Biotreatment of Brewery Wastewater using the Filamentous Cyanobacterium *Leptolyngbya* sp.

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Abstract
Brewery wastewater is generated from the beer brewing process in large amounts (4-8 m^3 per m^3 of beer produced). Brewery wastewater consists of high organic matter content, significant nitrogen and phosphorus concentrations and easily biodegraded compounds. Even though most biological treatment technologies applied to brewery wastewaters include the use of bacteria, cyanobacteria (photosynthetic microorganisms) constitute attractive means for sustainable and low cost wastewater treatment producing high biomass concentration. In this study, the capacity of a filamentous cyanobacterium *Leptolyngbya* sp. for the pollutants removal from brewery wastewater was investigated coupled with objective to determine its proteins, carbohydrates and lipids content after wastewater treatment. The experiments were conducted in batch mode under non-sterile conditions in lab-scale photobioreactors. The removal rates of nitrate, ammonium, orthophosphates, total phosphorus and chemical oxygen demand (COD) were almost 49.2%, 100%, 57.1%, 57.9% and 24.3%, respectively, within the first 7 days of cultivation. The maximum biomass concentration was 350 mg/L, while the biomass produced was consisted of approximately 53.5% carbohydrates, 20.2% proteins and 10% lipids. Therefore, the treatment of brewery wastewater using cyanobacteria species could be effective, while the cyanobacterial biomass could be used in numerous fields for diverse applications.
Keywords: Brewery wastewater; cyanobacteria; *Leptolyngbya* sp.; cyanobacterial biomass

1. Introduction

Microalgae and cyanobacteria are a diverse group of unicellular, photosynthetic organisms that thrives at water environments, using CO_2 as a carbon source and inorganic nitrogen and phosphorus sources from their aqueous media *(Raposo et al, 2010)*. Their capacity to accumulate large quantities of lipids, carbohydrates and proteins make algae a suitable substrate for a wide range of biotechnological applications (biodiesel, bioethanol, bioplastics, pigment, pharmaceutical substances production etc.) *(Koutra et al, 2018)*. A limiting factor for applying large-scale cultivation is the availability and the cost of nutrients *(Economou et al, 2015)*. A practical, sustainable way to overcome this obstacle is the utilization of streams of nutrient-rich wastewaters. Brewery wastewaters are produced in large quantities (4-8 m^3/m^3 of beer produced) *(Kebede et al, 2018)*. The significant nitrogen and phosphorus concentrations they consist of *(Raposo et al, 2010)*, coupled with the relatively high cost of conventional wastewater treatment methods used, could make cyanobacteria / microalgae cultivation an appealing, low-cost way of simultaneously treating the wastewater and extracting substances of commercial value.
In this study, a mixed culture, consisting mainly of the filamentus, alkaliphilic, photoautotrophic cyanobacterium *Leptolyngya* sp. *(Kim et al, 2015)*, is utilized in order to investigate its capacity to treat brewery wastewaters (Table 1.), in combination with accumulating proteins, carbohydrates and lipids.

Table 1. Brewery wastewater characteristics

Pollutant	Concentration (mg/L)
NO_3^--N	12.3
NH_4^+-N	4.6
Orthophospates (OP)	6.2
Total Phosphates (TP)	6.5
d-COD	175

2. Materials & Methods

2.1. Culture characterization

A microbial sample was collected from a local municipal wastewater treatment plant and was cultivated photoautotrpohpically in an aquatic medium consisting of (g L_{-1}) KNO_3 0.2, $MgSO_4 \cdot 7H_2O$ 0.1, $CaCl_2 \cdot 2H_2O$ 0.05,K_2HPO_4 0.108, και KH_2PO_4 0.056 at pH 7–7.5. After 30 days of cultivation, the microbial consortium was analyzed using the inverted microscopy method (Fig 1a.). The microbial population constisted mainly of the cyanobacterium Leptolyngbya sp. (>90%). (Fig.1 b). The culture was preserved in aquariums, using brewery wastewater as medium.

2.1. Experimental apparatus and methods

The experiments were conducted under non-sterile conditions in 1 L, batch, stirred photobioreactors containing brewery wastewater. Samples of 20 mL were collected on a daily basis for biomass quantification, biomass analysis (in terms of lipid, protein and carbohydrate concentration) and for measuring the concentration of pollutants (NO_3–N; NH_4–N; orthophosphate (OP, PO_4^{3-}); total phosphate (TP) and dissolved chemical oxygen demand (d-COD)). The experiments were conducted under three different pH conditions, 8, 9 and uncontrolled (ranging between 10-11). The pH was adjusted using 5M H_2SO_4 solution.

3. Results and Discussion

The evolution of biomass productivity is presented at Fig 1b. After 1-2 days of lagging, an exponential increase of biomass was observed, reaching a maximum concentration of 350 mg/L after 6-8 days. The optimum growth was achieved under uncontrolled pH conditions (i,e. ranging between 10-11), showcasing the alkaliphilic nature of the cyanobacterium. Moreover, this could be attributed to reduced microbial competition, as these highly alkaline conditions are considered hostile for most microbes. Moreover, the maximum removal rates achieved under these conditions for NO_3–N, NH_4–N, OP, TP and d-COD were 49.2%, 100%, 57.1%, 57.9% and 24.3% respectively. The produced biomass consisted of 10% lipids, 53.5% carbohydrates and 20.2% proteins under optimal conditions, at the end of cultivation (when nutrient depletion occurs). It is worth mentioning that the produced biomass formed aggregates when stirring was stopped (Fig 3c.). This could be an appealing attribute, as it could lower the cost seperating biomass from the aquatic medium significantly.

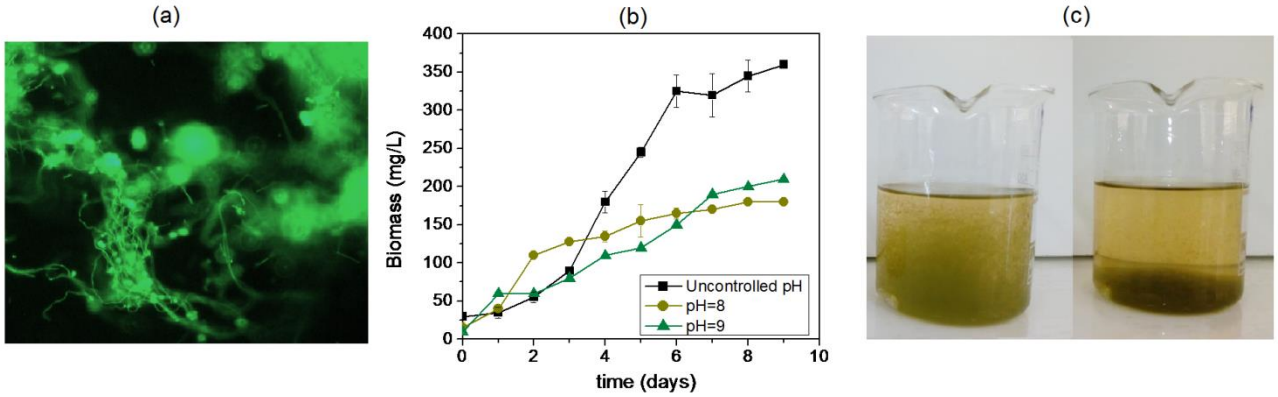

Figure 1. (a) Microsopy image of *Leptolyngya* sp filaments, (b) Biomass evolution under 3 different pH values, (c) Sample before and after 5 min of precipitation

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