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Cultivation of filamentous cyanobacteria (blue-green algae) in agro-industrial wastes and wastewaters: A review

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ABSTRACT

Recently research interest has focused on the production of biofuel from microalgae. Microalgae are photosynthetic microorganisms that grow utilizing solar energy, nevertheless, the quantities of fertilizers that should be used for their production are enormous. One alternative to the use of synthetic fertilizers is to employ wastes and wastewaters (W&WWs), especially from the agro-industrial sector which are rich in inorganic pollutants such as nitrogen and phosphorus, which can be recovered. Simultaneously with the cultivation of microalgae using wastes and wastewaters for biomass production, treatment of the wastes and wastewaters occur through removal of the pollutants. Filamentous cyanobacteria appear to be suitable candidates for cultivation in wastes and wastewaters because they produce biomass in satisfactory quantity and can be harvested relatively easily due to their size and structure. In addition their biomass composition can be manipulated by several environmental and operational factors in order to produce biomass with concrete characteristics. Herein we review the factors that affect the biomass composition of cyanobacteria and present several studies that discuss the culture of filamentous cyanobacteria in agro-industrial wastes and wastewaters, with special emphasis on *Spirulina*.

1. Introduction

Microalgae, a broad category encompassing eukaryotic microalgae and cyanobacteria, can be cultivated to produce biomass for a wide range of applications, including animal and human nutrition, the health sector, cosmetics and agriculture (biofertilizers) [1–4]. In parallel, an important application for the cultivation of microalgae is the production of biomass for energy purposes. Microalgae produce biomass, which can be converted into energy or an energy carrier through a number of energy conversion processes. They include thermochemical conversion (gasification, direct combustion and pyrolysis), biochemical conversion (anaerobic fermentation, anaerobic digestion and photobiological hydrogen production) and esterification of fatty acids to produce biodiesel [5–12].

Microalgae biomass contains considerable amounts of proteins [13] and on the basis of biomass composition the quantity of nitrogen (N) required as fertilizer is estimated to be 8–16 tons N/ha, which means that microalgae production involves enormous amounts of N fertilizers. The use of such large quantities of fertilizer for microalgae cultivation raises questions about their environmental impact [14,15]. Furthermore, the use of fertilizer contributes to the cost of algal biomass production. For example the use of fertilizer constitutes nearly half of the overall cost of *Spirulina* cultivation [16]. In order to reduce the use of fertilizer, wastewaters rich in N and phosphorus (P) can be used as a cultivation medium, while at the same time microalgae can be used to reduce the inorganic and organic load of these wastewaters, thereby providing a method of biological wastewater treatment [17–24].

A serious drawback to unicellular micro-algal cultivation is the harvesting of the biomass due to the microscopic dimensions of microalgae (0.5–30 μm) [25,26]. In essence, harvesting means that the algal biomass is separated from the liquid cultivation medium. As a result algal biomass is concentrated or dewatered, forming a slurry that consists of 5–15% dry solids [7]. Harvesting of biomass from the broth is thought to contribute 20–30% of the total cost of biomass production [25]. Filamentous cyanobacteria, with dimensions of around 200 μm can help reduce the harvesting problem because they may be harvested relatively easily by filtration. In addition, some filamentous cyanobacteria form aggregates and can be harvested by sedimentation or by flotation [27–29].

Today research is focused on the cultivation of microalgae rich in lipids in order to produce biodiesel [6,9,30]. Although cyanobacteria are not rich in lipids (up to 20%), they have relatively high biomass productivity. Microalgae with high biomass productivity may generate energy more efficiently by means of other types of energy conversion besides biodiesel production technology [10,11,14,31]. In addition, the majority of the existing techniques for lipid extraction, in order to produce biodiesel, require the drying of slurry harvested from the algal biomass [7,14,25]. The drying of the biomass

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is an energy consuming process and must be taken into consideration [32]. Therefore, using wet concentrated biomass may be a more cost-effective way to produce biofuels. Biomass energy conversion technologies in which wet algal biomass could be applied include anaerobic fermentation [11], anaerobic digestion [33] and thermochemical liquefaction [34].

Moreover, the composition of algal biomass in lipids, proteins and carbohydrates can be affected and consequently manipulated by various cultivation factors. Each biomass energy conversion technique is suitable to a specific type of biomass composition. For instance, anaerobic fermentation technology requires carbohydrates, which are fermented into alcohol [11]. Thus for this technology biomass with a high carbohydrate content is desirable. On the other hand, in anaerobic digestion the substrate must have a carbon to nitrogen ratio (C:N) suitable for the digestion process [33]. Therefore, in respect to biomass energy conversion, the biomass composition would be manipulated in order to produce the most suitable composition for each of the energy conversion technologies.

This paper aims to present basic knowledge for the culture of cyanobacteria, to review the factors that affect biomass composition and to give useful references for further research into this topic. This review concerning the cultivation of filamentous cyanobacteria focuses on two areas: the production of biomass using agro-industrial wastes and wastewaters as substrate and on the reduction of the organic and inorganic load of these agro-industrial wastes and wastewaters.

2. Photosynthesis and carbon metabolism

Photosynthesis is a process conducted by photoautotrophs in which inorganic compounds and light energy are converted to organic matter. Microalgae are oxygen producing photosynthetic organisms, which means that they use light energy to extract protons and electrons from water (H_2O) to reduce CO_2 in order to form organic molecules (glucose). The organic matter is formed according to the stoichiometric formula:

$$6H_2O + 6CO_2 + \text{light energy} \rightarrow C_6H_{12}O_6 + 6O_2 \quad (1)$$

The photosynthesis process can be divided into two reaction stages, namely the light and the dark stage. In the light reactions stage the light energy is absorbed by pigments of the photosystem antennae and converted into a biochemical reductant $NADPH_2$ and a high energy compound ATP [35]. The pigments that absorb the light energy are chlorophylls, which absorb the red light region (650–700 nm), carotenoids, which absorb the blue light region (400–500 nm), and phycobilins, which absorb the orange-red light region (600–650 nm). The latter are pigments which are present only in the cyanobacteria and the red-algae [35,36].

In the dark reactions stage the products of the light reactions are subsequently consumed by the reduction of CO_2 to carbohydrates [35]. In microalgae, as well as in higher plants, the light and dark reactions can operate independently. When the microalgae are subjected to illumination, the light reactions are automatically activated and are deactivated if the quality and quantity of light decreases below the threshold for photopigment stimulation or if organic substrates are present in adequate concentration. These organic substrates can be utilized as a carbon source and/or as an energy source to generate ATP [37].

In general microalgae are photoautotrophic organisms [38], but some microalgae species grow utilizing organic compounds as energy or/and carbon sources [39–42]. Since microalgae have this ability, their carbon metabolism can be divided into four types (1) photoautotrophy, (2) heterotrophy, (3) photoheterotrophy and (4) mixotrophy. In photoautotrophy the sole energy source for biomass production is light energy and the sole carbon source is inorganic compounds. In contrast, in heterotrophy, the cells utilize organic compounds as energy and carbon sources. In photoheterotrophy the energy source is light, which is required so that microalgae can utilize the organic compounds as a carbon source. Finally in mixotrophy the main energy source is light, although both organic compounds and CO_2 are essential [43]. Photoautotrophic, photeheterotrophic and mixotrophic growth are influenced by light intensity and by carbon source concentration, while heterotrophic growth is influenced only by the organic substance concentration [43–46]. Mixotrophy has several advantages over the other types of metabolism mentioned above. In mixotrophic cultures photoinhibition is reduced, growth rates are improved and biomass night losses due to respiration are less [45,47–51]. It is supposed that in mixotrophic cultures the specific growth rate is approximately the sum of the autotrophic and heterotrophic specific growth rate [47,52] but Chojnacka and Noworyta [48] suggest that the mixotrophic specific growth rate is not the simple combination of the autotrophic and heterotrophic specific growth rate and Vonshak et al. [53] suggest that the two metabolic processes affect each other.

3. Cyanobacteria genera

Cyanobacteria (or cyanophyceae) are non-motile, planktonic, occasionally forming blooms and belong to the kingdom of eubacteria and to the division of cyanophyta. They are also gram-negative and are common in some extreme environments. Cyanobacteria are a large and morphologically diverse group [3,54,55], which can thrive in all kinds of waters with some species thriving in freshwater while others thrive in brackish water or the marine environment. The habitats and the ecological requirements of cyanobacteria are diverse and depend on the genera and even on the strain. For example, *Spirulina platensis* shows growth at pH 9.0–10.0 and grows well at 11.5 but not at 7.0, while *Anabaena* sp. displays optimal growth at pH 7.4–8.4 and its productivity decreases significantly at pH values higher than 9 [56]. Vonshak and Tomaselli [57] have isolated several *Spirulina* strains, a number of which have a range of optimum temperatures between 24–28 °C and other up to 40–42 °C.

Therefore, an important factor in the production of algal biomass is the selection of the strain that is best suited to the environmental and cultivation conditions. For instance, Sheshardi and Thomas [58] found that the cultivation of a locally isolated species of *Spirulina* in Zarrouk medium supplemented with biogas effluent demonstrated higher productivity (12.39 g/m^2 d) compared to *S. platensis* (10.88 g/m^2 d).

Some of the filamentous cyanobacteria genera are: *Anabaena*, *Anabaenopsis*, *Aphanizomenon*, *Nadularia*, *Oscilatoria*, *Spirulina*, *Phormidium*, *Nostoc*, *Nostochpsis*, and *Scytonema*. In Table 1 the biomass concentration, the productivity and the biomass composition of selected cyanobacteria are listed. From the above genera, *Spirulina* sp. is by far the most researched cyanobacterium due to its importance for food and the production of several metabolites. Thus, this review will be based mainly on the knowledge that we have regarding this particular species.

4. Cultivation factors

In order for a culture to be successful, various environmental and operational factors, which affect the biology and habitats of the organisms, must be taken into account. These factors also affect cyanobacterial biomass productivity as well as biomass composition. The most important factors are: nutrients, pH and alkalinity,