Applied Energy 88 (2011) 3389–3401

Contents lists available at ScienceDirect

Applied Energy

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

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

Giorgos Markou *, Dimitris Georgakakis

Department of Natural Resources Management and Agricultural Engineering, Agricultural University of Athens, Iera Odos 75, 11855 Athens, Greece

ARTICLE INFO

Article history:
Received 7 September 2010
Received in revised form 22 November 2010
Accepted 9 December 2010
Available online 26 January 2011

Keywords:
Cyanobacteria
Blue-green algae
Spiraling
Wastewater
Biofuel
Biomass

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

* Corresponding author.
E-mail address: markoug@aua.gr (G. Markou).

0306-2619/$ - see front matter © 2010 Elsevier Ltd. All rights reserved.
doi:10.1016/j.apenergy.2010.12.042

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,

Table 1
Biomass concentration, productivity and biomass composition of selected cyanobacteria.

Species	Biomass concentration	Productivity	Protein (%)	Lipid (%)	Carbohydrates (%)	References
Anabaena cylindrica			43–56	4–7	25–30	[59]
Anabaena cylindrica				10.7–12.3		[60]
Anabaena sp.		9–35 g/m^2 d				[61]
Anabaena variabilis		6–13 g/m^2 d	51.9	22.7	9.2	[56]
Nostoc sp.	0.2–0.3 g/L				30.66–32.85	[62]
Oscillatoria rubescens	0.05–0.327 g/L		28–53.8	11.3–12.8		[63]
Oscillatoria sp.			49.3–79.4			[64]
Oscillatoria sp.	0.26 g/L				19.32	[62]
Phormidium angustissimum					28.48	[62]
Spirulina maxima		0.21–0.25 g/L d	60–71	6–7	13–16	[7,59]
Spirulina platensis			46–63	4–9	8–14	[62]
Spirulina platensis		0.06–0.42 g/L d				[65]

light and cultural cell density, temperature, and contamination by other microorganisms.

4.1. Nutrients

4.1.1. Carbon

Carbon is an essential nutrient for cyanobacteria cultivation and can be taken up from inorganic and organic forms. Inorganic carbon is utilized through the CO_2 concentrating mechanism, an active function that enables the cyanobacteria to acquire and concentrate inorganic carbon from the extracellular environment. Cyanobacteria have the ability to utilize both CO_2 and HCO_3^- as an inorganic carbon source. As intercellular carbon is in the form of HCO_3^-, it is converted to CO_2 by the enzyme carbonic anhydrase (CA) according to the reaction [66–69]:

$$CO_2 + H_2O \leftrightarrow HCO_3^- + H^+ \quad (2)$$

The CO_2 dissolved in water generates a weak acid/base buffer system, namely the bicarbonate–carbonate buffer system. The bicarbonate–carbonate buffer system is the most important buffer system generally present in natural waters and anaerobically digested wastes and forms with the phosphate, ammonium and various organic acids buffer subsystems a mixed weak acid/base system [70–73]. The formation of an inorganic carbon species is a function of pH and temperature. As is shown in Fig. 1, in pH values up to about 10.5, bicarbonate species dominate, while in higher pH values carbonate $\left(CO_3^{2-}\right)$ species dominate. Under high pH values the cyanobacteria calcify, promoting calcium carbonate ($CaCO_3$) precipitation. This calcification process:

$$Ca^{2+} + HCO_3^- \rightarrow CaCO_3 + H^+ \quad (3)$$

generates minerals and protons. The protons are used in photosynthesis for carbon and nutrient assimilation [74]. In general, calcification stimulates photosynthesis [74–76]. However, biomass productivity of *Spirulina* decreases in environments with pH values higher than 9, even though *Spirulina* thrives in media with pH values that range from 8 to 11.5 [77,78].

The bicarbonate–carbonate buffer system provides carbon for photosynthesis through the following reactions:

$$2HCO_3^- \leftrightarrow CO_3^{2-} + H_2O + CO_2 \quad (4)$$

$$HCO_3^- \leftrightarrow CO_2 + OH^- \quad (5)$$

$$CO_3^{2-} + H_2O \leftrightarrow CO_2 + 2OH \quad (6)$$

Although the dissolution of CO_2 in water results in acidification due to the forming of carbonic acid, the photosynthetic process of CO_2 fixation causes a gradual rise in pH due to accumulation of

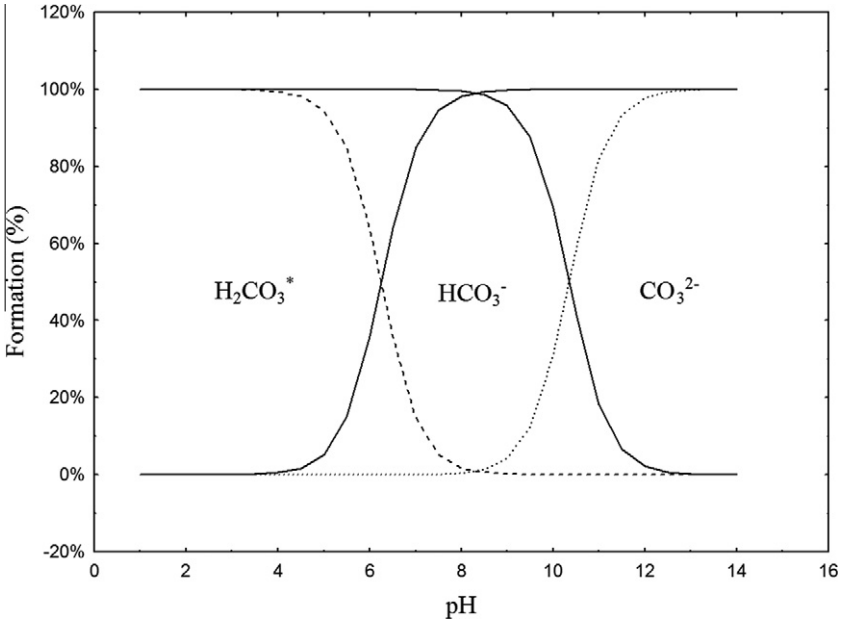

Fig. 1. Formation of inorganic carbon species as a function of pH ($H_2CO_3^*$, refers to $CO_{2(aq)} + H_2CO_3$).

OH^- [79,80]. Moreover, the tendency of pH to rise is related to photosynthetic activity, which means that pH becomes higher where photosynthetic activity is higher [45]. Rising pH can be regulated by the addition of mineral acids, such as HCl [81] or organic acids. The latter is advantageous in cultures based on mixotrophic metabolism, because organic acids can function as a carbon source [79].

Cyanobacteria and chlorophyceae can grow with up to 18% dissolved CO_2 in the cultivation medium, but cyanobacteria utilize CO_2 at a higher rate than chlorophyceae [80,82,83]. CO_2 fixation efficiency can amount up to 38% in *Spirulina* sp. cultures [77,80] and in *Anabaena* sp. ATCC 33047 CO_2 fixation rates achieve 1.45 g CO_2/L d [84].

CO_2 as a nutrient represents one of the most costly components in the cultivation of microalgae [85]. Therefore a system that couples a waste CO_2 source with the cultivation of CO_2 fixing organisms, like cyanobacteria, can not only reduce cultivation costs but also mitigate or remove CO_2, an environmental pollutant. Waste CO_2 can be provided by the flue gases from power plants [86] or as sodium bicarbonate ($NaHCO_3$) following the recovery of CO_2 by means of NaOH from combustion flue gasses [87]. In the agro-industrial sector, CO_2 can be provided by using biogas from the anaerobic digestion of agro-industrial waste [88], which contains 30–45% CO_2 [89] or by using CO_2 emissions from the aerobic composting of animal manure [16]. In the case of biogas from anaerobic digestion, there are two aims: on the one hand to provide the algal culture with carbon and on the other hand to purify the biogas by removing CO_2 from it. At the experimental level the CO_2 concentration in biogas after its removal by algal culture ranged from a negligible percentage up to 11.5% [88,90]. There would appear to be a linear relationship between the rate of algal growth and the removal of CO_2 from the biogas. Carbon utilization efficiency of biogas CO_2 for biomass production can reach 95% [90].

Bicarbonate alkalinity and carbon can be provided by the addition of sodium bicarbonate to the cultivation medium. In synthetic cultivation media for the cultivation of *Spirulina* sp. the most frequently used medium is Zarrouk medium, which provides carbon as sodium bicarbonate in the amount of 16.8 g/L [64]. In the earlier studies sodium bicarbonate was added to media containing anaerobically digested wastes in amounts in the range of 9–17 g/L, but 2–4 g/L is now considered as sufficient [91–93]. Lincoln et al. [94] suggest that supplementation of sodium bicarbonate does need not to exceed 0.5% of the culture medium to be adequate for *Spirulina* growth in effluents of anaerobically digested pig and cattle manure.

As mentioned above, a source of carbon for cyanobacteria growth can be organic substances, such as sugars, fatty acids and amino acids. However, the ability to grow heterotrophically or mixotrophically on one or other of the organic substrates is species dependent [40,41,95–99]. It has been found that *S. platensis* can be cultivated in high strength organic artificial wastewater, which contains acetate up to 10 g/L [100]. In Table 2 some species cultivated in organic substrates are listed.

4.1.2. Nitrogen

Nitrogen is also an important nutrient for the production of microalgal biomass. The nitrogen content of the biomass can range from 1% to more than 10% and is dependant upon the amount, the availability and the type of the nitrogen source [56,79,105]. Nitrogen can be utilized as NO_3^-, NO_2^- or NH_4^+ and also as N_2. Some cyanobacteria, such as *Oscilatoria*, *Anabaena* and *Spirulina*, are diazotrophic, which means that they are capable of utilizing elemental nitrogen as their sole nitrogen source by the reduction of N_2 to NH_4^+ [106]. This process is catalysed by the enzyme nitrogenase [107]. The order in which cyanobacteria prefer to utilize nitrogen is $NH_4^+ > NO_3^- > N_2$. When NH_4^+ is available, cyanobacteria do not utilize other nitrogen sources until all the ammonia is utilized [108]. In cyanobacteria the most important route for ammonium utilization is through the glutamine synthetase enzyme system. When only nitrate is available it is reduced intracellularly by nitrate reductase to nitrite and nitrite is reduced by nitrite reductase to ammonium [91,109,110]. The uptake of nitrate is light energy dependent, and since the reduction of nitrite consumes energy, cyanobacteria prefer to utilize already reduced nitrogen such as ammonium [111]. Hence, nitrate consumption in wastewater is reduced due to the high turbidity of the wastewater [112]. However, nitrate seems to be important for the cultivation of cyanobacteria [27]. High concentrations of ammonium will inhibit uptake of nitrate because ammonium represses the synthesis of nitrate reductase, while high nitrate concentration inhibits ammonia uptake [54,108,113]. When ammonia is used as the sole nitrogen source, the pH will drop due to the release of H^+ ions [79].

The form in which nitrogen is present in a solution is pH and temperature dependent. According to Fig. 2, in pH values higher than 9.25 free ammonia begins to dominate over ammonium. Also, high temperatures favour the formation of free ammonia. Free ammonia is generally toxic to photosynthetic organisms [114], but the toxicity appears to be reduced in alkalophilic species such as *S. platensis* [110,115]. In the presence of 5 and 10 mM of NH_4Cl, the photosynthetic activity of *S. platensis* retained 70% and 50% of its maximal photosynthetic capacity respectively [116]. However, the growth of *S. platensis* is nearly completely inhibited in wastewater, which contains 200 mg/L ammonia [100,117]. In other cyanobacteria such as *Anabaena* sp., the ammonia inhibition occurs in lower concentrations. The growth of *Anabaena* sp. is completely inhibited in 10 mM of NH_4Cl, while the photosynthetic activity of *Anabaena* sp. in 5 mM of NH_4Cl is 10% of its photosynthetic activity capacity [110]. The ammonia toxicity level for *Phormidium bohneri* is reported to be around 3.2 mM $N{-}NH_4^+$/L [28].

The composition of cyanobacterial biomass composition is affected by nitrogen nutrition. Crude protein biomass content appears to be related to the nitrogen available in the medium [118] and the variation of the nitrogen content of the cultivation media shows that the proteins contained in the cyanobacterial biomass increase to some extent with the increasing of the nitrogen content [63,119,120]. Thus, W&WW rich in nitrogen may be considered a suitable cultivation medium for protein production. For example, *Spirulina maxima* grown on fermented cattle and poultry manure, which is rich in nitrogen, had 60.1% and 71.8% protein content respectively [121].

The effect of variation in nitrogen concentration on biomass lipid content is not clear; according to Piorreck et al. [63], cyanobacteria do not significantly change their lipid composition, while Walach et al. [122] observed that under nitrate limitation the lipid content increased and Sassano et al. [120] observed that under nitrate limitation the lipid content decreased. However, according to Colla et al. [123], at 30 °C the protein and lipid content is not significantly affected by variation in nitrogen concentration, while at 35 °C an increase in proteins and lipids occurs.

In most of the research work studied *Spirulina* sp. is cultivated in Zarrouk medium, in which nitrogen is available as sodium nitrate ($NaNO_3$). Various researchers have tried to use different types of nitrogen sources such as ammonium chloride [105,111,124], ammonium sulphate [105,125] acid ammonium phosphate [105] and urea, the latter in order to reduce the costs of nitrogen as nutrient [111,126,127]. However, sodium nitrate is the most suitable nutrient for *Spirulina* cultivation [105].

4.1.3. Phosphorus

Phosphorous is also an essential macro-nutrient for microalgae growth. Although cyanobacterial biomass do not need large amounts of phosphorus, as it contains less than 1% of it, phosphorus

Table 2
Selected cyanobacteria cultivated in organic substrates.

Species	Organic carbon source	Cultivation type	Production	Cell concentration	References
Spirulina platensis	Glucose	Mixotrophic		2.66 g/L	[44]
Spirulina platensis	Glucose	Mixotrophic		2.521 g/L	[52]
Spirulina platensis	Glucose	Heterotrophic		0.827 g/L	[52]
Spirulina platensis	Glucose	Mixotrophic	84 mg/L d		[101]
Spirulina platensis	Acetate	Mixotrophic	76 mg/L d		[101]
Spirulina platensis	Propionate	Mixotrophic	60 mg/L d		[101]
Spirulina platensis	Acetate	Mixotrophic		1.81 g/L	[44]
Anabaena variabilis	Fructose	Mixotrophic		2.1 g/L	[102]
Anabaena variabilis	Glucose, Ribose, Maltose	Mixotrophic		0.22 g/L	[102]
Anabaena variabilis	Sucrose	Mixotrophic		0.26 g/L	[102]
Anabaena variabilis	Fructose	Heterotrophic		10 g/L	[103]
Nostoc sp. strain Mac	Glucose	Heterotrophic		0.37 g/L	[104]
Nostoc sp. strain Mac	Fructose	Heterotrophic		0.41 g/L	[104]
Phormidium sp.	Sucrose	Heterotrophic		1.18 g/L	[97]

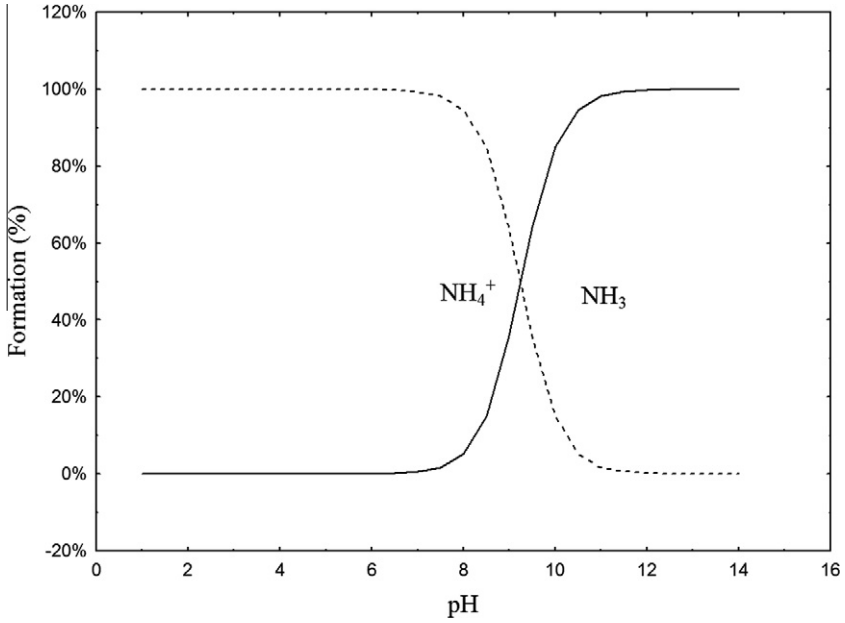

Fig. 2. Formation of ammonia/ammonium species as a function of pH.

is an important growth limiting factor, especially in natural environments where phosphorus is limited [79,128]. Low phosphorus concentration is related to low cell densities [129]. However, cyanobacteria are able to store excess P as polyphosphate reserves, which can be sufficient for prolonged culture in phosphorus deficient media [79,109]. Phosphorus deficient cells take up phosphates at higher rates than phosphorus sufficient cells do [130]. The form of phosphorus, which is utilized by microalgae, is the orthophosphate $\left(PO_4^{3-}\right)$ form. Fig. 3 shows the formation of phosphate species as a function of pH. In aquatic systems phosphorus occurs in pentavalent form as a mixture of dissolved and particulate types and the available organic phosphorus is hydrolyzed to PO_4^{3-} by extracellular enzymes [131].

The utilization of phosphate is energy dependent and its uptake rate is slower in dark than in light environments [132–134]. Moreover, the uptake of phosphate is influenced 1 by pH; uptake rates decrease in acid and relatively alkaline environments [133]. Additionally, the presence of ions also influences the uptake of phosphate; namely, lack of ions such as K^+, Na^+ and Mg^{2+} decreases the phosphate uptake rate [129,131–133].

The variation of phosphorus concentration in the culture medium influences biomass composition, but there is lack of research regarding this topic. Nevertheless, the decrease of phosphorus concentration in cultures of *Anabaena variabilis* causes an increase in carbohydrate and a decrease in protein content [79,135].

4.2. Other macro- and micro-nutrients

Besides the essential nutrients mentioned above cyanobacteria also require for their growth a number of other macro-nutrients in considerable amounts, including sulphur (S) [88], calcium (Ca) [136], magnesium (Mg) [137] and potassium (K) [138]. Micronutrients required include molybdenum (Mo) [139], iron (Fe) [133], nickel (Ni) [140], copper (Cu) [141], zinc (Zn) [142], cobalt (Co) [142], boron (B) [143], manganese (Mn) [144] and chloride (Cl) [145]. In Table 3 some of the macro- and micro-nutrients contained in selected agro-industrial W&WW are listed.

4.3. Temperature

Temperature is an important physical factor, which strongly influences the oxygen evolving activity of the photosystem II (PSII), has a number of effects on the cyanobacterial membranes and influences nutrient availability and its uptake [150–152]. There is

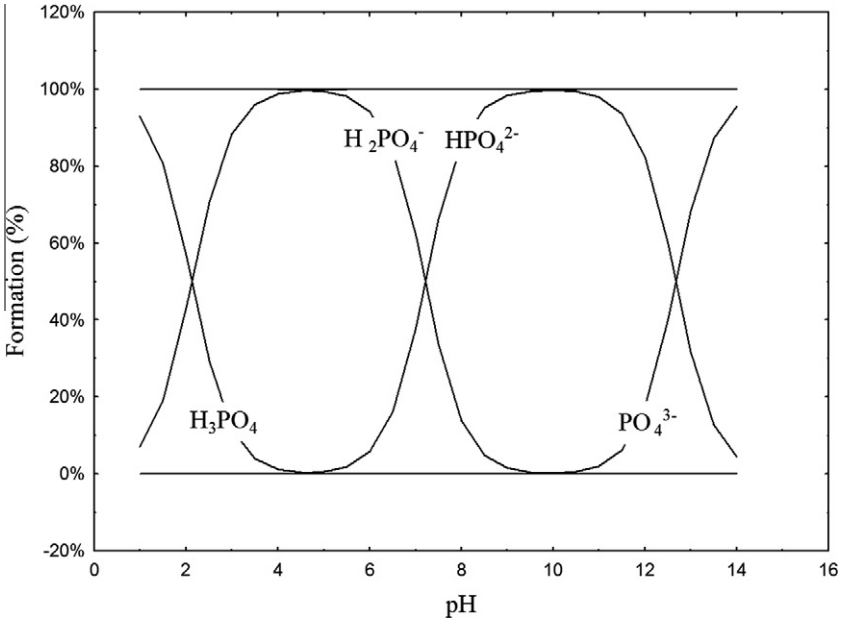

Fig. 3. Formation of phosphate species as a function of pH.

Table 3
Several macro- and micro-nutrients contained in selected agro-industrial W&WW.

W&WW	K	Na	Ca	Mg	Mn	Ni	Cu	Co	Fe	Zn	References
Digested swine manure	366 mg/L	111 mg/L	174 mg/L	225 mg/L	1.15 mg/L		0.02 mg/L	0.09 mg/L	38 mg/L	0.08 mg/L	[121]
Swine slurry	3–7.5 mg/L			0.6–1.5 mg/L			309 (mg/kg TS)				[146]
Poultry manure	12.5–32.5 mg/g manure	2–7.4 mg/g manure	36.2–59.6 mg/g manure	1.8–6.6 mg/g manure	259–378 μg/g manure		38–68 μg/g manure		8–560 μg/g manure		[147]
Digested poultry manure	592 mg/L	214 mg/L	42 mg/L	54 mg/L	0.1 mg/L		0.04 mg/L	0.12 mg/L	2.5 mg/L	0.1 mg/L	[115]
Digested cattle manure	116 mg/L	38 mg/L	171 mg/L	60 mg/L	0.12 mg/L		0.04 mg/L	0.02 mg/L	9.1 mg/L	0.44 mg/L	[115]
Dairy wastewater	8.6–155.5 mg/L	263–1265 mg/L	1.4–58.5 mg/L	6.5–46.3 mg/L	<1–835 μg/L	12–71 μg/L	<1–30 μg/L	<1–7 μg/L	39–4329 μg/L		[148]
Olive-oil mill wastewater		11–42 mg/L	2–64 mg/L	44–220 mg/L	1.1–6 mg/L		1.5–6 mg/L		18.3–120 mg/L		[149]

a connection between temperature, light and photoinhibition. At low temperatures cyanobacteria are photoinhibited by high light intensities and thus temperature can be considered as the most important limiting factor in outdoor cultivation during the winter. However, photoinhibition can be considerably reduced by an increase in temperature [152–154].

Optimum temperature for biomass production is genera and strain dependent [152,155]. For instance, optimal temperature for *A. variabilis* is 35 °C [56], while for *Spirulina* sp. it is in the range of 30–38 °C [57,78,156]. Nevertheless, even among the genera of *Spirulina* sp. some strains are more thermophilic than others [151]. In Table 4 the minimum and maximum temperature and the growth rate of some cyanobacteria are listed.

Temperature is a factor that also affects cyanobacterial biomass composition. Nevertheless contradictory results are reported in the literature. Some report that protein content decreases with an increase in temperature [157] while at the same time carbohydrate content increases [158] however others report exactly the opposite [51]. Ogbonda et al. [78] and Abu et al. [159] report a variation in biomass composition as the temperature rises. According to the

Table 4
Minimum and maximum temperature and growth rate of some cyanobacteria.

Species	T_{min} (°C)	T_{opt} (°C)	Growth rate μ_{max} (1/d)	References
Anabaena variabilis	–	35	–	[53]
Anabaena variabilis	<10	35	1.1	[161]
Oscillatoria	<15	27	–	[155]
Spirulina maxima	15	30–35	0.26–0.45	[152]
Spirulina platensis	15	25–30	0.46–0.58	[152]

same authors, the highest crude protein content in *Spirulina* sp. was achieved at 30 °C combined with a pH value of 9.0. The lipid content is also affected by temperature. It seems that it is favoured by an increase in temperature [78,123,158,160].

4.4. Light and cell density

As mentioned above, light is the primary energy source for cyanobacteria growth, enabling it to carry out all the necessary metabolic processes. Photosynthesis is strongly dependent on the quality and the quantity of light. The first is expressed as the wavelength of light and the latter as photon-flux-density (PFD) [36,162]. Cyanobacteria absorb light mainly in the 400–500 nm and 600–700 nm light wavelength range.

Cyanobacterial growth rates are enhanced by increasing light density up to the point of light saturation, at which point photosynthetic activity reaches its maximum [159,163]. At high light densities, the photosynthetic capacity decreases and cyanobacterial growth is inhibited. This phenomenon is called photoinhibition and occurs at light oversaturation, a situation in which the photosystem II (PSII) is negatively affected [154,164]. However, cyanobacteria are able to repair the damage caused from photoinhibition. Thus, photoinhibition is the balance between damage and its repair [165,166]. Photoinhibition depends mainly on light [164], temperature [148], strain [155,167] and cultivation type (indoor or outdoor) [167,168].

In contrast, at high cell densities, cell mutual shading takes place and light intensity decreases due to the increase in turbidity of the culture, causing a reduction in the photosynthetic activity [56,162]. The light intensity decreases exponentially according to the following equation:

$$I_L = I_o \cdot e^{(-\gamma L)} \quad (7)$$

where I_L is the light intensity at depth L, I_o is the initial light intensity and γ is the turbidity [156]. Especially in cultivation in wastewaters, which contain particulate matter and have higher turbidity, a further reduction in light intensity may occur [169]. Thus, the light/dark cycle, namely the speed of a cell moving from illuminated to darkened layers and vice versa, also appears to be an important factor affecting the photosynthetic efficiency in cultures with high cell density [170]. Therefore, mixotrophic cultures may be more advantageous because they are less sensitive to light oversaturation, require less light for growth and have higher metabolic activity [53]. Optimal light intensity for *S. platensis* is reported to be 72 μmol photon/m^2 s [124].

Biomass composition is affected by light intensity. In general, total lipid, fatty acids and protein content decreases as light intensity increases [160,163,171]. *Spirulina* sp. cultivated in seawater supplemented with anaerobically digested pig waste produced more lipids at low light intensities, while the protein content was nearly the same [93]. However, Tedesco and Duerr [160] report that, although the total lipids content decreased from 170 to 860 μmol photon/m^2 s, at the higher light density of 1400 μmol photon/m^2 s^1 the total lipids content was higher than that of 860 μmol photon/m^2 s, and that light intensity variation had little effect on the fatty acid content.

4.5. Contamination and competition

Outdoor cultures of microalgae suffer from contamination by other microorganisms such as bacteria, fungi, yeasts and other microalgae genera, the metabolites or growth rates of which may inhibit the growth of the cultivated microalgae [121,169]. Autochthonous microalgae survived better than *S. platensis* and even outcompeted it in cultures with a high percentage of waste in the growth medium [172]. With regard to wastewater treatment the purpose is not to produce well controlled biomass with a definite composition from a specific microalga but to remove pollutants efficiently from the wastewater [172,173] but from the energy production viewpoint, biomass with specific characteristics may be desirable. However, contamination can be eliminated or alleviated by chosen strains from extreme habitats, like *Spirulina*, which is strongly competitive in environments with high pH and relatively high ammonia concentrations [115,174]. It is known that in general cyanobacteria are competitive in environments with high pH (8.5–10) [168]. Another way to prevent contamination is to keep the culture under optimal conditions for the selected species [27], to cultivate diazotrophic species in nitrogen-free media, as these species can utilize elemental nitrogen in the air [61], or to immobilize the cyanobacterial filaments in various immobilization media, such as chitosan, alginate and agarose. [21–23,28]. De la Noüe and Bassères [28] found that the contamination of the cyanobacterium *P. bohneri* by other algal species occurred when the inoculum, which served as the cultivation starter, was too small. In addition, no supplementation with sodium bicarbonate in the culture of *Spirulina* caused chlorophytes to dominate [94].

5. Agro-industrial wastes and wastewaters

The agro-industrial sector generates considerable amounts of W&WW, most of which are rich in organic or/and inorganic pollutants. The intensification of agro-industrial production and the disposal to land of the W&WW generated have raised a number of environmental issues, including eutrophication, surface and ground water pollution, odour pollution, gas emissions etc. High concentrations of livestock in small areas generate enormous quantities of waste, which are insufficient for land disposal and land application because of soil saturation [175–179]. The major source of organic waste is animal manure, but there are considerable amounts of organic waste originating from the food industry, such as dairy, olive-oil mill, and winery. There are various biological, physicochemical, and mechanical methods to treat all these W&WWs [178–182], but the most common methods of treatment used today are aerobic and anaerobic digestion. However, these techniques achieve only secondary treatment, which means that they remove at most the organic pollutants and have almost minimal effect on the management of inorganic pollutants [183]. Inorganic pollutant removal requires very expensive physico-chemical methods [106], especially the removal of phosphorus, which is the most difficult pollutant to remove [23]. Mixotrophic cyanobacteria can be used for the removal of organic pollutants and in general microalgae can be employed as an alternative secondary or post-secondary treatment process at a lower cost [106,184]. As none of the cyanobacteria species can utilize all of the pollutants contained in W&WW at the same removal rate, higher total treatment efficiency can be achieved by polyculture, namely the use of a mixture of various species of microalgae or even the combination of microalgae and bacteria. Each of the microalgae species in the polyculture remove a specific amount of certain species of the pollutants and as a result the overall removal of pollutants may be higher. The effect of the synergism between the bacteria and the microalgae is that the latter produce oxygen, which is utilized by the bacteria, which decompose the various organic substances to CO_2, ammonia and phosphate, which in turn are essential for microalgae growth [100,172,185,186]. This oxidation of the organic pollutants may also be related to the oxidizing of odorous compounds and their deodorization [94].

However, removal of ammonia can occur as a result of the stripping and removal of phosphorus by precipitation and sinking. The latter method in particular is enhanced by photosynthesis, which

Table 5
Characteristics of several agro-industrial wastes and wastewaters.

W&WW	COD	BOD	TS	VS	TN	NH_4^+–N	Nitrates	PO_4^{3-}–P	TP	pH	References
Poultry manure					18.2–72 mg/g manure	0.21–29.9 mg/g manure	0.03–1.5 mg NO_3^-–N/g manure	4.3–5.2 mg/g manure	13.5–34 mg/g manure		[147]
Olive-oil mill wastewater	67–178 g/L	46–94 g/L	6.35–7.19%		0.62–2.1 g/L					4.2–5.17	[149]
Swine manure	68 g/L		4.8%			4300 mg/L	5 mg/L	783 mg/L		7.2	[28]
Anaerobically digested swine manure	7.7 g/L		0.89%			3294 mg/L	111 mg/L	277 mg/L		8.0	[27]
Swine liquid waste	19.7–21.2		18.7–19.7 g/L	15–15.4 g/L	974.8–1025.5 mg/L	301.2–486.5 mg/L				6.23–6.84	[190]
Anaerobically digested swine liquid waste	9.68–12.9 g/L		10.7–13.59 g/L	6.08–9.94 g/L	891.2–1015.2 mg/L	439.7–724.1 mg/L				7.0–7.7	[184]
Aerobically stabilized swine wastewater	343.6–840 mg/L		1.48–1.53 mg/L			42.43–125 mg/L	3.25–6.1 mg nitrates/L	24.0–49.4 mg/L	2.27–5.7 mg/L		[112]
Beef, cow		6–50%	14–15%	12%	3.8–5.9%			1.1–2.5%			[92]
Sheep			28%	23%	11.3%			2.1%			[86]
Swine slurry			7%	75–86% TS	6–18% TS	3–17% TS					[146]
Cattle slurry			8–11%	75–82% TS	2.6–6.7% TS	1–4% TS					[140]
Cheese-whey	61–68.8 g/L				980–1480 mg L				379–510 mg/L		[191]
Dairy cleaning wastewater	0.785–7.62 g/L	0.565–5.72 g/L	1837–14205 mg/L	562–11034 mg/L	14–140 mg/L	1–34.1 mg/L	0.6–80 mg NO_3^-–N/L and 0.3–34 mg NO_2^-–N/L	6–35 mg/L	29–181 mg/L	6.2–11.3	[148]

Abbreviations: COD, Chemical oxygen demand; BOD, Biochemical oxygen demand; TS, Total solids; VS, Volatile solids; TN, Total nitrogen; TP, Total phosphorus.

increases the pH values [187,188]. Ammonia stripping to the atmosphere may be the most important mechanism for nitrogen removal [189], which can reach up to 70% [91]. It is worth mentioning that livestock is responsible for 64% of anthropogenic ammonia emissions [178]. Thus, for an agro-industrial W&WW treatment method to be effective it is required to have the least possible ammonia emissions.

Some animal wastewaters, such as from pig and poultry, contain high concentrations of ammonia and although cyanobacteria can grow in a wide range of salinity, wastewaters must first be diluted before they can be used as cultivation media, especially for pH values exceeding 8.0, because the free form of ammonia is toxic for cyanobacteria growth [28,118]. However, one of the major advantages of cyanobacteria cultivation in digested animal waste is that the macro- and micro-nutrients (Table 3 and Table 5) required are provided by this kind of raw material [24].

A serious drawback to using W&WW for the cultivation medium is the wide variation in composition among the various types of W&WW and even in the same type, which depend on physiological and management variables (Table 5). This makes it difficult to manipulate the cyanobacteria biomass content without monitoring the W&WW composition and adding various substances to regulate the synthesis of the media and to adjust them according the needs of the species under cultivation. A further serious drawback is the seasonal nature and the seasonal variation in the amount of W&WW generated.

In what follows, we briefly describe research dealing with the cultivation of cyanobacteria using various W&WW as cultivation medium or as element of cultivation medium.

5.1. Swine W&WW

Pig waste consists mainly of faeces, urine and wash water, which forms a slurry material that contains high amounts of organic and inorganic pollutants. Pig waste together with cattle waste are the most studied among the agro-industrial W&WWs as a cultivation medium for cyanobacteria growth.

Chung et al. [192] cultivated *S. platensis* in effluent from anaerobically digested pig waste. The dilution used was in the range of 0.88–7.05%. They found that *Spirulina* growth increased with the increasing of $NaHCO_3$ up to 16.8 g/L and of $NaNO_3$ up to just 1 g/L. Even when $NaHCO_3$ was reduced to 1.7 g/L *Spirulina* growth in wastewater was better than in the synthetic medium, suggesting that the organic substances contained in wastewater are a good source of carbon. The yield of *S. platensis* biomass was 5 g/m^2 d and the efficiency of nitrogen recovery was 76%.

Pouliot et al. [27] investigated the effect of the type of agitation (stirring or aeration) on a culture of *Phormidium* sp. in a medium enriched with settled pig manure. They found that the best agitation mode was bubbling (aeration) on a 14–24 h basis. Stirring was not a suitable system for agitating filamentous cyanobacteria due to the low aeration of the culture, the breakage of algal cells and the accumulation of nitrate due to nitrification, which is an indicator of poor health in algal cultivation. Ammonia and phosphate removal was 95% and 62% respectively and the growth rate was 0.34 1/d.

De la Noüe and Basséres [28] used anaerobically digested manure at concentrations of 0.6–3% in the medium and cultivated *P. bohneri* at two different temperature regimes (19 and 20 °C). The cyanobacterium responded strongly to increasing temperature, with a 2.3-fold increase in biomass productivity from 10 to 20 °C. A higher COD reduction of about 80% was found at 20 °C and 2% manure concentration. Ammonium, nitrogen and phosphate were completely removed after 12 days at 0.6% manure concentration and 20 °C. At manure concentration of 0% and 2% respectively *Phormidium bonheri* was able to remove just half the amount of

$N—NH_4^+$ and $P—PO_4^{3+}$. However, in another study [174] *Phormidium* sp. was capable to grow in 10–50% diluted effluent of anaerobically digested pig waste. Ammonium, total phosphorus, $P—PO_4^{3-}$ and $N—NO_3^-$ removals were 48%, 68%, 100% and 87% respectively. The best removal efficiency was obtained at 25% dilution. The COD of the pig waste was reduced 91%, mainly due to the synergism between the microalgae and oxidative bacteria present in these cultures.

Chaiklahan et al. [91] calculated that the use of 20% diluted anaerobically digested pig waste supplemented with 4.5 g/L $NaHCO_3$ and 0.2 g/L urea was 4.4 times cheaper than the modified Zarrouk's medium. They also found that the average productivities were 19.9 and 12 g/m^2 d for laboratory and pilot scale conditions respectively. Nitrogen and phosphorus removal rates were 34 mg/L d and 4 mg/L d respectively.

Supernatant of aerated swine wastewater was used by Cañizares and Domínguez [18] as medium for the cultivation of *S. maxima*. *Spirulina* grew very well at any dilution tried. Even without dilution *Spirulina* grew well indicating its toleration to the concentration of inorganic pollutants contained in this waste. However, the maximal biomass concentration and maximal removal of $N—NH_4^+$ (75%) and of total phosphorus (53%) was obtained with a dilution of 50%. In another study of Canizares et al. [193] *S. maxima* was immobilized in K-carrageenan and was cultivated in the same medium as in the study mentioned above. The immobilization enhanced the removal of $N—NH_4^+$ and the total phosphorus. In 50% dilution the removal was 80% and 90% for $N—NH_4^+$ and total phosphorus respectively. Nevertheless, maximum removal was obtained by 25% dilution and was 90% for both ammonium nitrogen and total phosphorus. Biomass composition of *S. maxima* and *Phormidium* sp. was found by Cañizares-Villanueva et al. [112] to be varied in relation to the growth medium. In a medium with 50% diluted aerated pig wastewater the protein content was 36%, for lipids 6% and for carbohydrates 44% in *S. maxima* biomass, whereas in *Phormidium* sp. the protein content was 53.4%, for lipids 9.4% and for carbohydrates 27.5% [111].

Olguín et al. [24,189,194] have used in their studies seawater supplemented with effluent from anaerobically digested pig waste. They suggest that a supplementation of 2% offers the optimal concentration for the cultivation of *S. maxima* in high rate oxidation ponds. Total nitrogen removal was 76–96%, NH_3–N was almost completely removed (up to 100%) and phosphate removal was 99%. Volatile solids were reduced by 28% and COD was reduced by 25.2–52.2%. The productivity in outdoor conditions ranged between 3.6–15.1 g/m^2 d and varied with temperature, light intensity, pond depth and season. Protein content of biomass amounted to 17–71% [24,189,194] and it was found that enrichment of lipids or of polysaccharides was dependent on the specific light flux under nitrogen-deficiency accumulation [93].

5.2. Cattle W&WW

Worldwide there are around 1.3 billion head of cattle [195]. Cattle manure contributes to methane emissions as much as pig manure does (8 Mt of gas for the year 2004) [196]. Cattle manure possesses a considerable amount of nitrogen and has a high organic pollutant content.

Ayala and Vargas [118] cultivated *S. maxima* in a medium supplemented by effluent of anaerobically digested cow manure. Growth rates of 45.3 mg/L d were obtained.

Mitchell and Richmond [197] cultivated *Spirulina* in Zarouk medium enriched with cattle waste leachate. The highest cell density was obtained in the medium that contained 16.3% waste leachate and 83.7% Zarouk medium. However, the authors judged that a medium that contained 33.3% of waste leachate and 66.7% Zarouk medium had the greatest practical benefit. Higher output rates were obtained by the addition of $NaHCO_3$, nitrate and phosphate. The addition of 2 g/L $NaHCO_3$ gave three times higher output rate than that obtained without $NaHCO_3$ and the addition of nitrate and phosphate gave around 100% and 50% higher output rates respectively. In outdoor experiments, in which the only nutrients added to the leachate were $NaNO_3$ and $NaHCO_3$, the highest rate of production amounted to 14 g/m^2 d.

In a study by Lincoln et al. [94], *Spirulina* was cultivated in a medium with diluted (1:1) anaerobically digested cattle manure. Growth was rapid and no inhibition occurred in the presence of $N–NH_3$ at a concentration of less than 75 mg $N–NH_3$/L, but growth was inhibited at concentrations above 100 mg $N–NH_3$/L. However, $N–NH_3$ removal was 24 mg/L d and biomass productivity was 70 mg/L d for laboratory experiments and 24 mg/L d for outdoor cultivations.

Oron et al. [198] used raw cow manure to cultivate *S. maxima* under field conditions. They report a yield of *Spirulina* biomass of 3158 mg/L.

5.3. Poultry W&WW

The most significant wastes from poultry are litter, manure and slaughterhouse waste [147,199]. Poultry manure contains high amounts of total nitrogen (average 46 g/kg manure) and high amounts of ammonia nitrogen (average 14.4 g/kg manure) [141] and thus it must be diluted in order to be used as a cultivation medium for cyanobacteria. Data regarding the use of poultry manure for cyanobacteria cultivation is scarce. The only study located dealing with this topic is the one by Mahadevaswamy and Venkataraman [200]. These researchers diluted effluent from anaerobically digested poultry droppings and then cultivated *S. platensis* in the effluent. They found that the effluent with 2% dilution was the most suitable. The cell density of the culture on the effluent amounted to about 100 mg/L in 19 days, which was about 20% lower than the cell density achieved in the synthetic cultivation medium.

5.4. Dairy W&WW

Dairy industry generates high strength wastewaters characterized by high BOD and COD concentrations [190,201]. Dairy wastewaters contain ammonia, minerals and phosphates along with high levels of dissolved or suspended solids, such as lactose, fats and proteins, originating from the milk [202–204]. However, dairy industry produces various products such as milk, butter, and cheese, and the generated W&WWs vary widely [191]. Processwater used for cleaning the production apparatus also generates high amounts of wastewaters [148]. The dairy W&WW that contains the highest value of organic pollutants is cheese-whey, which usually contains 30–50 g/L BOD and 60–100 g/L COD [205]. Cheese-whey also contains considerably high levels of nitrogen and phosphorus [191], which can be recycled through the use of cyanobacteria. Thus, Blier et al. [206] used *P. bohneri* to tertiary treat the effluents of anaerobically digested cheese. The growth rate obtained was 0.62 1/d and the biomass yield was 329 mg/L. Ammonia removal was almost complete and after 4 days of cultivation no significant amounts was left in the medium. Phosphorus removal after 4 days exceeded 69%. The ammonia and phosphorus removal rate was 5.9 mg $N–NH_3$/L d and 2.9 mg $P—PO_4^{3-}$/L d respectively. In a further study by the same authors [207] *P. bohneri* was cultivated with three nitrogen dilutions (30, 40 and 50 mg $N–NH_3$/L). They found that the maximum biomass concentration and growth rate was achieved in the more diluted effluent (30 $N–NH_3$/L). Biomass concentration amounted to 565 mg/L after 16 days of cultivation and the growth rate was 0.58 1/d. Ammonia removal (2.9–3.1 mg $N–NH_3$/L d) was almost the same for all

dilutions but the highest removal of phosphorus (4.9 mg P—PO_4^{3-}/L d) was achieved in the least diluted effluent.

5.5. Other agro-industrial wastes and wastewaters

Studies which discuss the cyanobacterial treatment of agro-industrial wastes besides animal and livestock wastes are scarce. Ayala and Vargas [118] used waste from industrial yeast production to cultivate *Spirulina*, obtaining a growth rate of 85.7 mg/L d. Adrade and Costa [45] used molasses at a concentration of 0.25–0.75 g/L as a substrate for the cultivation of *S. platensis*. The biomass concentration that they obtained was 2.94 g/L and the specific growth rate was 0.147 1/d, while the productivity amounted to 0.32 g/L d on average. There was an increase of pH values during the light phase and a decrease during the dark period, in which heterotrophic growth occurred.

Bonemeal as a substitute of calcium and phosphate salts was used by Vankataraman et al. [16] and Sheshardi and Thomas [58]. The biomass production using bonemeal was almost the same with the one of the controls and 3% of bonemeal was considered to be adequate for completely replacing calcium and phosphate salts.

In the study by Vankataraman et al., [16] sheep's blood at a dilution of 1% increased the growth of cultures of *Spirulina* almost three times in comparison with the control.

Phang et al. [208] used wastewater from the production of sago starch to cultivate *S. platensis*. The specific growth rate was 0.51 1/d and the biomass productivity was 14.4 g/m^2 d. The highest protein, carbohydrate and lipid content of the biomass were 68%, 23% and 11% respectively. The removal of COD, N–NH_3 and phosphate reached 98%, 99.9% and 99.4% respectively.

6. Conclusions

Cyanobacteria can make a significant contribution to the treatment of agro-industrial W&WW, reducing considerably their inorganic and organic pollutants. The produced cyanobacterial biomass, which may be rich in carbohydrates and/or proteins can possibly contribute to the production of biofuels besides biodiesel. The manipulation of the composition of cyanobacterial in order to suit a specific biomass energy conversion is feasible by controlling the cultivation conditions. However, a serious drawback to the use of W&WW as cultivation media is their seasonal nature and the strong variation in their composition.

Acknowledgments

G. Markou worked on this study as part of his Ph.D. in process, which is partially funded founded by Greek State Scholarships Foundation (I.K.Y.). We thank Dr. Despo Kritsotaki for her help with English. The text was finally edited by Jonathan Smith, CORDIS Science Editor. This work is dedicated to newborn Sophia.

References

[1] Metting FB. Biodiversity and application of microalgae. J Ind Microbiol 1996;17:477–89.
[2] Spolaore P, Joannis-Cassan C, Duran E, Isambert A. Commercial applications of microalgae. J Biosci Bioeng 2006;101:87–96.
[3] Thajuddin N, Subramanian G. Cyanobacterial biodiversity and potential applications in biotechnology. Curr Sci 2005;89:47–57.
[4] Tan LT. Bioactive natural products from marine cyanobacteria for drug discovery. Phytochemistry 2007;68:954–79.
[5] Amin S. Review on biofuel oil and gas production processes from microalgae. Energy Convers Manage 2009;50:1834–40.
[6] Brennan L, Owende P. Biofuels from microalgae – a review of technologies for production, processing, and extractions of biofuels and co-products. Renew Sust Energy Rev 2010;14:557–77.
[7] Mata TM, Martins AA, Caetano NS. Microalgae for biodiesel production and other applications: a review. Renew Sust Energy Rev 2010;14:217–32.
[8] Vasudevan P, Briggs M. Biodiesel production—current state of the art and challenges. J Ind Microbiol. 2008;35:421–30.
[9] Demirbas A. Use of algae as biofuel sources. Energy Convers Manage 2010;51:2738–49.
[10] John RP, Anisha GS, Nampoothiri KM, Pandey A. Micro and macroalgal biomass: a renewable source for bioethanol. Bioresour Technol 2011;102:186–93.
[11] Harun R, Jason WSY, Cherrington T, Danquah MK. Microalgal biomass as a cellulosic fermentation feedstock for, bioethanol production. Renew Sust Energy Rev, in press [uncorrected proof].
[12] McKendry P. Energy production from biomass (part 2): conversion technologies. Bioresour Technol 2002;83:47–54.
[13] Becker EW. Micro-algae as a source of protein. Biotechnol Adv 2007;25:207–10.
[14] Sialve B, Bernet N, Bernard O. Anaerobic digestion of microalgae as a necessary step to make microalgal biodiesel sustainable. Biotechnol Adv 2009;27:409–16.
[15] Lardon L, Hélias A, Sialve B, Steyer J, Bernard O. Life-cycle assessment of biodiesel production from microalgae. Environ Sci Technol 2009;43:6475–81.
[16] Venkataraman LV, Madhavi Devi K, Mahadevaswamy M, Mohammed Kunhi AA. Utilisation of rural wastes for algal biomass production with *Scenedesmus Acutus* and *Spirulina platensis* in India. Agr Waste 1982;4:117–30.
[17] Martin C, de la Noüe J, Picard G. Intensive cultivation of freshwater microalgae on aerated pig manure. Biomass 1985;7:245–59.
[18] Cañizares RO, Domínguez AR. Growth of *Spirulina maxima* on swine waste. Bioresour Technol 1993;45:73–5.
[19] Mulbry W, Kondrad S, Pizarro C, Kebede-Westhead E. Treatment of dairy manure effluent using freshwater algae: algal productivity and recovery of manure nutrients using pilot-scale algal turf scrubbers. Bioresour Technol 2008;99:8137–42.
[20] Posten C, Schaub G. Microalgae and terrestrial biomass as source for fuels – a process view. J Biotechnol 2009;142:64–9.
[21] Hoffmann JP. Wastewater treatment with suspended and nonsuspended algae. J Phycol 1998;34:757–63.
[22] Mallick N. Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. BioMetals 2002;15:377–90.
[23] de-Bashan LE, Bashan Y. Immobilized microalgae for removing pollutants: review of practical aspects. Bioresour Technol 2010;101:1611–27.
[24] Olguín E, Galicia S, Camacho R, Mercado G, Pérez T. Production of *Spirulina* sp. in sea water supplemented with anaerobic effluents in outdoor raceway under temperature climatic conditions. Anglais 1997;48:242–7.
[25] Molina Grima E, Belarbi EH, Acién Fernández FG, Robles Medina A, Chisti Y. Recovery of microalgal biomass and metabolites: process options and economics. Biotechnol Adv 2003;20:491–515.
[26] Waterbury JB. The cyanobacteria—isolation, purification and identification. In: Dworkin M, editor. The prokaryotes – a handbook on the biology of bacteria, 3rd ed.; 2006. p. 1053–73.
[27] Pouliot Y, Buelna G, Racine C, de la Noüe J. Culture of cyanobacteria for tertiary wastewater treatment and biomass production. Biol Waste 1989;29:81–91.
[28] de la Noüe J, Bassères A. Biotreatment of anaerobically digested swine manure with microalgae. Biol Waste 1989;29:17–31.
[29] Hori K, Ishii Si, Ikeda G, Okamoto Ji, Tanji Y, Weeraphasphong C, et al. Behavior of filamentous cyanobacterium *Anabaena* spp. in water column and its cellular characteristics. Biochem Eng J 2002;10:217–25.
[30] Demirbas A, Fatih Demirbas M. Importance of algae oil as a source of biodiesel. Energy Convers Manage 2011;52:163–70.
[31] Pittman JK, Dean AP, Osundeko O. The potential of sustainable algal biofuel production using wastewater resources. Bioresour Technol 2011;102:17–25.
[32] Li Y, Horsman M, Wu N, Lan CQ, Dubois-Calero N. Biofuels from Microalgae. Biotechnol Progr 2008;24:815–20.
[33] Angelidaki I, Ellegaard L, Ahring B. Applications of the anaerobic digestion process. In: Ahring B, Ahring B, Angelidaki I, Dolfing J, Euegaard L, Gavala H, et al., editors. Biomethanation II. Berlin/Heidelberg: Springer; 2003. p. 1–33.
[34] Yang YF, Feng CP, Inamori Y, Maekawa T. Analysis of energy conversion characteristics in liquefaction of algae. Resour Conserv Recycl 2004;43:21–33.
[35] Masojidek J, Kobližek M, Torzillo G. Photosynthesis in microalgae. In: Richmont A, editor. Handbook of microalgal culture: biotechnology and applied phycology. Oxford: Blackwell Publishing Ltd.; 2004. p. 20–39.
[36] Janssen M. Cultivation of microalgae, effect of light/dark cycles on biomass yield [PhD thesis]. Wageningen: Wageningen University; 2002.
[37] Tuchman N. The role of heterotrophy in algae. In: Stevenson R, Bothwell M, Lowe R, editors. Algal ecology: freshwater benthic ecosystems. California: Academic Press Inc.; 1996. p. 299–319.
[38] Goldman JC. Outdoor algal mass cultures – I. Applications. Water Res 1979;13:1–19.
[39] Vonshak A. Recent advances in microalgal biotechnology. Biotechnol Adv 1990;8:709–27.
[40] Rippka R. Photoheterotrophy and chemoheterotrophy among unicellular blue-green algae. Arch Microbiol 1972;87:93–8.
[41] Smith A, London J, Stanier R. Biochemical basis of obligate autotrophy in blue-green algae and thiobacilli. J Bacteriol 1967;94:972–83.
[42] Smith AJ. Modes of cyanobacterial carbon metabolism. Ann I Pasteur Microbiol 1983;134:93–113.
[43] Chojnacka K, Marquez-Rocha FJ. Kinetic and Stoichiometric relationships of the energy and carbon metabolism in the culture of microalgae. Biotechnol Adv 2004;3:21–34.

[44] Chen F, Zhang Y, Guo S. Growth and phycocyanin formation of *Spirulina platensis* in photoheterotrophic culture. Biotechnol Lett 1996;18:603–8.
[45] Andrade MR, Costa JAV. Mixotrophic cultivation of microalga *Spirulina platensis* using molasses as organic substrate. Aquaculture 2007;264:130–4.
[46] Chen G-Q, Chen F. Growing phototrophic cells without light. Biotechnol Lett 2006;28:607–16.
[47] Lee YK. Algal nutrition: heterotrophic carbon nutrition. In: Richmond A, editor. Handbook of microalgal culture: biotechnology and applied phycology. Oxford: Blackwell Publishing Ltd.; 2004. p. 116–24.
[48] Chojnacka K, Noworyta A. Evaluation of *Spirulina* sp. growth in photoautotrophic, heterotrophic and mixotrophic cultures. Enzyme Microb Technol 2004;34:461–5.
[49] Chen F, Zhang Y. High cell density mixotrophic culture of *Spirulina platensis* on glucose for phycocyanin production using a fed-batch system. Enzyme Microb Technol 1997;20:221–4.
[50] Marquez FJ, Nishio N, Nagai S, Sasaki K. Enhancement of biomass and pigment production during growth of *Spirulina platensis* in mixotrophic culture. J Chem Technol Biotechnol 1995;62:159–64.
[51] Torzillo G, Sacchi A, Materassi R. Temperature as an important factor affecting productivity and night biomass loss in *Spirulina platensis* grown outdoors in tubular photobioreactors. Bioresour Technol 1991;38:95–100.
[52] Marquez FJ, Sasaki K, Kakizono T, Nishio N, Nagai S. Growth characteristics of *Spirulina platensis* in mixotrophic and heterotrophic conditions. J Ferment Bioeng 1993;76:408–10.
[53] Vonshak A, Cheung SM, Chen F. Mixotrophic growth modifies the response of *Spirulina* (Arthrospira) *platensis* (cyanobacteria) cells to light. J Phycol 2000;36:675–9.
[54] Darley WM. Algal biology: a physical approach. London: Blackwell Scientific Publications; 1982.
[55] Barsanti L, Gualtiery P. Algae: anatomy, biochemistry and biotechnology. New York: CRC Taylor & Francis; 2006.
[56] Fontes AG, Angeles Vargas M, Moreno J, Guerrero MG, Losada M. Factors affecting the production of biomass by a nitrogen-fixing blue-green alga in outdoor culture. Biomass 1987;13:33–43.
[57] Vonshak A, Tomaselli L. *Arthrospira* (*Spirulina*): systematics and ecophysiology biochemistry. In: Vonshak A, editor. *Spirulina platensis* (*Arthrospira*): physiology, cell-biology and biotechnology. London: Taylor & Francis; 2003. p. 505–22.
[58] Seshadri CV, Thomas S. Mass culture of *Spirulina* using low-cost nutrients. Biotechnol Lett 1979;1:287–91.
[59] Becker EW. Microalgae: biotechnology and microbiology. Cambrigde: Cambridge Univesrity Press; 1994.
[60] Sallal AK, Nimer NA, Radwan SS. Lipid and fatty acid composition of freshwater cyanobacteria. Microbiol 1990;136:2043–8.
[61] Moreno J, Vargas MÁ, Rodríguez H, Rivas J, Guerrero MG. Outdoor cultivation of a nitrogen-fixing marine cyanobacterium, *Anabaena* sp. ATCC 33047. Biomol Eng 2003;20:191–7.
[62] Rodjaroen S, Juntawong N, Mahakhant A, Miyamoto K. High biomass production and starch accumulation in native green algal strains and cyanobacterial strains of Thailand. KASETSART J Nat Sci 2007;41:570–5.
[63] Piorreck M, Baasch K-H, Pohl P. Biomass production, total protein, chlorophylls, lipids and fatty acids of freshwater green and blue-green algae under different nitrogen regimes. Phytochemistry 1984;23:207–16.
[64] Hashimoto S, Furukawa K. Nutrient removal from secondary effluent by filamentous algae. J Ferment Bioeng 1989;67:62–9.
[65] Reichert CC, Reinehr CO, Costa JAV. Semicontinuous cultivation of the cyanobacterium *Spirulina platensis* in a closed photobioreactor. Braz J Chem Eng 2006:23–8.
[66] Reuter W, Müller C. Adaptation of the photosynthetic apparatus of cyanobacteria to light and CO_2. J Photochem B: Biol 1993;21:3–27.
[67] Jaiswal P, Prasanna R, Kashyap AK. Modulation of carbonic anhydrase activity in two nitrogen fixing cyanobacteria, Nostoc calcicola and *Anabaena* sp.. J Plant Physiol 2005;162:1087–94.
[68] Badger MR, Palmqvist K, Yu JW. Measurement of CO_2 and HCO_3^- fluxes in cyanobacteria and microalgae during steady-state photosynthesis. Physiol Plant 1994;90:529–36.
[69] Badger MR. G.D. P. CO2 concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution. J Exp Bot 2003;54:609–22.
[70] Van Vooren L, Lessard P, Ottoy JP, Vanrolleghem PA. PH buffer capacity based monitoring of algal wastewater treatment. Environ Technol 1999;20:547–61.
[71] Loewenthal RE, Ekama GA, Marais GvR. Mixed weak acid/base sytems. Part 1 – mixture characterization, vol. 15. Water SA. 1989.
[72] Georgacakis D. VFA-NH_3 buffer relationships in anaerobic digesters [PhD thesis]. Columbia: University of Missouri; 1979.
[73] Georgacakis D, Sievers DM, Iannotti EL. Buffer stability in manure digesters. Agr Waste 1982;4:427–41.
[74] McConnaughey TA, Whelan JF. Calcification generates protons for nutrient and bicarbonate uptake. Earth Sci Rev 1997;42:95–117.
[75] Merz M. The biology of carbonate precipitation by cyanobacteria. Facies 1992;26:81–101.
[76] Riding R. Cyanobacterial calcification, carbon dioxide concentrating mechanisms, and Proterozoic–Cambrian changes in atmospheric composition. Geobiology 2006;4:299–316.
[77] Binaghi L, Del Borghi A, Lodi A, Converti A, Del Borghi M. Batch and fed-batch uptake of carbon dioxide by *Spirulina platensis*. Process Biochem 2003;38:1341–6.
[78] Ogbonda KH, Aminigo RE, Abu GO. Influence of temperature and pH on biomass production and protein biosynthesis in a putative *Spirulina* sp.. Bioresour Technol 2007;98:2207–11.
[79] Grobbelaar JU. Algal nutrition: mineral nutrition. In: Richmond A, editor. Handbook of microalgal culture: biotechnology and applied phycology. Oxford: Blackwell Publishing Ltd.; 2004. p. 97–115.
[80] de Morais MG, Costa JAV. Biofixation of carbon dioxide by *Spirulina* sp. and *Scenedesmus obliquus* cultivated in a three-stage serial tubular photobioreactor. J Biotechnol 2007;129:439–45.
[81] Sánchez-Luna LD, Bezerra RP, Matsudo MC, Sato S, Converti A, de Carvalho JCM. Influence of pH, temperature, and urea molar flowrate on Arthrospira platensis fed-batch cultivation: a kinetic and thermodynamic approach. Biotechnol Bioeng 2007;96:702–11.
[82] Richmont A, Karg S, Boussiba S. Effect of bicarbonate and carbon dioxide on the competition between *Chlorella vulgaris* and *Spirulina platensis*. Plant Cell Physiol 1982:1411–7.
[83] de Morais M, Costa J. Carbon dioxide fixation by *Chlorella kessleri*, *C. Vulgaris*, *Scenedesmus obliquus* and *Spirulina* sp. cultivated in flasks and vertical tubular photobioreactors. Biotechnol Lett 2007;29:1349–52.
[84] González López CV, Acién Fernández FG, Fernández Sevilla JM, Sánchez Fernández JF, Cerón García MC, Molina Grima E. Utilization of the cyanobacteria *Anabaena* sp. ATCC 33047 in CO_2 removal processes. Bioresour Technol 2009;100:5904–10.
[85] Borowitzka M. Algal biotechnology products and processes—matching science and economics. J Appl Phycol 1992;4:267–79.
[86] Benemann JR. CO_2 mitigation with microalgae systems. Energy Convers Mange 1997;38:S475–9.
[87] Jander F. Massenkultur von Mikroalgen mit pharmazeutisch nutzbaren Inhaltsstoffen unter Verwendung von CO_2 und $NaHCO_3$, gewonnen aus den Abgasen eines Blockheizkraftwerkes [PhD thesis]. Kiel: Christian-Albrechts-Universität Kiel; 2001.
[88] Travieso L, Sànchez EP, Benitez F, Conde JL. Arthospira sp. intensive cultures for food and biogas purification. Biotechnol Lett 1993;15:1091–4.
[89] Deublein D, Steinhauser A. Biogas from waste and renewable resources: an introduction. Weinheim: Willey-VCH; 2008.
[90] Converti A, Oliveira RPS, Torres BR, Lodi A, Zilli M. Biogas production and valorization by means of a two-step biological process. Bioresour Technol 2009;100:5771–6.
[91] Chaiklahan R, Nattayaporn C, Wipawan S, Kalyanee P, Boosya B. Cultivation of *Spirulina platensis* using pig wastewater in a semi-continuous process. J Microbiol Biotechnol 2010;20:609–14.
[92] Laliberté G, Olguin EJ, de la Noüe J. Mass cultivation and wastewater treatment using *Spirulina*. In: Vonshak A, editor. *Spirulina platensis* (*Arthrospira*): Physiology, cell-biology and Biotechnology. London: Taylor & Francis; 2003. p. 159–73.
[93] Olguín EJ, Galicia S, Angulo-Guerrero O, Hernández E. The effect of low light flux and nitrogen deficiency on the chemical composition of *Spirulina* sp. (*Arthrospira*) grown on digested pig waste. Bioresour Technol 2001;77:19–24.
[94] Lincoln EP, Wilkie AC, French BT. Cyanobacterial process for renovating dairy wastewater. Biomass Bioenergy 1996;10:63–8.
[95] Mühling M, Belay A, Whitton BA. Screening *Arthrospira* (*Spirulina*) strains for heterotrophy. J Appl Phycol 2005;17:129–35.
[96] Khoja TM, Whitton BA. Heterotrophy growth of blue-green algae. Arch Microbiol 1971;79:280–2.
[97] Khoja TM, Whitton BA. Heterotrophy growth of filamentous blue-green algae. Eur J Phycol 1975:139–48.
[98] Kenyon CN, Rippka R, Stanier RY. Fatty acid composition and physiological properties of some filamentous blue-green algae. Arch Microbiol 1972;83:216–36.
[99] Singh G, Kothari R, Sharma R, Ramamurthy V. Enhancement of *Spirulina* biomass productivity by a protein hydrolysate. Appl Biochem Biotechnol 1995;50:285–90.
[100] Ogbonna JC, Yoshizawa H, Tanaka H. Treatment of high strength organic wastewater by a mixed culture of photosynthetic microorganisms. J Appl Phycol 2000;12:277–84.
[101] Lodi A, Binaghi L, De Faveri D, Carvalho JCM, Converti A. Fed batch mixotrophic cultivation of *Arthrospira* (*Spirulina*) *platensis* (Cyanophycea) with carbon source pulse feeding. Ann Microbiol 2005;55:181–5.
[102] Haury JF, Spiller H. Fructose uptake on growth and nitrogen fixation by *Anabaena variabilis*. J Bacteriol 1981;147:227–35.
[103] Wolk PC, Shaffer PW. Heterotrophic micro- and macrocultures of a nitrogen-fixing cyanobacterium. Arch Microbiol 1976;110:145–7.
[104] Bottomley PJ, van Baalen C. Charasteristics of heterotrophic growth in the blue-green alga *Nostoc* sp. strain Mac. J Gen Microbiol 1978;107:309–18.
[105] Costa JAV, Cozza KL, Oliveira L, Magagnin G. Different nitrogen sources and growth responses of *Spirulina platensis* in microenvironments. World J Microbiol Biotechnol 2001;17:439–42.
[106] Benemann JR. Production of nitrogen fertilizer with nitrogen-fixing blue-green algae. Enzyme Microb Technol 1979;1:83–90.
[107] Stal LJ. Cyanobacterial mats and stromalites. In: Whitton BA, Potts M, editors. The ecology of cyanobacteria: their diversity in time and space. New York: Kluwer Academic Publishers; 2000. p. 61–120.
[108] Ohmori M, Ohmori K, Strotmann H. Inhibition of nitrate uptake by ammonia in a blue-green alga, *Anabaena cylindrica*. Arch Microbiol 1977;114: 225–9.

[109] Oliver RL, Ganf GG. Freshwaters blooms. In: Whitton BA, Potts M, editors. The ecology of cyanobacteria: their diversity in time and space. New York: Kluwer Academic Publishers; 2000. p. 149–94.
[110] Boussiba S, Gibson J. Ammonia translocation in cyanobacteria. FEMS Microbiol Lett 1991;88:1–14.
[111] Converti A, Scapazzoni S, Lodi A, Carvalho J. Ammonium and urea removal by *Spirulina platensis*. J Ind Microbiol Biotechnol 2006;33:8–16.
[112] Cañizares-Villanueva RO, Domínguez AR, Cruz MS, Ríos-Leal E. Chemical composition of cyanobacteria grown in diluted, aerated swine wastewater. Bioresour Technol 1995;51:111–6.
[113] Meeks JC, Wycoff KL, Chapman JS, Enderlin CS. Regulation of expression of nitrate and dinitrogen assimilation by *Anabaena* species. Appl Environ Microbiol 1983;45:1351–9.
[114] Abeliovich A, Azov Y. Toxicity of ammonia to algae in seawage oxidation ponds. Appl Environ Microbiol 1976;31:801–6.
[115] Belkin S, Boussiba S. Resistance of *Spirulina platensis* to ammonia at high pH values. Plant Cell Physiol 1991;32:953–8.
[116] Belkin S, Boussiba S. High internal pH conveys ammonia resistance in *Spirulina platensis*. Bioresour Technol 1991;38:167–9.
[117] de Godos I, Vargas VA, Blanco S, González MCG, Soto R, García-Encina PA, et al. A comparative evaluation of microalgae for the degradation of piggery wastewater under photosynthetic oxygenation. Bioresour Technol 2010;101:5150–8.
[118] Ayala F, Vargas T. Experiments on *Spirulina* culture on waste-effluent media and at the pilot plant. Hydrobiologia 1987;151–152:91–3.
[119] Piorreck M, Pohl P. Formation of biomass, total protein, chlorophylls, lipids and fatty acids in green and blue-green algae during one growth phase. Phytochemistry 1984;23:217–23.
[120] Sassano CEN, Gioielli LA, Ferreira LS, Rodrigues MS, Sato S, Converti A, et al. Evaluation of the composition of continuously-cultivated *Arthrospira* (*Spirulina*) *platensis* using ammonium chloride as nitrogen source. Biomass Bioenergy 2010;34:1732–8.
[121] Wu JF, Pond WG. Amino acid composition and microbial contamination *Spirulina maxima*, a blue-green alga, grown on the effluent of different fermented animal wastes. Bull Environ Contam Toxicol 1981;27:151–9.
[122] Walach MR, Bazin MJ, Pirt SJ, Balyuzi HHM. Computer control of carbon–nitrogen ratio in *Spirulina platensis*. Biotechnol Bioeng 1987;29:520–8.
[123] Colla LM, Oliveira Reinehr C, Reichert C, Costa JAV. Production of biomass and nutraceutical compounds by *Spirulina platensis* under different temperature and nitrogen regimes. Bioresour Technol 2007;98:1489–93.
[124] Carvalho JCM, Francisco FR, Almeida KA, Sato S, Converti A. Cultivation of *Arthrospira* (*Spirulina*) *platensis* (Cyanophyceae) by fed-batch addition of ammonium chloride at exponentially increasing feeding rates. J Phycol 2004;40:589–97.
[125] Soletto D, Binaghi L, Lodi A, Carvalho JCM, Converti A. Batch and fed-batch cultivations of *Spirulina platensis* using ammonium sulphate and urea as nitrogen sources. Aquaculture 2005;243:217–24.
[126] Sánchez-Luna LD, Converti A, Tonini GC, Sato S, de Carvalho JCM. Continuous and pulse feedings of urea as a nitrogen source in fed-batch cultivation of *Spirulina platensis*. Aquacult Eng 2004;31:237–45.
[127] Danesi EDG, Rangel-Yagui CO, de Carvalho JCM, Sato S. An investigation of effect of replacing nitrate by urea in the growth and production of chlorophyll by *Spirulina platensis*. Biomass Bioenergy 2002;23:261–9.
[128] McKinney R. Environmental pollution control microbiology: a fifty year perspective. New York: CRC Press; 2004.
[129] Seale DB, Boraas ME, Warren GJ. Effects of sodium and phosphate on growth of cyanobacteria. Water Res 1987;21:625–31.
[130] Prieto B, Pardo MA, Garbisu C, Llama MJ, Serra JL. Phosphate uptake by phosphorus-starved cells of the cyanobacterium *Phormidium laminosum*. World J Microbiol Biotechnol 1997;13:699–705.
[131] Corell DL. The role of phosphorus in the eutrophication of receiving waters: a review. J Environ Qual 1998;27:261–6.
[132] Falkner G, Horner F, Simonis W. The regulation of the energy-dependent phosphate uptake by the blue-green alga *Anacystis nidulans*. Planta 1980;149:138–43.
[133] Rigby CH, Craig SR, Budd K. Phosphate uptake by *Synechococcus leopoliensis* (Cyanophyceae): enhancement by calcium ion. J Phycol 1980;16:389–93.
[134] Ritchie RJ, Trautman DA, Larkum AWD. Phosphate uptake in the cyanobacterium Synechococcus R-2 PCC 7942. Plant Cell Physiol 1997;38:1232–41.
[135] Healey FP, Hendzel LL. Effect of phosphorus deficiency on two algae growing in chemostats. J Phycol 1975;11:303–9.
[136] Piccioni RG, Mauzerall DC. Increase effected by calcium ion in the rate of oxygen evolution from preparations of *Phormidium luridum*. Biochim Biophys Acta – Bioenerget 1976;423:605–9.
[137] Utkilen HC. Magnesuim-limited growth of the cyanobacterium *Anacystis nidulans*. J Gen Microbiol 1982;128:1849–62.
[138] Reed RH, Rowell P, Stewart WDP. Characterization of the transport of potassium ions in the cyanobacterium *Anabaena variabilis* Kütz. Eur J Biochem 1981;116:323–30.
[139] Reuter JG, Petersen RR. Micronutrient effects on cyanobacterial growth and physiology. New Zeal J Mar Freshwater Res 1987;21:435–45.
[140] Campbell PM, Smith GD. Transport and accumulation of nickel ions in the cyanobacterium *Anabaena cylindrica*. Arch Biochem Biophys 1986;244:470–7.
[141] Verma SK, Singh SP. Factors regulating copper uptake in a cyanobacterium. Curr Microbiol 1990;21:33–7.
[142] Cavet JS, Borrelly GP, Robinson NJ. Zn, Cu and Co in cyanobacteria: selective control of metal availability. FEMS Microbiol Rev 2003;27:165–81.
[143] Bonilla I, Garcia-González M, Mateo P. Boron requirement in cyanobacteria. Its possible role in the early evolution of photosynthetic organisms. Plant Physiol 1990;94:1554–60.
[144] Patrick R, Crum B, Coles J. Temperature and manganese as determining factors in the presence of diatom or blue-green algae floras in streams. Proc Natl Acad Sci 1969;64:472–8.
[145] McSwain BD, Tsujimoto HY, Arnon DI. Effects of magnesium and chloride ions on light-induced electron transport in membrane fragments from a blue-green alga. Biochim Biophys Acta – Bioenerget 1976;423:313–22.
[146] Schattauer A, Weiland P. Bescreibung ausgewählter Substrate. Handreichung Biogasgewinnung und – nutzung. Gülzow: Fachagentur Nachwachsend Rohrstoffe e.V.; 2006. p. 86–96.
[147] Edwards DR, Daniel TC. Environmental impacts of on-farm poultry waste disposal – a review. Bioresour Technol 1992;41:9–33.
[148] Danalewich JR, Papagiannis TG, Belyea RL, Tumbleson ME, Raskin L. Characterization of dairy waste streams, current treatment practices, and potential for biological nutrient removal. Water Res 1998;32:3555–68.
[149] Roig A, Cayuela ML, Sánchez-Monedero MA. An overview on olive mill wastes and their valorisation methods. Waste Manage 2006;26:960–9.
[150] Inoue N, Taira Y, Emi T, Yamane Y, Kashino Y, Koike H, et al. Acclimation to the growth temperature and the high temperature effects on photosystem II and plasma membranes in a mesophilic cyanobacterium *Synechocystis* sp. PCC6803. Plant Cell Physiol 2001;42:1140–8.
[151] Murata N. Low-temperature effects on cyanobacterial membranes. J Bioenerg Biomembr 1989;21:61–75.
[152] Vonshak A. *Spirulina*: growth, physiology and biochemistry. In: Vonshak A, editor. *Spirulina platensis* (*Arthrospira*): physiology, cell-biology and biotechnology. London: Taylor & Francis; 2003. p. 43–65.
[153] Vonshak A. Outdoor mass production of *Spirulina*: the basic concept. In: Vonshak A, editor. *Spirulina platensis* (*Arthrospira*): physiology, cell-biology and biotechnology. London: Taylor & Francis; 2003. p. 79–99.
[154] Jensen S, Knutsen G. Influence of light and temperature on photoinhibition of photosynthesis *Spirulina platensis*. J Appl Phycol 1993;5:495–504.
[155] Talbot P, Thébault JM, Dauta A, de la Noüe J. A comparative study and mathematical modeling of temperature, light and growth of three microalgae potentially useful for wastewater treatment. Water Res 1991;25:465–72.
[156] Rafiqul IM, Hassan A, Sulebele G, Orosco C, Roustaian P. Influence of temperature on growth, biochemical composition of *Spirulina platensis*, *S. Fusiformis*. Iran Int J Sci 2003;4:97–106.
[157] Talbot P, de la Noüe J. Tertiary treatment of wastewater with *Phormidium bohneri* (Schmidle) under various light and temperature conditions. Water Res 1993;27:153–9.
[158] de Oliveira MACL, Monteiro MPC, Robbs PG, Leite SGF. Growth and chemical composition of *Spirulina maxima* and *Spirulina platensis* biomass at different temperatures. Aquacult Int 1999;7:261–75.
[159] Abu GO, Ogbonda KH, Aminigo E. Optimization studies of biomass production and protein biosynthesis in a *Spirulina* sp. isolated from an oil-polluted flame pit in the Niger Delta. Afr J Biotechnol 2007;6:2550–4.
[160] Tedesco M, Duerr E. Light, temperature and nitrogen starvation effects on the total lipid and fatty acid content and composition of *Spirulina platensis* UTEX 1928. J Appl Phycol 1989;1:201–9.
[161] Robarts RD, Zohary T. Temperature effects on photosynthetic capacity, respiration, and growth rates on bloom-forming cyanobacteria. New Zeal J Mar Freshwater Res 1987;21:391–9.
[162] Chen C-Y, Yeh K-L, Aisyah R, Lee D-J, Chang J-S. Cultivation, photobioreactor design and harvesting of microalgae for biodiesel production: a critical review. Bioresour Technol 2011;102:71–81.
[163] Pandey JP, Tiwari A. Optimization of biomass production of *Spirulina maxima*. J Algal Biomass Utilization 2010;1:20–32.
[164] Agel G, Nultsch W, Rhiel E. Photoinhibition and its wavelength dependence in the cyanobacterium *Anabaena variabilis*. Arch Microbiol 1987;147:370–4.
[165] Murata N, Takahashi S, Nishiyama Y, Allakhverdiev SI. Photoinhibition of photosystem II under environmental stress. Biochim Biophys Acta – Bioenerg 2007;1767:414–21.
[166] Samuelsson G, Lönneborg A, Lönneborg A, Gustafsson P, Öquist G. The susceptibility of photosynthesis to photoinhibition and the capacity of recovery in high and low light grown cyanobacteria, *Anacystis nidulans*. Plant Physiol 1987;83:438–41.
[167] Vonshak A, Chanawongse L, Bunnag B, Tanticharoen M. Light acclimation and photoinhibition in three *Spirulina platensis* (cyanobacteria) isolates. J Appl Phycol 1996;8:35–40.
[168] Vonshak A, Guy R. Photoadaptation, photoinhibition and productivity in the blue-green alga, *Spirulina platensis* grown outdoors. Plant Cell Environ 1992;15:613–6.
[169] Larsdotter K. Wastewater treatment with microalgae – a literature review. Vatten 2006;62:31–8.
[170] Vonshak A, Abeliovich A, Boussiba S, Arad S, Richmond A. Production of *Spirulina* biomass: effects of environmental factors and population density. Biomass 1982;2:175–85.
[171] Cohen Z, Vonshak A, Richmond A. Fatty acid composition of *Spirulina* strains grown under various environmental conditions. Phytochemistry 1987;26:2255–8.
[172] Gantar M, Obreht Z, Dalmacija B. Nutrient removal and algal succession during the growth of *Spirulina platensis* and *Scenedesmus quadricauda* on swine wastewater. Bioresour Technol 1991;36:167–71.

[173] de la Noüe J, Laliberté G, Proulx D. Algae and waste water. J Appl Phycol 1992;4:247–54.
[174] Cañizares-Villanueva RO, Ramos A, Corona AI, Monroy O, de la Torre M, Gomez-Lojero C, et al. *Phormidium* treatment of anaerobically treated swine wastewater. Water Res 1994;28:1891–5.
[175] European Environment Agency. EEA Signals. Key Environmental issues facing Europe; 2009.
[176] Zilberman D, Templeton SR, Khanna M. Agriculture and the environment: an economic perspective with implications for nutrition. Food Policy 1999;24:211–29.
[177] Lacroix A, Beaudoin N, Makowski D. Agricultural water nonpoint pollution control under uncertainty and climate variability. Ecol Econ 2005;53:115–27.
[178] Bernet N, Béline F. Challenges and innovations on biological treatment of livestock effluents. Bioresour Technol 2009;100:5431–6.
[179] Imbeah M. Composting piggery waste: a review. Bioresour Technol 1998;63:197–203.
[180] Arvanitoyanis IS, Kassaveti A, Ladas D. Food waste treatment methologies. In: Arvanitoyanis IS, editor. Waste management for the food industries. Burlington: Elsevier; 2008. p. 345–410.
[181] Burton CH. The potential contribution of separation technologies to the management of livestock manure. Livestock Sci 2007;112:208–16.
[182] Burton CH, Turner C. Manure management—treatment strategies for sustainable agriculture. 2nd ed. Wrest Park: Silsoe Research Institute; 2003.
[183] Westerman PW, Bicudo JR. Management considerations for organic waste use in agriculture. Bioresour Technol 2005;96:215–21.
[184] Park J, Jin H-F, Lim B-R, Park K-Y, Lee K. Ammonia removal from anaerobic digestion effluent of livestock waste using green alga *Scenedesmus* sp.. Bioresour Technol 2010;101:8649–57.
[185] Travieso L, Benítez F, Sánchez E, Borja R, Martín A, Colmenarejo MF. Batch mixed culture of *Chlorella vulgaris* using settled and diluted piggery waste. Ecol Eng 2006;28:158–65.
[186] de la Noüe J, de Pauw N. The potential of microalgal biotechnology: a review of production and uses of microalgae. Biotechnol Adv 1988;6:725–70.
[187] White JS, Bayley SE, Curtis PJ. Sediment storage of phosphorus in a northern prairie wetland receiving municipal and agro-industrial wastewater. Ecol Eng 1999;14:127–38.
[188] de Pauw N, Van Vaerenbergh E. Microalgal wastewater treatment-systems: potentials and limits. In: Ghetti PF, editor. Phytodepuration and employement of the biomass produced. Reggio Emilia; 1983. p. 211–87.
[189] Olguín EJ, Galicia S, Mercado G, Pérez TJ. Annual productivity of *Spirulina* (*Arthrospira*) and nutrient removal in a pig recycling process under tropical conditions. J Appl Phycol 2003;15:249–57.
[190] Hill DT, Bolte JP. Methane production from low solid concentration liquid swine waste using conventional anaerobic fermentation. Bioresour Technol 2000;74:241–7.
[191] Demirel B, Yenigun O, Onay TT. Anaerobic treatment of dairy wastewaters: a review. Process Biochem 2005;40:2583–95.
[192] Chung P, Pond WG, Kingsbury JM, Walker Jr EF, Krook L. Production and nutritive value of *Arthrospira platensis*, a spiral blue-green alga grown on swine wastes. J Anim Sci 1978;47:319–30.
[193] Cañizares RO, Rivas L, Montes C, Domínguez AR, Travieso L, Benitez F. Aerated swine-wastewater treatment with K-carrageenan-immobilized *Spirulina maxima*. Bioresour Technol 1994;47:89–91.
[194] Olguín EJ, Hernández B, Araus A, Camacho R, González R, Ramírez ME, et al. Simultaneous high-biomass protein production and nutrient removal using *Spirulina maxima* in sea water supplemented with anaerobic effluents. World J Microbiol Biotechnol 1994;10:576–8.
[195] FAOSTAT. <http://faostat.fao.org/>.
[196] Martinez J, Dabert P, Barrington S, Burton C. Livestock waste treatment systems for environmental quality, food safety, and sustainability. Bioresour Technol 2009;100:5527–36.
[197] Mitchell SA, Richmond A. Optimization of a growth medium for *Spirulina* based on cattle waste. Biol Waste 1988;25:41–50.
[198] Oron G, Shelef G, Levi A. Growth of *Spirulina maxima* on cow-manure wastes. Biotechnol Bioeng 1979;21:2169–73.
[199] Salminen E, Rintala J. Anaerobic digestion of organic solid poultry slaughterhouse waste – a review. Bioresour Technol 2002;83:13–26.
[200] Mahadevaswamy M, Venkataraman LV. Bioconversion of poultry droppings for biogas and algal production. Agr Waste 1986;18:93–101.
[201] Orhon D, Görgün E, Germirli F, Artan N. Biological treatability of dairy wastewaters. Water Res 1993;27:625–33.
[202] Perle M, Kimchie S, Shelef G. Some biochemical aspects of the anaerobic degradation of dairy wastewater. Water Res 1995;29:1549–54.
[203] Sarkar B, Chakrabarti PP, Vijaykumar A, Kale V. Wastewater treatment in dairy industries – possibility of reuse. Desalination 2006;195:141–52.
[204] Vidal G, Carvalho A, Méndez R, Lema JM. Influence of the content in fats and proteins on the anaerobic biodegradability of dairy wastewaters. Bioresour Technol 2000;74:231–9.
[205] Gelegenis J, Georgakakis D, Angelidaki I, Mavris V. Optimization of biogas production by co-digesting whey with diluted poultry manure. Renew Energy 2007;32:2147–60.
[206] Blier R, Laliberté G, de la Noüe J. Tertiary treatment of cheese factory anaerobic effluent with *Phormidium bohneri* and *Micractinum pusillum*. Bioresour Technol 1995;52:151–5.
[207] Blier R, Laliberté G, de la Noüe J. Production of the cyanobacterium *Phormidium bohneri* in parallel with epuration of a dairy anaerobic effluent. Process Biochem 1996;31:587–93.
[208] Phang SM, Miah MS, Yeoh BG, Hashim MA. *Spirulina* cultivation in digested sago starch factory wastewater. J Appl Phycol 2000;12:395–400.