Algal Research 24 (2017) 368–377

Contents lists available at ScienceDirect

Algal Research

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

Effect of organic carbon enrichment on the treatment efficiency of primary settled wastewater by *Chlorella vulgaris*

Laurence Evans[a], Sebastian J. Hennige[b], Nik Willoughby[c], Adebayo J. Adeloye[d], Michael Skroblin[e], Tony Gutierrez[a],*

[a] *Institute of Mechanical, Process and Energy Engineering, School of Engineering and Physical Sciences, Heriot-Watt University, Edinburgh EH14 4AS, UK*
[b] *School of Geosciences, The King's Buildings, University of Edinburgh, Edinburgh EH9 3FE, UK*
[c] *Institute of Biological Chemistry, Biophysics and Bioengineering, School of Engineering and Physical Sciences, Heriot-Watt University, EH14 4AS, UK*
[d] *Institute for Infrastructure and Environment, School of Energy, Geoscience, Infrastructure and Society, Heriot-Watt University, Edinburgh EH14 4AS, UK*
[e] *Veolia Water Outsourcing Ltd., Newbridge Wastewater Treatment Works, Lochend Road, Edinburgh EH28 8SY, UK.*

ARTICLE INFO

Keywords:
Primary settled wastewater (PSW)
Static culturing
Carbon enrichment
Chlorella vulgaris
Mixotrophic microalgae
Bioremediation

ABSTRACT

This work evaluated the performance of a microalgae treatment process for settled municipal wastewater in a laboratory setting under static culturing conditions, as an alternative to traditional, energy intensive secondary and tertiary wastewater treatment systems. Primary tank settled wastewater (PSW) was first enriched with small quantities of glucose ($< 300\ \mathrm{mg\ L^{-1}}$) as an organic carbon source to facilitate the bioremediation by the mixotrophic microalga *Chlorella vulgaris*. Characterisation of the wastewater revealed significant reductions in NH_3-N (from 28.9 to 0.1 $\mathrm{mg\ L^{-1}}$) and PO_4-P (from 3.2 to 0.1 $\mathrm{mg\ L^{-1}}$) in just 2 days. Additionally, the exogenous glucose appeared completely removed from the wastewater after the first day. These achieved levels of treatment in respect of both the NH_3-N and PO_4-P were much higher than those recorded without *C. vulgaris* treatment with or without glucose enrichment. This would mean that the microalgae were chiefly responsible for removing the inorganic nitrogen and phosphorus, while the naturally occurring heterotrophic organisms had consumed the carbonaceous matter. The reliability of this process was evaluated across a further three independent batches of PSW with varying compositions of these inorganics and chemical oxygen demand using alternative organic (glycerol) and inorganic (CO_2) carbon sources. The efficiency of the microalgae treatment process at reducing NH_3-N and PO_4-P was consistent in PSW enriched with organic carbon, resulting in $> 90\%$ reduction of the inorganic compounds in each batch. The results demonstrate that microalgal culturing processes to treat PSW in bioreactors without aeration are a key area to develop as an alternative biological treatment option.

1. Introduction

Wastewater treatment is necessary to limit the potential impacts of pollution and eutrophication on receiving aquatic systems. Its main aim is towards the significant reduction of carbonaceous (organic) materials and, where sensitive surface waters are involved, nutrients (i.e. phosphorus (P) and nitrogen (N) compounds). The main phase of wastewater treatment is biological, essentially performed by microorganisms, such as in the activated sludge process or the biological nutrient removal process; these processes are conventionally termed the secondary treatment phase [1]. These secondary treatment processes are dependent on oxygen (O_2) to enable the endogenous microorganisms present to breakdown and assimilate the organic and inorganic matter. This stipulation for O_2 comes at a high cost with wastewater treatment consuming approximately 1 to 3% of the total electricity generated in developed nations of which 40 to 60% is expended on supplying air to the aeration basin [2–4]. This is important considering the cost to treat wastewater is projected to rise as a result of growing urbanisation and the proposition of more stringent effluent requirements. For example, the enactment of the Urban Wastewater Treatment Directive sets European discharge limits at 2 or 1 $\mathrm{mg\ L^{-1}}$ total phosphorus (TP) for population equivalence of < 100 k or > 100 k, respectively [5]. These discharge limits contribute considerably to the natural P concentrations in riverine and estuarine environments [6], and decreasing inputs of P to receiving systems is considered key to reducing eutrophication [7]. In order to limit phytoplankton growth and thus eutrophication in receiving waters, discharge TP concentrations of $< 0.5\ \mathrm{mg\ L^{-1}}$ is necessary and currently under consideration [8].

In recent decades, policies to safeguard water resources have

---

* Corresponding author.
E-mail address: tony.gutierrez@hw.ac.uk (T. Gutierrez).

http://dx.doi.org/10.1016/j.algal.2017.04.011
Received 7 October 2016; Received in revised form 11 April 2017; Accepted 18 April 2017

influenced the development of wastewater treatment systems and its management, including a focus on energy consumption and the sustainable performance of these industrial processes. Given the importance of wastewater treatment, a key question is how to reduce energy consumption of this process without affecting performance in respect to meeting water discharge limits. One direction towards making wastewater treatment more sustainable is to recover the resources that it holds, such as water, nutrients (e.g. P and N) and energy. Verstraete et al., (2009) estimated the total value of resources which could be recovered from wastewater at € 0.35 per m^3 based on 2009 market prices [9]. The shift of wastewater treatment from being an end-of-pipeline process to a resource has seen the development and operation of technologies such as sludge digestion for methane production, the integration of energy capturing technology utilising the wastewater treatment infrastructure and nutrient recovery aimed at P and N [2,10].

One particular option for the remediation and capture of inorganic N and P from wastewater is using microalgae. The rationale behind this approach lies in the ability of mixotrophic microalgae to utilise organic and inorganic carbon, as well as the N and P in wastewater for their growth, hence leading to a reduction in the concentration of these substances that will meet discharge limits. Simultaneously, energy-rich microalgal biomass is produced that could be recovered and utilised for the generation of energy or other products following further processing. The remediation potential of this approach has been evaluated for use in an array of wastewater types with promising results [11,12]. A further benefit of microalgae incorporation into wastewater treatment is their generation of dissolved O_2 via photosynthesis. Photosynthetic oxygenation has the potential to meet dissolved O_2 needs to a treatment system without the use of mechanical aeration or mixing, thereby reducing the energy demand for the treatment process. To exemplify, Karya et al. (2013) employed a sequence batch design with *Scenedesmus* sp. and nitrifying bacteria isolated from activated sludge to evaluate whether this co-culture system can support nitrification [13]. Without mechanical aeration, the process was shown successful in reducing 81 to 85% of ammonium-nitrogen through its conversion to nitrate-nitrogen by nitrification, for which the O_2 for this process had been generated by the microalga. Further support for a microalgae-based wastewater treatment approach as a viable biological system relates to its general improved performance in the presence of bacteria. Although considered unavoidable and a major challenge because of the potential to out-compete algae, the presence of bacteria in co-culture with mixotrophic microalgae has been shown to respond better in treating wastewater compared to the use of axenic cultures [14,15]. This affect has been attributed to the exchange of co-factors between the microalgae and bacteria, which include growth promoting compounds and vitamins [16]. Furthermore, when compared to current secondary treatment systems, microalgae also provide a potential system for sequestering carbon as well as removal of micro-pollutants and toxic metals [17].

Despite these advantages, there are various practical and economical challenges that still limit the implementation of microalgae-bacteria co-cultures for wastewater treatment. One such challenge is the cultivation process. As with most conventional wastewater treatment operations, aeration systems are used in microalgae culturing to provide mixing for improving the exchange of O_2 and carbon dioxide (CO_2) to maintain an optimal environment for their performance. However, mixing provided by recirculation pumps in tubular photobioreactors (PBR) and baffles in high rate algae ponds would further increase the energy requirement. A case study carried out in Almería, Spain analysing the cost of operating a 30 m^3 PBR plant found that the use of recirculation pumps and aeration pumps to be, respectively, the first and second highest energy expenders in the operation [18]. A further aspect of a microalgae treatment process is the stage in the treatment train it is introduced. Traditionally, microalgae remediation has been restricted to polishing secondary treatment effluent – i.e. after the energy intensive secondary treatment stage. Therefore, the introduction of microalgae in such a situation would not result in the much-desired reduction in overall energy demands of wastewater treatment. As described above, this is largely a direct result of additional mixing and aeration provided. In addition, the added cultivation cost is not feasible if the biomass does not compensate for the energy utilised throughout the process. As a result, a more effective treatment process would be to integrate a microalga secondary treatment phase, herein for treating primary settled wastewater (PSW) directly while meeting effluent standards. The application of microalgae would therefore be an alternative biological treatment process to current conventional secondary processes, not just for enhanced removal of N and P. The potential cultivation of microalgae for PSW treatment has, however, not been fully studied in this respect, and a static culturing system could provide a direction for the development of a low energy microalgae treatment system.

In this study, we explore the potential for using the microalga *Chlorella vulgaris* to treat municipal PSW and evaluate its efficiency in removing NH_3, PO_4 and chemical oxygen demand (COD) under static culture conditions. To improve the availability of carbon and to overcome potential light limitations caused by the opaque nature of wastewater, the effects of exogenous organic and inorganic carbon on microalgae growth and remediation performance were also evaluated. To validate the efficiency and reproducibility of this process that takes into account natural fluctuations in the composition (biological/chemical) of wastewater, we further conducted three independent batch studies with PSW obtained on different days of the year.

2. Materials and methods

2.1. Microalgae strain, medium and maintenance

Chlorella vulgaris strain CCAP 211/79 was used in all experiments. This is a non-axenic freshwater microalga that was originally isolated from a waste solvent bio-filter at Heriot-Watt University, Edinburgh, UK [19]. All manipulations of the stock culture were carried out under sterile conditions in a biological laminar flow hood to limit the contamination of the culture with other microorganisms.

Strain CCAP 211/79 was maintained in a modified Bold basal medium (BBM, Table S1 and S2) adjusted to pH 7.2 and heat sterilised (121 °C, 15 min).

Seed cultures used as the inoculum for all experiments were maintained in 350 mL BBM cultured in 500 mL glass bottles which were aerated continuously with atmospheric air through a sterile In-Line HEPA filter (Ø 53 mm, pore size $\geq 0.3\ \mu m$, Whatman International, Ltd., UK) at a volumetric flow rate of 0.15 of air volume per volume of liquid per minute (V/Vm). The cultures were grown in batch mode and sub-cultured at late exponential phase (7 to 9 days). Seed cultures for all experiments were grown for 7 days prior to use as inocula. Environmental growth conditions were the same for both the stock cultures and the experimental runs. These were fixed at 15 ± 1 °C and a 12:12 light-dark cycle (Fluora, Osram, Germany) at a photon flux of 100 $\mu mol\ m^{-2}\ s^{-1}$ (US-SQS/L probe, Walz, Germany).

2.2. Wastewater source

Primary settled wastewater was obtained from Seafield Wastewater Treatment Plant located in Edinburgh, UK. The facility treats predominantly municipal wastewater from Edinburgh City and the surrounding area via a combined sewer catchment. The site treats an average flow of 283 ML day^{-1} with a population equivalent of approximately 800,000, treated to comply with the carbonaceous treatment standards required by the Urban Wastewater Treatment Directive with a final effluent biological oxygen demand (BOD) and COD of $< 25\ mg\ L^{-1}$ O_2 and 125 $mg\ L^{-1}$ O_2 respectively [5]. The treatment process comprises of 10 preliminary screens, 4 grit removal tanks, 4 primary settlement