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Growth of microalgae using nitrate-rich brine wash from the water industry

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ABSTRACT

Safe and accepted limits for nitrates in drinking water are exceeded in around one-third of the groundwater bodies in Europe. Whilst anion exchange (AEX) is an effective technology to strip nitrates, the regeneration of AEX resins using saturated sodium chloride (brine) results in a significant quantity of nitrate-rich saline waste, which is currently disposed of at a substantial cost to the water industry. The aim of this research was to evaluate the viability of using AEX brine wash as a nutrient source to support microalgal growth. Experiments were carried out at laboratory and pilot scales to test which algal species were able to grow on brine wash, to determine the optimal nitrate concentration within modified growth media, and to identify whether the origin of the brine wash affected the nitrate uptake potential. In small scale laboratory experiments, five marine algal species were able to grow in modified *f/2* growth media containing nitrate sourced from the brine wash. Further experiments showed that three species could grow on the modified media at nitrate concentrations from 5 to 274 mg L^{-1}. *P. tricornutum* could remediate up to 6.5 mg nitrate in 50 mL cultures in laboratory scale experiments, up to 570 mg at 10 L scale and 1700 mg at 100 L scale. We found that the origin of the brine wash did not significantly affect the growth of the cultures or the amount of nitrate removal from the modified media. The algal biomass could be used effectively in biogas production in small-scale trials, although with < 10% the yield from *P. tricornutum* biomass from standard f/2 medium. Our results suggest that it may be possible to derive value from brine wash as a sustainable source of nitrate for the growth of microalgae in bulk after optimisation.

1. Introduction

An important source of drinking water throughout Europe is from groundwater. For example, in the UK and Scandinavia, groundwater provides up to two-thirds of the drinking water, whereas Lithuania and Austria are almost entirely dependent on groundwater [1]. However, nitrate (NO_3^-) contamination of groundwater is common, particularly in Central and Western Europe, most likely due to the use of fertilisers in the intensive farming of arable lands [2–4]. As a consequence approximately 60% of all groundwater sources exceeded the EU limit (50 mg L^{-1}) in 2015 ([5–7]) and similarly in the U.S.A. 22% of domestic groundwater wells exceed local nitrate limits [8]. To meet safe drinking water standards, excess nitrate must be diluted or removed from groundwater before it is fed into the drinking water supply such as anion exchange [9]. However, the anion exchange process yields a hyper-saline (~52 g NaCl L^{-1}), nitrate-rich (~4–20 g nitrate L^{-1}) wash that must be disposed of safely [10,11].

Due to the substantial cost and environmental impact of these methods [12,13], much research has been carried out into alternative approaches for the treatment and subsequent use of the used anion exchange resin [14–16]. One approach would be to use the brine wash as a nutrient source for microbial growth, such as photosynthetic microalgae [17–20]. The microalgae cannot yet be used to desalinate or reduce the volume of the brine wash, but they could be used to remove nitrate effectively. Additionally, the algal biomass can be further valorised, for example for the production of biogas via anaerobic digestion [21–23], or be used for higher value applications such as fertiliser, animal feed or source of natural products [24–26].

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In this study, we investigated the potential of microalgae to grow on nitrate sourced from brine wash from an industrial anion exchange system from a local water company, Cambridge Water Ltd., UK. A number of marine algal species were tested for their ability to grow on liquid growth media with the brine wash as the sole source of nitrate. Experiments were carried out at both laboratory and pilot scale, to establish the optimal concentration of brine wash for biomass production, the effectiveness of nitrate remediation, and whether the origin of the brine wash affected the nitrate uptake potential. Finally, we investigated the biogas potential of algal biomass to quantitatively evaluate the conversion of chemical energy, from CO_2 fixation via photosynthesis, to useful energy such as methane.

2. Materials and methods

2.1. Brine wash

Material for the study was provided by Cambridge Water Ltd. who produce approximately 5.69 m^3 day^{-1} of nitrate-rich brine wash using an IONEX counter-current ion exchanger system (Puritech, Belgium). Samples (5–10 L) of crude brine wash were collected from two Cambridge Water Ltd. treatment plants for borehole drinking water (FDWTW and BABWTW site codes, Cambridgeshire, UK) during 2013–2016. The nitrate concentration of the brine wash was measured for each batch, and found to range from 5 to 42 g L^{-1} brine wash (the nitrate concentration is dependent on when the brine was taken during the wash cycle). A detailed analysis of one batch of brine wash was undertaken by Cambridge Water Ltd., and was found to contain, in addition to the nitrate (42 g L^{-1} as NO_3^--N), 52 g L^{-1} chloride, 44 g L^{-1}, sodium, 0.29 g L^{-1}, phosphate (PO_4-P), 1.8 g L^{-1} sulphate, 148 mg C L^{-1} total organic carbon, pH 7.64.

2.2. Algal species selection and growth conditions

Five marine algal species were selected for use in the study: *Nannochloropsis oceanica* (CCAP 211/46), *Phaeodactylum tricornutum* (UTEX 646), *Tetraselmis suecica* (CCAP 66/22), *Isochrysis galbana* (CCAP 927/14), and *Pavlova lutheri* (CCAP 931/1, now referred to as *Diachromena lutheri*). Marine species were selected over freshwater species as they are better adapted to deal with the elevated salinities that could potentially arise from the use of brine wash in growth media. All cultures were grown on sterile *f/2* medium [27] (details of the growth media can be found at www.ccap.ac.uk). For brine tolerance, growth and nitrate removal experiments, both standard and modified *f/2* recipes were used as the basis for media formulations, without above ambient CO_2 supplementation. For the modified medium, sodium nitrate was excluded and replaced with a volume of crude brine wash to provide a range of nitrate concentrations, including the equivalent of standard growth media (*f/2* media = 55 mg L^{-1}). All other components, apart from salinity remained the same as in standard growth media (Tables 1 and 2). The growth media, with their nitrate sources substituted with brine wash, are hereafter referred to as "modified *f/2*". Experiments carried out at the laboratory scale used modified media with nitrate sourced from the BABWTW borehole site. Algae were grown in either 6 × 10 mL well Corning® Costar® plates or 50 mL plastic Nunc Easyflasks (Thermo Scientific) using a 1:10 culture:media ratio. All laboratory experiments were conducted in INFORS HT Multitron closed incubation shakers (Infors, Basel, Switzerland) maintained at 20 °C, 100 rpm, with 150 μmol m^{-2} s^{-1} illumination (12/12 h light/dark cycle). Experiments at the pilot scale (10 to 100 L) were conducted in the University of Cambridge Algal Innovation Centre housed at the Cambridge University Botanic Gardens, UK using nitrate sourced from either the FDWTW or BABWTW borehole sites. Algae were grown in either 10 L vertical bubble column bioreactors (Anaero Technologies, Cambridge, UK) during August and September 2015 or in a 100 L horizontal bioreactor (designed and assembled by Steve Skill, co-author) during February 2016.

Table 1
Initial (T0) concentrations of nitrate (mg L^{-1}) in standard *f/2* growth media, crude (undiluted) brine wash and modified media at the start of preliminary brine wash tolerance experiments. The nitrate concentration range in the brine wash experimental flasks was set to provide nitrate below, above and equal to the concentration provided by standard growth media.

	Treatment	*f/2* (mg nitrate L^{-1})
1	Standard media – control	55
2	Undiluted brine wash – control	14,000
3	Modified media 1/10 NO_3^-	5
4	Modified media 1/2 NO_3^-	28
5	Modified media 1 × NO_3^- [a]	55
6	Modified media 2 × NO_3^-	110
7	Modified media 5 × NO_3^-	275

[a] Comparable to standard media.

2.3. Growth measurements

Algal growth was measured by optical density (OD) at 600 nm (Thermo Spectronic UV1, Thermo Fisher, Hemel Hempstead, UK) and by cell counts using a Coulter particle counter (Beckman Coulter Z2 coulter particle count and size analyser). Algal dry cell weight was obtained by gravity filtration of a known volume (50 mL) of culture through a pre-dried, pre-weighed GF/C filter (Whatman GF/C, 47 mm), followed by rinsing twice with 0.65 M ammonium formate (99%, Acros Organics) to remove media salts whilst also preventing cell lysis due to osmotic shock. Filters were then dried at 80 °C for at least 48 h prior to re-weighing.

Determination of nitrate and phosphate (as orthophosphate, PO_4^{3-}) concentration was performed colorimetrically using a Hach Lange DR 3900 spectrophotometer with the appropriate test kits (Nitrate Kit LCK 339, range 1 to 60 mg nitrate L^{-1}; Phosphate Kit LCK 349, range 0.15 to 4.5 mg phosphate L^{-1} Hach Lange, Manchester, UK). Algal cultures and brine wash were filtered through 0.45 μm syringe filters and the resulting filtrate was used for the analysis. For experiments where *f/2* growth medium had been used, the filtrate for nitrate analysis was also treated with a chloride elimination kit (LCW 925, Hach Lange) to remove any analytical interference from the chloride ions in the media. However, untreated filtrate was used for phosphate analysis as the salts did not interfere with the reaction chemistry. Where nitrate concentrations exceeded the range of the kit, samples were diluted with deionised water. Salinity and pH were measured using a VWR CO 300 Digital Conductivity Meter and a Hach Lange Pocket Pro pH meter (Hach Lange, Düsseldorf, Germany).

2.4. Anaerobic digestion

The Bio-Methane Potential (BMP) of *Phaeodactylum tricornutum* grown in standard and modified *f/2* containing brine wash from two bore hole sites FDWTW and BABWTW was measured using 15 × 1-L HDPE reactor bottles submerged in a water bath maintained at 35 °C (Anaero Technology Ltd., Cambridge, UK). The working volume in the reactors was 700 mL. Samples were pre-treated at 90 °C for 12 h followed by an inoculum to substrate ratio of 8 g volatile solids (VS) inoculum to 1 g VS substrate. All reactors were mixed at 45 rpm by a single internal paddle mixer and gas flow was monitored and logged continuously and converted automatically to standard temperature and pressure (STP) by continuous monitoring of temperature and atmospheric pressure. Total and volatile solids were determined according to the standard methods [28]. Samples were then processed for dry solids (DS) in a Memmert oven at 105 °C for 6 h. Volatile solids were heated overnight at 550 °C. The biogas potential of substrates was calculated as a cumulative measure of the average daily biogas yields in L kg^{-1} VS