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RESEARCH ARTICLE

Domestication and Proliferation of Algae Cultures for Boosting Efficiency of Waste-water Treatment through Symbiosis

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Abstract

Native algae strains have been domesticated and stimulated in activated sludge wastewater treatment operations. The term of domestication indicates that we did not add any selected algae to the wastewater, but made use of species existing in the system. The term of proliferation indicates a stimulation of the biological oxidation process by provision of CO_2 with the air stream and illumination of the reactor. The idea of domestication of algae present in communal wastewater systems was demonstrated. Stimulation of the system with domesticated algae community did improve efficiency of the treatment process. Removal of organic components in terms of reduction of chemical and biological oxidation demands (dissolved COD, BOD) as well as nitrogen and phosphorous contents was superior to extent of removal in conventional activated sludge system. We did conclude that conventional systems lack available light and carbon resources for these microorganisms. Upon providing these, symbiotic operation can contribute to reducing greenhouse gas emissions and increase of the rate of pollutants removal kinetics. Symbiotic operation increased the production of biomass expressed in terms of total suspended solids. Biodiesel potential of the filterable biomass was in the range of 8-18%. Because of technical difficulties in manipulation of the excess sludge other than biodiesel synthesis processing scheme has been recommended for economically viable processing.

Keywords

algae, wastewater, biodiesel

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1 Introduction

1.1 Algae in wastewater treatment

We have started to deal with algae by being deeply motivated by enthusiasm of providing feedstock in abundance for biofuels and avoiding competition with food cycle. We had to revisit this and to refine our interest for direct production of algae for biodiesel processing. Literature data show that algae concentrations do not exceed a level of 0.7-1.0 g/l in water. This concentration of algae is very low for practical applications. This has led to a conclusion that under market realities algae cannot be a panacea to supply feedstock for feasible biodiesel production. Lundquist et al. [1] revealed on the basis of detailed de novo analysis, that project costs are too high for microalgae in facilities designed primarily for supplying feedstock for biofuel. Even with low capital charges, it is not possible to produce microalgae biofuels cost "competitively with fossil fuels, or even with other biofuels, without major advances in technology. Difficulties are related to control uniformly warm temperature conditions and ample sunshine availability in addition to adequate expertise. Cost model of Slade and Bauen [2] concluded to similar recommendations. For the sake of dramatic improvements that are required for both productivity and energy efficiency it is to greatly reduce the cost of biomass production with demanding requirement on cost of carbon source (CO_2) and nutrients.

There seems to be a consensus in the field that algae production can only be viable under warm climatic conditions. Algae production in indoor bioreactor facilities must aim the production of precious commodities of cosmetics, pharmaceuticals and nutraceuticals [2]. Wastewater borne algae do not meet hygienic criteria for such. This is why we have turned our interest toward algal based wastewater treatment (WWT). This practice does not compete with food cycles but makes use of waste components and in addition can extend the basis for production of biofuels feedstocks. Only a few optimistic companies have stayed in the business of algal biofuels.

Symbiosis of activated sludge and algae in WWT has been reported as early as in 1950's [3]. Under autotrophic conditions 1 g of microalgae assimilate 1.88 g CO_2 from the atmosphere and provides oxygen for turning the atmosphere into cleaner air to breath. The idea is brilliantly ecologic: algae produce what facultative microorganisms need for proliferation and facultative microorganisms produce what is necessary for algae. Nutrients (C, P, N, K, etc.) are abundantly present in most wastewater effluents. Because of provision of readily dissolved oxygen by algae facultative microorganisms access this basic component to biochemical oxygen with much higher efficiency than from oxygen having been present in relatively large air bubbles. Because of proliferation of facultative microorganisms the essential component to photosynthesis of algae is present in abundance and by such algae grow faster than under carbon dioxide starvation conditions, with the conditions of being illuminated adequately. Algae assisted WWT has been restricted to warm climatic conditions. There have been limited efforts to employ algae under colder climates. Thorin et al. [3] presented results on symbiosis in co-digestion of sewage sludge and microalgae with some degree of synergies under mesophilic and thermophilic conditions.

Aeration in biochemical degradation consumes high specific energy in form of electric energy by compressors WWT to provide. By the use of symbiotic operation Aquanos was able to achieve a 57% reduction in total operating costs when compared with activated sludge [4]. It remains to life cycle analysts to estimate the efficiency of competing against global warming. We are not convinced that it is necessary to select a given strain of algae [5] for significantly positive result from the point of view of biofuels production. By operating with "native" strains of algae in wastewater we have a chance to promote the most ecologic solution. In this type of approach those native algae strains will be domesticated this constitutes a key idea of present work. We have also been interested to learn about technical aspects of symbiotic WWT and the potential of use of the sludge for biodiesel production under colder climate conditions. Criteria if circular economy have also been on the motivation list to recycle valuable components to replenish fertility of soils. Employing efficient unit operations in biochemical and colloid chemical processes in WWT has also been on the list of motivations.

We have had two prior experiments to learn about the role of illumination (this has been considered to be a process of stimulation). An activated sludge system with facultative microorganisms in a WWT unit clearly produced phototrophic responses. We did observe that the energy of the incident light boosted microbial activity in activated sludge of WWT equipped with our bio-membrane-stripper [6], without the addition of any other nutrient, other than present in the waste stream. While performing this research, we have concluded that light promotes proliferation of activated sludge. Alas, we have failed to pay attention to potential benefit of presence of native algae strains and to associate the improvement with symbiotic effects of boosting activity of the aerobic microorganism community. Biodegradation efficiency was improved by 10-30% when the system was illuminated, achieving a faster biochemical oxidation of COD compounds. Removal efficiency of phosphorous was increased by 20-30% to a level of 70-80%, without the use of any added chemical. In our actual experiments we have refreshed this idea and conditions have been adjusted to needs and conditions for symbiotic communities of algae and activated sludge.

It has been a motivation factor in an efforts to learn about the impact of illumination on efficiency of anaerobic degradation (AD) of food waste [7]. If everything was similar, biogas production activity of the microbial colony of was much reduced (Fig. 1) under illuminated conditions. Similarities have been determinant to continue with symbiotic experiments in WWT.

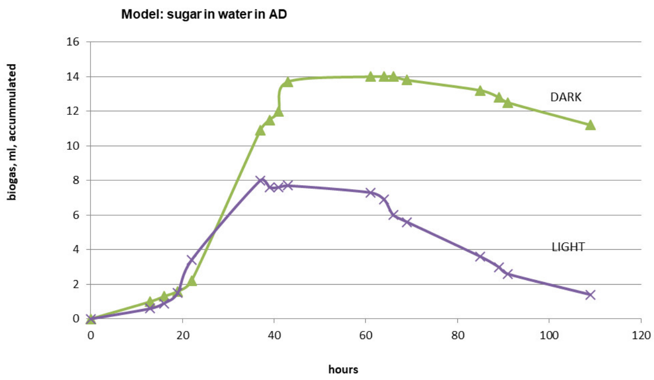

Fig. 1 illumination does inhibit anaerobic microorganisms to produce biogas [7]

1.2 Biodiesel synthesis from algal sources

There have been scientific and technical reviews dealing with algal based biofuels production [8, 9, 10]. The latest in this series, the Review of DOE of Algal Biofuels Technology confers the message that algae are potential resources for biofuels production, because their physiology makes that under normal or stress conditions biochemistry responses result in production of triacylglycerides (TAG) or carbohydrates. Storage of these and other components within cells predestinate algae for production of different fuels through different conversion routes. Their composition makes possible to produce biogas, bioethanol or biodiesel or bio-combustible fuel oil or combination of these. We have maintained our interest to biodiesel and therefore the objective of our study has been limited to specifics to biodiesel. Efforts to improve efficiency of WWT operations and to learn about conversion specifics have been the overall framework.

Most recent treaties have focused on biodiesel to process low cost refuse feedstocks in second generation technologies. Problems in conversion of such refuse stocks by transesterification and in handling and processing byproducts turned engineers to prefer the route of severe catalytic hydrotreatment (SCH) and isomerization to produce excellent characteristic hydrodiesel fuel. The preference for SCH is similar to what petroleum engineers do practice by employing SCH to improve product qualities for the last 20-30 years. In SCH pressures above 8 MPa, temperatures above 300 °C and fast deactivation of the catalyst make that scale to economy of hydrodiesel production exceeds by an order of magnitude the scale to economy of conversion technology by second or advanced generation transesterification schemes (150-200 kt/y vs 3-35 kt/y). To match supply- demand characteristics and closing the gap between fossil and renewable energy feedstocks it has been proposed [11] to co-process secondary feedstocks and vacuum distillates in SCH. The quality of hydroprocessed diesel (HD) is net superior to trans-esterified diesel (TE) because of specifics of rearrangement and cracking of the deoxygenated, saturated hydrocarbons into i-alkanes, on the expense of diesel yields [12]. By taking into account ecologic and rural role of TE biodiesel we have opted to explore further the TE conversion with the sole criteria of meeting quality specifications of EN 14214 [13].

Alternative use of refuse biomass in liquefaction is also associated with severe technological regimes, pressure: up to 20 MPa, temperatures up to 375 °C. Thermal treatment of refuse stocks and biodiesel byproduct glycerol phase in delayed coking has also been considered [14, 15]. On this track we have opted for coking the algal WWT sludge biomass to improve the light oil and coke yields. Benefits have been lower in extent than expected.

2 Materials and methods

2.1 Algal wastewater treatment and analysis

The technique of domestication is a mode of operation in which those microorganisms that are present in wastewater system are grown and employed within the system. No other micro-organism species are added to the system. Conditions of treatment proliferate activity of these.

Proliferation is a mean of stimulated mode of operation. Special conditions are provided to support domestication and high activity for symbiotic operation. The technique provides access to oxygen, carbon source and illumination for the benefit of more efficient degradation of pollutant components.

The wastewater stock was sampled from anoxic chamber of a communal system that has just been put in operation in Tarhos, Hungary. System performance analysis was the duty of our company and the feedstock was sampled under real conditions and circumstances and no other nutrient components were added to the system in due course of biochemical oxidation with the exception of air, CO_2, and light. Feedstock characteristics are summarized in Table 1.

Together with the frame of analysis matrix of physical and chemical tests. TSS, total suspended matter was determined by filtering 7.5 ml sample through a dried and tared Whatman glass microfilter 934-AHTM of Sigma Aldrich. The final vacuum was 100 mbar.

Table 1 Analysis matrix of physical and chemical tests, characteristics of the stock sampled from anoxi chamber to be explored in biochemical oxidation tests

Test	Method used	Unit	Feedstock
TSS, total suspended matter	ASTM D5907 - 13	mg/l	785
pH	KEM- AT-510	-	7.86
Chemical Oxygen Demand, dissolved	LCK 314, LCK 614	mgO_2/l	278
Biological Oxygen Demand, dissolved	Oxytop and microcosm	mgO_2/l	181
Ammonia, dissolved	LCK 303	mg/l	25.1
Total nitrogen, dissolved	LCK 238	mg/l	41.5
Phosphorous, dissolved	LCK 349, LCK 350	mg/l	8.20

Dissolved chemical and biological oxygen demands (COD, BOD), ammonia, total nitrogen (TN) and phosphorous (P) contents of the filtrates and bacterial colonies have been measured from the filtrate. The cake was dried in oven at 105 °C and weighed on an analytical balance. Biomass production has been assessed by comparing actual TSS values to values under "condition 1" without employing any other stimulation than provision of oxygen.

Bacterial colonies showed no specific characteristics under the microscope (Fig. 2).

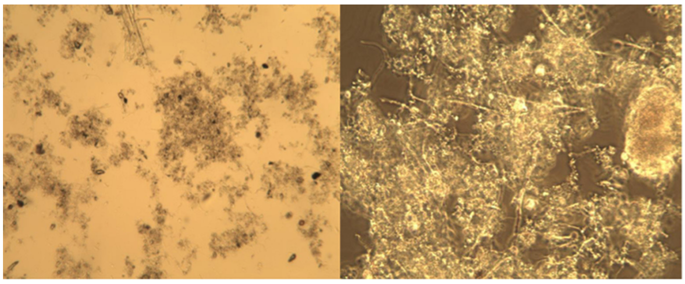

Fig. 2 microbial colonies in the feedstock of the experiment under microscope objectives (magnification: 100x and 400x)

There were the following species present: Rotatoria sp., Pyxidicula sp., Epystilis sp., Aspidisca sp., Arcella sp., Euglypha sp., Vorticella sp. (Courtesy to Alföld Víz) in preponderance. pH was tested by the use of a KEM AT-510, potentiometric titrator. Compositional analysis was tested by the use of test kits of Hach with a DR 2800 spectrophotometer.

Biochemical oxidation tests have been performed in a 500 ml capacity stirred vessel made of stainless steel. Illumination was provided by the use of 3W led light, with a light pass less than 30 mm in water. Mixing was provided by 12 V motor, equipped with a propeller having a variable stirring rate of revolution in the range of 75-300 RPM. Temperature was controlled by circulated water in the jacket of the vessel. pH was read and monitored by an immersed probe connected to Boeco pH-meter, BT-600. Metering of food grade CO_2 was controlled by a precision metering valve of Fitok (MSSS-MLV-6-V). The stream of CO_2 was mixed with air stream delivered by a mini compressor and controlled by a valve and rotameter settings Sampling was done by the use of an automatic pipette.

2.2 Biodiesel synthesis

Conversion of algal feedstocks have been done by Co-Sol technique [16] in aliphatic hydrocarbon solvent (hexane) under homogeneous single phase reaction mixture in 50 ml three neck flask under total reflux and continuous stirring with a magnet bar.

The biomass was separated from the water phase by filtration, on similar filter media as for the TSS analysis of the treated water. The filtered material was dried in oven at 105 °C to constant weight on an analytical balance. The dried biomass constituted the substrate for the synthesis to FAME. For the sake of controlling the rates of reagents and catalyst the biomass was supposed to testing saponification number. This test shows the amount of free fatty acids and hydrolyzed fatty acids in terms of mg KOH/g necessary for forming soap. 56.1 mg KOH/g is equivalent to 32 mg MeOH in esterification +transesterification. Here are the reactions involved in testing and conversion to biodiesel (Table 2).

Sequence of operations in biodiesel conversion: 1st step (esterification and cell wall destruction): 0.6% H2SO4 catalyst, 2 times excess of stoichiometric amount of MeOH, hexane solvent and the dry substrate biomass are mixed under reflux for 30 min at a temperature of about 70 °C. Time to cool is 10 min before transferring the reaction mixture to a separatory funnel. After 10 min of settling the lower phase was separated for mass balance analysis and eventual for recycle of the reagent. The upper phase was returned to the 50 ml three neck flask. 2nd step (transesterification): 0.8% KOH catalyst, 1.5 times excess of stoichiometric amount of MeOH was added. The reaction mixture was boiled under reflux at a temperature of about 65 °C for 20 min. Cooling and settling was similar to step 1. The upper phase was washed with distilled water and settled for 10 mins for two times. Because of low amounts the refined biodiesel upper phase was tested for composition and yield by the use of an ACME 6100 gas chromatograph according to EN 14103:2011 [17] suitable to determine methyl esters between C6 and C24. For reference Supelco® 37 Component FAME Mix of Sigma Aldrich was used. The column was Zebron 5HT, 30 m x 0.25 mm x 0.25 µm. Injection volume: 3 µl, temperature of injection was 300°C, split rate 10:1, carrier gas: He @1 ml/min. Detector: FID@350°C. Oven program: 80 °C (1 min) to 180 °C, ramp 15 °C/min, final 350 °C, ramp of 7 °C/min, final hold 9 min.

Table 2 Reactions involved in testing saponification number and in conversion to biodiesel (fame= fatty acid methyl ester)

Saponification:	
of free fatty acids	$FA + KOH \Leftrightarrow FAK + H_2O$
of triglycerides	$TG + 3KOH \Leftrightarrow 3FAK + G$
Esterification	$FA + MeOH \Leftrightarrow FAMe + H_2O$
Transesterification	$TG + 3MeOH \Leftrightarrow FAMe + G$

3 Results

3.1 WWT Performances

Properties of the treated water streams in function of time and conditions are summarized in Table 3.

Even though there are many open questions, these experiments provided results to conclude:

- The extent of biological oxidation can be boosted by higher availability of oxygen in the system;
- Lighting gives boost to biological oxidation. Starvation of light resulted in net reduction in extent of removal of the desired components;
- The wastewater and the treatment process show that there is starvation on carbon sources. Upon sparkling carbon dioxide into the system the efficiency of biological oxidation was boosted;
- Addition of carbon dioxide into the system resulted in net increase in volume of biomass, but the order of magnitude of biomass production was not changed;

Table 3 Waste water treatment efficiencies in the experimental biochemical oxidation

Conditions	0	1	2	3	4	5	6	7	8	9
air		+	+	+	+	+	+	++	++	++
CO_2		-	+	+	-	+	++	-	++	++
light		-	-	+	+	-	+	-	+	+
Time, days		5	5	5	5	5	5	5	5	5
Tests										
TSS, mg/l	785	286	290	371	299	421	512	283	531	492
pH	7.86	7.38	7.01	6.9	7.41	6.92	6.75	7.33	6.72	6.76
dissolved, COD, mgO_2/l	278	77	57.8	40.1	43.8	62	35.8	71	38-3	37.2
dissolved, BOD, mgO_2/l	181	52	39	30	29	44	22	43	26	25
dissolved, TN, mg/l	41.5	17.8	8.8	3.1	13.8	11.0	2.51	10.4	6.3	5.8
dissolved, P, mg/l	8.20	3.24	2.98	2.35	3.01	2.76	2.10	3.1	2.15	2.37

- Addition of carbon dioxide into the system resulted in net symbiosis in terms of efficiency of biological oxidation measured in removal of dissolved COD, BOD, N2 and P containing components;
- There are reserves in the system as indicated by the rate of BOD/COD at a level of about 0.6.
- There is also room for further improvements and refinements in the experimental system. Even though the results are convincing experiments in the improved system must be repeated to conclude to statistical analysis.

On examining dynamics of COD and P removal during the 5 days test conclusion of having reserves in biochemical oxidation must be revisited. P removal patterns are similar for stimulated and domesticated cases and can be approached by exponential equation with good match (Fig. 3).

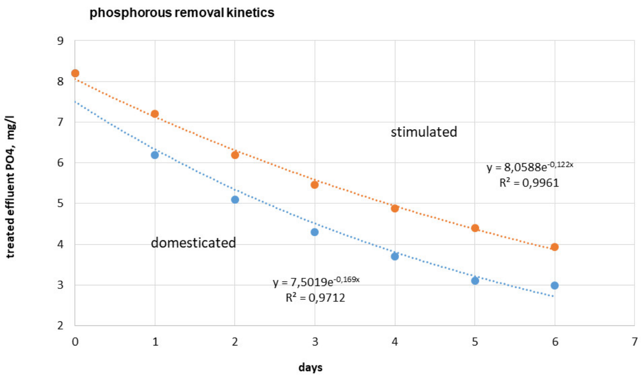

Fig. 3 phosphorous removal kinetics under stimulated and domesticated conditions

What cannot be seen by simple approximation is that in case of domestication there are signs for exhaustion of microorganism community after the day five treatment period. This can be associated to scarcity of carbon source in the system. There are clearer signs for exhaustion in COD removal kinetics (Fig. 4).

One can also approach this pattern as a matter of colloid chemistry problem, by better dispersion of those small diameter microorganisms (supposedly algae) in the water phase that can pass through the network of the filter used. For the domesticated system COD removal is very fast at start and shows exhaustion after day 2. It seems that the process of COD removal is a more complicated bio- and chemical process than removal of phosphorous. If approximation is applied there is a 2nd order polynomial approximation for the case of domesticated and 3rd order polynomial approximation for the domesticated and stimulated system. This is good for biofuels business. The better the phosphorous removal, the higher the biomass production. Because of the limited number of data points, a straightforward linear relationship can be associated for P-content and biomass production, but with more modest reproducibility (Fig. 5) than between P-content and time of treatment.

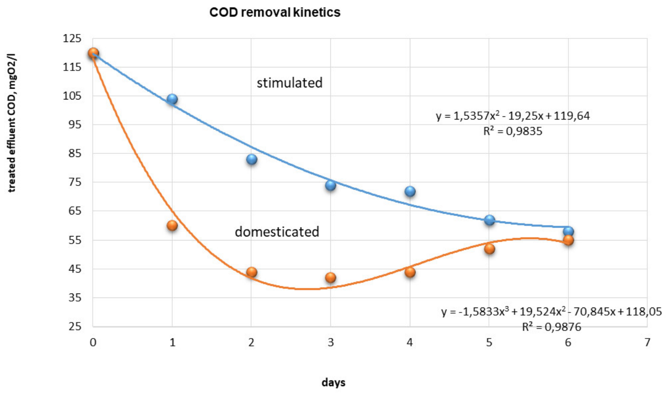

Fig. 4 COD removal kinetics by stimulation and domestication

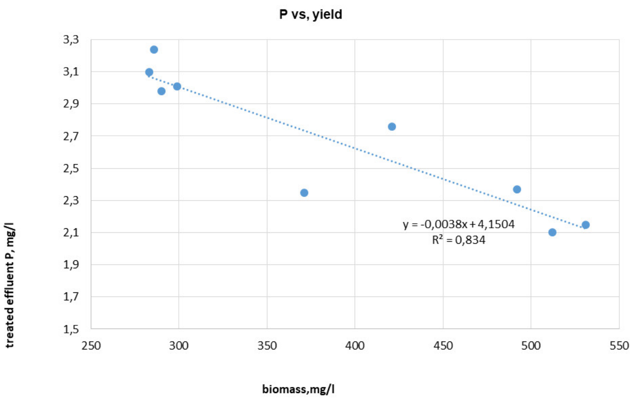

Fig. 5 Phosphorous removal vs biomass production

But it can be stated that addition of carbon dioxide clearly contributed to increase of the yield of biomass and to reduction of phosphorous content in the treated stream. There are two distinct regions: [high P content – low biomass production] for scarcity of light with [low P content – higher biomass production] indicates the importance of illumination, hence positive impact of photosynthesis in a symbiotic system. In making conclusions to biomass production it is to bear in mind the extent of operation of the experimental system. Upon complete removal of P the biomass production approaches the value known as optimal production of biomass in algal systems, i.e. 1 g/l, but for this the experiment had to be run for more weeks.

3.2 Biodiesel processing

The limited scale of the experiments did not make possible a full analysis of the biodiesel, other than estimation of yield and composition of the FAME fraction.

The immediate observation of experiments is the difficulty in handling the phase of polars. In the settled phase solids stubbornly adhered to the wall of the flask. This point makes us to revisit the series of technique employed in the process of conversion of the biomass to biodiesel and makes us to understand why actual efforts follow the route of either conversion of the biomass to biogas or treatment in thermal processes, such as hydrothermal liquefaction or delayed coking. Differences in compositions have been visually evident in settling funnels of the refined biodiesel phases (Fig. 6) in function of illumination conditions in biochemical oxidation.

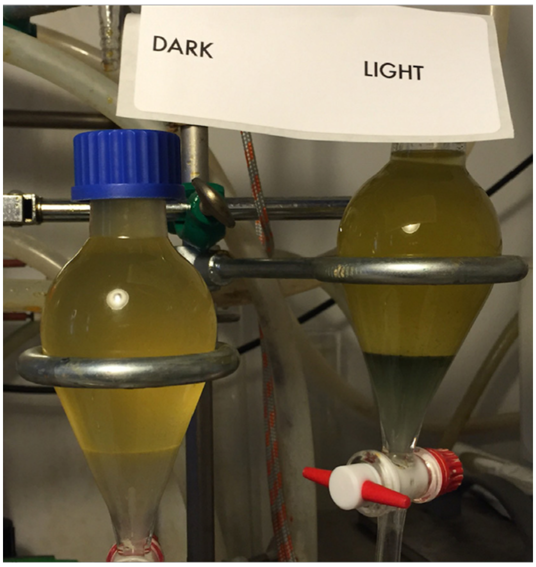

Fig. 6 Comparison of biodiesel phase refining, influence of illumination in algae culture, algae grown in light produced denser polar phase

Color of the refined and settled phases clearly indicate that there were markant differences in composition of algae grown in light or in dark if other conditions were similar. Similar difference could have been observed in the reaction flask too.

Table 4 reports conversion data of the filtered and dried biomass after 5 days of biochemical oxidation.

Numbers of experiments in the row of "conditions" are similar to those in Table 3. Here again, the limited amount of sample and experimental data points make that we can only state that there is potential in conversion of biomass of symbiotic WWT treatment.

Differences are not only evident in appearance of the settled phases, but in composition of the biofuels too. The main components are C_{16}, C_{18} fatty acids in both samples. The rate of unsaturation is higher in both presented biodiesel samples than in customary FAME fuels. There are fatty acid peaks outside the accepted range of [14-24] carbon atoms in molecule (C_{14}-C_{24}), within the retention time window of [2.7:20.0 min] (Fig. 7).

Without exact listing of FAME components, it is of importance that there are more and accentuated components in in the biodiesel synthesized from biomass that was cultivated in light (upper) than in the biodiesel from biomass cultivated in dark. Illumination has markedly promoted the biosynthesis of shorter fatty acid containing components. The presence of light components indicate that biodiesel produced from such algal systems must be treated in a downstream operational process, to remove components with less than 14 carbon molecule in the fatty acid side of the ester, by vacuum distillation. This can make the entire process even more difficult to finance. This is another reason to consider processing of the biomass in thermal or severe catalytic hydrotreatment operations.

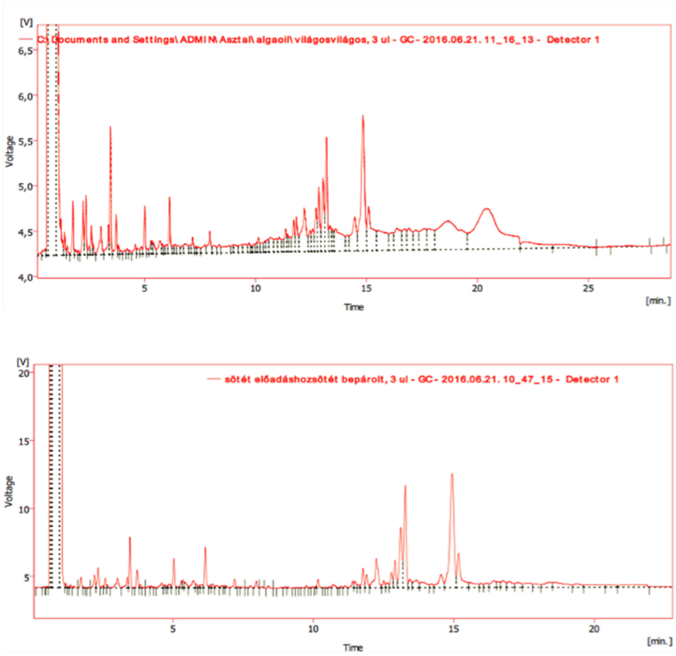

Fig. 7 FAME pattern of a biodiesel converted from algal biomass

4 Conclusions

Beside those know positive facts, as lower greenhouse gas production, better specific energy consumptions, treating wastewater in symbiotic WWT operation with native algae species under domesticated conditions net superior efficiencies have been recorded, in comparison to conventional WWT figures. Accordingly, for a given level of treatment the time on stream can be reduced. This makes possible to increase processing capacities of a facility without extensive investment for larger volume processing units.

There is a FAME production potential of 8-18% in the biomass of the (excess) sludge. Processing difficulties of this phase makes to consider other alternative routes. Hydrothermal or biogas conversion processes can be the preferred upgrading schemes of the settled sludge phase, rather than FAME synthesis for automotive fuels production.

Table 4 Fame yields, synthesis data

Conditions	0	1	2	3	4	5	6	7	8	9
Biodiesel yield, mg/l	62.8	31.5	26.1	51.9	32.9	37.9	92.2	19.8	95.6	88.6
Biodiesel yield, %	8	11	9	14	11	9	18	7	18	18

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