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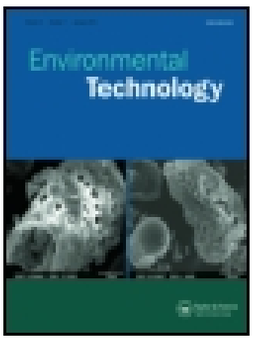

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Evaluation of microalgae production coupled with wastewater treatment

Davide De Francisci [a1], Yixi Su[a], Arvo Iital[b], Irini Angelidaki[a].

a. *Technical University of Denmark, Department of Environmental Engineering, Building 113, DK-2800 Kgs. Lyngby, Denmark.*
b. *Tallin University of Technology, Department of Environmental Engineering, Ehitajate tee 5, 19086 Tallinn, Estonia.*

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[1] Corresponding author: *Tel:* +45 45251680

E-mail address: dadf@env.dtu.dk (D. De Francisci)

Evaluation of microalgae production coupled with wastewater treatment

Davide De Francisci [a2], Yixi Su[a], Arvo Iital[b], Irini Angelidaki[a].

a. Technical University of Denmark, Department of Environmental Engineering, Building 113, DK-2800 Kgs. Lyngby, Denmark.

b. Tallin University of Technology, Department of Environmental Engineering, Ehitajate tee 5, 19086 Tallinn, Estonia.

In the present study the feasibility of microalgae production coupled with wastewater treatment was assessed. Continuous cultivation of *Chlorella sorokiniana* with wastewater was tested in lab-scale flat panel photobioreactors. Biomass productivity was determined for four dilution rates (4.32 d^{-1}, 3.6 d^{-1}, 1.8 d^{-1} and 0.72 d^{-1}). The productivity peak was 1.524 g $l^{-1}d^{-1}$ at the dilution rate of 2.41 d^{-1}. Nitrogen and phosphorus removals were found to be inversely proportional to dilution rates, while COD removal was found to be 50% at all the tested conditions. The biomass obtained at the highest dilution rate was characterized for its content of lipids, proteins and pigments. The average yields of fatty acid methyl esters (FAME), protein, lutein, chlorophylls and β-carotene was 62.4 mg, 388.2 mg, 1.03 mg, 11.82 mg and 0.44 mg per gram dry biomass, respectively. Economic analysis revealed that potentially more than 70 % of revenue was from the production of pigments, i.e. chlorophyllin (59.6%), lutein (8.9%) and β-carotene (5.0%) while reduction in discharging costs of the treated wastewaters could account for 19.6% of the revenue. Due to the low yield of FAME and the low market price of biodiesel, the revenue from the above was found to be the least profitable (1.4%). Even when taking into account all these different revenues combined, this cultivation strategy was found with the current prices to be uneconomical. Power consumption for artificial light was responsible for the 94.5% of the production costs.

Keywords: *Chlorella sorokiniana*, biorefinery, wastewaters, photobioreactors, economic analysis

[2] Corresponding author: *Tel:* +45 45251680

E-mail address: dadf@env.dtu.dk (D. De Francisci)

Introduction

Increasing concerns about climate change and sustainability of fossil fuels based economies have brought interest to microalgae for potential to establish bio-based economy, mainly due to their higher areal productivity over traditional biomasses [1]. Nevertheless, algal biomass production cost is still one major obstacle for commercialization of algae-derived products, especially for the low-value ones such as biofuels. As a consequence, current application of algal biomass is centered on high-value products (i.e. health, cosmetics, nutraceutical and food) [2]. In order to make the production of algal biomass profitable, efforts can be made on process integration, algal biology and cultivation system design [1, 3]. First, it is strongly recommended to produce biofuel simultaneously with value-added co-products, following a biorefinery strategy [4]. Furthermore, the combination of microalgae production with wastewater treatment for removal of nutrients and hazardous compounds can lead to a further step towards a cost-effective process, by saving the costs for N and P fertilizers when using nutrient rich streams [5, 6]. Moreover, revenue from wastewater treatment would help the overall process economy.

In this context, selection of appropriate algal species is pivotal: the ability of the species to grow in specific wastewaters and then generate biomass suitable for further transformation to high value products has a direct impact on the potential revenues. Furthermore, the use of wastewater as the culturing media adds stricter requirements for robustness of microalgae against adverse conditions, such as contamination with possible toxic compounds and competition with undesired microorganisms [7, 8]. Zhou et al. [9] isolated multiple species from natural environments and screened five potential high lipid producers in concentrated municipal wastewater by DNA sequencing: *Auxenochlorella protothecoides*, *Hindakia* sp., *Scenedesmus* sp. and two *Chlorella* sp.

A similar work found two *Chlorella* species, *C. protothecoides* and *C. kessleri* were growing better in wastewater compared to 14 other algal strains [10]. Additionally, several studies dealing with algal consortia suggested *Chlorella* sp. and *Scenedesmus* sp. as relatively robust species that can grow in wastewater [11-13].

Apart from the selected species, biomass production coupled with wastewater treatment depends on a variety of operation parameters such as type of wastewater, light intensity and cycle, pH, temperature, dilution rate, etc. [14]. Flow rate of medium, that determines the rate of nutrient supply, largely impacts the growth rates of the microorganisms. Biomass concentration at steady state depends on the equilibrium between specific growth rate and the imposed dilution rate [15]. Dilution rate is following the growth rate of algae up to maximum growth rate whereafter at higher dilution rates wash out would happen. As a consequence, the maximum biomass productivity would be reached at a specific dilution rate which is close (but lower) to the maximum growth rate of the algae at that specific condition. Previous studies investigated the effect of dilution rates on the overall productivity and observed that the optimal productivity corresponds to medium values of the dilution rates. This is probably due to less optimal growth conditions which not support maximum rates of the algae, such nutrients deficiency or content of potential inhibitors [16, 17].

Reducing production cost and/or increasing productivity are possible ways to improve the economics of algal biomass production. The present study aims to further investigate and assess the biomass productivity and the biomass composition of selected microalgae species grown in wastewater, instead of widely used synthetic media for supply of nutrients. Use of wastewater would reduce cost for nutrients (necessary for the cultivation) into revenue deriving from the removal of the same nutrients as environmental service. In this context, the algal biomass was used as a source for high

added value products and biofuels to offset the production costs. Additionally, attempts to improve the productivity via strain selection and optimization of cultivation-operation were made. Based on the data generated, the economics of algal biomass production was assessed in four scenarios considering an annual production of 330 days.

Materials and methods

Algal strains, medium and wastewater

Microalgal species *Chlorella sorokiniana* and *Scenedesmus obliquus* were chosen for the initial screening because they are frequently found in different wastewaters [11-13] and thus are expected to show robust growth in such environments. The strains were obtained from SCAAP (Scandinavian Culture Collection of Algae & Protozoa, Denmark) and cultivated in sterilized Woods Hole medium (MWC) [18] containing selenium.

Mixed influent industrial/municipal wastewater from Kohtla-Järve, Estonia was selected for testing with algae based on the assumption that it represents typical conditions in larger municipalities where industrial and municipal wastewaters as well as storm water are mixed and then treated together. The mixed industrial/municipal probe represented time–adjusted average water sample collected over 24 hours. The water sample has been analysed by the Estonian Environment Research Centre and the list of substances for the analyses involved COD_{Cr}, TOC, BOD_7, NO_2-N, NO_3-N, NH_4-N, N_{tot}, PO_4-P and P_{tot}. A number of hazardous compounds were present in the wastewater and were analysed by Kohtla-Järve WWTP using standard procedures (Table S1 in Supplementary Material). Part of the collected water sample was frozen (-20°C) and transported to Danish Technical University for further tests with microalgae.

For all the cultivation experiments, wastewater underwent sedimentation to remove the majority of solid particles. Sedimentation is considered an economic method in large scale applications for gross separation of larger particles and therefore it was chosen as separation methodology. Analysis of nutrients and organic compounds of the supernatant after sedimentation was performed at the Technical University of Denmark.

Due to storage and sedimentation of the wastewater samples, some changes in the water quality occurred, resulting in lower COD, N_{tot}, and P_{tot} concentrations and higher NH_4-N content (Table 1).

Microplate screening

Screening for the best performing algal strain in the wastewater was carried out in 24-well microplates (PE VISIPLATE, 24 well black-walled, clear bottomed). The microplates were incubated at room temperature, illuminated by LED at 400±50 µmol photons m^{-2} s^{-1} and shaken at 140 rpm with a 50 mm throw. Growth was monitored by fluorescence (440 nm emission, 690 nm detection) using a Synergy Mx microplate reader (BioTek Instruments, Inc., USA).

Cultivation procedures, well-top membranes, growth rate calculations, and detection limits were as described in recent study [19]. Each of the strains was inoculated in triplicates in 100% wastewater or mixtures of wastewater and MWC + se medium with varying percentages of wastewater (75%, 50% and 25%). Culture volume in each well was 2 ml. The screening was repeated for two generations for both species.

Photobioreactor cultivation

A flat-panel photobioreactor (Algaemist reactor, Wageningen University) was used to cultivate *C. sorokiniana* with the wastewater pretreated by sedimentation. Undiluted

wastewater was used for this set of cultivation experiments due to the positive results obtained from the microplate screening where cultivation in undiluted wastewater supported algal growth (see Results and discussion: Microplate screening).

The cultivation was initiated in batch mode. Parameter settings in this experiment are listed in Table 2, and were chosen according to the optimal growth condition for this species [20-22]. When the growth reached early stationary phase, the cultivation was switched to continuous mode. The dilution rate was set to 4.32 d^{-1}, which was close to the maximum specific growth rate observed during the exponential phase in batch mode. Thereafter, the dilution rate was stepwise decreased to 3.6 d^{-1}, 1.8 d^{-1} and 0.72 d^{-1}. Optical density (OD_{750}) throughout the cultivation was monitored. Moreover, biomass was collected for each dilution rate when the OD value was stable. The temperature of the effluent was maintained at 4°C to inhibit algae metabolism and growth after harvest.

Analytical methods

The samples obtained from the highest dilution rate was subject to lipid, protein and pigment quantification.

Cell growth and dry cell weight

Cell growth of algae was monitored by measuring optical density at 680 and 750 nm using a Hach Lange DR2800 spectrophotometer. The correlation between optical density (OD) and dry weight (DW) concentration of samples (C_x) was determined as described in Van Wagenen et al. [17]. The correlation curve between OD_{750} of cell suspensions and dry weight of the biomass resulted to be linear, $C_X = 0.31 OD_{750} - 0.04$ with a $R^2 > 0.95$.

177

178 *Lipid determination*

179 The procedure for the quantification of fatty acid methyl esters (FAMEs) was based on
180 the modified Folch method [23]. 10 mg of freeze-dried and powdered biomass was
181 mixed to a solvent mixture of chloroform: methanol (2 mL, 2:1, v/v) in duplicate. After
182 vortexing for 20 minutes, FAMEs were formed by addition 1 mL of methanol and 300
183 µL of H_2SO_4 and incubation at 100°C for 20 minutes. After cooling down, 1 mL of
184 distilled water was added to the sample, which was then vortexed for 5 minutes and
185 centrifuged at 4,000 rpm for 10 minutes. The lower layer including the organic solvent
186 was analysed with gas chromatography (HP 5890, Agilent, USA) with a flame ionized
187 detector (FID) and INNOWAX capillary column (Agilent, USA). The GC column
188 temperature was programmed as follows: (1) initial column temperature at 50 °C, hold
189 for 1 min, (2) increase to 200 °C at a rate of 15 °C min^{-1}, hold for 9 min, and (3)
190 increase to 250 °C at a rate of 2 °C min^{-1}, maintain for 2 min. Individual FAME
191 component was identified and quantified by comparing the retention times and peak
192 areas with those of the FAMEs standard solutions, respectively. The internal standard
193 was Supelco 37 Component FAME Mix, item no. 47885- U, Sigma−Aldrich.

194

195 *Protein determination*

196 For protein hydrolysis, duplicates of 50 mg biomass were suspended in 6 ml of 6N HCl
197 and transferred in close vessels. The vessels were flashed with nitrogen to prevent
198 oxidative degradation of some oxygen/sensitive amino acids. The vessels were then
199 microwaved for 30 min at 150 and 500W (Multiwave 3000, Anton Paar). Samples were
200 then freeze-dried to remove HCl. The residues were resuspended in 400 milliQ H_2O
201 and filtered through 0.22 syringe filters before the protein quantification by in-needle

derivatization HPLC-FLD (Dionex UltiMate 3000, Thermo Scientific). Amino-acids were separated in a c18 reversed phase column (Eclipse Plus C18, Agilent Technologies, USA) with an in-line guard column (EC 4/2 Universal RP, Macherey-Nagel, Germany) and mobile phases A (10mM Na_2HPO_4, 10 mM $Na_2B_4O_7$) and B (methanol: acetonitrile: water, 45:45:10). The flow rate was 0.420 mL min^{-1}. Quantitative analyses were performed by means of calibration curves using a commercial amino-acid mix standard (AAS18 Fluka).

Pigments determination

Two milligrams of freeze-dried biomass were mixed with 3 ml of 90% acetone in duplicates. Well mixed samples were sonicated in ice bath for 10 min (Branson 3510MT). The supernatant was separated from the residual biomass by centrifugation at 13,000 rpm for 10 min. A Zorbax Eclipse plus C8 RRHD 1.8 µm 3.0×150 mm column was used for UHPLC separation at 60 °C with a 75 min separation time. Detection utilized UV–VIS at 450 nm. Quantification was done relative to individual pigment standards obtained from DHI, Hørsholm, diluted from 15 to 1500 µg L^{-1}.

Nutrient measurements

Samples corresponding to each dilution rates were centrifuged in order to harvest biomass. The supernatants were collected for nutrient composition analysis. Contents of COD, total nitrogen (N_{tot}), total phosphorus (P_{tot}) and ammonium were determined for the supernatant using Hach Lange Cuvette Kits. (LCK314, LCK238 and LCK348, while Spectroquant® ammonium test (Merck Millipore) was used for the measurement of ammonium.

Estimation of biomass market value

Evaluation of economic potential of algae biomass was performed by calculating the gross profit, taking into account only the difference between revenue and the operating cost, without deducting costs for overhead, payroll, taxation and interest.

Specifically, a value of unit biomass was calculated as sum of revenues from all products of interest, including biodiesel, proteins and pigments (e.g. lutein, chlorophylls and β-carotene) as well as benefit for removing COD, N and P from the wastewater. Market value for each bioproduct obtained per unit biomass can be calculated from the experimentally obtained yields, i.e. FAME (C_f), amino acid (C_{aa}) and pigments (C_p). Prices of desirable products (Table 3) were obtained from an e-commerce website: www.alibaba.com. Specifications of the benchmark products can be found on the company pages. The revenue from bio-products is the sum of production of each product (P_i) multiplied with its price, shown in the following equation.

$$Revenue_b = \sum_i C_i \cdot Price_i$$

Estimation of production cost was based on data from literature. Aim with this preliminary economic assessment was to estimate which costs – revenues are more important for the operational cost balance. The estimation only includes operation costs and not initial investment costs. The rationale behind this was to generate a dataset that could serve as a preliminary assessment of the profitability of this specific concept. In case the process resulted to be not economically feasible based on operational costs and revenues, it would be logical to assume investments for facilities construction would make the economic prospects even more difficult. CO_2 supply was the only input needed cost, while nitrogen and phosphorus were considered free as present in the wastewater. Power consumptions for light, CO_2 sparging and harvesting were considered main items of production cost for algal biomass. Additionally, cationic

coagulant was chosen for the estimation of the harvesting costs due to its effectiveness and low cost compared to others [24]. Detailed calculation can be found in supplementary material.

Scenarios for potential cost reduction

A basic economic analysis was conducted to evaluate potential cost reduction opportunities. In addition to the base case (where costs for CO_2 and LED were both taken into account), three alternative scenarios were proposed. Case (1) assumed industrial flue gas containing CO_2 was provided freely e.g. from a nearby power plant without significant influence on cell growth and composition. In case (2), the cost for power of lighting was eliminated by substituting artificial light with natural light source (i.e. sunlight). Because of the unstable supply as a consequence of day-night cycle and seasonal variation, specific growth rate and cell density was assumed to decrease by 14% and 31%, respectively [25]. In the third scenario, assumptions in case (1) and (2) were combined.

Statistics analysis

IBM SPSS Statistics (Version 22) was used for statistical analysis. Data comparison was performed using one way ANOVA test and unpaired t-test with 95% confidential intervals.

Results and discussion

Microplate screening

Based on specific growth rate (Figure 1), *C. sorokiniana* shows higher robustness in this wastewater over *S. obliquus* at all conditions. The highest specific growth rates are 2.40

276 d^{-1} and 2.04 d^{-1} for *C. sorokiniana* and *S. obliquus*, respectively, which are obtained in a
277 mixture with 50% wastewater in the second generation. Acclimation in the second
278 generation was observed for both species. Furthermore, when wastewater concentration
279 was higher than 50%, growth rates were inversely proportional to wastewater
280 concentration for both species, which suggests possible inhibitory effects of wastewater
281 on the algal growth.

282 This could be due to presence of hazardous compounds from the oil-shale
283 industry in the KJ wastewater, which can potentially be harmful to microalgae species.
284 At the same time, undiluted wastewater contains the highest concentration of nutrients
285 and therefore leads to the highest cell density of *C. sorokiniana* (Figure 2), even with a
286 lower growth rate. The same tendency was observed in a previous study, where 100%
287 wastewater resulted in initial inhibition to algae, but eventually it resulted in the highest
288 algae density compared to diluted concentrate [26]. Based on these results and on
289 considerations that dilution of wastewater would be more technical complex and costly,
290 undiluted wastewater was used for the photobioreactor (PBR) experiments.

291

292 ***Algae productivity***

293 Average biomass productivities and biomass concentration measured at steady states of
294 four dilution rates are shown in Figure 3. The cultivation was initiated with the dilution
295 rate (4.32 d^{-1}) close to the maximal specific growth rate (4.56 d^{-1}) observed in a batch
296 cultivation in the same wastewater. This dilution rate led to the lowest biomass
297 concentration (0.18 g l^{-1}) and, as a consequence, to the lowest productivity (0.8 g $l^{-1}d^{-1}$).
298 With the decrease of dilution rates, biomass concentration rose to 1.44 g l^{-1}, (dilution
299 rate of 0.72 d^{-1}) corresponding to low productivity (0.95 g $l^{-1}d^{-1}$). The highest biomass
300 productivity (1.46 g $l^{-1}d^{-1}$) was exhibited at a dilution rate of 1.8 d^{-1}. The curve

301 describing the correlation between dilution rate and biomass productivity was fitted to a
302 binomial equation, and the highest productivity was estimated to be 1.524 g $l^{-1}d^{-1}$ at a
303 dilution rate of 2.41 d^{-1}, corresponding to a cell density of 0.63 g l^{-1}.

304 The trend seen with decrease of cell concentration with increasing dilution rates
305 is contradictory to the theoretical expected. The expected trend would be that the cell
306 concentration was stable with increasing dilution rate, until initiation of wash out which
307 would correspond to a sharp decrease the cell concentration.

308 The explanation to the observed relationship could be due to the spontaneous
309 flocculation and wall attachment occurred during the cultivation (Figure 4). The
310 calibration curve (section Analytical methods) used to calculate cell concentration was
311 generated using homogeneously suspended cells, and therefore OD measurements do
312 not reflect cell concentrations of flocculant cell associations. High flow rates (high
313 dilution rates) in upflow reactor systems are causing selection pressure to the cells. Only
314 cells managing to create flocs are resisting wash out, by creating flocs presenting larger
315 diameter than the single cells and thereby having a higher sedimentation rate, while the
316 suspended cells are washed out of the reactor. Therefore high dilution rates are
317 promoting flocculation and thereby OD measurements at these high rates are giving an
318 underestimation of the cell concentration.

319 Previous studies employed the same photobioreactor system (flat plate) used in
320 the current one [16, 17] and have found similar trends. The operation conditions and
321 growth data achieved in these previous publications listed in Table 4 for comparison. In
322 Van Wagenen et al. [17] parallel experiments were conducted with a high light intensity
323 (2100 μmol m^{-2} s^{-1}) and a low light intensity (200 μmol m^{-2} s^{-1}). The operating
324 conditions of the present study (wastewater instead of synthetic media and low light
325 intensity) are very similar.

However, even if the light intensity in the present work was twice as much as the low light experiment in Van Wagenen et al. [17], lower biomass density and productivity were obtained. A reason for this difference could be the different nutrient supplements in the media used. The nutrient content, especially nitrogen in Kohtla-Järve influent wastewater was considerably lower compared to the aforementioned study (Table 5). It has been proven that biomass concentration and NO_3-N supply are positively correlated, up to a saturation level of about 30 mg NO_3-N l^{-1} (further increase of cell density was limited, which may be caused by the limitation of other nutrients) [27]. The positive effect of increasing nitrogen and phosphorus concentration on algal growth was also reported, demonstrating that the highest level of algal biomass corresponded to the highest initial N_{tot} of 25 mg l^{-1} [28].

Nutrient removal

Nitrogen and phosphorus concentrations were determined for the treated wastewater and for the resulting biomass after harvesting. Nutrient contents of the treated wastewater were compared with the composition of untreated wastewater.

Removal efficiencies at different dilution rates are shown in Figure 5. Overall, the highest removal efficiencies (> 90%) were observed at the lowest dilution rate (0.72 d^{-1}). With the decrease of dilution rate, the removals of total nitrogen, total phosphorus and ammonium were steadily increased. However, the removal of COD for all dilution rates remained around 50%. Limited COD reduction was also previously reported [29, 30]. This indicates that the residual ~50% of COD consisted by organics not degradable by microalgae. This also shows that organic carbons were consumed very quickly in these experiments and therefore were the preferred carbon source by *C. sorokiniana* over CO_2 (heterotrophy/mixotrophy). This is in agreement with a previous study, in

351 which batch cultivations of *C. sorokiniana* were conducted at increasing concentration
352 of organic carbon, with the highest growth rate corresponding to the highest
353 concentration [31].

354 Van Wagenen et al. [17] observed very high removal efficiencies for PO_4-P in
355 all the tested dilution rates. In the present work phosphorus removal rate was instead
356 increased with dilution rate. An explanation for this could be the fact that phosphorus
357 was in excess in the wastewater used in this previous study (N/P ratio was 36.5:1 in Van
358 Wagenen et al. [17] while it was only 14.9:1 in the Kohtla-Järve influent wastewater
359 which we used in this study).

360 Finally, average concentrations of mineral elements present in the algal biomass
361 are 8.87 % N and 1.04 % P, which partly represent the nutrients transferred from
362 wastewater to biomass. Similar N and P contents were also reported when microalgae
363 were grown in dairy manure and obtained biomass consisting of 7 % N and 1% P [32].

364

365 ***Biomass characterization***

366 Compositional analysis of the algal biomass grown in wastewater is listed in Table 6.
367 Palmitic acid (16:0), palmitoleic acid (16:1), oleic acid (18:1) and linolenic acid (18:3)
368 were found to be the most abundant fatty acids present in the algal biomass (Table 7).
369 This is in agreement with typical fatty acid composition of *C. sorokiniana* found in
370 literature [33-36].

371 Fatty acid content in *C. sorokiniana* can vary from 0.6% to 47.51% depending
372 mainly on the growth conditions (Table 8). FAME yield of current study is relatively
373 low compared to fatty acid contents of *C. sorokiniana* reported in literature.Nitrogen
374 starvation has been widely recognized as a stress condition which stimulates the
375 accumulation of lipids. Li et al. [46] showed that the initial nitrogen concentration in the

medium was positively correlated with the growth of *C. sorokiniana,* but reversely correlated with the lipid content. Lipid accumulation is believed to be a consequence of the inhibition of proteins and starch biosynthesis which usually occurs in stationary phase [47].

Furthermore, composition of the lipid profile is in general correlated to culturing conditions, and this may be another reason for low fatty acid content in the algal biomass produced in the present work. In contrast to polar lipids (e.g. membrane components), neutral lipids are responsible for energy storage in cells and are precursors for FAME production. It has been shown that different nutritional conditions can affect the percentage of neutral lipids within the total lipid content varying from 2.9% to 60% [36]. In addition, low irradiation, as in the present study, induces the formation of polar lipids, whereas the formation of triacylglycerols is favoured at high light intensity conditions [48]. Also, although results show that available organic carbon source was consumed, nitrogen and phosphorus were still abundant in the effluent of culture (Figure 5). Therefore, microalgae in this condition were not stressed by nutrient limitation and thus tended to invest carbon and energy for cell growth. The high protein content 38.82% (w/w) in the algal biomass is an indicator for the active proliferation. In conclusion, in the present work the high growth rate (supported by sufficient nutrient supplement) was probably the reason for the relatively low fatty acid yield. Clearly, there is a tradeoff between biomass productivity and lipid content that cannot be achieved simultaneously. This is why two-phase cultivation strategies are a possible solution for the economics of algae cultivation [49, 50].

Estimation of biomass value and economic potential

400 The revenue generated from cultivationg *C. sorokiniana* in this specific wastewater is
401 estimated to be 3.27 € kg^{-1} dry biomass, which includes 2.63 € kg^{-1} (80.4%) from the
402 production of valuable bioproducts and 0.64 € kg^{-1} (19.6%) from removal of nutrients
403 from wastewater as an environmental service (Table 9).

404 More specifically, chlorophyllin accounts for 59.7% of the total value, whereas
405 the share of biodiesel is negligible (1.4%) as a consequence of the low FAME yield. As
406 per kilo of microalgae produced, roughly 1580 L wastewater can be treated at a dilution
407 rate of 2.41 d^{-1}, which makes significant contribution (19.6%) to the overall revenue.
408 However, the nutrient removal efficiencies in this condition are unsatisfactory for
409 treating wastewater. Removal efficiencies of only 52.1% for COD, 57.5% for nitrogen
410 and 68.8% for phosphorus were achieved. The cost for producing a kilo of microalgae
411 was estimated to be 12.46 € kg^{-1} comprising 94.5% for power for illumination, whereas
412 the remaining 5.5% was for CO_2 supply (2.7%), cost of cationic flocculant (0.4%),
413 power for harvest (2.1%) and aeration (0.3%).

414 As already mentioned, biodiesel is the least remunerative product. Despite the
415 fast growth of *C. sorokiniana*, the parallel low FAME production largely affects the
416 economics of the strategy presented in this study. Furthermore, coupling biomass
417 production and wastewater treatment contributes to the total revenue. However, the
418 COD and nutrients removal efficiencies at the dilution rate, 2.41 d^{-1} were poor.
419 Consequently, the resulted wastewater may not fulfill the quality for reuse and may
420 require additional steps for further treatment.

421 Finally, the economic potential in the case of utilizing artificial light is -9.19 €
422 kg^{-1}-biomass, showing economically unsustainable production.

423

424 ***Scenarios for potential cost reduction***

Economics of algal biomass production was assessed in four scenarios considering an annual production of 330 days. The results indicate the economic potential can be positive only when the cost for artificial light is eliminated (Figure 6). Results show that the substitution of artificial light with sunlight can reduce production cost by 96.0%, whereas the reduction resulted from using free CO_2 is 2.7%. The elimination of CO_2 cost has relatively little effect (+3.6%) on the overall cost reductions. By contrast, economical potential can be increased by 116.1% and become positive as a result of considerable drop in cost for artificial light.

On the other hand, the substitution of artificial light by sunlight hypothetically causes 14% and 31% reduction in specific growth rate and cell density, respectively [25], resulting in 40.7% reduction in biomass productivity. As a consequence, annual revenue is reduced by 39.6%. In addition, because nitrogen removal is 56% less in a light-dark cycle condition in comparison with continuous illumination [51], the shorter illumination period leads to further decrease in nitrogen removal efficiency to 26.8%.

This analysis highlights that excluding use of artificial light is an imperative to enable sustainable production of algal biomass for any purpose. In the base case, at least 76.5% of the cost for artificial light needs to be reduced to ensure breakeven for the necessary utilities for biomass production (e.g. electricity, flocculant and CO_2). In the case that excludes the costs for CO_2 and light, biomass cost is reduced to 424 € t^{-1}, which is substantially lower than 5,960 € t^{-1} as reported in [52] and 2,340 $ t^{-1} reported in [53]. Exclusion of capital cost and operational cost such as labour and general plant overhead is one major reason for the underestimation in our estimation. Furthermore, some basic assumptions for the calculation are different. For example, aeration power accounted for the biggest fraction of cost in Norsker et al.'s calculation, which is relatively low in the present work.

Conclusion

This work demonstrated that microalga *C. sorokiniana* can well adapt to the wastewater chosen for this assessment and thus exhibits high biomass productivity. The cultivation led to a significant but not optimal removal of COD, N and P. Nitrogen and phosphorus removals were observed to be inversely proportional to dilution rates, while COD removal was found to be constant. Microalgae cultivation can therefore be considered a promising tool for partial nutrient recovery from wastewaters, but not yet an ideal tool to meet wastewater treatment plants requirements. In this context, the nutrient recovery translates in the production of valuable biomass that could make the entire process profitable. The composition of the resulting biomass was determined in respect to lipids, proteins and pigments content. The economic assessment performed on the entire process showed that pigments in particular could play a pivotal role in economics of algae production and should be the primary goal to pursue. It is noteworthy that the cultivation conditions in the present study were generally chosen to ensure optimal microalgae growth and optimal biomass productivity. However, the same conditions translate in poor content of high value products in the same biomass. For this reason it is advisable to develop two-phase cultivation strategies, in which microalgae are first kept in optimal growth conditions to generate high biomass yield, and then stressed to increase the high added value products content in the same biomass.

Finally the economic assessment performed on this specific species/wastewater combination proved this cultivation strategy to be uneconomical, mostly due to the energy consumption for artificial light, which accounts for 94.5% of the production costs.

Word count: 5,113

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Table 1 Composition of KJ wastewater.

Indicator	Before sedimentation	After sedimentation
COD	442 mg O_2 l^{-1}	386.9 mg O_2 l^{-1}
N_{tot}	117 mg N l^{-1}	48.6 mg N l^{-1}
P_{tot}	10.5 mg P l^{-1}	7.2 mg P l^{-1}
NH_4-N	34.7 mg N l^{-1}	46.7 mg N l^{-1}

685 Table 2. Parameter settings for PBR cultivation

Parameter	Setting
Temperature	37℃
pH	7.0
Light intensity	400 μmol m^{-2} s^{-1}
Air flow rate	160 ml min^{-1}
CO_2 flow rate	40 ml min^{-1}

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709 Table 3. Specifications and market prices of desirable products.

Product	Specification	Price	Reference
FAME	B100 biodiesel	734 € t^{-1}	Keysun Bio-Tech Co.Ltd
Amino acids	AA content: 54.4%	426 € t^{-1}	Seek Bio-Technology Co.Ltd
Lutein	80%	284 € kg^{-1}	Xi'an Lyphar Biotech Co.Ltd
Chlorophyllin	95%	165 € kg^{-1}	Xi'an Lyphar Biotech Co.Ltd
β-carotene	95%	411 € kg^{-1}	Xi'an Lyphar Biotech Co.Ltd

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Table 4. Comparison of experimental conditions and growth performance of *C. sorokiniana* in flat panel PBR. PFD = photon flux density, D= dilution rate, C_X = biomass concentration and P_b = biomass productivity.

Medium	PFD	D	C_X	P_b	Reference
	(μmol m^{-2} s^{-1})	(d^{-1})	(g l^{-1})	(g l^{-1} d^{-1})	
M8a	2100	5.76	2.2	12.2	[16]
IC effluent	2100	3.6	1.56	5.87	[17]
IC effluent	200	1.44	1.09	1,67	
KJ influent	400	2.41	0.60	1.52	This study

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761 Table 5. Comparison of media used for continuous cultivation of *C. sorokiniana* in flat
762 panel PBR.

Indicator	Unit	M8a	IC effluent	KJ influent
COD	mg O_2 l^{-1}	-	590	386.9
N_{tot}	mg N l^{-1}	1680	190	48.6
P_{tot}	mg P l^{-1}	641	11-12	7.2
NH_4-N	mg N l^{-1}	-	-	60.1

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Table 6. Productivities of desired bioproducts.

Product	Yield (%, w/w)	Productivity (mg l^{-1} d^{-1})
Biomass		1524
FAME	6.24	95
Protein	38.82	592
Lutein	0.103	1.57
Chlorophylls	1.182	18.01
β-carotene	0.044	0.671

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812 Table 7. Fatty acids profile of *C. sorokiniana*

Type of fatty acid		Percentage
Total FAs (% dw.)		6.24
Fatty acid (% total FAs)	Palmitic acid (C16:0)	20.22
	Palmitoleic acid (C16:1)	9.51
	Oleic acid (C18:1)	19.82
	Linolenic acid (C18:3)	8.39

813 Table 8. Characterization of *C. sorokiniana* biomass in literatures.

Research focus	Growth performance (d^{-1}/ g L^{-1} d^{-1})	Lipid content (%, w/w)	FAME yield (%, w/w)	Protein content (%, w/w)	Reference
Effect of temperature	-	~ 10%	1.3 – 6.1%	-	[35]
Effect of C/N ratio	-	13 – 46%	2.1 – 7.3%	-	[33]
Pigment composition	5.76 d^{-1}	10.0%	-	68.5%	[37]
Effect of biochemical stimulants	42 mg $l^{-1}d^{-1}$	5 – 7%	-	45 – 60%	[38]
Mixotrophic growth	0.44 d^{-1}	20 – 50%	-	10 – 32%	[39]
Effect of inoculum size	0.89 d^{-1}	-	-		[40]
Photoautotrophic/ heterotrophic growth	-	21 – 26% (P) 20 – 56% (H)	0.6 – 0.8% (P) 12 – 33.6% (H)	12 – 13% (P) 6.2 – 13% (H)	[36]
Cultivation with deep sea water	176.6 mg $l^{-1}d^{-1}$	51.7%	47.51%	-	[41]
Cultivation in cattle manure	12.77 mg $l^{-1}d^{-1}$	25 – 35%	12%	34%	[42]
Fed-batch cultivation	3.29 d^{-1}	14.5 – 38.7%	12.8 – 34.1%	-	[43]
Photoautotrophic/ heterotrophic/ mixotrophic growth	0.68 d^{-1} (P) 2.07 d^{-1} (H) 3.40 d^{-1} (M)	-	9.0% (P) 6.2 – 17.6% (H) 13.4 – 34.7% (M)	-	[34]
Cultivation in domestic wastewater	220 mg $l^{-1}d^{-1}$	48.31%	-	-	[44]
Mixotrophic growth	1.602 d^{-1}	20 – 27%	-	-	[45]
Effect of nitrogen limitation	3.21 d^{-1}	20 – 51%	-	-	[46]
Continuous cultivation	2.41 d^{-1}, 1.52 g $l^{-1}d^{-1}$		6.24%	38.8%	This study

814 (P: photoautotrophic; H: heterotrophic; M: mixotrophic)

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818 Table 9. Estimation of biomass value.

Product	Yield	Productivity	Revenue
Biomass		1.524 g $l^{-1}d^{-1}$	
FAME (B100)	0.0624 g g^{-1}	0.095 g $l^{-1}d^{-1}$	0.46 € kg^{-1}
Amino acid fertilizer (54.4%)	0.3882 g g^{-1}	0.592 g $l^{-1}d^{-1}$	0.162 € kg^{-1}
Lutein (80%)	1.03 mg g^{-1}	1.565 mg $l^{-1}d^{-1}$	0.292 € kg^{-1}
Chlorophyllin (95%)	11.81mg g^{-1}	18.014 mg $l^{-1}d^{-1}$	1.950 € kg^{-1}
β-carotene (95%)	0.44 mg g^{-1}	0.671 mg $l^{1}d^{-1}$	0.181 € kg^{-1}
Sum			2.630 € kg^{-1}
Wastewater treatment	Removal	Quantity	Revenue
Wastewater		1581.4 $L^{-3}kg^{-1}$	
COD	52.1%	0.319 kg kg^{-1}	0.042 € kg^{-1}
Nitrogen	57.5%	0.044 kg kg^{-1}	0.356 € kg^{-1}
Phosphorus	68.8%	0.008 kg kg^{-1}	0.242 € kg^{-1}
Sum			0.640 € kg^{-1}
Total revenue			3.271 € kg^{-1}

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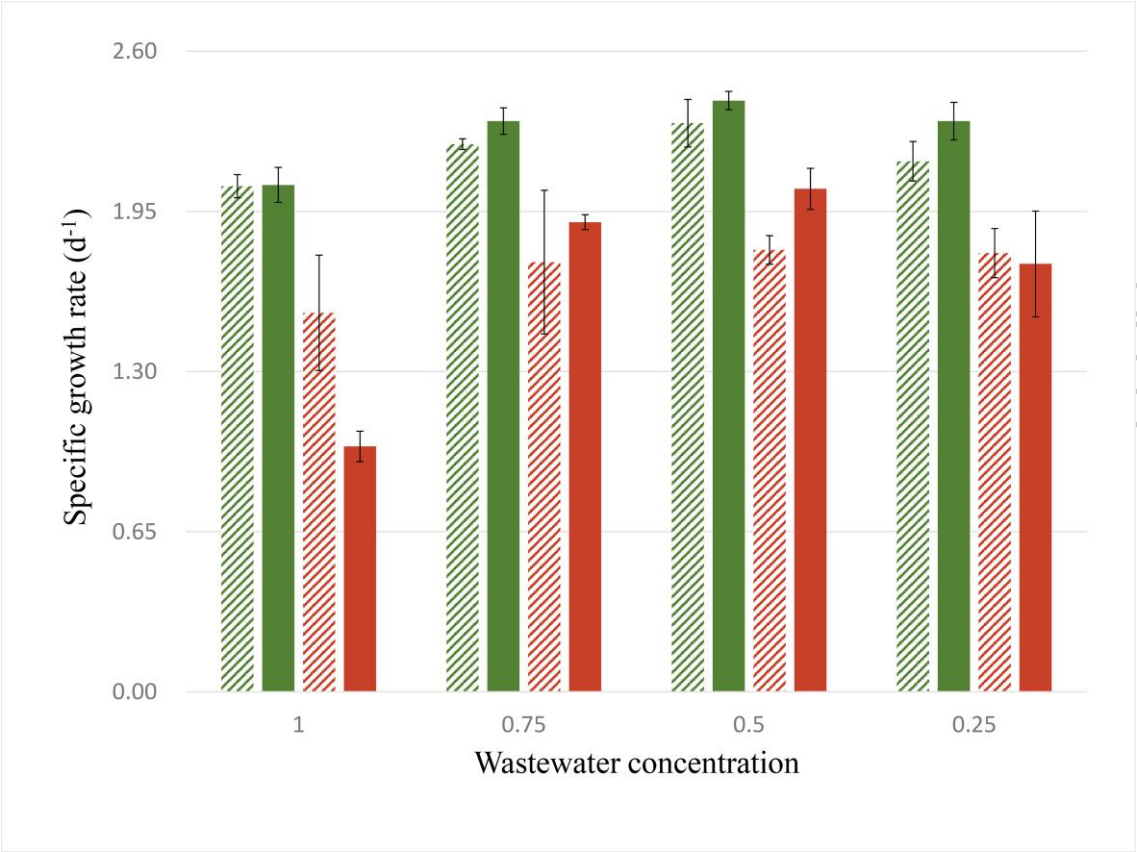
List of figures



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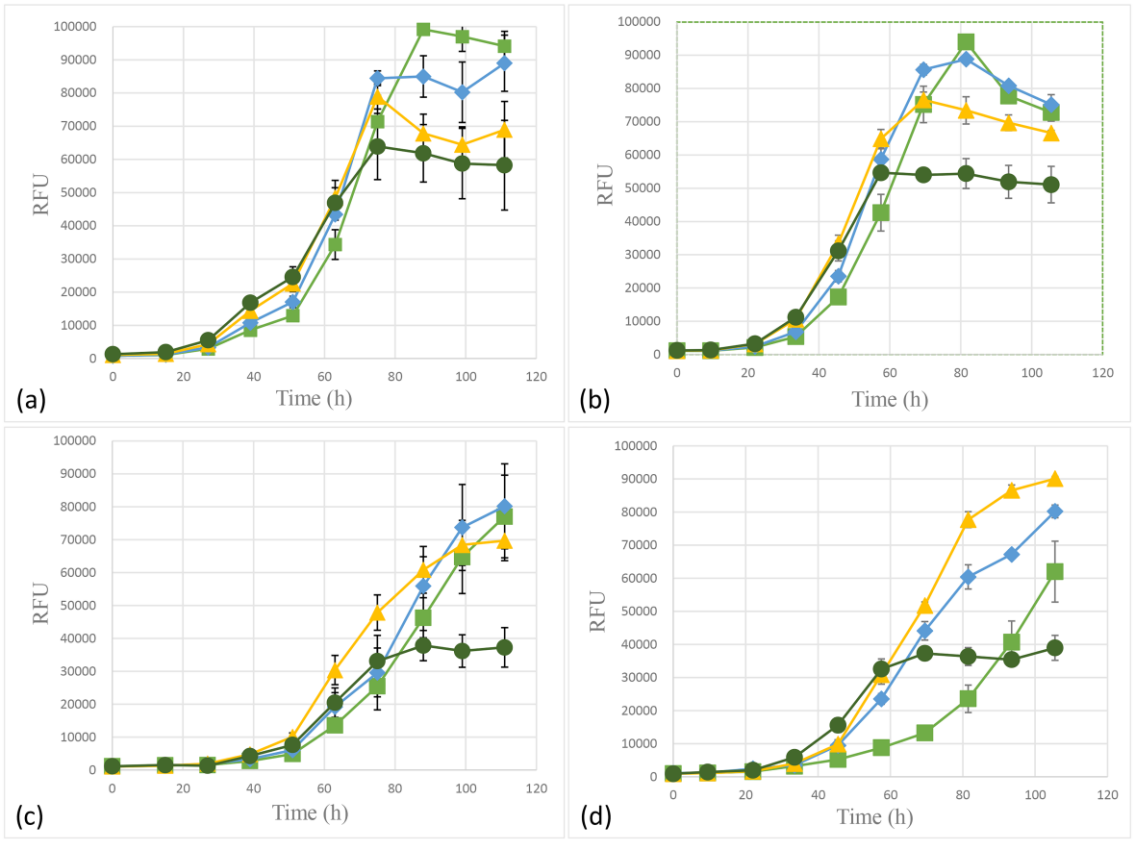

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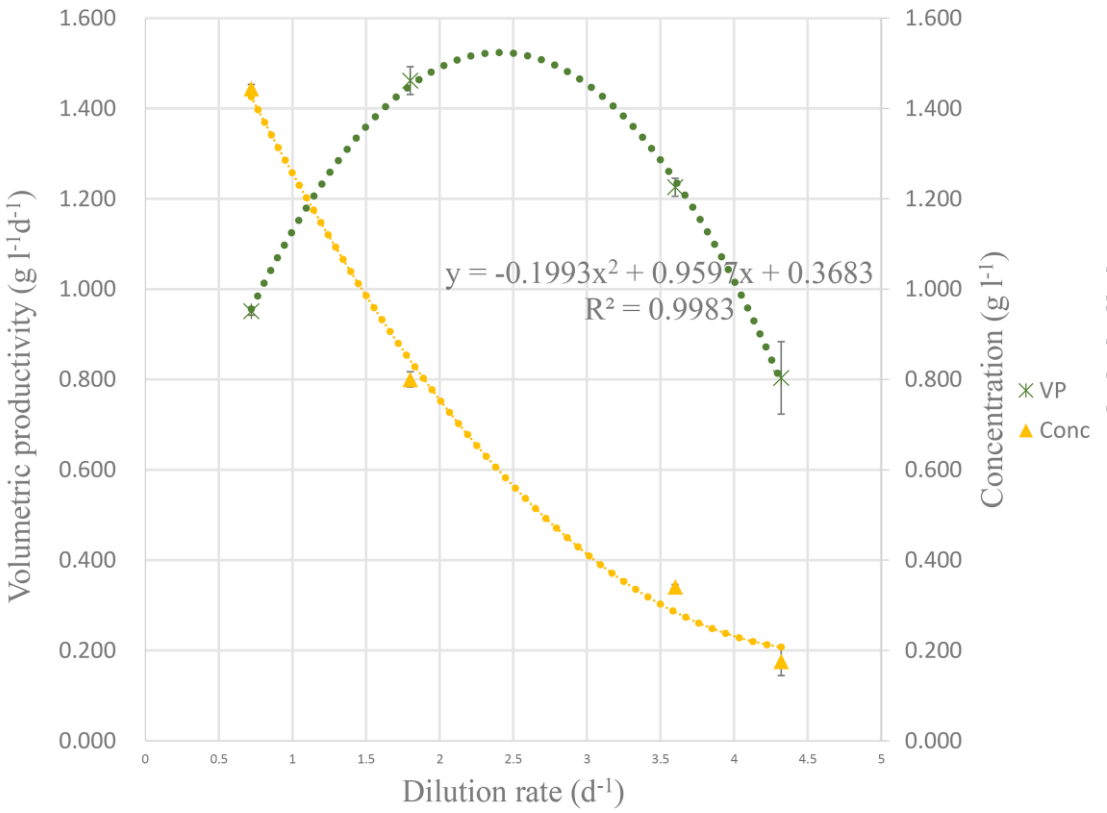

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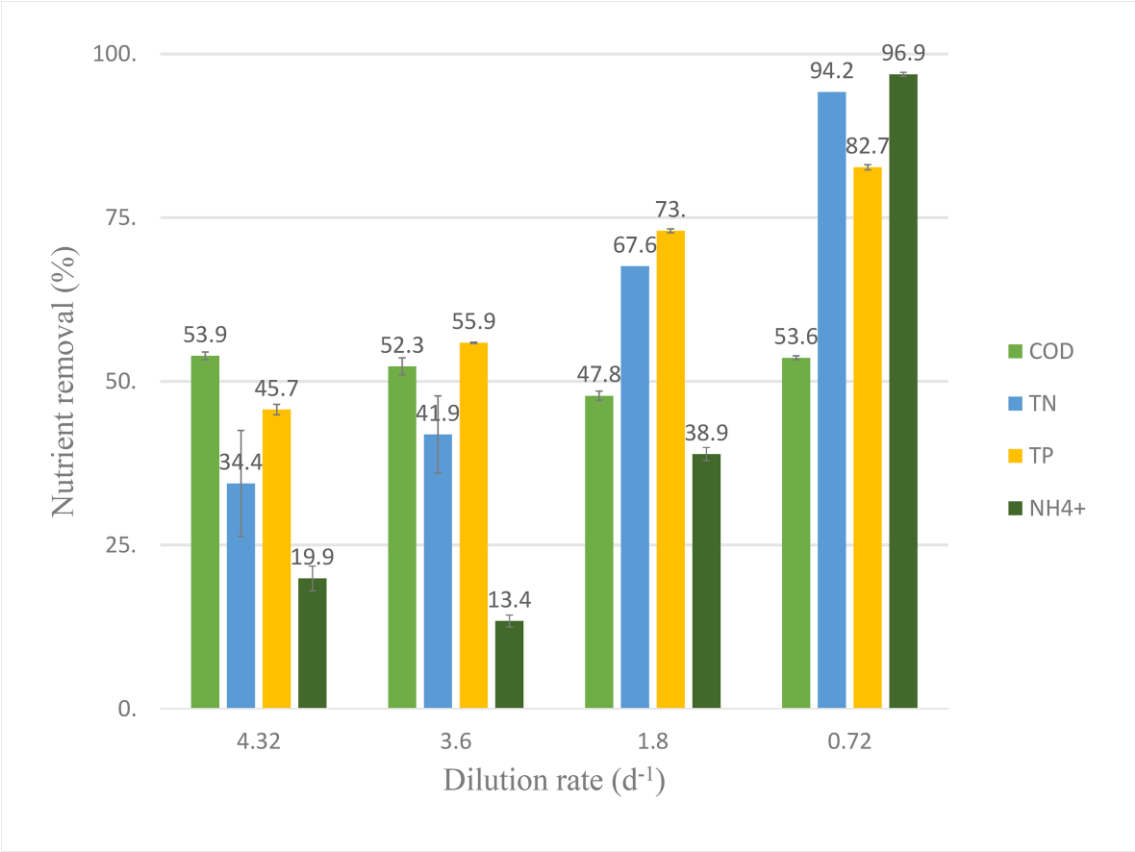

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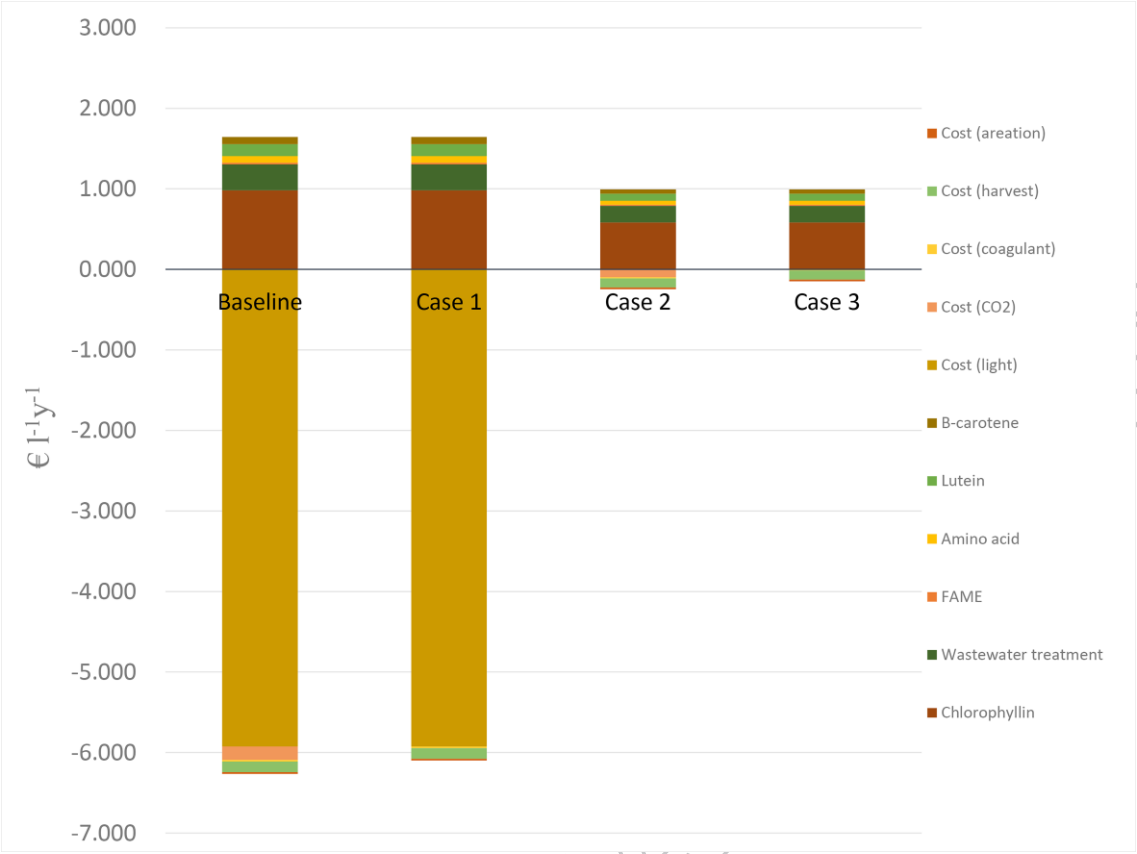

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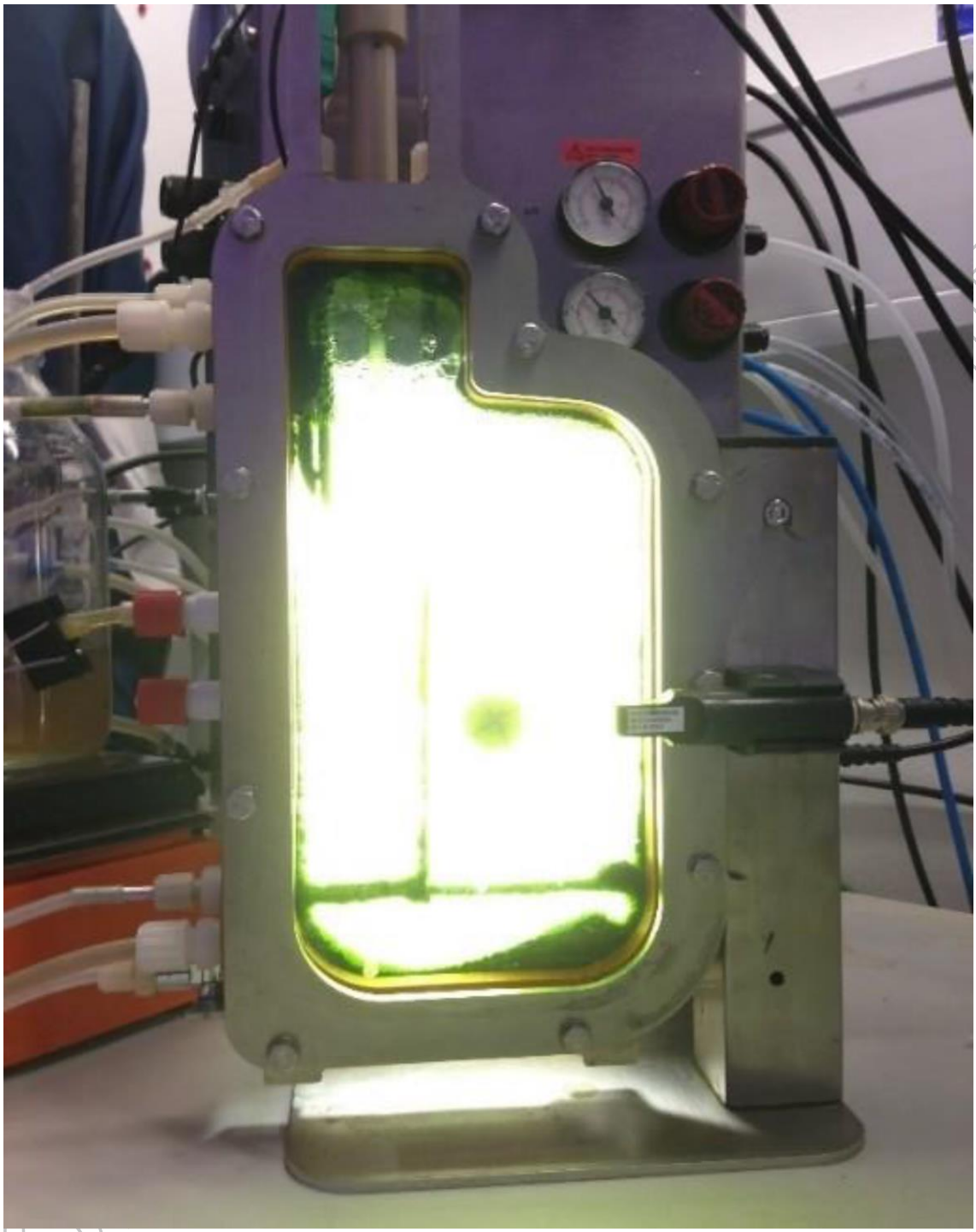

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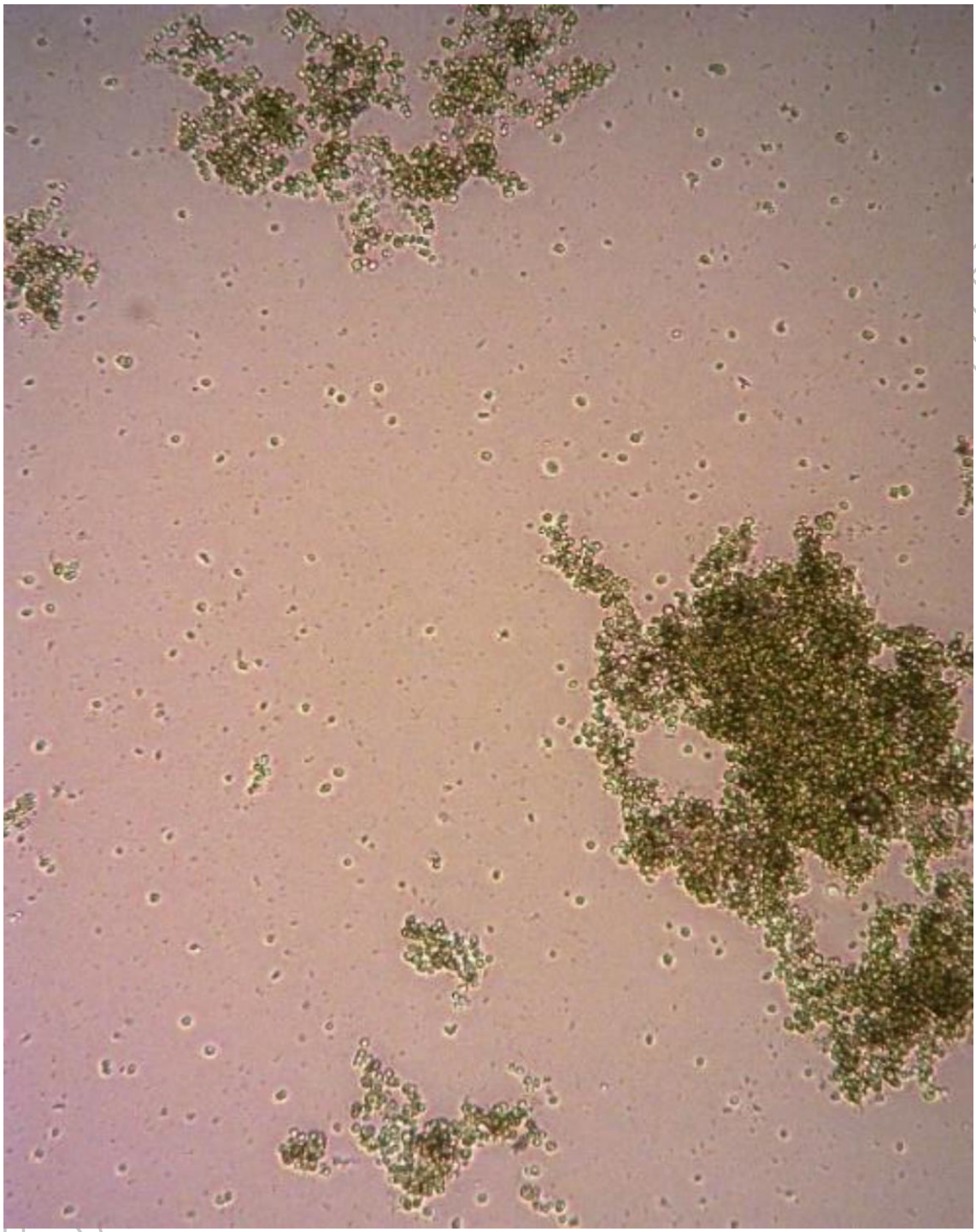

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