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Ammonium removal from anaerobically treated effluent by *Chlamydomonas acidophila*

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HIGHLIGHTS

- Anaerobically treated effluent to grow *Chlamydomonas acidophila* was investigated.
- A decrease in the pH of effluent to 3 was necessary for adequate microalgae growth.
- *C. acidophila* was able to grow at concentrations up to 1000 mg NH_4–N L^{-1} in media.
- The microalga removed 88 mg L^{-1} of NH_4–N in 10 days from media containing 966 mg L^{-1}.
- *C. acidophila* appears a promising agent for removal of NH_4–N from effluents.

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ABSTRACT

Several batch culture studies were carried out to evaluate an anaerobically treated effluent as a low-cost growth medium for the microalga *Chlamydomonas acidophila* and to study the effectiveness of the microalga in removing NH_4–N from the effluent. An initial decrease in the effluent pH to 3 was required for adequate growth of *C. acidophila* and removal of NH_4–N. Growth of the microalgae was inhibited at high light intensity (224 μmol photons m^{-2} s^{-1} at the surface of the vessels). However, the growth was not greatly affected by the high solid content and turbidity of the effluent. The microalga was able to grow in media containing NH_4–N at concentrations of up to 1000 mg L^{-1} (50% of effluent) and to remove 88 mg of NH_4–N L^{-1} in 10 days. *C. acidophila* therefore appears a promising agent for the removal of NH_4–N from anaerobically treated effluents.

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1. Introduction

The contaminants that most frequently impair the quality of water resources in Europe are phosphorus (P) and nitrogen (N). In most temperate estuaries and coastal ecosystems, N is the element most limiting to primary production and is a major cause of eutrophication. Swine manure exerts serious environmental impacts because of the large amounts of organic matter, N and P that it contains (de Godos et al., 2010). Improperly managed livestock manure can have damaging effects on the environment. Although anaerobic digestion is often considered an acceptable method of removing most of the organic matter from piggery and cattle waste, safe disposal of the resulting effluent remains a problem. Further action is required in cases where there are not sufficient grounds for the application of excess nitrogen to land, in accordance with the European Nitrates Directive (91/676/EEC) (concerning the protection of waters against pollution caused by nitrates derived from agricultural sources), which limits the contribution to 170 kg N ha^{-1} $year^{-1}$ in nitrate-vulnerable zones.

Anaerobically treated effluent can be used as a nutrient supplement for growing algae, thus avoiding the nutrient runoff and eutrophication of surface waters that can result from spreading the effluent on land. The algae can then be used for different purposes: as feedstock for producing biogas, as third generation biofuel, for commercial uses and for recycling on farms as feed, compost or fertilizer (Brennan and Owende, 2010). Anaerobically treated effluents contain all major nutrients and potentially represent an excellent low cost cultivation medium for algae.

Microalgae have proven efficient in removing nutrients from different effluents such as municipal waste (Li et al., 2011; Martínez et al., 2000), agricultural waste (Mulbry et al., 2008) and industrial wastewater (Valderrama et al., 2002). Livestock waste and the resulting anaerobically treated effluents have been used successfully to grow *Spirulina* spp. (de Godos et al., 2010), *Scenedesmus* spp. (Franchino et al., 2013; Park et al., 2010), *Neochloris oleabundans* (Franchino et al., 2013), *Nannochloris* spp. (Jimenez-Perez et al., 2004), *Chlorella* spp. (Franchino et al., 2013;

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Yan et al., 2013) and *Rhizoclonium* spp. (Mulbry et al., 2008). In microalgal cultures, biomass growth leads to an increase in pH (Franchino et al., 2013). The pH of most microalgal cultures is between 7 and 9, since photosynthetic CO_2 fixation causes a gradual increase in the pH of the medium due to the accumulation of OH^- (Martínez et al., 2000). There is a pH-dependent equilibrium between the soluble ammonium ion (NH_4^+) and dissolved molecular ammonia (NH_3). High pH favours volatilization of ammonia by driving the equilibrium between NH_3 and NH_4^+ to molecular ammonia. In the atmosphere, NH_3 can react with nitrate and sulfate to form particulate matter, which can contribute to acidic deposition. The efficiency of NH_4^+ removal from any system operating at high pH and open to the atmosphere must be considered to be a result of NH_3 formation (Martínez et al., 2000). Therefore, for successful growth of microalgae, aeration with CO_2 is required to maintain the pH of the medium below 8.

Cultivation of extremophilic microorganisms has gained interest in recent years due to the ability of these microorganisms to accumulate and produce valuable compounds such as metabolites, enzymes and surfactants. *Chlamydomonas acidophila*, a unicellular green alga, is a dominant phytoplankton species in acidic mining lakes such as the Rio Tinto (the "red river") (Huelva, Spain), so-called because of the high concentration of iron in the water, the pH of which remains constant at between pH 2 and 3 along a stretch of 80 km (Cuaresma et al., 2006). The extremely oxidizing conditions of this environment suggest that the microorganisms growing in the river should express different antioxidant mechanisms to protect themselves from oxidative stress (Cuaresma et al., 2011). *C. acidophila* accumulates high concentrations of lutein, a well-known antioxidant, which has recently become a focus of interest for the treatment of oxidative diseases such as macular degeneration (Cuaresma et al., 2011). This microalga can adapt to acidic environmental stress (acidification and heavy-metal toxicity) (Nishikawa et al., 2003), and as it is able to withstand extreme conditions, it may be able to grow in the presence of residual effluents – thus preventing the loss of NH_3 via volatilization.

The aims of this study were to investigate the growth of *C. acidophila* in an anaerobically treated effluent and to determine the ability of the species to remove NH_4–N from the effluent. Specifically, the effects of initial pH and solid content of the effluent, as well as the effects of initial cell density, light intensity and different concentrations of effluent on the growth of *C. acidophila* were studied.

2. Methods

2.1. Microorganisms and culture conditions

The strain of *C. acidophila* used in the study was obtained from the Göttingen collection of algae for culture (Sammlug Von Algenkulturen Göttingen [SAG], Germany). Cells used for the inoculum were grown in a square aquarium of constant working volume 7 L (ensured by daily addition of distilled water to replace water lost by evaporation), which was maintained at ambient temperature, bubbled with air (5 L min^{-1}) and continuously illuminated with fluorescent lamps (114 μmol photons m^{-2} s^{-1} at the surface of the aquarium). The irradiance was measured by a photosynthetically active radiation (PAR) sensor (JYP-1000). The microalgae were maintained by periodic transfer to the culture medium containing the following ingredients: $(NH_4)_2SO_4$ (1000 mg L^{-1}), K_2HPO_4 (20 mg L^{-1}), $MgSO_4$ (20 mg L^{-1}), Na_2EDTA (130 mg L^{-1}), $FeSO_4{\cdot}7H_2O$ (80 mg L^{-1}), $MnCl_2{\cdot}4H_2O$ (0.18 mg L^{-1}), $ZnCl_2$ (0.052 mg L^{-1}), $Na_2MoO_4{\cdot}2H_2O$ (0.063 mg L^{-1}) and $CoCl_2{\cdot}6H_2O$ (0.018 mg L^{-1}). Once cell growth reached the exponential phase, the cells were harvested and separated from the media by centrifugation at 3000 rpm for 3 min. The separated cells were washed 3 times with distilled water and the centrifugation step was repeated to remove the culture medium. The concentrated cells of *C. acidophila* were then used in the different assays.

2.2. Anaerobically treated effluent

The effluent used in the assays was generated by the anaerobic digestion of a mixture comprising 60% pig slurry, 30% cheese whey and 10% sheep manure (wet weight). The mixture was digested in 8 L volume continuously stirred-tank reactors maintained at 35 °C and with a loading rate of 1.88 g L^{-1} d^{-1} of volatile solids (VS) and a hydraulic retention time (HRT) of 30 days. The total solids (TS) and VS contents of the anaerobically treated effluent (ATE) were 4.0% and 2.7%, respectively. To minimize the solid content and turbidity of the culture media, the ATE was centrifuged at 3500 rpm for 3 min, and the resulting supernatant (ATES) was used for algal growth studies. The TS and VS contents of the ATES were 1.1% and 0.5%, respectively; the pH of the supernatant was 7.88 and the NH_4–N content, 1.9 g L^{-1}.

2.3. Chemical analysis

Determination of NH_4–N in the samples was carried out according to a colorimetric method based on the Berthelot reaction (Nelson, 1983). The pH, TS content and VS content were determined according to the Standard Methods for the Examination of Water and Wastewater (APHA, 1995). The biomass concentration and growth in the assays was measured indirectly, as the optical density of the culture at a wavelength of 800 nm (OD_{800}), in a spectrophotometer (Shimadzu UV-1800).

2.4. Experimental set-up

Several batch laboratory scale growth studies were conducted to evaluate the use of the anaerobically treated effluent as a low-cost growth medium for *C. acidophila* and to study the effectiveness of this microalga in removing NH_4–N from the effluent. Specifically, the effects of initial pH and solid content of the effluent, as well as the effects of initial cell density, light intensity and different effluent concentrations, on the growth of *C. acidophila* were evaluated.

The assays were carried out in two different incubation systems, both maintained at 26 ± 2 °C. The first system was an orbital incubator shaker (Sartorius Certomat BS1), in which cultures of volume 50 mL were stirred at 220 rpm in 125 mL glass Erlenmeyer flasks. The surfaces of the flasks were continuously illuminated, at a rate of approximately 29 μmol photons m^{-2} s^{-1}, by several fluorescent lamps. The second system was a plant growth chamber (Fitotron) in which air was bubbled (5 L min^{-1}) through cultures held in 1000 mL working volume glass bottles. As before, constant illumination was provided at the surface of the bottles by fluorescent lamps, in this case at a rate of 113 μmol photons m^{-2} s^{-1}.

The culture medium was a maintenance medium, whereas a modified medium in which $(NH_4)_2SO_4$ was substitute by anaerobically treated effluent as nitrogen source was used as the growth medium. All media in flasks and bottles were inoculated from the seed culture in exponential growth phase, and each inoculum was adjusted to an OD_{800} of approximately 0.350. In all assays, the NH_4–N concentration and the OD_{800} of the culture were checked every 3–4 days throughout the incubation periods. Before removing the subsamples required for these tests, the water lost by evaporation was replaced by adding distilled water to the cultures.

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