Bioresource Technology 189 (2015) 273–278

Contents lists available at ScienceDirect

Bioresource Technology

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

Algal-based, single-step treatment of urban wastewaters

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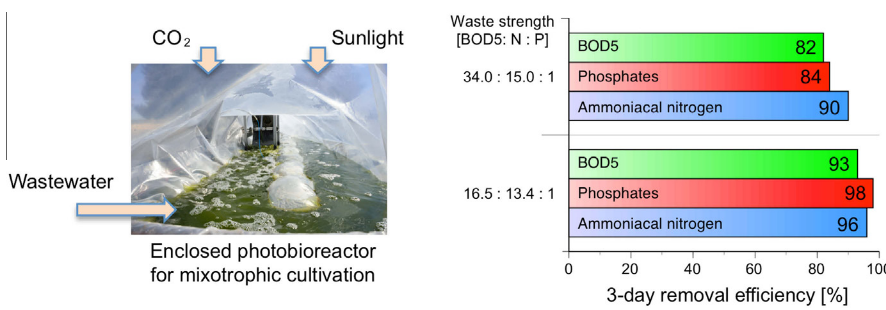
HIGHLIGHTS

- Demonstrated wastewater treatment by mixotrophic metabolism.
- Demonstrated single-step removal of BOD and nutrients.
- Demonstrated higher BOD removal by mixotrophic metabolism.
- Demonstrated comparable nutrient removal by mixotrophic metabolism.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 28 January 2015
Received in revised form 24 March 2015
Accepted 25 March 2015
Available online 6 April 2015

Keywords:
Mixotrophic metabolism
Wastewater treatment
Galdieria sulphuraria
Organic removal
Nutrient removal

ABSTRACT

Currently, urban wastewaters (UWW) laden with organic carbon (BOD) and nutrients (ammoniacal nitrogen, N, and phosphates, P) are treated in multi-stage, energy-intensive process trains to meet the mandated discharge standards. This study presents a single-step process based on mixotrophic metabolism for simultaneous removal of carbon and nutrients from UWWs. The proposed system is designed specifically for hot, arid environments utilizing an acidophilic, thermotolerant algal species, *Galdieria sulphuraria*, and an enclosed photobioreactor to limit evaporation. Removal rates of BOD, N, and P recorded in this study (14.93, 7.23, and 1.38 mg L^{-1} d^{-1}, respectively) are comparable to literature reports. These results confirm that the mixotrophic system can reduce the energy costs associated with oxygen supply in current UWW treatment systems, and has the potential to generate more energy-rich biomass for net energy extraction from UWW.

1. Introduction

Urban wastewater (UWW) treatment plants in current use have been designed and operated solely for the purpose of meeting the mandatory discharge regulations to protect receiving waters and public health. Technologies deployed in today's wastewater treatment plants to meet these regulations consume significant electrical energy and dissipate valuable carbon- and nutrient-content of the wastewater into the environment. For example, organic-content of UWW is aerobically mineralized to gaseous carbon dioxide and discharged into the atmosphere; ammonia-content is converted by nitrification/denitrification process to inert dinitrogen and discharged into the atmosphere. In recent years, there has been a shift in this paradigm where UWWs are being recognized as a renewable resource from which water, energy, nutrients, and useful chemicals could be reclaimed for beneficial use.

This study proposes an approach based on mixotrophic metabolism for energy-efficient and sustainable treatment of UWW. The

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http://dx.doi.org/10.1016/j.biortech.2015.03.120
0960-8524/

premise of this approach is that, mixotrophic metabolism driven by sunlight and BOD oxidation can simultaneously remove BOD, N and P in UWWs to the required effluent standards. Results of this study demonstrate the feasibility of BOD, N and P removal in a single-step process to generate more energy-rich biomass than by current methods. The higher biomass yield enables energy extraction as gaseous or liquid biofuels via catalytic hydrothermal gasification (Elliott, 2008), anaerobic digestion (McCarty et al., 2011), or hydrothermal liquefaction (Biller and Ross, 2011; Chakraborty et al., 2012).

Critical to the success of the proposed approach is a low-cost, enclosed photobioreactor (PBR) developed by us, that minimizes evaporative water loss and retains metabolic gases (O_2 and CO_2) enabling mixotrophic oxidation of organic carbon for maximal conversion to biomass with fewer input requirements. Another embodiment in the proposed approach is hydrothermal liquefaction (HTL) of the biomass to extract its energy-content as biocrude with concomitant solubilization of its nutrient-content. Upon separation of the biocrude from the products of HTL, the nutrient-rich aqueous phase could be recycled to the cultivation step to increase biomass productivity as discussed later.

Previous studies by the authors (Selvaratnam et al., 2014a,b) have documented the feasibility of a thermo-tolerant, acidophilic, heterotrophic/photoautotrophic alga, *Galdieria sulphuraria* (here after *G. sulphuraria*) as a successful and robust algal species for efficient N and P removal. The choice of *G. sulphuraria* in this study was motivated by its metabolic versatility that includes the ability to grow on the largest known range of organic substrates known among photosynthetic microorganism (Schonknecht et al., 2013). It is also an acidophile, growing between pH 1–4, conditions that rapidly inactivate plant and animal pathogens found in wastewater. The ability of *G. sulphuraria* to naturally acidify its growth medium from neutrality to optimum levels under heterotrophic conditions (Oesterhelt et al., 2007) makes it an ideal strain for mixotrophic treatment of UWW. This study demonstrates the ability of *G. sulphuraria* in removing BOD from UWW as well as nutrients to validate the premise that this species can be successfully cultivated in UWWs for energy-positive wastewater treatment.

A central design advantage of the mixotrophic system over traditional WWT systems stems from the fact that stoichiometric carbon-to-nitrogen (C:N) ratio in UWW is closer to that of algal biomass composition than to that of heterotrophic bacteria (Fig. 1). Even more important is that CO_2 capture via photosynthesis corrects the stoichiometric imbalance between C:N:P ratios in WW relative to either type of biomass to afford single-step biological treatment that can simultaneously achieve discharge standards for all three components. This offers a significant advantage over

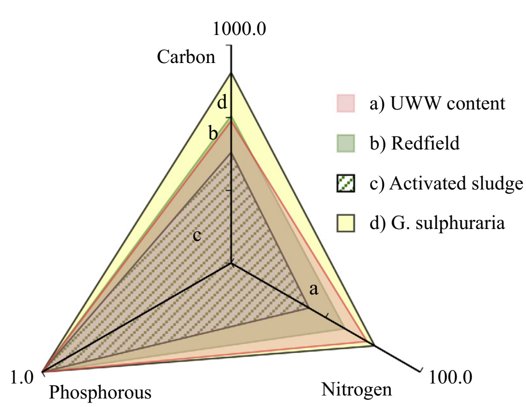

Fig. 1. C:N:P ratio in urban wastewater (UWW) (a) compared to composition of various biomasses cultivated with UWW: algal biomass according to Redfield formula (b); activated sludge (c); and *G. sulphuraria* (d).

the traditional practice that necessitates a two-step process including energy-intensive aeration:aerobic oxidation for BOD removal followed by nitrification/denitrification for N removal with external carbon supply to bridge the C:N imbalance.

The higher energy-efficiency of the mixotrophic system over the traditional system for wastewater treatment is due to the fact that the former is driven by photosynthesis, whereas the latter requires electrical energy to provide the necessary dissolved oxygen. Both processes are capable of generating biomass that can be converted to useable energy; for example, by anaerobic digestion to produce methane as energy carrier. An energetic comparison of the wastewater-to-biomass-to-methane conversion pathways has shown that the mixotrophic pathway can yield more than double the net electrical energy than the traditional pathway (Selvaratnam et al., 2014b). Sturm and Lamer (2011) have reported similar advantage of algal-based UWW treatment systems.

Several recent studies have built on the pioneering efforts of Oswald (1962, 1988), Oswald et al. (1953) to develop improved mixed algal/bacterial systems for UWW treatment with minimal energy input. While early studies had focused on using algal systems for polishing the secondary effluent to prevent eutrophication of receiving waters, later studies have demonstrated the feasibility of algal systems in treating the primary effluent as well as side-streams from various wastewater treatment process (Cho et al., 2011; Dalrymple et al., 2013; Wang et al., 2010). Wang et al. (2010) have demonstrated feasibility of algal treatment of four different side-streams at a wastewater treatment plant (wastewater before/after primary settling, wastewater after activated sludge tank, and centrate). More recent studies have extended the feasibility of algal systems to wastewater treatment and simultaneous energy generation (Lardon et al., 2009). This paper reports on the rates and efficiencies of removal of BOD, N, and P from primary-settled urban wastewater by *G. sulphuraria*.

2. Methods

The algal culture used in this study, *G. sulphuraria* CCMEE 5587.1, was obtained from Culture Collection of Microorganisms from Extreme Environments (University of Oregon). Bacteria-free colonies were picked from glucose-containing agar plates to establish axenic stock cultures and cultivated in 16-mm borosilicate glass tubes (culture volume = 6 mL) with a parafilm wrap around the closure to minimize evaporative losses while permitting O_2 and CO_2 diffusion. In all the tests, initial pH level of the media was adjusted to 2.5 by adding 10 N sulfuric acid. The tubes were placed in a tissue-culture roller drum (New Brunswick Scientific Co., Edison, NJ, USA) housed in a CO_2-enriched (2–3% vol/vol) incubator (Percival, USA) and maintained at 40 °C in a 14-h/10-h light–dark cycle mimicking outdoor conditions. Biomass growth was analyzed daily, based on measurements of optical density at 750 nm (OD 750) using Beckman DU-530 UV/Vis spectrophotometer and converted to ash-free dry weight (AFDW, g L^{-1}) using the correlation established in the previous study (Selvaratnam et al., 2014b).

2.1. Test I – growth of G. sulphuraria in UWW

The goal of Test I was to demonstrate the ability of *G. sulphuraria* to grow in a medium representative of typical UWW (BOD:N:P ratio of 16.5:13.4:1) and assess its BOD, N, and P removal capabilities. In this test, the standard Cyanidium recipe for *G. sulphuraria* (Selvaratnam et al., 2014a) was modified as follows: instead of using deionized water to prepare the standard growth medium, filter-sterilized (0.45 μm filter unit, Thermo Scientific Inc.) primary effluent obtained from the Las Cruces Municipal Wastewater