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Solving Nuisance Cyanobacteria Eutrophication Through Biotechnology

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Abstract: Management of nutrient inputs and usage of nuisance biomass as feedstock for bioenergy may be the solution of coastal lagoons eutrophication. We studied the species composition, photosynthetic pigments (Chl-a and Chl-c) and performance (OJIP-kinetics and JIP-test parameters), biochemistry (lipids and carbohydrates composition), and hydrogen production potential of *Limnoraphis* (*Lyngbya*) nuisance biomass collected from Lafri lagoon (1.24 km^2) in Greece. The results showed that the removal of algal biomass from Lafri lagoon before its sedimentation, characterized by low Fv/Fm (0.42) and PItotal (2.67) values, and transfer of this in a simple, closed bioreactor, has the potential to produce hydrogen (H_2), a renewable CO_2-neutral energy that can directly be converted into electricity. The free carbohydrates of the lagoon water and that from the algal cells (42g glucose analogs per m^3) could be also transferred to alcohols (biofuels), while the rest of the biomass could be used as organic fertilizer. The total lipid content (2.51%) of dry biomass composed primarily by palmitic acid was low. However, the presence of eicosapentaenoic (3.5%), and docosahexaenoic (1.7%), polyunsaturated fatty acids is worth mentioning. By harvesting and conversion of this coastal lagoon nuisance algal biomass to energy or other products, one could improve its water quality and, therefore, biodiversity and fish production; that is a sustainable solution of eutrophication necessary for the ongoing climatic change.

Keywords: coastal lagoon; Lyngbya; hydrogen production; algal biotechnology; climatic change

1. Introduction

The use of fertilisers in agriculture [1] and the atmospheric precipitations [2] have increased nitrate and phosphate concentrations in rivers, lakes, and coastal lagoons in the last decades [3]. As a result, undesirable algal growth (bloom), frequently cyanobacteria have been observed [4,5], a phenomenon that may further be promoted by global warming [6,7]. The algal blooms often cover the surface and increase the turbidity of waters, inhibiting the solar irradiance from supplying energy to the benthic zone and the primary producers of photosynthesis, which then disappear [8,9]. When the algal material dies and the decomposition process starts to induce oxygen depletion (anoxia), and in turn may lead to the death of aquatic lives and to a range of other effects that reduces biodiversity [10].

Excessive algal growth (blooms) in several coastal lagoons due to an excess of nutrient loads is seen as a problem by many stakeholders, such as fisheries, municipal administration, and nature conservationists [11,12]. Although efforts should be undertaken in reducing nutrient loads, to avoid long recovery periods the floating algal biomass should be harvested [13]; it could also help to reduce nutrient water concentration, especially phosphate and nitrogen, which in turn, would improve water quality and minimize eutrophication [10,14,15]. The control of algal blooms could help to ensure adequate oxygen levels for the water fauna and maintain biodiversity and fish production, thereby keeping the ecosystem functioning and productive [16]. As far as is concerned, the harvested algal biomass is important to further explore the existing technologies as for example composting [17], bioethanol [18] and biomethane [19] production which economic feasibility has not yet been confirmed [20].

The third generation of biofuels, based on improvements in the production of biomass, is taking advantage of specially engineered energy crops such as algae which are harvested or cultivated to act as a low-cost, high-energy and entirely renewable feedstock. They have been successfully used as feedstock in biohydrogen (H_2) production recently, because of their rich carbohydrate content [21–23]. Algae can fix CO_2 in the form of carbohydrates in the cytoplasm and in the form of lipids in cell membranes [24]. Carbohydrates are stored in the form of glycogen in cyanobacteria, starch in green and red algae, and β-glucans in brown algae [25,26]. The use of algae could help to reduce the dependency on ground-based biomass, especially energy crops, and help diversify the possible input materials for bioenergy production [27,28].

It is known that molecular hydrogen will be the optimal energy carrier of the future and contribute to the growth of the world's economy by facilitating a stable supply of energy. Hydrogen combustion yields only water thus reducing emissions of carbon dioxide in the atmosphere [29]. At present, most of the world's hydrogen is produced by reforming fossil fuels, which is accompanied by the release of carbon into the environment. However, hydrogen might be produced from non-fossil renewable resources. In this respect, the light-induced conversion of water into hydrogen and oxygen is preferable. One of the prospective ways of obtaining hydrogen from water at the expense of solar energy is to use photosynthetic microorganisms capable of hydrogen production, such as cyanobacteria and green algae [21,30–32].

The aim of this paper was to study the species' composition, photosynthetic pigments (Chl-a and Chl-c) and performance (OJIP-kinetics and JIP-test parameters), biochemistry (lipids and carbohydrates composition), and hydrogen production potential of *Limnoraphis* (*Lyngbya*) biomass collected from a hyper-eutrophicated lagoon belonging in the Porto-Lagos Lagoon and Vistonis Lake complex, Thrace Region, in Greece. The ultimate aim was to propose a eutrophication solution through biotechnology that converts the nuisance algal biomass to renewable and CO_2 neutral energy in the form of hydrogen and biofuels.

2. Materials and Methods

2.1. Sample Collection

Microalgal bloom biomass and water collected from totally six different sites of the Lafri lagoon, three on 11 July 2016, and three on 21 July 2016. The suspensions were transferred to the laboratory in three each time closed plastic containers within an hour after the sampling. The material from each of six plastic containers (replicates) was divided into parts for the analyses. The main quantity was kept in a cultivation room (21–22 °C, 40–60 μmol photons m^{-2} s^{-1}, 14 h light per day) for taxonomical observations and pigment and photosynthetic performance analyses. The rest from different replicates of each sampling effort was mixed and packed into two each time, 1.5 L plastic bottles and sent within 24 h to a cooler box using express mail service to the University of Crete for carbohydrate analyses and biotechnological applications for hydrogen production.

2.2. Species Composition

Fresh bloom biomass samples were examined under a light microscope (Eclipse 50i, equipped with a DS-Fi1 power camera, 5 megapixel CCD, Nikon, Tokyo, Japan) and species were identified using the taxonomic suggestions [33,34].

The traditional oscillatorialean genus *Lyngbya* belonging to the Section 3 of the cyanobacteria [35] was identified using the taxonomic keys [36]. However, with a polyphasic evaluation (molecular, morphological and ecological data) this genus was found to be polyphyletic and all planktic and aerotopated *Lyngbya* species have been transferred in the new genus *Limnoraphis* after re-classification according to Komárek et al. [33,37], for taxonomic revision of the genus see Komárek.

2.3. Fluorescence Induction Measurements of Chlorophyll a

The chlorophyll fluorescence (OJIP) transient of algal biomass was measured by a double-modulation fluorometer (FL 3500/F, PSI, Drasov, Czech Republic) in the 10 µs to 1 s time region using a logarithmic time scale. Samples were dark incubated for 15 min before the measurements were taken. The origin of the fluorescence induction curve is a base value, designated as the minimum fluorescence yield (F0; measured after 20 µs). Fast fluorescence induction curves were normalized on both F0 and Fm to better illustrate the reduction status of the J (Vj) and I (Vi) transients. From fluorescence data, key JIP-test parameters have been estimated [38].

2.4. Pigments, Dry Weight, and Organic Matter Estimations

Chl a and Chl c content of algal biomass was measured according to the methodology [39]. A UV-1800 spectrophotometer from Shimadzu Corporation (Kyoto, Japan) was employed. The fresh material was rinsed in tap water, and all epiphytes and debris cleaned thoroughly and put on absorbent paper to remove excess water to assess wet biomass (WB) to the nearest milligram. Drying of wet material to constant biomass at 50 °C allowed the determination of dry biomass (DB). Organic matter (OM) was estimated as the loss upon oxidation of the DB in a muffle furnace at 400 °C for 12 h.

2.5. Fatty Acid Analysis and Methyl Esters Preparation

For fatty acid analysis, lipids were extracted from about 2.5 g lyophilized, homogenized dry material [40], twofold extraction with chloroform-methanol, 1:2 *v/v* and was further prepared for fatty acid analysis according to the procedure [41]. Methyl esters (FAMEs) were prepared by hot saponification (100 °C for 1 h) with 0.5 N NaOH and methylation with 14% boron trifluoride-methanol [42]. FAMEs were extracted using three times 1 mL volumes of iso-octane after quenching the cooled reaction mixtures with saturated NaCl solution (5 mL). From the organic fraction, iso-octane was removed using a steam of dry argon, and the samples were stored at −28 °C until analysis. At the day of analysis 50 µL of iso-octane were used for reconstitution of each sample. One (1.0) µL of this final solution was taken for the injection of the sample in the gas chromatographic apparatus.

2.6. Gas Chromatographic (GC-FID) Analysis of Fatty Acid Methyl Esters

Fatty acid methyl esters were analyzed by a 5890-Series II gas chromatograph (Hewlett Packard, Palo Alto, CA, USA) equipped with a flame ionization detector, capillary column (SGE-BPX 70; 50 m length, 0.22 mm diameter, 0.25 µm film thickness), and a split/splitless capillary inlet (19251 A). Helium was used as carrier and nitrogen as auxiliary gas with a flow rate of 36 mL/min. Flow rates of hydrogen and compressed air for the FID were 30 and 330 mL/min, respectively. Samples were injected in a split mode, using a split ratio of 1:100, and the volume injected was 1 µL. The operating conditions were 320 °C injection port, 300 °C flame ionization detector, and column temperature was programmed to hold at 50 °C for 2 min, then increases linearly, with a rate of 20 °C/min, to 150 °C followed by a 1.5 °C/min increase to 230 °C with 3 min final time at 230 °C. For peak identification,

solutions of reference substances (Supelco 18919-1 amp FAME mix C4-C24 and FAMEs purchased from Sigma-Aldrich, St. Louis, MO, USA) were analyzed under the same conditions, and their retention times (RT) and chromatograms were compared to those of the samples. The contribution of each identified compound was expressed as a percentage (%) of its peak area to the total area of all peaks eluted in each chromatogram. The precision of the results was always better than ±5%.

2.7. Carbohydrates Estimation

For carbohydrate extraction, samples were centrifuged in 1500 g for 5 min in order to separate cells (pellet) from the lagoon water. The pellet was solubilized in 2 mL of dH_2O and exposed to microwave energy (~600 W) three times, for 10 sec every time, with intermediate ice breaks to return them to room temperature in order to divide the soluble intracellular carbohydrate fraction from the insoluble one. Under these conditions, parallel to the free carbohydrates in lagoon water, we have two additional separate carbohydrate samples: the solubilized cell carbohydrates and the insoluble cell carbohydrates that have been polymerized. For the quantification of carbohydrates, we used the Molisch test. The Molisch test is a sensitive chemical test for the presence of carbohydrates, and it involves dehydrating carbohydrates with sulfuric acid to produce an aldehyde, which condenses with two molecules of α-naphthol, resulting in a red- or purple-colored compound. In 100 μl of each sample, which contained carbohydrates, 1 mL sulfuric acid (H_2SO_4) and 10 μL a-naphthol (3% *w/v* in ethanol) were added, and it was incubated at room temperature for 20 h. The samples have a violet color, which is proportional to the concentration of carbohydrates. Quantification is done by measuring the absorption at 565 nm, which is represented in μmol/L based on the reference curve designed with known concentrations of glucose.

2.8. Experimental Procedure for Hydrogen (H_2) Production

In all experiments we used 50 mL lagoon water with the algal biomass (0.5 g WB/50 mL) and 250 mg glucose into 125 mL hermitically sealed bottles (diameter 5 cm, height 9.5 cm) with septum. The rest of the bottle volume was atmospheric air at the beginning of the experiment. The experiments were performed in a temperature-controlled chamber (30 °C) at a light intensity of approximately 100 μmol photons m^{-2} s^{-1}. Sampling took place daily, at the same time, using gas-tight needles without opening the bottles. The cultures were manually shaken to achieve complete solubility of the microorganisms in the culture medium before each sampling. Although the carbohydrates synthesized by photosynthesis and exist in the lagoon samples can effectively be utilized for H_2 production [43], in this treatment used additional glucose to check the possibility of eutrophic cyanobacteria, in relative short incubation periods, to produce continuously H_2.

2.9. Gas Chromatographic (GC-TCD) Measurements of H_2, O_2, and Methane

Hydrogen, oxygen, and methane measurements were made utilizing gas chromatography with a thermal conductivity detector (GC-TCD) (GC 2010 Plus, Shimadzu, Kyoto, Japan). To separate H_2 and O_2, argon was used as the carrier gas under pressure of five bars and at oven temperature of 120 °C. The column used was a capillary Vici Metronics MC (Poulsbo, WA, USA) with length 30 m (diameter: 0.53 mm) and film thickness 20 μm. The temperature of TCD was set at 200 °C for the detector and 180 °C for the injector. The quantification of hydrogen, oxygen, and methane was done by injecting known quantities in the GC-TCD.

3. Statistical Analysis

Six replicates (n = 6) were used for the fluorescence induction measurements of Chlorophyll a, and fatty acids estimations. Two replicates (n = 2) were used for the carbohydrates estimation and the hydrogen, oxygen, and methane production. The analysis of each replicate was repeated three times.

Data analysis and presentation were carried out using and the STATISTICA v.7.1 (StatSoft, Tulsa, OK, USA) and ORIGIN v. 9 (OriginLab Corporation, Northampton, MA, USA) software packages, respectively.

4. Results

4.1. Species Composition

The biomass from the Lafri lagoon consisted mainly of cyanobacteria comprised more than 95% of the total bloom biomass. A total of four cyanobacterial taxa were identified: *Limnoraphis* (*Lyngbya*) cf. *robusta*, *Limnoraphis* (*Lyngbya*) cf. *birgei*, *Microcystis aeruginosa* and *Anabaena* sp. *Limnoraphis* (*Lyngbya*) cf. *robusta* made almost all the cyanobacterial biomass (>95%). Filaments showed a wide spectrum of coloration changing from blue-green-green to orange-brownish when carotenoids cover the other pigments. The brownish color is well recognizable in massive water blooms and floating mats of the species (Figure 1A). Morphology of the bloom-forming cyanobacterium and dimensions are presented in Figure 1B–F. Associated to the cyanobacteria, a diverse community of epiphytic and benthic diatoms was identified that consisted of *Nitzschia*, *Navicula*, *Synedra*, *Cocconeis*, *Epithemia*, *Cymbella*, *Achnanthes*, and *Pleurosigma*. In addition, the planktic diatoms *Biddulphia obtusa* (also a facultative epiphyte) and *Cyclotella* sp. were present, together with cryptophytes and prasinophytes.

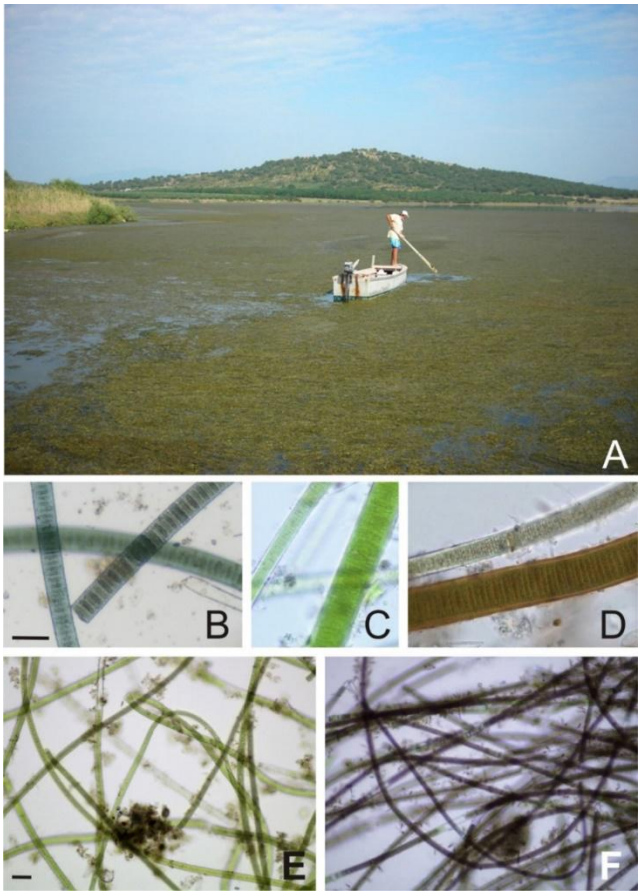

Figure 1. (**A**): An overview of *Limnoraphis* (*Lyngbya*) heavy bloom in Lafri coastal lagoon, in June. (**B**–**F**): Light micrographs of *Limnoraphis* (*Lyngbya*) filament morphology and coloration in the bloom biomass. Scale bars 20 μm for (**B**–**D**) and 30 μm for (**E**,**F**).

4.2. Chlorophyll Fluorescence-Pigment Analysis-Water Content

The fast phase of fluorescence induction kinetics from the eutrophicated algal biomass represents the health situation of the algal biomass. The high variability in the induction curves from different lagoon places and the variance of the estimated JIP-test parameters (Figure 2) showed a physiologically heterogeneous or different stressed material. When the decomposition process started, the mean value of maximum quantum yield (Fv/Fm) from the eutrophication biomass was about 0.42. Less variation in the algal biomass was found in pigments (Chl a = 0.52 ± 0.05 SE mg/L, Chl c = 0.02 ± 0.003 SE mg/L), water (76.1% of WB ± 2.1 SE), and organic matter (46% of DB ± 4 SE) content.

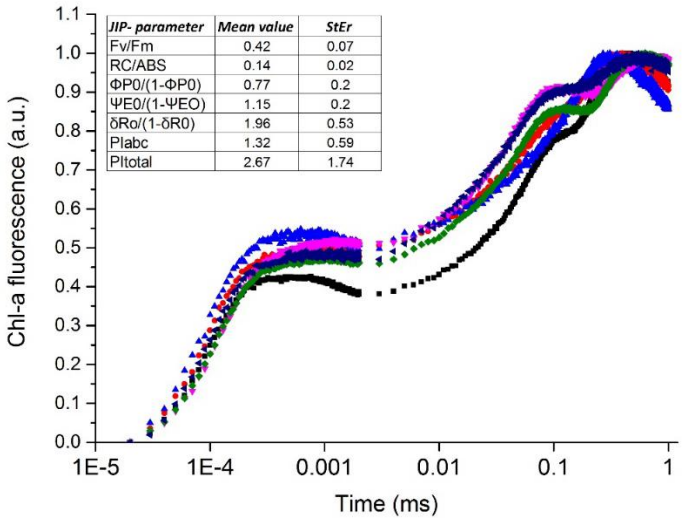

Figure 2. Rapid chlorophyll fluorescence induction curves (n = 6 replicates are shown in different colors) of bloom biomass and mean values and Standard E rror (±) of the most important JIP-test parameters. F_0: Minimal fluorescence intensity when all PSII reaction centers (RCs) are open; F_m: Maximal fluorescence intensity when all PSII RCs are closed; F_v/F_m: Maximum quantum yield of PSII; RC/ABS: Density of RCs; ΦP0 = TR_o/ABS: Maximum quantum yield for primary photochemistry (at t = 0); ΨE0 = ET_0/TR_0: Probability that a trapped exciton moves an electron into the electron transport chain beyond QA (at t = 0); δR0 = RE_0/ET_0: Probability with which an electron from the intersystem electron carriers is transferred to reduce end electron acceptors at the PSI acceptor side (RE); PI_{abc}: Performance index for energy conservation from photons absorbed by PSII antenna, to the reduction of QB; PI_{total}: Performance index for energy conservation from photons absorbed by PSII antenna, until the reduction of PSI acceptors.

4.3. Lipids

The total lipid content was 2.51% ± 0.42 SE of dry biomass composed primarily by C16:0 (25.02% ± 2 SE) and C16:1 n–9 (14.7 ± 4.59 SE) fatty acids (Figure 3). The ratio of saturated to unsaturated lipids was 0.96 ±0.16 SE.

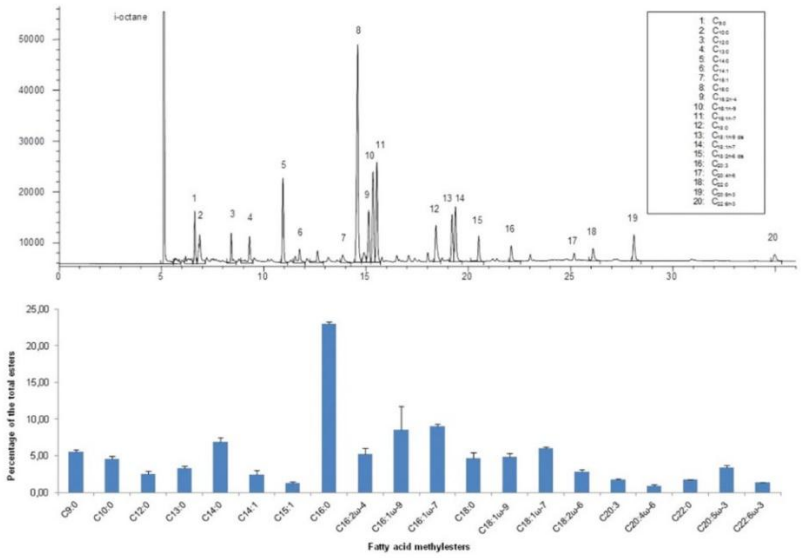

Figure 3. Composition and content (%) of fatty acids in algal biomass mean values (n = 6; ±SE).

4.4. Carbohydrates

The total carbohydrate content of the algal biomass and lagoon water was relatively high (Figure 4). There were 42 g (glucose analogs) per m^3. Of these, 19.5 g/m^3 (47%) were freely dissolved in water, 11.1 g/m^3 (27%) were cell carbohydrates solubilized, and the other 11.0 g/m^3 (26%) were polymerized in cell structures (mainly as glycogen, starch, and cell walls).

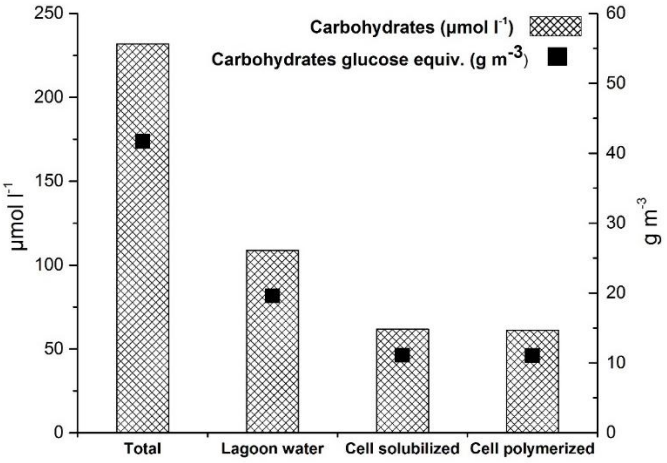

Figure 4. Mean values (n = 2; three repetition of analysis in each sample) of content of carbohydrates free dissolved in lagoon water, solubilized in cells and polymerized in cell structures, measured as glucose analogs.

4.5. H_2 Production

Incubation of algal biomass and lagoon water in a closed system showed that the culture reduced the O_2 concentration gradually and produced a relatively high rate of H_2, reaching in nine days the amount of 20 L H_2 Kg^{-1} biomass precipitate (Figures 5 and 6). Additionally, traces of methane were measured (Figure 5). It should be understood that the rate of H_2 production shown in Figure 6 is the coincidence of a series of H_2 production mechanisms, with participant nitrogenases and hydrogenases of different cyanobacteria and green algae, which form the eutrophic biomass of our example (in Lafri lagoon). The results of Figure 5 show clearly that after the establishment of anoxic conditions (day 2), the rate of hydrogen production remains continuously constant (about 2.8 L H_2 $Kg^{-1}day^{-1}$).

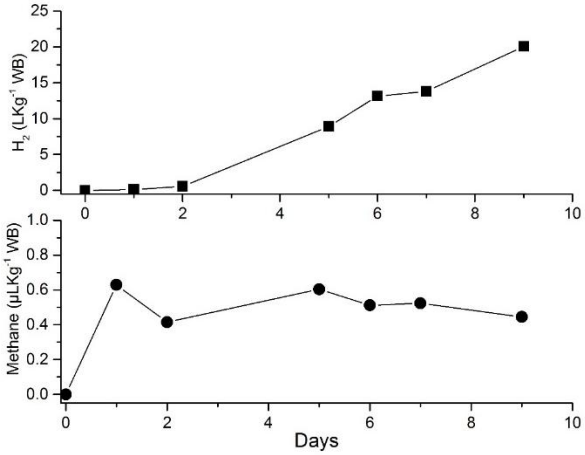

Figure 5. Kinetics of hydrogen and methane production in a closed system mean values (n = 2; three repetition of analysis in each sample). WB = wet biomass.

Additionally to the above, GC-TCD profiles from Figure 6 shows clearly a reduction of N_2 peak during the incubation time and that confirm the N_2 fixation of the microalgal biomass that consists mainly from cyanobacteria. This is also an indication for the participation also of nitrogenases in the H_2 production.

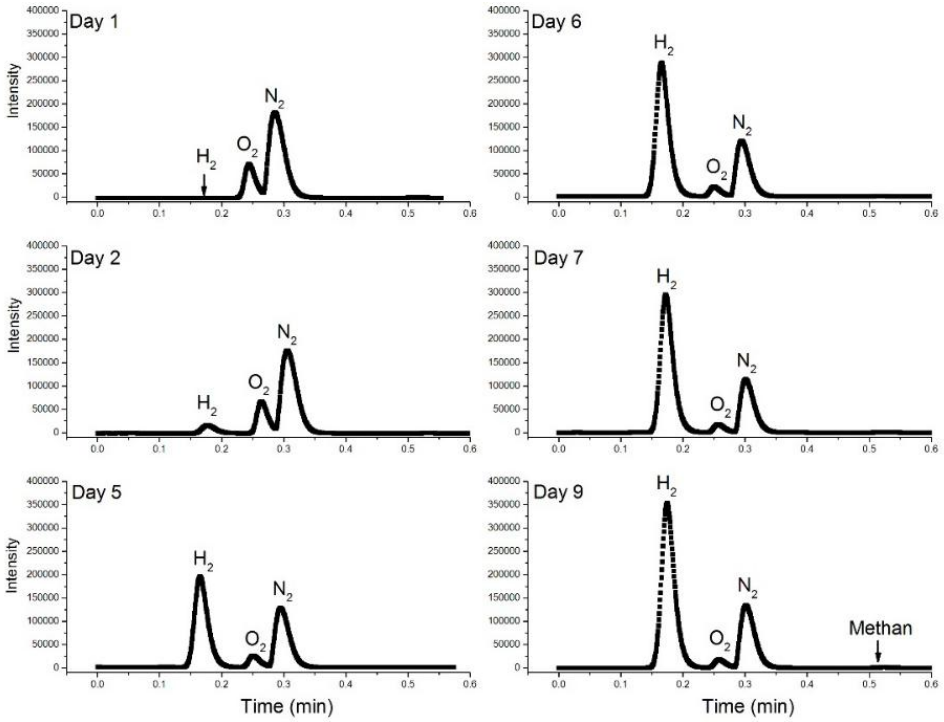

Figure 6. GC-TCD profiles during the incubation time – Changes in H_2, O_2 and N_2.

5. Discussion

Intensive algal blooms in coastal lagoons are indicative of the eutrophication phenomenon, which is related to water nutrient levels. The most cases of eutrophication in coastal lagoons are the result of cyanobacteria blooms, like our paradigm in the Lafri Lagoon. These cyanobacterial blooms seem to prevent optimal ecosystem function, as indicated by water quality assessments [44] and the relatively low fish production [45]. The cyanobacterial biomass tolerates high light intensities and performs photoadaptation to high light conditions [46]. The variability of the fluorescence induction measurements showed a physiologically heterogeneous or differently stressed material possibly because of the onset of decomposition procedure and sedimentation. This is also in agreement with relatively low values of maximum quantum yield (Fv/Fm = 0.42) and of photosynthetic performance indices (PI_{abc} = 0.32, PI_{total} = 2.67) of algal biomass in the Lafri lagoon (Figure 2).

The sedimentation of the algae may enhance the establishment of anoxic conditions in the lagoon water; this may also be because of the high H_2 production, which expels the rest of oxygen from the water phase [47]. Our results in Figures 5 and 6 indicate that the cyanobacterial bloom biomass had the ability to establish hypoxic conditions and to produce H_2 in a closed illuminated system that mimics natural conditions.

Two mechanisms have been proposed in green algae for H_2 production. The first is the photosystem II (PSII) dependent pathway, which under anoxic conditions transfers electrons from water through photosystem II (PSII) and photosystem I (PSI) to ferredoxin (Fd), and at the end to hydrogenase that reduces protons to H_2 [30,48]. The second pathway is the PSII independent pathway that depends on the metabolic oxidation of organic compounds (glucose and other carbohydrates) coupled to PSI through the plastoquinone (PQ) pool and results in H_2 production [49]. Except for the above photobiological hydrogen production pathways, there is also another known as dark fermentation. The majority of microbial hydrogen production through dark fermentation is driven by the anaerobic metabolism of pyruvate, formed during the catabolism of various organic substances [50,51].

In cyanobacteria, three different hydrogenase enzymes have been identified: a nitrogenase, a reversible bidirectional hydrogenase, and an uptake hydrogenase [31,52]. Nitrogenase produces hydrogen as a side reaction while fixing N_2. Our results (Figure 6) clearly show that the increase in H_2 production is combined with N_2 fixation, which confirms (in our paradigm) the participation also of nitrogenases in the procedure of H_2 production from the particular algal bloom biomass.

Reprogramming of the above eutrophication procedure could be a biotechnological application schematically presented in Figure 7. The absolute negative impact of eutrophication consists of different steps, some of which, if we observe them individually, have a positive impact and could be important for biotechnological applications: 1) The excess amount of nutrients and fertilizers that flush from land to lagoon will be transformed by the algae into massive algal biomass that biofilters and improves the water quality of the lagoon [15,53]. 2) Because of the hypertrophication, there is no limitation of nutrients that lead to a high production of organic matter in algal biomass in the form of carbohydrates (sugars) and lipids (Figures 4 and 5) that could be easily converted into biofuels.

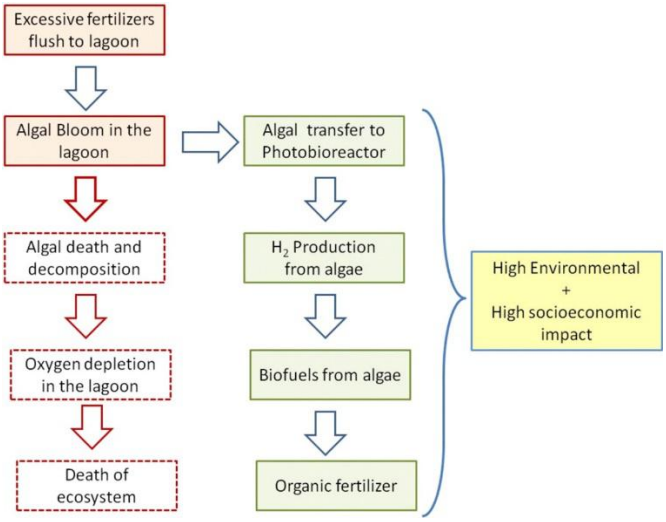

Figure 7. Simplified model based on the separation of eutrophication into two distinct steps (before and after the algal sedimentation), and transferring the second step process to a bioreactor, we can protect the water quality and biodiversity of the lagoons and keep the socioeconomic impact on fish production and community positive. In addition, the environmental impacts will find a sustainable solution through a "smart" biotechnological application that leads to high yield production of hydrogen and biofuels.

These two steps of the eutrophication procedure are very important for the water quality but also for biotechnological applications, especially for the production of 3rd generation biofuels, without any cost.

The last step of algal blooms is the decomposition and sedimentation of the algal material. Biomass sedimentation in the lagoon establishes anoxic conditions that could, combined with the high concentration of organic matter, induce methane and/or H_2 production (Figures 5 and 6). This decomposition process may deplete oxygen (establishment of anoxic conditions), and in turn may lead to fish kills and in a range of other effects reducing biodiversity [10]. In order to overcome this negative impact of eutrophication, we propose the removal of the algae from the lagoon before the onset of sedimentation and transferring them to simple, closed reactors for H_2 and biofuel production according to the simplified scheme of Figure 7.

Indeed, the total carbohydrate content estimated in this study (Figure 4) in water and in algal biomass was relatively high and in the same range of other published works [23].

Another promising downstream pathway from algal biomass is the lipid extraction for biodiesel. The technology of turning algae lipids to biodiesel is, in principle, relatively easy, but the algal biomass should contain high quantities of fatty acids, something that does not happen in this particular case of our paradigm. As far as the fatty acid composition of *Limnoraphis* biomass is concerned, it showed high levels of saturated fatty acids (51.5 ± 3.9% of the total), whereas the levels of mono-unsaturated and poly-unsaturated fatty acids (PUFA) were 48.5 (±2.6%) of the total (Figure 3). Similar results were also obtained [54,55]. Moreover, in agreement with other studies [54,56] the palmitic acid (PA:C16:0) has been documented as the most prevalent fatty acid of cyanobacteria biomass. The presence of eicosapentaenoic (C20:5ω–3), EPA 3.5 ± 0.9%, and docosahexaenoic (C22:6ω–3), DHA 1.7 ± 0.3%, polyunsaturated fatty acids (PUFAs) in the particular algal biomass is worth mentioning. Although the major sources of both acids EPA and DHA, on a worldwide basis, are still marine fish, microalgae and cyanobacteria, exhibiting competitive advantages as sources of PUFAs because fish have typically lower contents (on a mass basis), are subject to seasonal variations in fatty acid profile, and may be significantly contaminated with heavy metals owing to pollution throughout the food chain [57,58]. Besides confirming the appropriateness of some cyanobacteria for wide use as feed in the aquaculture

industry, they have been used for functional food formulation aimed at therapeutically enhanced and nutritional features [59]. Additionally, biomass rest could be excellent organic fertilizer, mainly because of their N_2 fixation procedure [60].

Since harmful algal blooms also have a wide range of detrimental impacts through the production of toxins, care should also be undertaken in the case of the Lafri lagoon. The toxicity of species of genus *Limnoraphis*, between others the synthesis of the liver toxin microcystin seems to depend on the strain and environmental triggers [33,61], and therefore the evaluation of biomass for toxins has to be determined [62]. In general, the potential applications of nuisance microalgae blooms is still largely unconsidered and unexplored [63].

In conclusion, by separating the natural process of eutrophication into two distinct steps (before and after the algal sedimentation), and transferring the second step process to a bioreactor, we can protect the water quality and biodiversity of the lagoons and keep the socioeconomic impact on fish production and community positive. Therefore, the environmental impacts of such disentanglement of eutrophication phenomenon will find a sustainable solution through a biotechnological application that leads to high yield production of H_2 and biofuels in dependence on the composition of the eutrophic algal biomass.

Author Contributions: S.O. and K.K. conceived and designed research, analyzed and interpreted data, wrote the article; N.S. analyzed and interpreted the lipids data and contributed to the writing of the article; A.Par., M.S.M., S.Z., P.P., M.P., O.N. and A.P. conducted experiments and analyzed data; A.Pap. collected the material; M.M-G. interpreted the species composition analysis and contributed in a manuscript review. All authors read and approved the manuscript.

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