Bioresource Technology 244 (2017) 15–22

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Short and long-term experiments on the effect of sulphide on microalgae cultivation in tertiary sewage treatment

J. González-Camejo[a], R. Serna-García[b], A. Viruela[a], M. Pachés[a], F. Durán[a], A. Robles[b], M.V. Ruano[b], R. Barat[a,*], A. Seco[b]

[a] *CALAGUA – Unidad Mixta UV-UPV, Institut Universitari d'Investigació d'Enginyeria de l'Aigua i Medi Ambient – IIAMA, Universitat Politècnica de Valencia, Camí de Vera s/n, 46022 Valencia, Spain*

[b] *CALAGUA – Unidad Mixta UV-UPV, Departament d'Enginyeria Química, Universitat de València, Avinguda de la Universitat s/n, 46100 Burjassot, Valencia, Spain*

ARTICLE INFO

Keywords:
Chlorella
Microalgae
Scenedesmus
Sewage
Sulphide

ABSTRACT

Microalgae cultivation appears to be a promising technology for treating nutrient-rich effluents from anaerobic membrane bioreactors, as microalgae are able to consume nutrients from sewage without an organic carbon source, although the sulphide formed during the anaerobic treatment does have negative effects on microalgae growth. Short and long-term experiments were carried out on the effects of sulphide on a mixed microalgae culture. The short-term experiments showed that the oxygen production rate (OPR) dropped as sulphide concentration increased: a concentration of 5 mg S L^{-1} reduced OPR by 43%, while a concentration of 50 mg S L^{-1} came close to completely inhibiting microalgae growth.

The long-term experiments revealed that the presence of sulphide in the influent had inhibitory effects at sulphide concentrations above 20 mg S L^{-1} in the culture, but not at concentrations below 5 mg S L^{-1}. These conditions favoured *Chlorella* growth over that of *Scenedesmus*.

1. Introduction

Anaerobic membrane bioreactors (AnMBRs) have been reported as a more promising technology for wastewater treatment than conventional aerobic treatments for their several advantages: i) higher energy recovery from organic matter as biogas, ii) reduced power consumption, and iii) up to 90% reduction in sludge production (Giménez et al., 2011). However, AnMBRs are not able to remove nutrients from wastewater (Aiyuk et al., 2006), which means some post-treatment is required before discharging wastewater in sensitive areas (European Directive 91/271/CEE). In this respect, microalgae cultivation appears to be a sustainable technology for treating AnMBR effluent, allowing not only nutrient removal but also the possibility of moving towards water resource recovery in the sewage treatment field (Ruiz-Martínez et al., 2012; Viruela et al., 2016).

Autotrophic microalgae are photosynthetic microorganisms which use light energy and inorganic carbon (CO_2 and $HCO_3{}^-$) to grow. They also require high amounts of inorganic compounds, such as ammonium (NH_4^+) and phosphate (PO_4^{3-}), which can be obtained from a nutrient-rich wastewater stream (Tan et al., 2016). The microalgae biomass generated can be used as an energy source, since it can be converted into biogas, biodiesel, biohydrogen, fertilizers and high-value products (Maroneze et al., 2016). The combination of an AnMBR and a microalgae cultivation system is therefore a win-win strategy, since it would be feasible to recover both nutrients and other resources such as energy and water from the wastewater. However, among other issues, it must be taken into account that sulphate is reduced to sulphide in an AnMBR by means of sulphate reducing bacteria (SBR). In acid sulphate soils, such as those typically found in the Mediterranean Basin, water (and therefore wastewater) contains high concentrations of sulphate. AnMBR effluent is thus expected to have high sulphide concentrations but low sulphate concentrations (Giménez, 2014).

Sulphide has been reported to inhibit the photosynthesis process of microalgae, as it reduces the electron flow between the photosystem II (PSII) and photosystem I (PSI) (Pearson et al., 1987; Miller et al., 2004). By way of example, Küster et al. (2005) studied the toxicity of the *Scenedesmus* microalgae through the inhibition of cellular reproduction during a one-generation cycle lasting 24 h. Their results showed 50% inhibition when the sulphide concentration was around 2 mg S L^{-1}. González-Sánchez and Posten (2017) studied the deployment of a *Chlorella* sp. culture for biogas upgrading and found that these microalgae were inhibited at sulphide concentrations higher than 16 mg S L^{-1}. However, as sulphur acts as macronutrient for microalgae growth, the absence of sulphide or sulphate in the medium can also

---

* Corresponding author.
E-mail address: rababa@dihma.upv.es (R. Barat).

http://dx.doi.org/10.1016/j.biortech.2017.07.126
Received 5 June 2017; Received in revised form 18 July 2017; Accepted 21 July 2017

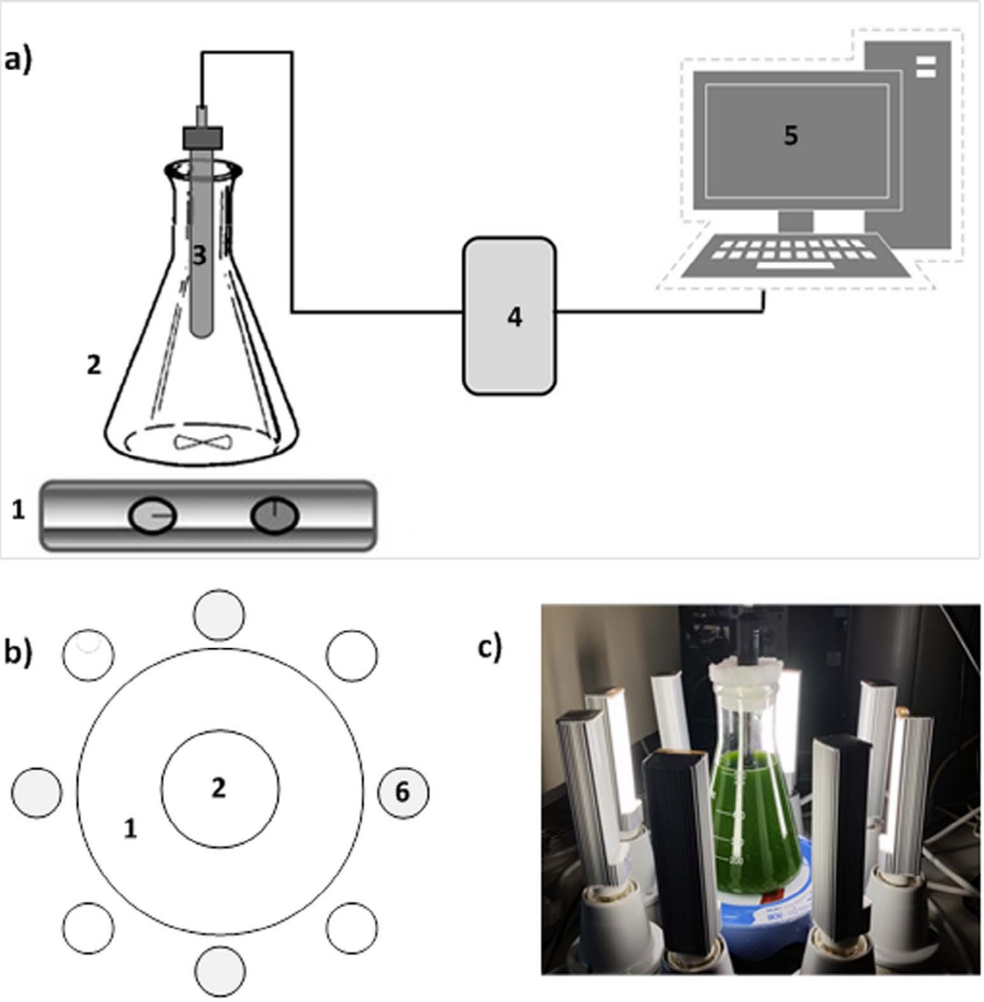

Fig. 1. General view: a) Front view; b) Top view; c) Experimental set-up. Nomenclature: 1: Magnetic stirrer; 2: Erlenmeyer flask; 3: Oxygen and temperature probe; 4: Oximeter; 5: Biocalibra software; 6: Led lamp on.

limit microalgae growth (González-Sánchez and Posten, 2017). This means that before setting up a microalgae culture to treat sewage on an industrial scale, it will be necessary to analyse the effects of introducing sulphide into the system, such us inhibition, nutrient limitation, species distribution in the culture, etc.

The aim of this work was thus to study the effect of sulphide on mixed microalgae culture in tertiary sewage treatment. Short-term experiments were carried out on a bench-scale and long-term pilot-scale experiments in an outdoor membrane photobioreactor (MPBR) using as growth medium the nutrient-loaded effluent from an AnMBR plant at the Carraixet full-scale WWTP (Giménez et al., 2011).

2. Material and methods

2.1. Microalgae substrate

The microalgae substrate used for both the short and long-term experiments was the nutrient-rich effluent from an AnMBR plant, which is described in detail in Giménez et al. (2011) and Robles et al. (2013). The AnMBR influent was from the pre-treatment of the Carraixet WWTP (Valencia, Spain): screening, degritter and grease removal. The average nutrient concentrations of the microalgae substrate during the experimental period were: ammonium 58.4 ± 4.8 mg N L^{-1} and phosphate 7.5 ± 0.5 mg P L^{-1}, with an N:P molar ratio of 17.3 ± 1.3. Nitrite and nitrate concentrations were negligible. The substrate also had a total COD concentration of 57 ± 8 mg COD L^{-1}, alkalinity of 810 ± 47 mg $CaCO_3$ L^{-1}, VFA of 1.5 ± 0.6 mg HAc L^{-1}, and sulphide of 112.7 ± 13.8 mg S L^{-1}. Sulphate was detected in negligible concentrations. This microalgae substrate was expected to favour microalgae growth over other organisms as it contained low amounts of COD and TSS but high concentrations of nutrients.

The variability of the nutrient load during the experimental period was associated with variations in both WWTP and AnMBR performance.

2.2. Microalgae inoculum

The microalgae used in this study were originally collected from the walls of the secondary clarifier in the Carraixet WWTP (Alboraya, Spain). The inoculum consisted of a culture dominated by *Scenedesmus* ($>$ 99% eukaryotic cells), but it also contained other genera such as *Chlorella*, *Monoraphidium*, as well as diatoms, bacteria and cyanobacteria in negligible concentrations. This inoculum was used because these microalgae had already been adapted to the outdoor conditions (light, temperature, etc.) of the location.

Prior to the inoculation of the photobioreactors (PBRs) in the MPBR plant, the culture was adapted to the microalgae substrate (see Section 2.1) under laboratory conditions as described in González-Camejo et al. (2017). After this pre-cultivation step, a start-up phase was carried out in the MPBR pilot plant, which consisted of the following: i) inoculation of the PBR with the microalgae culture from the laboratory (pre-cultivation: 10% of the total working volume with a biomass concentration between 300 and 500 mg VSS L^{-1} and 90% of the total working volume with microalgae substrate: AnMBR effluent); ii) conditioning stage in batch mode until reaching pseudo-steady state conditions (i.e. reaching stable microalgae biomass concentration); and iii) semi-batch mode