[69]	Batch	Sediment	Iron rod	Carbon felt	one	II	2	98
[70]	Continuous	Activated sludge	Carbon rod	Carbon rod	one	III	3.4	98.3 g
[70]	Continuous	Activated sludge	Carbon rod	Carbon rod	one	III	3.4	88.4 g
[40]	Fed-Batch	Denitrifying microbes	Combination	Combination	two	II	2.5	100 h
[40]	Fed-Batch	Denitrifying microbes	Combination	Combination	two	II	2.5	55 h
[40]	Fed-Batch	Denitrifying microbes	Combination	Combination	two	II	2.5	94 h
[40]	Fed-Batch	Denitrifying microbes	Combination	Combination	two	II	2.5	50 h
[71]	Batch	Activated sludge	Carbon fiber	Carbon fiber	two	II	2.24	95 i
[71]	Batch	Activated sludge	Carbon fiber	Carbon fiber	two	II	2.24	95 i
[71]	Batch	Activated sludge	Carbon fiber	Carbon fiber	three	II	2.24	95 i
[72]	Batch	CW Sediment	None	Carbon felt	one	II	2	53.88 j
[72]	Batch	CW Sediment	Carbon felt	Carbon felt	one	II	2	48.39 j
[72]	Batch	CW Sediment	Carbon felt	Carbon felt	one	II	2	41.35 j
[72]	Batch	CW Sediment	Carbon felt	Carbon felt	one	II	2	41.52 j
[72]	Batch	CW Sediment	Carbon felt	Carbon felt	one	II	2	41.18 j
[72]	Batch	CW Sediment	Stainless Steel	Carbon felt	one	II	2	40.34 j
[72]	Batch	CW Sediment	Copper	Carbon felt	one	II	2	40.55 j
[72]	Batch	CW Sediment	Plastic	Carbon felt	one	II	2	74.61 j
[57]	Batch	Activated sludge	Carbon fiber	Combination	3D-BES	II	3	77.12 k
[57]	Batch	Activated sludge	Carbon fiber	Combination	3D-BES	II	3	97.58 k
[57]	Batch	Activated sludge	Carbon fiber	Combination	3D-BES	II	3	96.36 k
[57]	Batch	Activated sludge	Carbon fiber	Combination	3D-BES	II	3	88.48 k
[73]	Batch	Activated sludge	Combination	Carbon rod	one	II	2.35	96.55
[58]	Fed-Batch	Denitrifying microbes	Carbon cloth	Carbon fiber	one	I	0.028	98.92 l
[58]	Fed-Batch	Denitrifying microbes	Carbon cloth	Carbon fiber	one	I	0.028	97 l
[58]	Fed-Batch	Denitrifying microbes	Carbon cloth	Carbon fiber	one	I	0.028	48.98 l
[59]	Batch	Activated sludge	Carbon paper	Stainless Steel	two	I	0.1	54 m
[59]	Batch	Activated sludge	Carbon paper	Stainless Steel	two	I	0.1	40 m
[59]	Batch	Activated sludge	Carbon paper	Stainless Steel	two	I	0.1	16 m
[60]	Batch	Activated sludge	Carbon fiber	Carbon fiber	two	I	0.43	96
[61]	Fed-Batch	Denitrifying microbes	Carbon rod	Carbon felt	two	I	0.1	96.8
[62]	Continuous	Activated sludge	Carbon felt	Carbon granules	CW-BES	III	25	58.9 g
[62]	Continuous	Activated sludge	Carbon felt	Carbon granules	CW-BES	III	25	91.26 g
[63]	Batch	Activated sludge	Stainless Steel	Stainless Steel	one	III	3.57	99
[64]	Continuous	Activated sludge	Carbon fiber	Combination	three	II	1.07	72.9

significant enhancement of the *nirS* and *nosZI* genes is indicative of the presence of autotrophic denitrifiers in BES [20,72]. *hzsB* and ANAMMOX-specific 16S rRNA genes represent the prevalence of the ANAMMOX (anaerobic ammonium oxidation) process in these systems [68,72]. Ammonia oxidizing genes such as *amoA* and *comammox amoA* genes were found in higher numbers on the anode or the counter electrode, thus anode is responsible for oxidation or donation of electrons [20,76].

3.4. Importance of factors influencing NO_3 removal

The feature selection was carried out to determine the importance of varying factors on the rate of NO_3 removal (mg liter^{-1} day^{-1}) and percentage (%) removal using a random forest classification algorithm. The analysis for the rate of removal (mg liter^{-1} day^{-1}) deemed all the components and operational parameters important, the inoculum was deemed as a tentative parameter which was rendered important after rough fixing of the model (Fig. 5a). In contrast to random forest

Table 3
The table displays predominant Phylum, Class and Species observed in BES utilized for NO_3 removal. (ND – no data).

Citation	Predominant phylum	Class	Species	Rate of nitrate removal (mg liter^{-1} day^{-1}) or % nitrate removal
[34]	*Proteobacteria, Bacteroidetes, Actinobacteria, Firmicutes*	*Gammaproteobacteria*	*Proteiniphilum acetatigenes, Weeksella virosa, Pseudomonas stutzeri, Thauera aromatica, Bacillus novalis*	196 mg liter^{-1} day^{-1}
[70]	*Proteobacteria, Firmicutes, Actinobacteria*	*Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Epislonproteobacteria, Clostridia, Bacilli*	ND	93.35 %
[66]	*Bacterioidetes, Proteobacteria. Firmicutes, Actinobacteria*	*Alphaproteobacteria, Betaproteobacteria, Gammaproteobacteria, Epislonproteobacteria, Clostridia, Bacilli*	*Thauera terpenica*	77 %
[17]	ND	ND	*Pseudomonas oleovorans, Aeromonas, Curtobacterium*	1.53 mg liter^{-1} day^{-1}
[43]	ND	*Betaproteobacteria*	*Thiobacillus thiophilus*	2.34 mg liter^{-1} day^{-1}
[37]	ND	ND	*Geobacter*	89.51 mg liter^{-1} day^{-1}
[69]	ND	ND	*Lysinibacillus, Ochrobactrum, Pseudomonas, Aeromonas*	98 %
[71]	*Proteobacteria, Chloroflexi, Bacteroidetes, Firmicutes*	ND	ND	95 %
[65] [a]	*Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Tenericutes, Chloroflexi, Acidobacteria, Fibrobacteres, Saccharibacteria*	*Gammaproteobacteria, Betaproteobacteria, Alphaproteobacteria, Bacilli, Clostridia, Erysipelotrichia, OPB54, Actinobacteria, Flavobacteriia, Spingobacteriia, Mollicutes, Anaerolineae, Acidobacteria, Fibrobacteria, Saccharibacteria_norank*	*Pseudomonas, Halomonas, Thauera*	87.71 %
[65] [a]	*Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Tenericutes, Chloroflexi, Chrysiogenetes, SHA-109*	*Gammaproteobacteria, Betaproteobacteria, Alphaproteobacteria, Bacilli, Clostridia, Erysipelotrichia, OPB54, Flavobacteriia, Mollicutes, Anaerolineae, TK10, KD4–96, Chloroflexi_uncultured*	*Pseudomonas, Halomonas, Thauera*	97.21 %
[65] [a]	*Proteobacteria, Firmicutes, Actinobacteria, Bacteroidetes, Tenericutes, Fibrobacteres, Verrucomicrobia, Chrysiogenetes*	*Gammaproteobacteria, Betaproteobacteria, Alphaproteobacteria, Proteobacteria_unclassified, Bacilli, Clostridia, Erysipelotrichia, OPB54, Flavobacteriia, Sphingobacteriia, Cytophagia, Mollicutes, Thermomicrobia, Chloroflexi_uncultured, Opitutae*	*Pseudomonas, Halomonas, Thauera*	93 %
[64]	*Firmicutes, Actinobacteria, Armatimonadetes, Chloroflexi, Planetomycetes, Acidobacteria, Nitrospirae, Patescibacteria, Proteobacteria, Bacteroidetes, Epsilonbacteraeota*	*ND*	*ND*	2.83 mg liter^{-1} day^{-1}
[62]	*Proteobacteria, Euryarchaeota, Firmicutes, Elusimicrobia, Actinobacteria, Latescibacteria, Bacteroidetes, Ignavibacteriae, Planctomycetes, Candidatus Saccharibacteria, Chloroflexi, Gemmatimonadetes, Armatimonadetes, Fibrobacteres, Acidobacteria, Chlamydiae, Verrucomicrobia, Parcubacteria, Nitrospirae, Pacearchaeota, Spirochaetes, Candidatus Saccharibacteria, Verrucomicrobia*	*Gammaproteobacteria, Phycisphaerae, Betaproteobacteria, Acidobacteria_Gp4, Alphaproteobacteria, Acidobacteria_Gp16, Bacilli, Cytophagia, Epsilonproteobacteria, Opitutae, Actinobacteria, Nitrospira, Clostridia, Anaerolineae, Spingobacteriia, Erysipelotrichia, Deltaproteobacteria, Planctomycetia, Flavobacteriia, Chlamydiia, Bacteroidia*	*ND*	4.5 mg liter^{-1} day^{-1}
[63]	*Proteobacteria, Bacteroidetes, Firmicutes*	*ND*	*ND*	0.27 mg liter^{-1} day^{-1}

[a] Difference in pH.

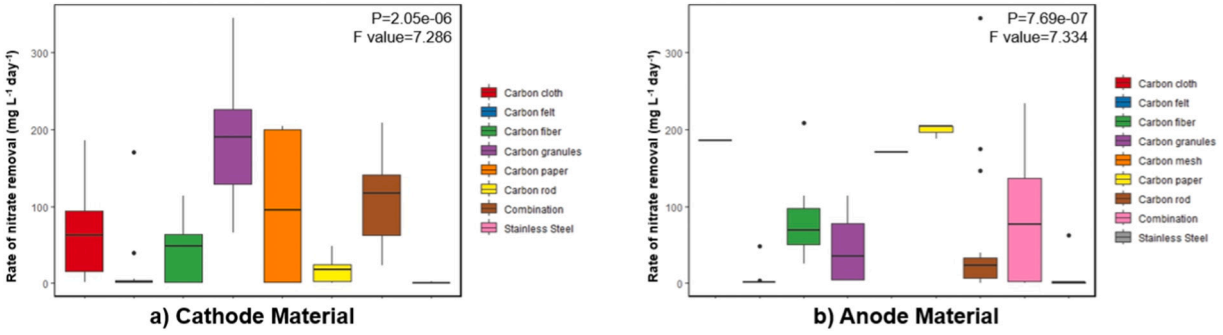

Fig. 2. Box plots representing different cathode (a) and anode (b) materials compared to the NO_3 removal rate.

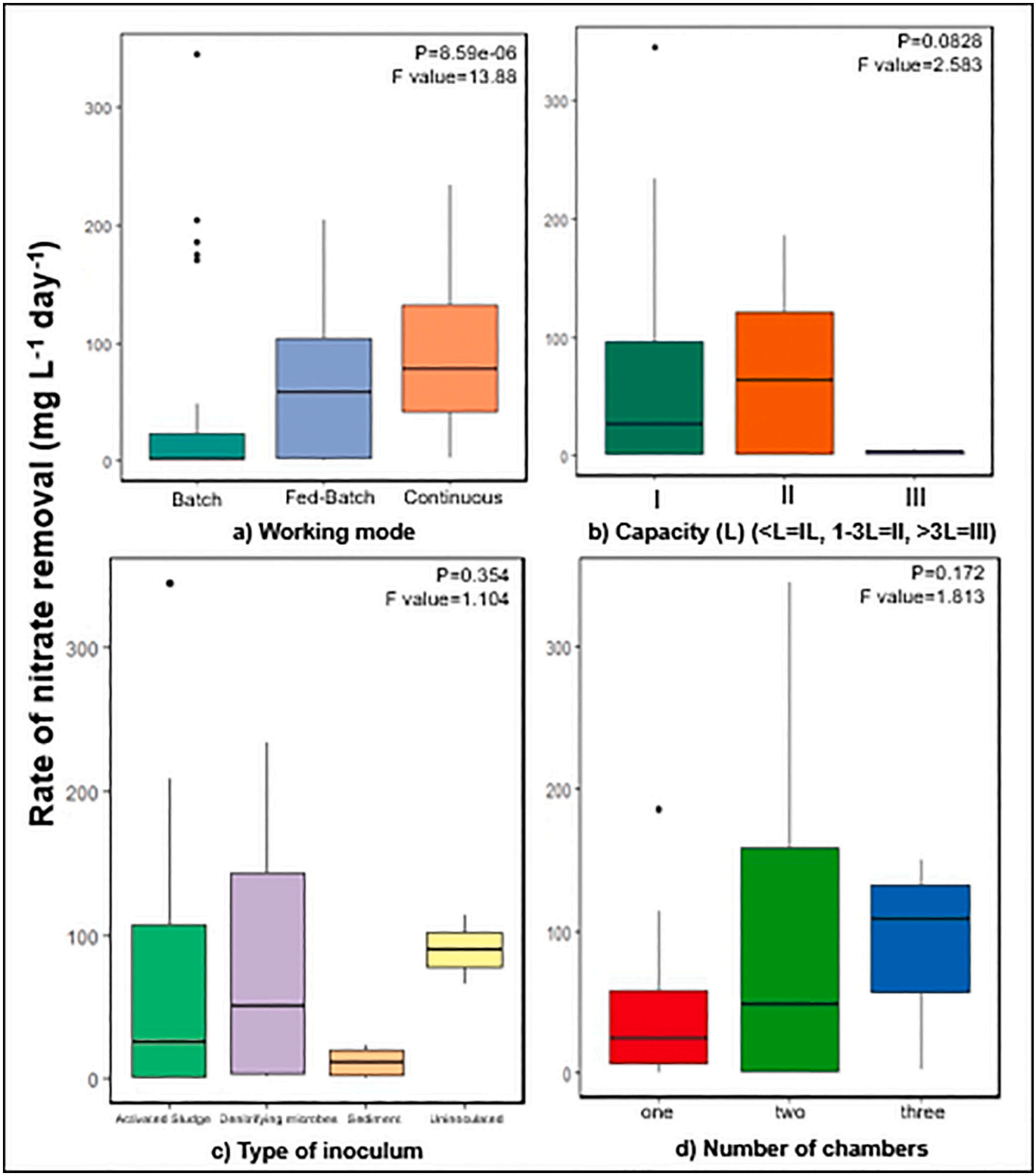

Fig. 3. The boxplots display operational parameters working mode (a), capacity (b), type of inoculum (c), and the number of chambers (d) compared to the NO_3 removal rate.

classification analysis performed on the rate of removal (mg L^{-1} day^{-1}), the number of chambers was deemed to be unimportant for the NO_3 percentage removal (%). Random forest classification analysis for the percentage of removal (%) deemed the factors of electrode materials, working mode, inoculum type, and capacity was considered important by the analysis (Fig. 5b).

4. Discussion

The operational parameters significantly influence the efficiencies of the systems; this analysis help in understanding which attributes have contributed to higher NO_3 removal rates. The most crucial parameters for NO_3 removal rates determined by the study were number of

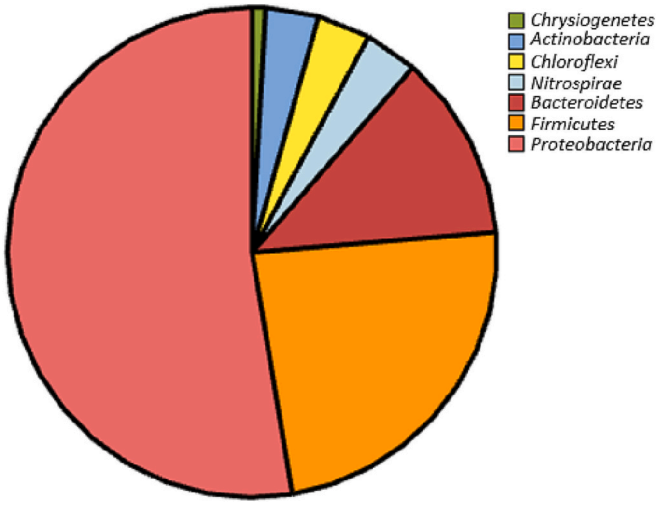

Fig. 4. The pie chart displays the proportion of phyla observed in BES utilized for NO_3 removal.

chambers, electrode materials, working mode, capacity and the type of inoculum in the order of importance. Electrode materials play a crucial role in harboring microbial communities as well as the transfer of electrons, which enhance NO_3 reduction. Working electrodes have been displayed to inhabit the N-transforming microbial communities, which aids in the enhancement of the reduction process, whereas the strengthening of cathode leads in enhanced electron transfer [77]. Granular carbon imparts a higher surface area which would aid microbial activity compared to other electrode materials. The working mode of the systems determines the concentrations of nutrients and essential cofactors through the BES operation. The inoculum adds to the microbial community structure in BES and also acts as a starter culture. Denitrifying sludge has been observed to potentially enhance the biocathode activity [78]. A distant shift in the microbial community structure was observed in systems with a biocathode, and the last is an electrode with a microbial population present at the electrode or in the electrolyte that catalyzes the cathodic reaction [66]. Chambers aid in maintaining the pH of the system by utilizing the cation exchange membrane.

The two-chamber systems maintain the pH systematically and impart equal partitioning of the cations; granular carbon and carbon paper offer a higher surface area compared to other materials; the continuous working mode enables a supply of essential nutrients; and the carbon source can be used to introduce complete denitrifiers into the system via inoculating denitrifying microbes. Therefore, these parameters have shown optimal removal efficiencies compared to other materials and factors. The type of inoculum and the capacity of the system were deemed of the least importance compared to other parameters, as the characteristics of the wastewater also have a crucial role in determining the microbial community structure. Whereas, the capacity of the systems didn't have significant differences in their removal efficiencies and therefore, the scalability of the systems can be successfully carried out by focusing on other crucial design and operational parameters of the BES. The removal efficiencies are an amalgamation of various factors which can be visualized by observing the changes observed by tweaking on parameter, the feature selection helps us determine the relative importance of these parameters (Tables 1, 2).

Feature selection using random forest classifier has deemed different parameters important among percentage removal (%) and rate of removal (mg L^{-1} day^{-1}), this display that they signify different attributes of the data set. The rate at which NO_3 is removed, is also an essential component in studying the system's efficiency; therefore, neglecting the time parameter while calculating the NO_3 removal rate would not give an optimum understanding of the efficiency of the system. Therefore, it is essential to prefer standardized removal rates over percentage removals (%) to understand the true efficiencies of the BES (Fig. 5).

Various BES systems such as CW-BES, MFC, and MESs can be employed for different characteristics of wastewater (Fig. 1). For example, agricultural runoff consists of large volumes of NO_3 polluted water, which is convenient to be treated with constructed wetlands due to the enormous volumes of water, but as the water is treated and the COD (Chemical Oxygen Demand) reduces through the course of the constructed wetland, the NO_3 concentration remains high as seen in [14] that is when CW conjugated with BES can aid in treating this complex agricultural runoff and treat the carbon content and nitrogen load of the runoff simultaneously. MFC technology can also be employed for more specific utilization, such as point sources of NO_3 effluent and industrial wastewater, which are high in NO_3 (e.g., fertilizer companies). MESs have the utmost potential when employing the BES in the field as they have the most potential for scalability due to their more straightforward design. For example, a simpler BES design, such as the single-chambered BES, can treat wastewater with low carbon concentrations.

Other relevant parameters observed in varying studies, such as the voltage hike from 0.7 to 0.9 V, enhance NO_3 removal efficiencies [17].

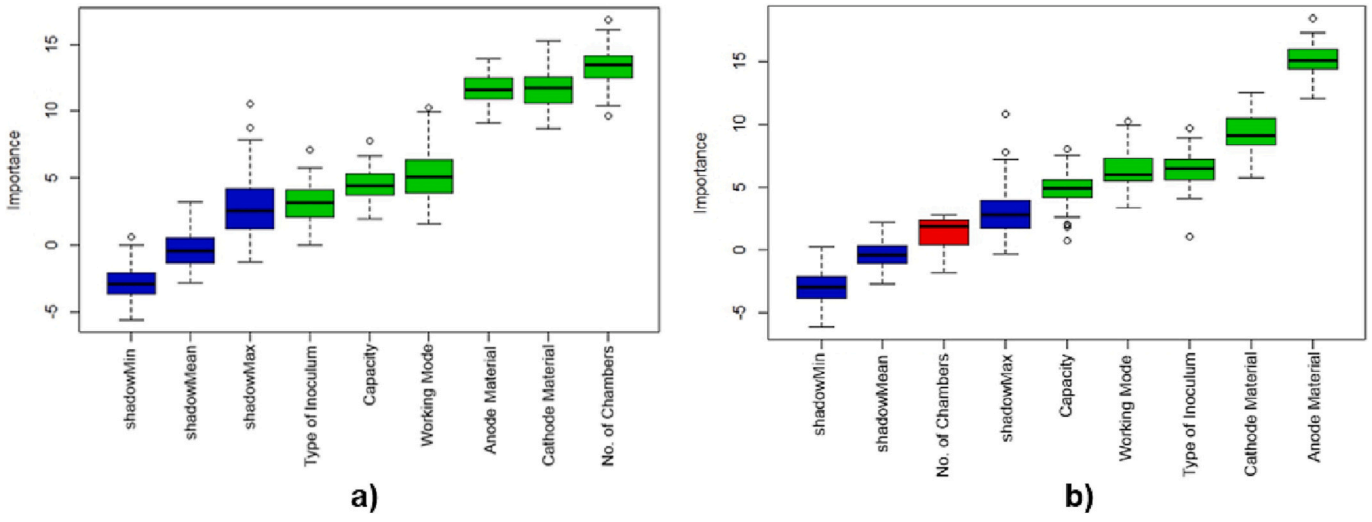

Fig. 5. Feature selection using random forest classifier of various factors on their rate of NO_3 removal (mg $liter^{-1}$ day^{-1}) (a) and NO_3 removal percentage (%) (b).

The migration transformation removal described that the applied voltage enhances the nitrogen species, which aids in NO_3 removal [64]. An external power supply can improve electrogenesis in the BES [48]. Complex carbon sources have improved removal rates compared to simpler carbon substrates [79]. Rice husk acts as a slow-release carbon source and provides surface area for microbial activity in BES [63]. Complex sources would be fodder for varied microbial communities, which would aid in processes resulting in an enhanced reduction of NO_3. Complex wastewater and effluents can also be exploited as carbon substrates in BES to aid heterotrophic denitrification.

The activity of the microbial community dictates the processes in BES; the study of BES for NO_3 removal is incomplete without studying the community structure thoroughly. Biological N removal processes are determined by C/N ratio as different enzymes have different affinities and feedbacks [80]. Autotrophic denitrification largely depends on the C/N ratio in the systems; the rise in the C/N ratio increases the NO_2 accumulation and declines autotrophic denitrification in BES. NO_3 triggers enhanced autotrophic denitrification [54]. Therefore, high NO_3 effluents with low carbon content can be efficiently treated with BES [43]. The C/N ratio has not been observed to influence the anode transforming efficiency [45]. The employment of autotrophic denitrification for NO_3 treatment has shown removal efficiencies at par with conventional systems [33]. Electroactive microbes enhance bidirectional electron transfer between anode and cathode, thus enhancing the reduction of NO_3 [40]. Polarity reversal has been displayed to improve NO_3 conversion to N_2, aiding complete denitrification [81]. Biocathodes can employ various processes for NO_3 removal in comparison to abiotic cathodes [38].

The community of the BES related to the nitrogen cycle is shown to be quite diverse in the examined studies. Autohydrogenotrophic denitrification was observed to be supported by *Pseudomonas, Halomonas, Rhodocyclaceae, Paracoccus, Dethiobacter* and *Thauera* species [65,75]. The family *Gallionellaceae* was observed in BES, which supports autotrophic denitrification [53]. Inoculating complete denitrifying bacterium such as *Thiobacillus denitrificans* and *Pseudomonas stutzeri* also aids in enhanced denitrification [20,82]. The most abundantly observed species in CW-BES are *Thiohalophilus* and *Clostridium* [62]. *Pseudomonas, Arenimonas, Flavobacterium, Bacillus, Rhodobacter* representating the heterotrophic denitrifiers were enhanced in BES [83]. Electroactive microbes are observed to enhance the reductive ability of the BES, and electroactive species such as the *Geobacter* were abundantly observed [40,83]. They have been responsible for the removal of organic carbon and N from the system [84].

The majority of the studies were conducted between temperatures 20°-30 °C, which supports optimum microbial activity though there is evidence of denitrification prevalent at temperatures below 10 °C. The species supported at low temperatures in BES are *Thermomonas, Arenimonas, Gallionella,* and *Thiobacillus* species [44]. The dominance of *Proteobacteria* and *Firmicutes* found in microbial community structure of NO_3-removing BES coincides with the dominant phyla in the benthic sediments of other wetlands [85]. The analysis of the community structure states that these systems support complete denitrifiers.

Different processes were studied through the abundance and prevalence of functional genes in the examined studies. *nirS* and *nosZI* genes were found to be responsible for the production and consumption of the N_2O fluxes [72]. Besides the denitrification process supported by BES, there is evidence of electrochemical support for ANAMMOX [20,68,72] and DNRA processes [20,72].

ANAMMOX process is suppressed with a high C/N ratio, i.e. 5.8 g COD/N promotes nitration, whereas ~3.5 g COD/N supports the process [80]. Marine ANAMMOX bacteria have been observed to be enhanced after stimulation externally ~1.5 V [86]. N^{15} tracer ex-situ experiments showed that BES support heterotrophic denitrification and ANAMMOX process for NO_3 removal [87].

DNRA process is mainly responsible for N recovery, to utilize the NH_4 as fertilizer and has displayed potential for complete removal of NO_3 [12,81]. With the $C/N = 6$, DNRA process has been shown to lose its electron output capability [21]. Periodic polarity reversal has been displayed to enhance DNRA process and increase N recovery [81]. *nrfA* genes are responsible for DNRA process and are upregulated with a decrease in NO_3 concentration, whereas *nirS* genes are downregulated in low NO_3 concentration [28].

The microbial community in BES is potent to perform autotrophic denitrification and ANAMMOX for NO_3 removal and displays the potential for complete denitrification to N_2 (Fig. 1). The proportions of *nir* and *nosZ* genes observed in BES were 83 % and 15 %, respectively, whereas *ANAMMOX 16S rRNA* and *nrfA* genes were <1 % of the prokaryotic proportions [20,72].

Design advances are highly responsible for enhancing denitrification, two reactor systems for complete denitrification [88], employment of simultaneous anaerobic carbon and N removal system for energy conservation [50], membrane-less reactor [51] and snorkel systems which employ concentrated electrons from sediment to reduce overlying NO_3 polluted water [69]. Discontinuous operation or fluidized biocathodes can enhance electron storage and transfer [53]. A tubular denitrifying reactor with a tubular membrane was employed, which significantly enhanced NO_3 removal this was the highest observed through various studies. The study recommends scaling the reactor by connecting various reactors in series with a low hydraulic retention time (HRT) [40]. Though, HRT largely influences microbial activity as the decline in HRT from 24 to 8 h has displayed a decline in EPS detection, which represents a decline in microbial load [84].

BES have been recognized as a tool for implementing a circular economy via generating electricity and value-added byproducts such as fertilizers by treating wastewater [89]. Circular economy has been employed via large-scale utilization of BES at the Tokyo Bay, where five sediment MFCs were employed to treat a highly eutrophied coastal bay, 11.5 ± 0.89 mW/m^2 power was generated with a hike in oxidation-reduction potential [90].

This study of analysis of varying parameters to determine the optimum one significantly influencing the NO_3 removal rates in BES is the first of its kind. This analysis can be further elaborated to not only build efficient models on BES but also design and operate sustainable, scalable, efficient systems for mitigating NO_3 pollution.

5. Conclusions

Overall, BES can be utilized as a potent system for NO_3-polluted water, it can aid various N-transforming processes and enhance denitrification. The choice of electrode material has a significant influence on the removal of NO_3 in BES, and a higher surface area yields higher microbial activity. Granular carbon and carbon paper were observed to show higher removal efficiencies. The inoculum type does not necessarily dictate the community structure; the water to be treated extensively decides the communities in the BES and is consequently responsible for the prevalent N transforming process in the systems. The volume of the system has shown lesser effect on the removal efficiencies which is assuring to build and utilize BES as a scalable system for NO_3 removal. The working mode of the system determines the concentration of essential nutrients for the maintenance of the microbial community structure and, therefore, a crucial operational parameter. There was no significant difference between the removal rate of the number of chambers, but it has been deemed an important parameter in feature selection. Chambers aid the partitioning of protons and, therefore, maintain the pH, which affects microbial activity. Systems are run for a couple of days to months in various studies, so determining the system's potency becomes difficult. This study helps take further steps toward treating NO_3-polluted water systemically and aids in restoring ecological imbalance. We need standardized units to represent NO_3 removal rate. Our analysis suggests that an external power supply, i.e. microbial electrosynthesis (e.g., MES, CW-BES), can aid in accelerating nitrate reduction and has displayed higher removal rates compared to MFC. We

suggest that BES as a continuous two-chamber system with cathode materials of granular carbon and carbon paper, and denitrifying microbes as inoculum type would also enhance the process, as it would altogether show optimum NO_3 removal efficiencies.

CRediT authorship contribution statement

Sharvari Sunil Gadegaonkar: Conceptualization, Methodology, Investigation, Data curation, Visualization, Formal analysis, Writing – Original draft preparation; **Ülo Mander:** Funding acquisition, Supervision, Writing – Review & Editing; **Mikk Espenberg:** Conceptualization, Methodology, Funding acquisition, Supervision, Project administration, Writing – Review & Editing.

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.

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