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Original Research

Direct ammonium oxidation to nitrogen gas (Dirammox) in *Alcaligenes* strain HO-1: The electrode role

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ABSTRACT

It has been recently suggested that *Alcaligenes* use a previously unknown pathway to convert ammonium into dinitrogen gas (Dirammox) via hydroxylamine (NH_2OH). This fact alone already implies a significant decrease in the aeration requirements for the process, but the process would still be dependent on external aeration. This work studied the potential use of a polarised electrode as an electron acceptor for ammonium oxidation using the recently described *Alcaligenes* strain HO-1 as a model heterotrophic nitrifier. Results indicated that *Alcaligenes* strain HO-1 requires aeration for metabolism, a requirement that cannot be replaced for a polarised electrode alone. However, concomitant elimination of succinate and ammonium was observed when operating a previously grown *Alcaligenes* strain HO-1 culture in the presence of a polarised electrode and without aeration. The usage of a polarised electrode together with aeration did not increase the succinate nor the nitrogen removal rates observed with aeration alone. However, current density generation was observed along a feeding batch test representing an electron share of 3% of the ammonium removed in the presence of aeration and 16% without aeration. Additional tests suggested that hydroxylamine oxidation to dinitrogen gas could have a relevant role in the electron discharge onto the anode. Therefore, the presence of a polarised electrode supported the metabolic functions of *Alcaligenes* strain HO-1 on the simultaneous oxidation of succinate and ammonium.

1. Introduction

Electricity-driven ammonium (NH_4^+) oxidation to dinitrogen gas (N_2) has been recently proved using ANAMMOX bacteria [1]. A similar process has been observed in the presence of feammox [2,3] or nitrifying bacteria, even though the mechanism is still mostly unknown in this latter case [4–9]. However, the electrical stimulation of heterotrophic nitrifying microorganisms has not been evaluated yet [10,11]. The most studied heterotrophic nitrifier is *Alcaligenes* [12,13]. Initial testing suggested that *Alcaligenes* used a two-step process involving the oxidation of NH_4^+ to nitrite/nitrate, followed by a reduction to N_2 [12,13]. However, it has been recently suggested that *Alcaligenes* use a previously unknown pathway to convert ammonium into N_2 (Dirammox route) [14,15]. According to whole genome annotation data, *Alcaligenes* strain HO-1 encodes a complete denitrification pathway, but it lacks genes coding for ammonia monooxygenases or hydroxylamine oxidoreductases homologs. However, a combination of inhibition experiments and isotopic labelling suggested that strain HO-1 had a novel genetic cluster for ammonia oxidation, encoding for a previously unknown pathway able to shortcut the conventional nitrogen cycle. In the Dirammox route, NH_4^+ is firstly oxidised to hydroxylamine (NH_2OH) and then to N_2 [14]. This enzyme (encoded by the DnfA) has finally been identified and characterised by isotopic experiments. It has been described as the first enzyme to be discovered, which is able to catalyse hydroxylamine oxidation to N_2 [16]. Recently, a second *Alcaligenes* species (strain JQ135) presenting the Dirammox route has also been reported [15].

The Dirammox pathway represents a considerable decrease in the number of electrons required to convert NH_4^+ to N_2 and, thus, a

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decrease in the organic matter and aeration needed for conventional nitrification-denitrification (e^- equivalents). In order to convert 1 mol of ammonium into N_2, conventional nitrification-denitrification requires a total of 13 mol of electrons (8 mol e^- for nitrification and 5 mol e^- for denitrification). On the contrary, the Dirammox route only requires 3 mol e^-. This fact alone significantly decreases the aeration requirements for the process, but the process would still be dependent on external aeration, usually the highest cost of a wastewater treatment plant [17].

Microbial electrochemical technologies (METs) are potential alternatives to suppress aeration in wastewater treatment plants due to the ability of some microorganisms to use an electrode as an electron acceptor instead of oxygen [1,8,18]. Considering the pivoting role of hydroxylamine in electricity-driven nitrification [5,8], one might hypothesise those heterotrophic nitrifying microorganisms such as *Alcaligenes*, could also be able to use an electrode as the terminal electron acceptor. Using an electrode instead of oxygen could suppress external aeration while being a barrier for competing for non-electroactive microorganisms. This work studied the potential use of a polarised electrode as an electron acceptor for ammonium oxidation using the recently described *Alcaligenes* strain HO-1 as a model heterotrophic nitrifier [14].

2. Materials and methods

2.1. Experimental set-up

Experimental tests were performed using a culture of *Alcaligenes* strain HO-1 [14] provided by the Institute of Microbiology, Chinese Academy of Sciences (IMCAS, Beijing, China). Once arrived at the University of Girona, *Alcaligenes* HO-1 was maintained in solid media Luria-Bertani at 30 °C.

Electrochemical experiments were performed in duplicates using 250 mL bottles equipped with a carbon cloth (25 cm^2, NuVant's ELAT LT2400W, FuelCellsEtc USA) as a working electrode, a graphite rod as a counter electrode and an Ag/AgCl sat. KCl reference electrode (+0.197 V vs. SHE) (Fig. SI1). Systems were kept at 30 °C and fed with the medium described by Wu et al. [14]. In all tests, the reactor medium was composed of 0.66 g L^{-1} $(NH_4)_2SO_4$, 0.66 g L^{-1} succinate, 0.5 g L^{-1} KH_2PO_4, 1.25 g L^{-1} Na_2HPO_4 $12H_2O$, 0.2 g L^{-1} $MgSO_4$ $7H_2O$, 10 g L^{-1} NaCl, and 2 mL L^{-1} of a trace elements solution [19].

Electrochemical reactors were inoculated using high-density cell suspensions collected after a plate-wash protocol. Briefly, *Alcaligenes* HO-1 cells grown on LB plates for 24 h were harvested by adding 1 mL of freshly prepared reactor medium and detaching cells from the plate surface with a spreader. Cell suspensions were collected afterwards and inoculated directly into the reactors. Initial cell densities were above 10^8 cells mL^{-1} in all cases (Optical density at 600 nm > 0.1).

A total of ten different replicates were evaluated under different conditions (Table 1).

2.2. Electrochemical characterization of Alcaligenes strain HO-1

Several tests were performed to evaluate the effect of electrode polarisation in selected steps of the ammonium oxidation route (Table 1). In some tests, aeration was applied. A basic aquarium pump was used. Air was filtered through a 0.22 μm pore diameter nylon filter (Isopore) before entering the reactor to prevent contamination of *Alcaligenes* cultures by air-borne microbes. Test 1 was devoted to determining whether NH_4^+ oxidation to N_2 and cell growth could be supported by a polarised electrode alone (i.e., can *Alcaligenes* grow without aeration?). Two reactor replicates were inoculated and operated under chronoamperometry mode without aeration. The working electrode was poised at +0.2 V vs. Ag/AgCl following the standardised *Geobacter* methodology (model anode-respiring bacteria) [18]. After 21 days of operation, aeration was introduced into the system at a periodicity of 1 h per day. The process ensured the O_2 saturation of the medium. This experimental period lasted a total of 35 days.

In Test 2, the systems were operated under different polarisation and aeration regimes to assess the effect on the growth and the activity of cells, as well as to better understand the possible role of the polarised electrode under the optimal conditions for *Alcaligenes* growth. Four reactor replicates were operated for 18 days. All replicates were run with intermittent aeration (12 h per day from day 2). Two replicates were operated under chronoamperometry at a working electrode potential of +0.2 V vs. Ag/AgCl. Two replicates were operated under open-circuit conditions (i.e., no electrode polarisation).

The effect of aeration in the presence of a polarised electrode was studied in Test 3. Two reactors were inoculated. During the first 11 days, the reactors were operated in the presence of a polarised electrode at a working electrode potential of +0.2 V vs. Ag/AgCl and aeration (12 h per day). From days 11–20, aeration was stopped to better elucidate the dynamics of the nitrogen compounds in the absence of oxygen.

Some individual steps in the oxidation reactions were tested in Test 4 for bioelectrochemical enhancement. In particular, the transformation of hydroxylamine (as a potential intermediate of the nitrogen cycle) was considered a relevant step to further evaluate. Two reactors in the presence of a polarised electrode at +0.2 V vs. Ag/AgCl and intermittent aeration were set up. On days 6 and 13, hydroxylamine, succinate, and ammonium were spiked (10 mg $N–NH_2OH$ L^{-1}, 1.2 g C-Succinate L^{-1}, and 50 mg $N–NH_4^+$ L^{-1}, final concentrations, respectively), and the dynamics of nitrogen compounds, the consumption of succinate, and the current density were evaluated on a time basis.

2.3. Analyses and calculations

Samples were regularly taken to measure pH, nitrite ($N–NO_2^-$), nitrate ($N–NO_3^-$), and ammonium ($N–NH_4^+$) following the

Table 1
Summary of tests performed. Legend: CA, Chronoamperometry with the working electrode poised at +0.2 V vs. Ag/AgCl; OCV, Open cell voltage.

Test	Question to be answered	Replicates	Aeration	Electrochemical operation
Test 1	Can it grow without aeration?	2	No (Days 0–21) Yes, intermittent (Days 21–35)	CA
Test 2	Can a polarised electrode increase the nitrogen removal rates?	4	Yes, intermittent	CA (two replicates) OCV (two replicates)
Test 3	Can nitrogen be removed without aeration but in the presence of a polarised electrode?	2	Yes, intermittent (Days 0–11) No (Days 11–20)	CA
Test 4	How does hydroxylamine behave during the process in the presence of a polarised electrode?	2	Yes, intermittent	CA

American Public Health Association (APHA) standards [20]. Nitrous oxide and dissolved oxygen (DO) were measured using an N_2O liquid-phase microsensor (Unisense, Denmark) and an oxygen sensor (Unisense, Denmark). The concentration of hydroxylamine (NH_2OH) was determined colourimetrically [21]. Biomass was measured by optical density at 600 nm (OD_{600}) using a spectrophotometer (Hach DR3900, USA). Succinate concentration was measured using ion chromatography (Dionex, ThermoFisher, USA). Electrode potential was poised at the working electrode using a potentiostat (Bio-logic, France).

Cyclic voltammetries were regularly recorded in a potential window between −0.6 and + 0.6 V vs. Ag/AgCl at a scan rate of 1 mV s^{-1} [18].

3. Results and discussion

3.1. Test 1: can Alcaligenes strain HO-1 be grown with a polarised electrode without aeration?

The initial hypothesis considered the possibility of the HO-1 strain using a polarised electrode instead of O_2 as the sole electron acceptor. Thus, in Test 1, the growth and electrical response of *Alcaligenes* were evaluated under anaerobic and aerobic conditions (Fig. 1). Two reactors were initially operated under chronoamperometric mode without aeration. From days 0–21, the biomass content (OD_{600}) remained mostly unchanged (no growth), but fluctuations in the current density profile could be observed. After 21 days of operation, aeration was introduced in the system (1 h per day). *Alcaligenes* started to grow, and the OD_{600} increased from 0.22 ± 0.03 on days 16–21 to 0.64 ± 0.12 on days 27–33, while the current density increased over time. Cyclic voltammetries were recorded before and after introducing aeration in the reactors to better understand the increase in current density (Fig. SI2). However, no relevant voltammetry signals could be detected. Taking it all together, our results indicated *Alcaligenes* did not grow, and low current densities were recorded with the sole presence of a polarised electrode as an electron acceptor (absence of aeration). Nevertheless, an increase in the current density was detected in the presence of aeration, suggesting that *Alcaligenes* could partly divert electrons to a polarised electrode.

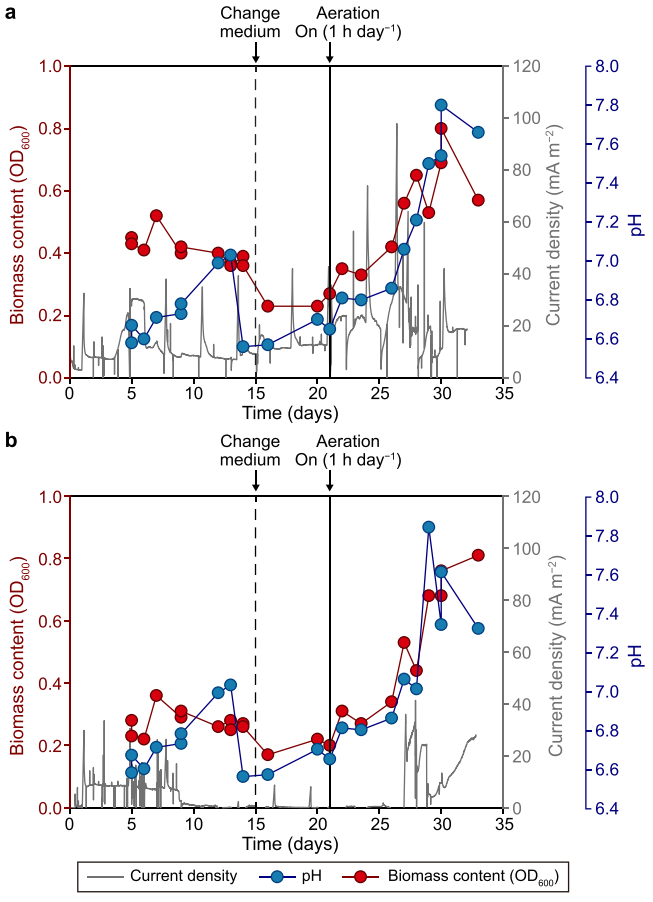

Fig. 1. Evolution of pH, current density, and biomass content (OD_{600}) on reactor replicates A (**a**) and B (**b**).

3.2. Test 2: can a polarised electrode increase the nitrogen removal rates of Alcaligenes?

Four reactors were set up and run as replicates (Test 2; Fig. 2) to better elucidate the role of a polarised electrode on *Alcaligenes* nitrogen metabolism. All replicates were run with intermittent aeration (12 h per day). No significant differences were observed in biomass growth between the polarised vs. non-polarised electrodes. Similarly, succinate, ammonium, nitrite, and nitrate concentrations varied in the two operational modes (with and without current). Different activities were observed in the two batch runs due to the higher biomass content in the second run. However, the values obtained were similar under the two conditions tested. Ammonium was removed in the first and second runs at 17 and 40 mg N L^{-1} d^{-1} in the reactors operated with a polarised electrode and 15 and 45 mg N L^{-1} d^{-1} in the reactors operated with a non-polarised electrode. Succinate and ammonium were depleted at similar rates in the two operational conditions. Similarly, no significant accumulations of nitrite (<2 mg N–NO_2^- L^{-1}) or nitrate (<3 mg N–NO_3^- L^{-1}) were detected under both experimental conditions. These results confirmed the capacity of *Alcaligenes* strain HO-1 to fully remove ammonium without the accumulation of intermediates, as previously described [14]. Complementary cyclic voltammetries were recorded in the presence and absence of aeration (Fig. SI3). Some redox peaks could be observed at −0.1 V vs. Ag/AgCl when aeration was Off. Those were peaks characteristic of a non-turnover condition (i.e., absence of substrate); thus, they could not be linked to a specific activity towards succinate or ammonium oxidation.

Considering the results observed in Test 2, it can be assumed that the usage of a polarised electrode did not provide any measurable increase in either succinate or ammonium removal rates in the presence of aeration.

3.3. Test 3: can Alcaligenes strain HO-1 remove nitrogen in the absence of O_2 under the presence of a polarised electrode?

The results obtained in Test 1 confirmed that *Alcaligenes* strain HO-1 could not grow using a polarised electrode as the sole electron acceptor, whereas Test 2 demonstrated that the ammonium removal rate did not significantly increase in the presence of a polarised electrode and aeration. In Test 3, the ammonium removal under anaerobic conditions (i.e., in the absence of O_2 and, thus, the absence of cell growth) was evaluated. Two reactor replicates were inoculated (Test 3; Fig. 3). During the initial 11 days, reactors were operated in the presence of a polarised electrode (+0.2 V vs. Ag/AgCl) and intermittent aeration. From days 11–20, aeration was stopped. In the presence of aeration and a polarised electrode, both succinate and ammonium were removed without a significant accumulation of nitrite, nitrate or nitrous oxide. At the working conditions (30 °C and atmospheric pressure), O_2 saturation occurs at 7.54 mg O_2 L^{-1}, enabling a maximum NH_4^+ removal of

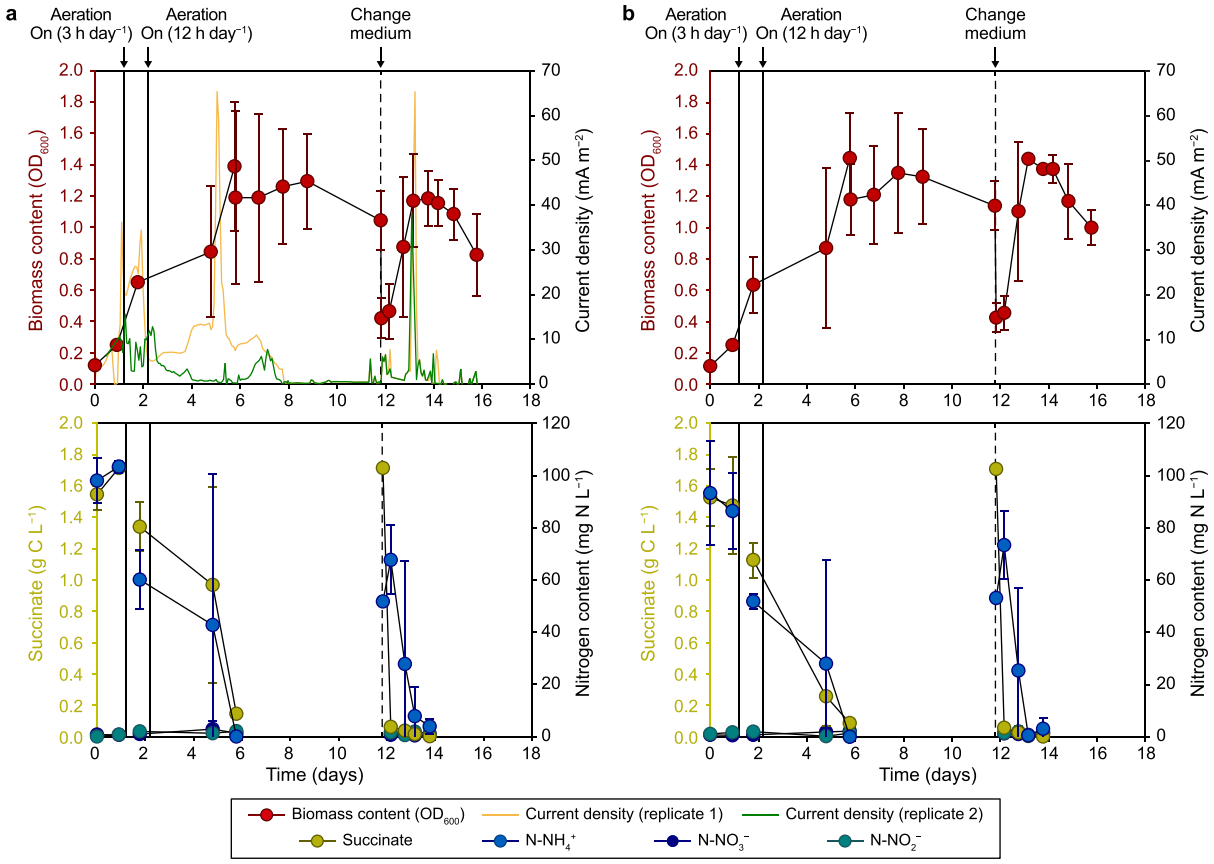

Fig. 2. Evolution of current density, biomass content (OD_{600}), succinate, ammonium ($N{-}NH_4^+$), nitrite ($N{-}NO_2^-$), and nitrate ($N{-}NO_3^-$) content on reactor replicates operated under closed circuit conditions (**a**) and open circuit conditions (**b**).

4.4 mg N L^{-1}. This was the maximum removal capacity when aeration was cased (first anaerobic batch). However, a higher removal was observed in this batch (41.8 mg N L^{-1}). In the presence of intermittent aeration, succinate and ammonium were removed at a rate of 0.5 ± 0.1 g C L^{-1} d^{-1} and 48 ± 9 mg N L^{-1} d^{-1}, respectively. Without aeration, succinate and ammonium were removed at a rate of 0.1 ± 0.1 g C L^{-1} d^{-1} and 11 ± 5 mg N L^{-1} d^{-1}.

It is worth noting that the current density fluctuated along with the experimental runs. When aeration was 'On' (days 0–11), current density peaks were detected at the mid-term of each feeding batch (values around 40 mA m^{-2}). Succinate and ammonium were removed following a linear trend. In contrast, the peak formation in the current density profile indicated that electricity was generated during the formation of a transient intermediate metabolite. Being succinate to fumarate a single-step reaction and ammonium to N_2 in a two-step reaction, current density was most likely related to the NH_4^+ removal route. It can be hypothesised that *Alcaligenes* produced/released an intermediate metabolite from the NH_4^+ removal process, a metabolite that presented electroactive activity. When aeration was 'Off' (days 11–20), *Alcaligenes* activity decreased, and the formation of transient peaks in current density evolution was less evident, but a continuous increase of current density was observed instead. The transient increase of current density peaked at around 20 mA m^{-2}.

During *Alcaligenes* growth, succinate and ammonium were removed without a noticeable production of nitrite, nitrous oxide or nitrate. However, this process generated current density at a working electrode polarised at +0.2 V vs. Ag/AgCl. The accumulated charge was 8.2 ± 0.8 C, representing a ratio concerning ammonium removed of 0.26 ± 0.02 C per mg $N{-}NH_{4\,removed}^+$. In the absence of aeration, ammonium was removed at a lower rate (11 ± 5 mg N L^{-1} d^{-1}), together with a slower current peak production (suggesting a lower accumulation rate of a putative electroactive intermediate) but representing a charge to ammonium removed up to three times higher (1.14 ± 0.55 C per mg $N{-}NH_{4\,removed}^+$). Hence, a comparatively higher generation and oxidation of electroactive intermediates were observed. Taking into account that ammonium removal in *Alcaligenes* occurs via hydroxylamine [14] and the electroactivity of NH_2OH [5,8], it was considered that the transient accumulation of hydroxylamine and its consequent oxidation could be responsible for current density generation. Considering the NH_2OH oxidation to N_2 ($n = 1e^-$), the current density observed represents 3.8 ± 0.3% of the ammonium removed in the presence of aeration and 10.9% and 22.3% in the first and second batch in the absence of aeration, hence representing significant support to the overall ammonium oxidation process. The electricity-driven oxidation of hydroxylamine by *Alcaligenes* was further investigated in Test 4.

3.4. Test 4: which is the role of hydroxylamine during the NH_4^+ removal carried out by Alcaligenes HO-1 in the presence of a polarised electrode?

The fate of hydroxylamine was evaluated in two reactor replicates inoculated with *Alcaligenes* HO-1 in the presence of a polarised electrode (Test 4). Hydroxylamine was spiked together with succinate and ammonium to evaluate the dynamics of nitrogen

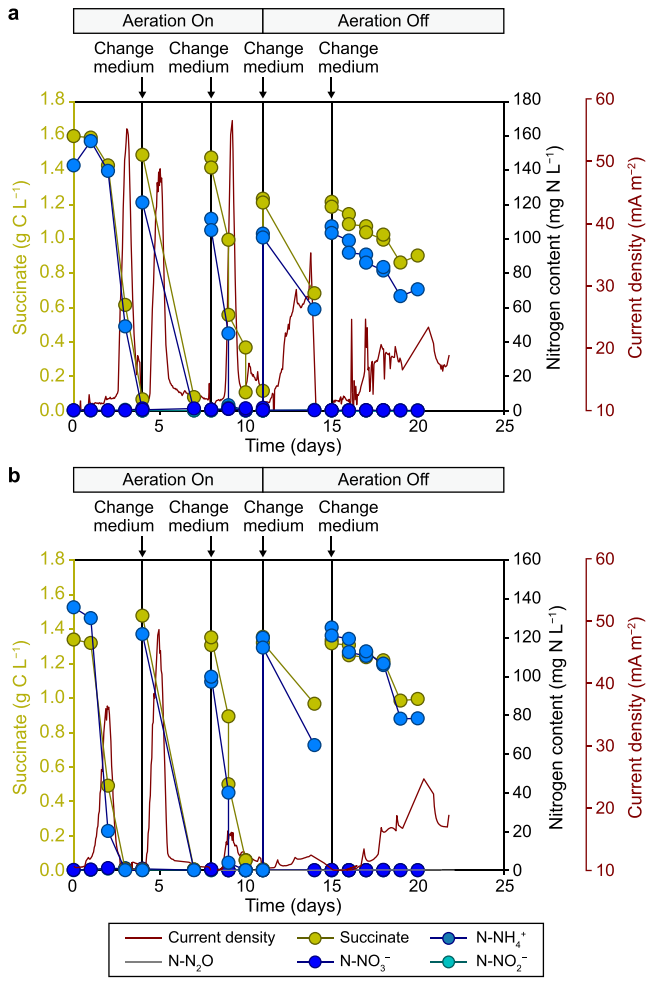

Fig. 3. Evolution of current density, succinate, ammonium ($N–NH_4^+$), nitrite ($N–NO_2^-$), and nitrate ($N–NO_3^-$) content on reactor replicates A (**a**) and B (**b**) operated under the presence of a polarised electrode.

compounds (and succinate) and the evolution of the current density in two different runs (Fig. 4).

At the start of the test, aeration was on mode 'On', and it was later stopped for better observation of the trends, according to the results obtained in Test 3. A performance similar to previous tests was observed in terms of ammonium and succinate removal rates and the lack of nitrite and nitrate accumulation (<4 mg N L^{-1}, data not shown) despite the presence of hydroxylamine at a relatively high concentration (around 10 mg $N–NH_2OH$ L^{-1}). The addition of hydroxylamine caused a sudden increase in the current density in the two runs (up to 40–100 mA m^{-2}). Current density decreased concomitantly with hydroxylamine depletion. These results supported the hypothesis that the current density generation detected in previous tests was related to the transient accumulation of hydroxylamine in the media.

In addition, an accumulation of N_2O has observed along that process, which was more significant than those observed in Test 3. A maximum accumulation of 0.2 and 2.4 mg $N–N_2O$ L^{-1} was observed for the first and second run, respectively, implying 2% and 23% of the total hydroxylamine removed. N_2O was only detected when O_2 was fully consumed. Nitrous oxide production from hydroxylamine oxidation had been previously observed in *Alcaligenes faecalis* [22]. It is worth noting that N_2O was finally removed at the end of the batch test without external input.

Taking it all together, *Alcaligenes* HO-1 could not sustain its metabolic activities by using an electrode poised at +0.2 V vs. Ag/AgCl as the sole electron acceptor. Aeration was required. However, even under aerated conditions, the polarised electrode could be used as a terminal electron acceptor, and the generation of current density was detected. Hydroxylamine accumulation and later oxidation could be responsible for this current generation. Whether this process was carried out by using external soluble mediators or direct electron transfer is unclear. According to the draft genome of *Alcaligenes* strain HO-1 (CP049362.1), this strain contains multiple cytochromes of the b, c, d and P450 families, some of which could likely be present as outer membrane cytochromes, but none of them has been annotated as such. Additional testing at, for example, other anode potentials, different succinate to ammonium ratios, O_2 set-points or the addition of external soluble mediators are required in future experiments to further elucidate the possible interactions between Dirammox *Alcaligenes* HO-1 and electrodes.

3.5. Perspectives for electrode-assisted biologic processes

Alcaligenes strains oxidise organic matter and ammonium to CO_2 and N_2 under aerobic conditions [14]. This work surveyed a possible electroactivity of *Alcaligenes* strain HO-1 (Table 2).

A polarised electrode could ideally replace O_2 as a terminal electron acceptor in the ammonium oxidation process [18,23]. However, no growth and lower NH_4^+ removal rates were observed when an electrode poised at +0.2 V vs. Ag/AgCl was used as the sole electron acceptor. These results suggested that the electroactivity was insufficient to consider a process where *Alcaligenes* works using an electrode as the sole terminal electron acceptor or to evaluate complementary benefits such as cathode H_2 production or electricity production from the reactor device. In this sense, the possible electrification of a heterotrophic nitrifying process dominated by *Alcaligenes* would be out of the current standards of electro-bioremediation, where the target pollutant is fully removed by an electrode-dependent process [24].

Nevertheless, the different tests performed in this study revealed an electroactivity role of *Alcaligenes* strain HO-1. Electron discharge on the anode electrode was detected either in the presence or the absence of aeration but was more intense and constant over time under oxygen-limiting conditions. Besides, *Alcaligenes* did not have an apparent increase of ammonium removal activity in the presence of an electrode, and electrons were discharged on the electrode during the NH_4^+ removal process. The results suggested that hydroxylamine, an intermediate of ammonium conversion into dinitrogen gas [14], could be involved in the electrochemical signal, a process suggested previously with nitrifying-enriched communities [5,8]. If NH_2OH oxidation to N_2 is considered a potential oxidation reaction, current density would then represent about 3.8% of the NH_4^+ removed in the presence of aeration and 16.6% in the absence of aeration. However, complement reactions cannot be fully discarded, nor the release of an electroactive compound different from hydroxylamine. For example, a metabolic surplus derived from periods of oxygen limitation [25], could occur transiently during an aerobic batch test or constantly in longer anaerobic periods. In any of the two conditions, using a polarised electrode could assist the long-term operation of the bio-reactor [26,27]. From an application point of view, these results suggest an alternative pathway for research on microbial electrochemical technologies, where bacteria and electrodes no longer have a relationship of obligate dependency but mutual assistance. In the context of biological nitrogen removal, electrode-dependent processes might have a chance to succeed in waters where organic matter is scarce, because the usage of electricity supply instead of chemical addition or aeration can become an important defensive

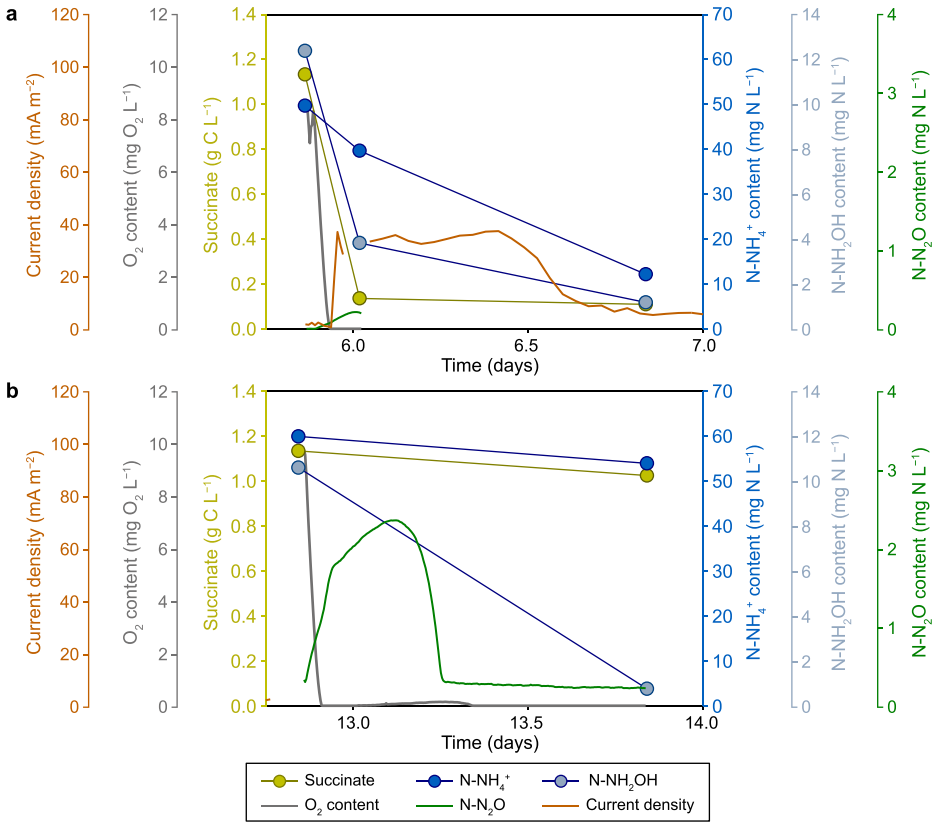

Fig. 4. **a**–**b**, Evolution of current density, succinate, ammonium (N–NH_4^+), hydroxylamine (N–NH_2OH), and nitrous oxide (N–N_2O) content on reactor operated under closed circuit conditions and two different hydroxylamine spikes.

Table 2
Summary of main results obtained in the different tests performed. Legend: **, results related to NH_2OH.

Test	Aeration Electrochemical mode	Peak current density	NH_4^+ removal rate	Nitrogen intermediates	
		(mA m^{-2})	(mg N L^{-1} d^{-1})	(mg N L^{-1})	(% total NH_4^+ removed)
Test 1	No aeration CA	20	-	-	-
	Aeration CA	40–60	-	-	-
Test 2	Aeration CA	60	17–40	NO_3^- < 3 NO_2^- < 2	<5
	Aeration OCV	-	15–45	NO_3^- < 3 NO_2^- < 2	<5
Test 3	Aeration CA	50	48 ± 9	NO_3^- < 1.5 NO_2^- < 1.5 N_2O < 0.2	<2
	No aeration CA	20	11 ± 5	NO_3^- < 0.2 NO_2^- < 0.3 N_2O < 0.2	<1
Test 4	Aeration CA	40–100	6–39 12–10**	N_2O < 2.4	2–24**

moat for the technology [1,8]. However, as the carbon-to-nitrogen ratio increases, the competition for the electrode surface between nitrifiers and heterotrophs might reduce the chances for a proper ammonium removal process, which would then require the integration with other technologies [28,29]. The share of electrode relevance in the process would gradually decrease at higher carbon-to-nitrogen ratios, up to a point where the electrode has the sole function of assisting bacteria in specific, but still relevant, metabolic processes, such as the electrically assisted hydroxylamine oxidation proven for *Alcaligenes* HO-1.

4. Conclusions

(1) *Alcaligenes* strain HO-1 metabolic dependence on aeration cannot be sustained with a polarised electrode. However, the concomitant elimination of succinate and ammonium was observed when operating a previously grown *Alcaligenes* strain HO-1 culture in the presence of a polarised electrode and without aeration.

(2) The usage of a polarised electrode together with aeration did not increase the succinate nor the nitrogen removal rates observed with aeration alone. However, current density generation was observed along a feeding batch test

representing an electron share of 3.8% of the ammonium removed in the presence of aeration and 16.6% without aeration.

(3) Hydroxylamine oxidation to dinitrogen gas could have a relevant role in the electron discharge onto the anode.

(4) The presence of a polarised electrode assisted the metabolic functions of *Alcaligenes* strain HO-1 on the simultaneous oxidation of succinate and ammonium, supporting that integrating electrodes into bio-processes could assist the overall system.

Credit authorship contribution statement

Narcís Pous: Conceptualization, Data curation, Investigation, Methodology, Writing - original draft. **Lluis Bañeras:** Conceptualization, Investigation, Methodology, Supervision, Writing - review & editing. **Philippe F.-X. Corvini:** Investigation, Writing - review & editing. **Shuang-Jiang Liu:** Investigation, Writing - review & editing. **Sebastià Puig:** Conceptualization, Funding acquisition, Methodology, 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.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ese.2023.100253.

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