Science of the Total Environment xxx (2017) xxx–xxx

Contents lists available at ScienceDirect

Science of the Total Environment

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

# Cultivation and selection of cyanobacteria in a closed photobioreactor used for secondary effluent and digestate treatment

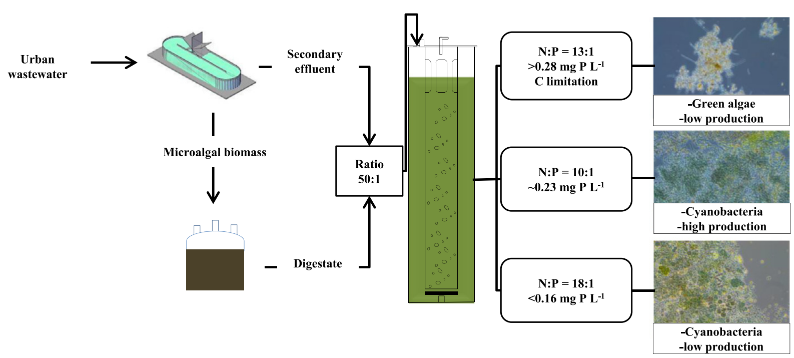
Dulce María Arias, Enrica Uggetti, María Jesús García-Galán, Joan García *

GEMMA - Environmental Engineering and Microbiology Research Group, Department of Civil and Environmental Engineering, Universitat Politècnica de Catalunya-BarcelonaTech, c/Jordi Girona 1-3, Building D1, E-08034 Barcelona, Spain

## HIGHLIGHTS

- Cyanobacteria were selected from a mixed microalgae consortium in a photobioreactor.
- Suitability of urban secondary effluent and digestate as nutrient sources was assessed.
- The effect of N and P concentration ratio on microalgae dominance was investigated.
- Low phosphorous loads promoted cyanobacteria dominance over green algae.
- P limitation increases N removal but decreases the biomass production.

## GRAPHICAL ABSTRACT




## ARTICLE INFO

*Article history:*
Received 19 December 2016
Received in revised form 10 February 2017
Accepted 10 February 2017
Available online xxxx

Editor: D. Barcelo

*Keywords:*
High rate algal ponds
Algae
Centrate
Bioproducts
Bioenergy

## ABSTRACT

The main objective of this study was to select and grow wastewater-borne cyanobacteria in a closed photobioreactor (PBR) inoculated with a mixed consortium of microalgae. The 30 L PBR was fed with a mixture of urban secondary effluent and digestate, and operated in semi-continuous mode. Based on the nutrients variation of the influent, three different periods were distinguished during one year of operation. Results showed that total inorganic nitrogen (TIN), inorganic phosphorus concentration ($PO_4^{3-}$), phosphorus volumetric load ($L_V$-P) and carbon limited/non-limited conditions leaded to different species composition, nutrients removal and biomass production in the culture. High TIN/$PO_4^{3-}$ concentrations in the influent (36 mg N $L^{-1}$/3 mg P $L^{-1}$), carbon limitation and an average $L_V$-P of 0.35 mg P $L^{-1}d^{-1}$ were negatively related to cyanobacteria dominance and nutrients removal. On the contrary, cyanobacteria predominance over green algae and the highest microbial biomass production (averaging 0.084 g Volatile Suspended Solids (VSS) $L^{-1}d^{-1}$) were reached under TIN/$PO_4^{3-}$ concentrations of 21 mg N $L^{-1}$/2 mg P $L^{-1}$, no carbon limitation and an average $L_V$-P of 0.23 mg P-$PO_4^{3-}$ $L^{-1}d^{-1}$. However, although cyanobacteria predominance was also favored with a $L_V$-P 0.15 mg $L^{-1}d^{-1}$, biomass production was negatively affected due to a P limitation in the culture, resulting in a biomass production of 0.0.39 g VSS $L^{-1}d^{-1}$. This study shows that the dominance of cyanobacteria in a microalgal cyanobacterial community in an agitated PBR using wastewater as nutrient source can be obtained and maintained for 234 days. These data can also be applied in future biotechnology applications to optimize and enhance the production of added value products by cyanobacteria in wastewater treatment systems.



* Corresponding author.
*E-mail address:* joan.garcia@upc.edu (J. García).

http://dx.doi.org/10.1016/j.scitotenv.2017.02.097
0048-9697/© 2017 Elsevier B.V. All rights reserved.

## 1. Introduction

Cyanobacteria (blue-green algae) are prokaryotic aerobic photosynthetic microorganisms with a long history of adaptive and evolutionary diversification, which has also conferred them the capacity to synthesize a large variety of bioactive compounds and other valuable by-products (Mimouni et al., 2012). During the last two decades, the industrial production of cyanobacteria has arisen special interest since they have been identified as one of the most promising group of organisms for the isolation of novel and biochemically active natural products such as antibiotics, antifungal or antivirus (Abed et al., 2009; Shalaby, 2011). Unlike eukaryotic algae, cyanobacteria have also the potential to assimilate and store glycogen, cyanophycin, polyphosphates and polyhydroxyalkanoates (Stal, 1992).

Studies related to the production of cyanobacteria and their metabolites generally employ pure or genetically modified cultures (Miyake et al., 2000). However, cultivation of cyanobacteria is not easy, even if pure cultures are submitted to strictly controlled processes using sterile medium substrates. In most of the cases contamination with other types of algae, in particular green algae (*Chlorophyta*), cannot be avoided (Drosg, 2015). Moreover, the use of these strictly controlled pure cultures in industrial applications lead to high production costs, and subsequent relatively expensive products (Samantaray and Mallick, 2012). Indeed, growth medium pure culture expenses correspond to 33% of the operational costs (Piccolo, 2012).

However, in the case of non-food applications such as the production of bioenergy or biofuels production, or bioplastics generation, a strict sanitary control is not required. In these cases, an alternative approach for the production of cyanobacteria could be the use of wastewater-borne cyanobacteria cultures, using non-sterile waste streams as substrate. In fact, wastewater treatment technologies are considered as the most promising and sustainable alternative to reduce additional production costs associated with nutrients and water in cyanobacteria cultures (Samantaray et al., 2011; Zhou et al., 2012). Indeed, the use of inexpensive substrates requiring lower energy inputs and cheaper equipment could reduce the production costs compared to pure culture processes. However, maintaining a dominant population of cyanobacteria in wastewater treatment systems is still limited to a few successful case-studies (Van Den Hende et al., 2016a, 2016b) and therefore remains as a challenging task (de Godos et al., 2014). Certainly, one of the problems most frequently encountered is that of cyanobacteria being out-competed by green algae in wastewater borne cultures; the factors that control these competence relationships are not well understood.

Most of the information available regarding the different factors that control growth and predominance of cyanobacteria found in literature comes from fresh water ecosystems, such as lakes and reservoirs. Cyanobacteria development in these environments depends on complex interactions among a great number of physical and chemical factors such as light intensity, temperature, turbulence, pH, and other biotic factors (Ahn et al., 2002; Dolman et al., 2012; Levich, 1996; Marinho and Azevedo, 2007; Reynolds, 1987). However, among all these factors, most of the studies agree that the nitrogen and phosphorus ratio (N:P) and their absolute concentration levels are the two key factors determining the competition capacity of cyanobacteria (Cai et al., 2013; Cottingham et al., 2015; Levich, 1996; Levine and Schindler, 1999; de Tezanos Pinto and Litchman, 2010; Talbot and de la Noüe, 1993). In this context, because cyanobacterial blooms frequently develop in eutrophic water ecosystems, it was firstly assumed that they required high N and P concentrations (Pick and Lean, 1987; Reynolds, 1987). However, later studies demonstrated that their dominance was related to a higher affinity than that of many other photosynthetic organisms for N and P (Monchamp et al., 2014; Mur et al., 1999). In addition to this high nutrient affinity, cyanobacteria have a substantial storage capacity for both these nutrients (Flores and Herrero, 2014), and some types of cyanobacteria have the capacity of fixing atmospheric N (Levine and Schindler, 1999; Schindler, 1977). This way, they can outcompete other microalgae under conditions of N and/or P limitation (Cottingham et al., 2015; Kim et al., 2007; Marinho and Azevedo, 2007). For this reason, cyanobacteria dominance has been reported under a wide range of N:P ratios, from 0.5:1 (N limitation) to >64:1 (P limitation) (Chislock et al., 2013; Levine and Schindler, 1999; Pick and Lean, 1987; Stocknerl and Shortreed, 1988), and even in fresh water ecosystems, where the concentrations of nutrients found are usually at least three orders of magnitude lower than those found in urban, agricultural or industrial wastewaters (de la Noüe et al., 1992). In this context, higher nutrient concentrations in wastewater promote higher algal photosynthesis, oxygen production and biomass concentration (Ahmadi et al., 2005). For instance, in a lake ecosystem, total inorganic nitrogen (TIN) reached a value of 1.167 mg $L^{-1}$ and Inorganic Phosphorus Concentration, (P-$PO_4^{3-}$) 0.107 mg $L^{-1}$, corresponding to 2.15 mg $L^{-1}$ of biomass concentration (Beaulieu et al., 2013), whereas it has been shown that open ponds fed with a secondary effluent from industrial wastewater (9.31 mg $L^{-1}$ of TIN and 2.37 mg $L^{-1}$ of P-$PO_4^{3-}$) have reached a biomass concentration of 668 mg $L^{-1}$ (Van Den Hende et al., 2016a). Therefore, it seems reasonable that cyanobacteria selection in wastewater cultures should be conducted considering the same determining factors, especially in terms of nutrients interaction. In the field of wastewater technology, several species of cyanobacteria have been successfully cultivated at experimental scale using both primary and secondary treated wastewaters (urban and industrial) as feedstock (Kamilya et al., 2006; Renuka et al., 2013; Van Den Hende et al., 2016a; Vijayakumar, 2012). The use of anaerobic digestate as nutrient source has also been evaluated (Markou and Georgakakis, 2011). However its use is conditioned by their high ammonium ($NH_4^+$), organic carbon and solids content, and most the studies included a dilution with tap water (Prajapati et al., 2014). Hence, the use of digestate diluted in another minor nutrient source (e.g. secondary effluent) could provide enough nutrients to fullfil the requeriments of cyanobacteria production and the possibility of their selective growth.

All in all, the objective of this study was to select and grow wastewater-borne cyanobacteria from a consortium of microalgae in a closed photobioreactor (PBR) fed with a mixture of secondary effluent and digestate. This work aimed to a dual benefit, considering the concomitant treatment of these waste streams. The study was carried out throughout 1 year in order to consider all the potential variations and variables affecting the PBR during a long term operation and therefore obtaining a realistic knowledge of the system functioning.

## 2. Methodology

### 2.1. Experimental set-up

The closed PBR was located indoors and consisted in a cylindrical tube made of polymethyl methacrylate (5 mm thickness) with a total volume of 35.8 L and a culture working volume of 30 L (Fig. 1). It was initially filled with 20 L of tap water and 10 L of inoculum obtained from the mixed liquor of an experimental high rate algal pond (HRAP) (1.54 $m^2$, 470 L) treating primary settled urban wastewater. A detailed description of the high rate algal pond system can be found elsewhere (García et al., 2006; Gutiérrez et al., 2015). The inoculum (105 mg TSS $L^{-1}$) consisted in a community of microalgae, bacteria, protozoa and small metazoa. Microscope observations (not shown) indicated that most of the biomass corresponded to microalgae, which is in accordance with previous publications (García et al., 2006; Gutiérrez et al., 2016; Nurdogan and Oswald, 1996). Microalgae consortium was mostly composed by green algae (genus *Chlorella* and *Stigeoclonium*) and cyanobacteria (cf. *Oscillatoria*).

The culture in the PBR was continuously maintained in alternate light:dark periods of 12 h. Illuminance during the light phase was supplied by a 600 W external metal halide lamp equipped with a digital ballast (model 5500k, Sunmaster, USA) placed at a 70 cm distance from the

Please cite this article as: Arias, D.M., et al., Cultivation and selection of cyanobacteria in a closed photobioreactor used for secondary effluent and digestate treatment, Sci Total Environ (2017), http://dx.doi.org/10.1016/j.scitotenv.2017.02.097