Microalgae treatment and water quality control

Deliverable 5.3

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Imprint

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Summary

Wastewater composition is a very important factor when considering microalgae treatment as a potential step within a wastewater treatment process. Therefore, use of microalgae species for waste water treatment requires selection of suitable microalgae-wastewater combinations. Mixed industrial/municipal wastewaters from Kohtla-Järve, Estonia were selected for testing with algae based on the assumption that they represent typical conditions in larger municipalities where industrial and municipal wastewaters as well as storm water are mixed and then treated together.

The final tests and treatment of collected wastewaters were performed on the microalgal species *Chlorella sorokiniana* which appeared to be more promising for the treatment of wastewaters based on the microplate screening tests. The study applied four dilution rates (i.e. four different retention periods for the wastewater) for testing the performance of this selected species.

The lowest dilution rate (0.72 d^{-1}) provided the highest biomass concentration up to 1.44g l^{-1}. The highest biomass productivity (1.46 g $l^{-1}d^{-1}$) is exhibited with a dilution rate of 1.8 d^{-1}. The highest removal efficiencies (> 90%) of pollutants are also observed at the lowest dilution rate (0.72 d^{-1}). However, the removal of COD for all dilution rates is only around 50%. The removal efficiencies of zinc at different dilution rates varied considerably being more than 30% at the lowest dilution rates of 0.72 and 1.80 d^{-1}.

The algae treatment at the lowest dilution rates are comparable to the treatment efficiencies of the conventional removal of total phosphorus. The removal of TN and COD did not perform that well and provided 65 to 42% weaker results, respectively, compared to the conventional treatment.

1. Introduction – microalgae cultivation systems

Microalgae are single-cell algal species that have certain potential advantages compared to e.g. macrophytes, bacterial communities and macroalgae. They are fast growing and cultivations are relatively easy to control. Therefore, lots of experiments have been carried out over the past decades to produce microalgae biomass for its practical use. Possible bio-products from that biomass involve biofuel, food, medicine, cosmetics, fertiliser, etc. At the same there are lots of challenges to make these productions cost-effective. Cultivation and harvesting of microalgae is still rather costly and economically unfeasible, except production of biomass for some high value products e.g. medicine and cosmetics.

Another option is mass production of algal biomass on waste streams for simultaneous treatment of wastewater that is quite an old idea. This kind of use requires selection of the most suitable species and conditions as well as the types of wastewaters to be treated. The cost-effectiveness of wastewater treatment by microalgae is also still questionable, as well as industrialization of the experimental results. Thus limitations for an industrialization have not been technical but in most cases nontechnical, i.e. a problem of commercialization.

Two types of microalgae cultivation systems are mainly used: open raceway ponds and closed photobioreactors. Raceway pond (Figure 1) is a mature technology that has been applied for algae cultivation since the 1950s. It is characterized by a shallow water (up to 0.5 m) that allows sufficient sunlight to facilitate photosynthesis and a paddle wheel that ensures mixing of water to prevent algae settlement and to enhance gas exchange.[1,2] The edge of raceway open systems is the relatively low cost for establishment, maintenance and scale-up. However, since culturing conditions of open systems are less controllable, the raceway pond suffers from low productivity. Furthermore, low biomass density of the culture greatly increases the costs for harvesting that is crucial for waste water treatment in order to remove nutrients and other substances. Additionally, the open systems encounter a major problem of contamination by fast growing competitors in the environment.

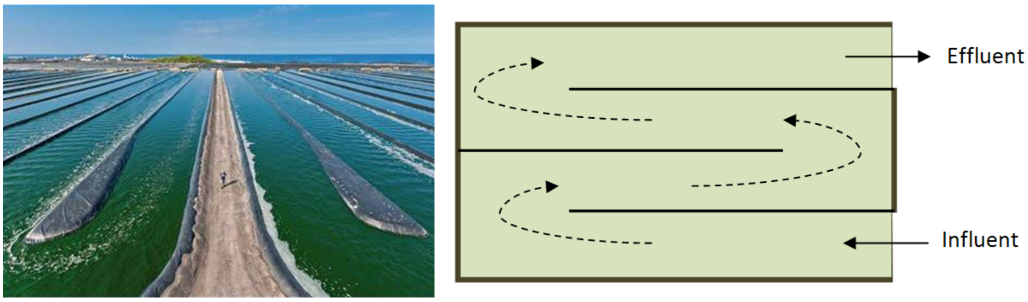

Figure 1: Open raceway pond system for microalgae cultivation *(Photo credit: Nature Magazine)*

Different forms of closed photo-bioreactors (PBRs) are also quite widely used, including vertical, horizontal, tubular, flat-panel and plastic bag designs (Figure 2). By contrast to open pond, closed systems of PBR enable better controlling of the culturing conditions and substantially reduce the chance of contamination by unneeded species, thereby providing higher productivity over the open systems. Documented biomass volumetric productivities of open pond range from 0.05 - 0.32 g l^{-1} d^{-1}, while productivity of PBR can reach up to 3.8 g l^{-1} d^{-1}, being usually 2- 2.5 folds higher than in high rate algae pond.[3,4]

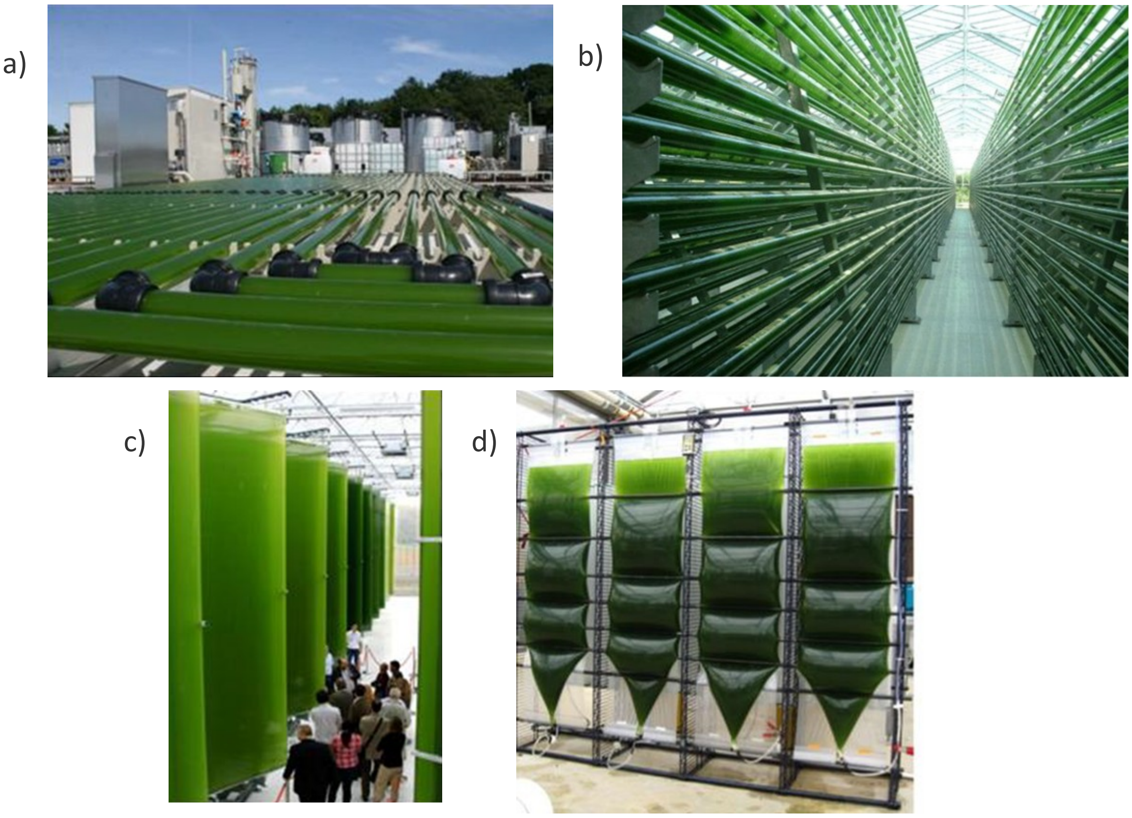

Figure 2: Examples of the different forms of closed photo-bioreactors for microalgae cultivation: a) tubular horizontal photo-bioreactor, b) horizontal tubes photo-bioreactor, c) vertical flat panel photo-bioreactors, d) closed hanging plastic bag cultivation system *(Photo credits: Wageningen university, Netherland; Abdel-Raouf et al., 2012; Ecoduna, Austria; Joel Cuello, Arizona university)*

To attain maximum productivity, the design of PBR configuration needs to fulfill certain requirements for light, temperature, pH, nutrient supply, mixing as well as oxygen degassing. Each of the common photobioreactor designs has certain advantages and disadvantages (Table 1). None of them is able to provide cost-effective control of all parameters simultaneously that remains a major challenge for economic production of microalgae.[5]

Table 1: Advantages and limitations of open pond and photobioreactors[3]

Production system	Advantages	Limitations
Raceway pond	Inexpensive construction Easy clean and maintenance Low energy inputs Low operational costs	Poor biomass productivity Easily contaminated Poor mixing, light and CO_2 utilization Loss water by evaporation
Tubular PBR	Large illumination surface Relatively cheap	Some degree of wall growth Fouling Requires large land space Gradients of pH, CO_2 along the tubes
Flat plate PBR	High biomass productivity Easy to sterilize Low oxygen build-up Good light path Large illumination surface	Difficult scale-up Difficult temperature control Some degree of wall growth
Column PBR	High mass transfer Low energy consumption Good mixing Easy to sterilize Reduced photoinhibition Reduced photooxidation	Small illumination area Expensive construction Shear stress

A big step towards more efficient cultivation systems has been the construction of pilot scale research facilities around the world where new designs are tested and optimized in order to solve a number of scaling up challenges, mainly contaminations and varying environmental conditions.

Although more concentrated algae cultures produced by PBR system is advantageous for reducing the energy consumption in dewatering, the energy consumption for pumping culture medium and overcoming friction is much higher than open pond at the cultivation stage.[6] Furthermore, toxic influence of accumulated oxygen is considered one of the most difficult problems of closed systems. Overheating and biofouling in a closed system are also problematic.[7] Additionally, capital investment is the dominated cost for cultivation with PBR, which is too high to be viable for algae production, even for high value products.[6,8,9] A financial analysis demonstrated that capital expense and operation expense need to be reduced by at least 80% and 90%, respectively, for PBRs to achieve a 95% probability of economic success.[9] Because of this, open ponds are preferred and currently used by most algae producers.

A large number of parameters are relevant to optimize microalgal cultivation including light-use efficiency, growth rate, biomass productivity, biomass composition and nutrient removal rate and nutrient concentration in effluent. For a specific algal strain and wastewater these parameters will depend on dilution rate (in continuous cultivation), nutrient composition and concentration of wastewater, pH, temperature, light incident intensity and distribution, CO_2 availability, reactor design, etc. With exception of wastewater nutrient composition and perhaps incident light intensity, these parameters are very much controllable using existing methods. Optimization of these parameters is therefore a very logical next step towards economically viable algae production.

2. Testing of microalgae

Wastewater composition is a very important factor when considering microalgae treatment as a potential step within a wastewater treatment process. Therefore, potential use of microalgae species for waste water treatment required selection of suitable microalgae-wastewater combinations and optimal conditions for both the biomass production as well as the treatment of wastewater. It involved several stages starting from the algae microtiter screening by using microplates and the development of a single stage continuous bioreactor configurations and optimization of the conditions to promote the transformation of the organic compounds and nutrients present in the wastewater into microalgal biomass. The reactor was designed to be scalable, so that data gathered could be applied to an industrial scale PBR scenario.

Several different operational conditions were studied (temperature, pH, CO_2 and organic loading rate, nutrients levels etc.) and process was optimized to improve the growth performance of the cultures (and the concomitant nutrients removal) and the composition of the generated microalgae biomass.

3. Influent wastewater quality

Based on the study on typical composition of wastewaters and considering a specific interest of stakeholders, a municipal WWTP in Kohtla-Järve, Estonia, has been selected for testing its influent wastewaters. It has been assumed that these wastewaters represent typical conditions in larger municipalities where different industrial wastewaters, municipal sewage and storm water are often treated together. The mixture of different wastewaters could also provide more suitable conditions, e.g. N and P mass ratios for the algae biomass production.

The list of chemical parameters for the analyses was defined based on the previous study results on the wastewater quality. Thus, possibly more problematic compounds exceeding the detection limit were selected. Specific interest of the stakeholders has also been considered when selecting the parameters for further analysis. The results of the chemical analysis of mixed industrial and municipal influent wastewater at Kohtla-Järve WWTP are provided in tables 2 and 3.

Table 2: The content of nutrients and organic compounds in mixed industrial and municipal influent wastewater at Kohtla-Järve WWTP

		Concentration	
Parameter	**Unit**	**Mixed industrial and municipal**	**Municipal wastewater**
COD_{Cr}	mg O_2 l^{-1}	437	338
TOC	mg O_2 l^{-1}	111	75.2
BOD_7	mg O_2 l^{-1}	196	103
NO_2-N	mg l^{-1}	< 0.001	< 0.001
NH_4-N	mg l^{-1}	34.3	26.7
NO_3-N	mg l^{-1}	< 0.13	< 0.13
Ntot	mg l^{-1}	40	58
PO_4-P	mg l^{-1}	2.08	3.24
Ptot	mg l^{-1}	3.19	4.77

Table 3: The content of hazardous compounds in mixed industrial and municipal influent wastewaters at Kohtla-Järve WWTP

		Concentration		
Parameter	**Unit**	**Mixed wastewater**	**Industry I**	**Industry II**
Bi-base phenols	µg l^{-1}	1500	1300	2700
Monobasic phenols	µg l^{-1}	350	48	5000
Pentachlorophenols	µg l^{-1}	<0.1	<0.1	<0.1
Naphthalene	µg l^{-1}	11	0.01	58
Acenaphthylene	µg l^{-1}	0.61	<0.01	5.7
Acenaphtene	µg l^{-1}	0.66	0.35	4.3
Fluorene	µg l^{-1}	0.53	1.2	3.5
Phenanthrene	µg l^{-1}	0.86	0.01	4.9
Anthracene	µg l^{-1}	0.29	<0.01	2.1
Fluoranthene	µg l^{-1}	0.14	0.45	1.1
Pyrene	µg l^{-1}	0.19	<0.01	1.8
Benzoanthracene	µg l^{-1}	0.06	0.58	0.61
Chrysene	µg l^{-1}	0.06	<0.01	0.31
Benzo(b)fluoranthene	µg l^{-1}	0.01	<0.005	0.09
Benzo(k)fluoranthene	µg l^{-1}	0.03	<0.005	0.10
Benzo(a)pyrene	µg l^{-1}	0.03	<0.005	0.25
Indeno(1,2,3-cd)pyrene	µg l^{-1}	0.01	<0.005	0.07
Benzo[ghi]perylene	µg l^{-1}	0.01	<0.005	0.05
Dibenzoanthracene	µg l^{-1}	<0.005	<0.005	0.02
PAH sum	µg l^{-1}	14	2.6	83
Benzene	µg l^{-1}	28	190	630
Chloroform	µg l^{-1}	0.38	<0.1	0.68
1,2, di*chloroethane*	µg l^{-1}	<0.1	<0.1	0.71
Di*chloromethane*	µg l^{-1}	<0.1	<0.1	<0.1
Hg	µg l^{-1}	<0.015	0.089	0.098
Ni	mg l^{-1}	<0.02	0.043	<0.02
Zn	mg l^{-1}	0.046	0.025	0.023
PCB-sum	ng l^{-1}	<5	<5	<5

The wastewater treatment process at Kohtla-Järve involves conventional primary treatment and activated sludge process for enhanced biological phosphorus and nitrogen removal. Based on the data from the national water use database the mean treatment efficiency by using conventional treatment for BOD$_7$, COD, SS, Ntot and Ptot has been 98%, 93 %, 97%, 93.6% and 92%, respectively, in 2010-2013.

4. Treatment of wastewater by algae

4.1 Algae screening by using microplates

The selection of the best microalgae-wastewater combinations required several stages starting from the algae microtiter screening by using microplates (Figure 3), followed by the cultivation of selected agae species in photo-bioreactor for further comparison of yields and treatment efficiencies of wastewater (Figure 4). Collected data allowed assessment of the biomass yields and performance of microalgae when removing pollutants from the wastewater.

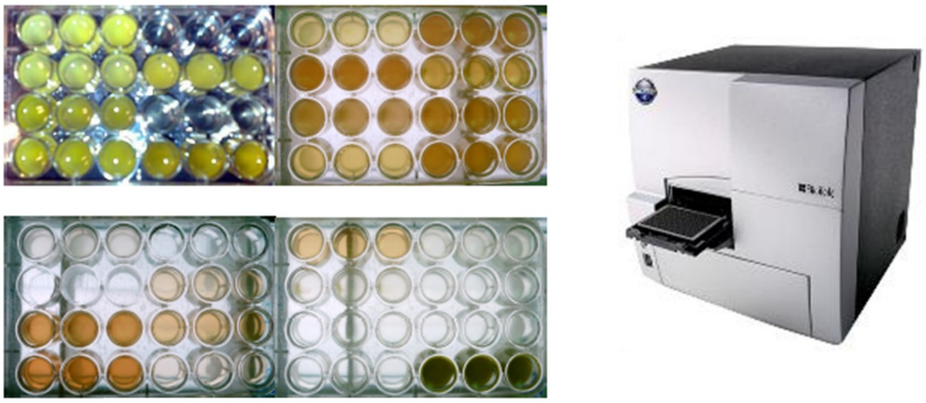

Figure 3: Microtiter screening by using microplates *(left)* and multi-mode microplate reader *(right)*

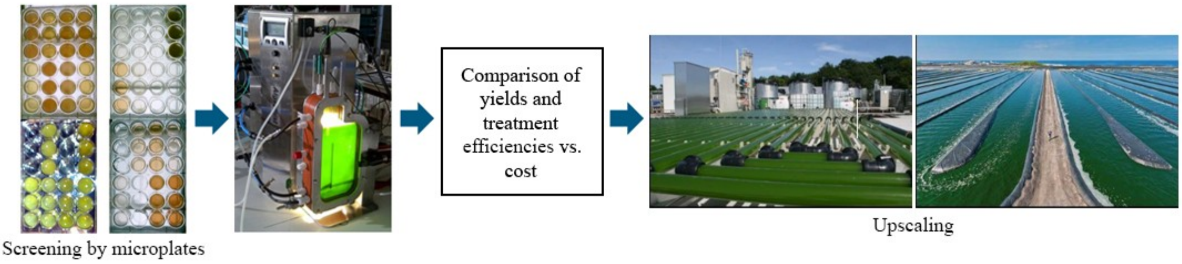

Figure 4: Basic stages for selection of optimal microalgae species providing higher yields, *versus* sufficient wastewater treatment and further upscaling to pilot scale

Screening in microplates enables reduction of the cost, space and time required to evaluate stains and thus improve the effectiveness of screening efforts. The results can not automatically provide answers regarding the best performing wastewater/species combinations for upscaling.

Microalgal species *Chlorella sorokiniana* vs *Scenedesmus obliquus* (Figure 5) were tested in microplates for their potential to grow in the wastewaters and the effect of pre-treatment of wastewaters against different parameters (temperature, pH, nitrogen and phosphorus dosage, organic matter, CO_2 and light intensity) was investigated. Comparison was made among wastewaters treated by filtration, centrifugation, sedimentation and the original wastewater (control) without any processing. Considering the effects of the pretreatment, removal of large particulates from the original wastewater (sedimentation) appears to have the most impact on algae growth. The data was then translated into growth curves (Figure 6) and the best species/wastewater combinations were determined.

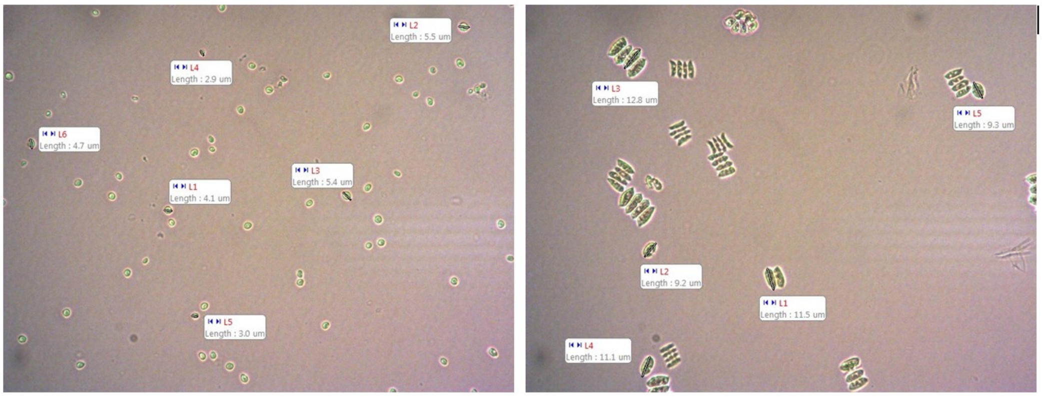

Figure 5: Microscopic image of *Chlorella sorokiniana (left) and Scenedesmus obliquus (right)*

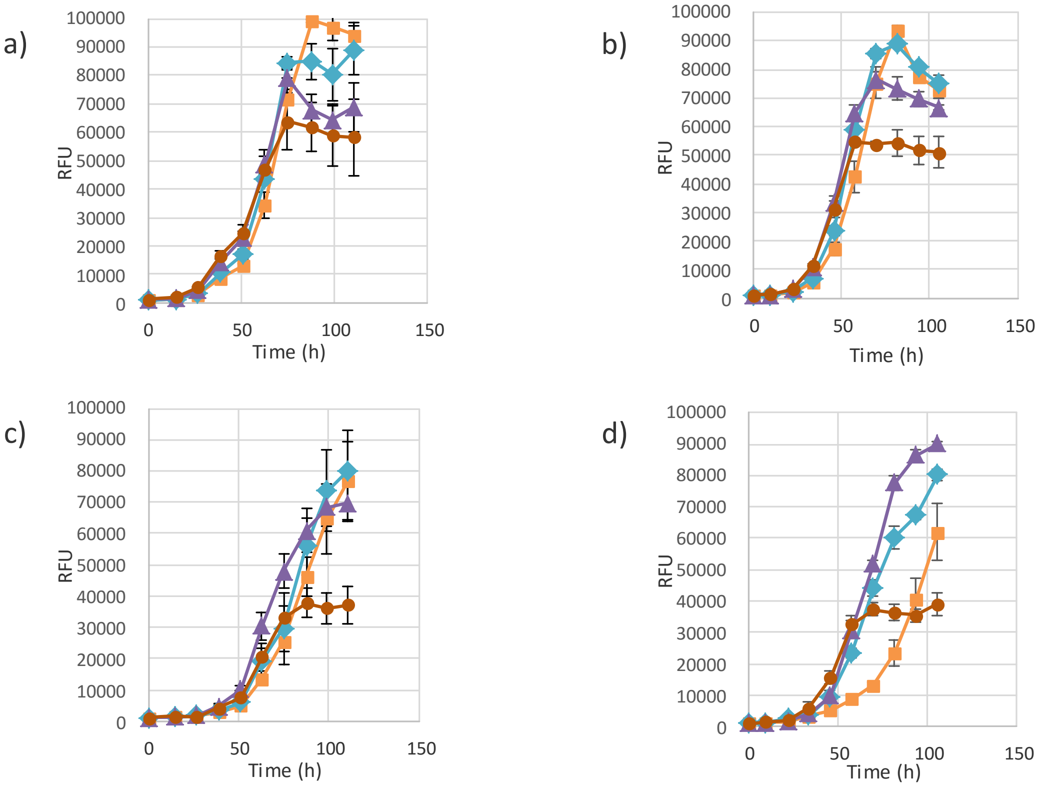

Figure 6: Growth curves: (a) *C. sorokiniana,* first generation, (b) *C. sorokiniana,* second generation, (c) *S. obliquus,* first generation, (d) *S. obliquus,* second generation (wastewater concentration: square- 100%, diamond-75%, triangle-50%, circle-25%)

Nutrient composition of the supernatant after sedimentation was measured and represented in Table 4. These results differ to some extent from the previous analysis made right after the wastewater sampling. The reason could be sedimentation of suspended solids in samples (Figure 7) as well as the changes occurred during the storage and handling of wastewaters.

Table 4: Composition of the influent wastewater at Kohtla-Järve after sedimentation of the water sample

Indicator	Quantity
COD	386.9 mg O_2 l^{-1}
Ntot	48.6 mg N l^{-1}
Ptot	7.2 mg P l^{-1}
NH_4-N	46.7 mg l^{-1}

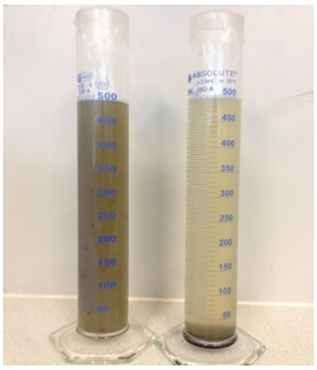

Figure 7: Removal of suspended solid by sedimentation

4.2 Photo-bioreactor cultivation

In order to validate the data coming from the microplate screening method, it is necessary to test these combinations in lab-scale photo-bioreactors which act like an intermediate step between the microplate scale where the volume of each well is only about 2 ml and the pilot scale facility where it can be several cubic meters. A flat-panel photo-bioreactor (Figure 8) was used to cultivate *C. sorokiniana* with the pretreated wastewater in order to assess the performance of algae growth. These species were selected as more promising considering the growth rate with different dilutions and the treatment of specific industrial/municipal wastewaters.

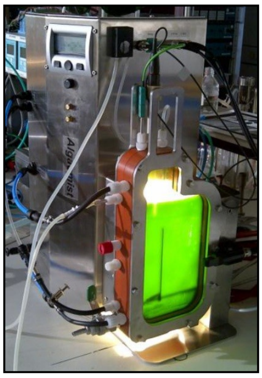

Figure 8: Closed laboratory scale flat panel photo-bioreactor *(Photo credit: Wageningen university, Netherland)*

5. Algae productivity

The lowest dilution rate (0.72 d^{-1}) provided the highest biomass concentration up to 1.44g l^{-1}. The highest biomass productivity (1.46 g $l^{-1}d^{-1}$) is exhibited with a dilution rate of 1.8 d^{-1}. The curve describing the correlation between dilution rate and biomass productivity indicates the peak point to be approximately 1.52 g $l^{-1}d^{-1}$ at a dilution rate of 2.41 d^{-1}.

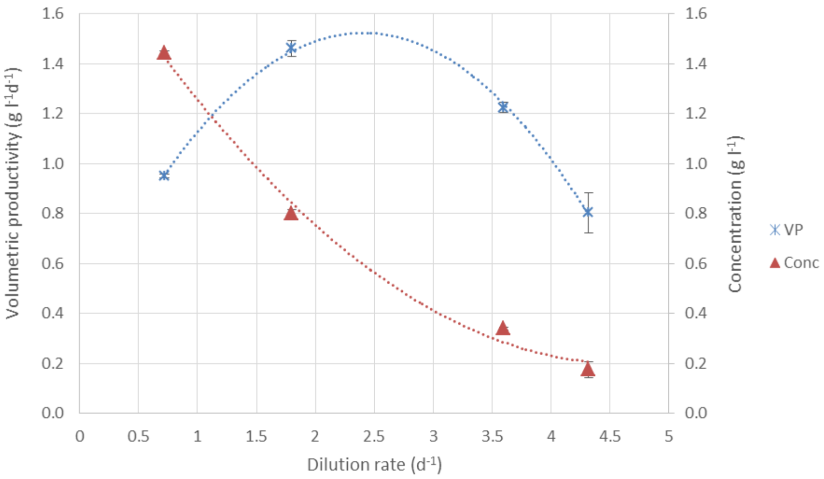

Figure 9: Effect of dilution rates on cell concentration and volumetric productivity

However, the biomass density and productivity obtained in present study were lower compared to the experimental work by Van Wagenen et al.[10] even the light intensity was twice as much. This can be attributed to the lower nutrient content, especially nitrogen in the influent wastewater from Kohtla-Järve, knowing that nitrogen is an essential nutrient for algal growth.

Generally, temperatures between 15°C and 25°C are the more suitable for most of the algae species. The light condition and temperatures during winter season could considerably impact the algae growth in the Nordic climate, even if the strains tolerate low temperatures. Nevertheless, those cold tolerant species may be used in wastewater treatment processes, even though the productivity can be relatively low.

6. Removal efficiency of pollutants

The treated wastewater was collected, analyzed and compared with the composition of untreated wastewater. The analysis of COD, TN, TP, NH_4-N and zinc were performed after the treatment process. The concentration of phenols and benzene in the influent wastewaters were also relatively high compared to the limit values, but analysis of these compounds in wastewaters used for algae treatment was omitted. Phenols and benzene are rather volatile and considerable amounts can be lost to the environment during and after the treatment process that could impact the reliability of the results. The treatment efficiencies by microalgae are presented in Figure 10.

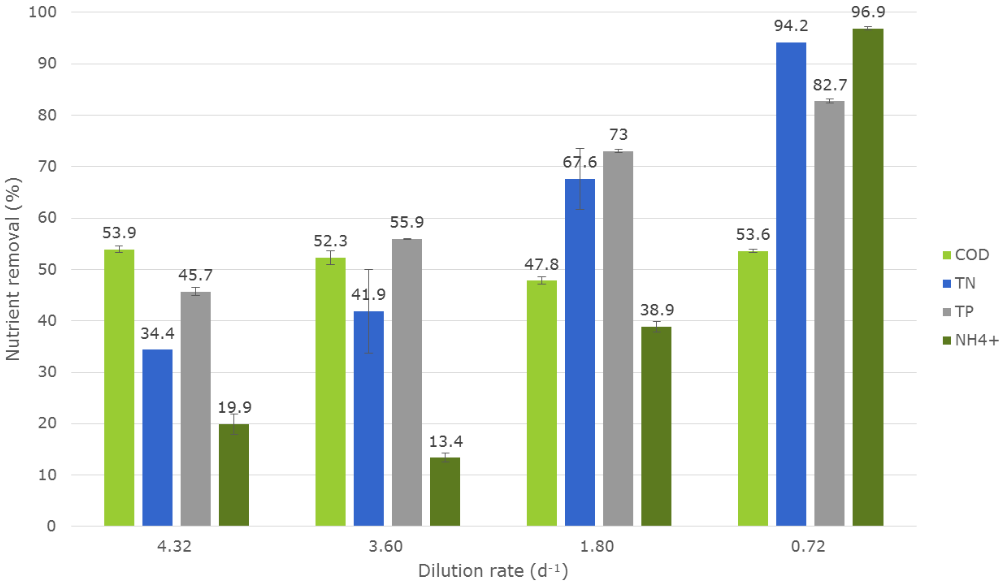

Figure 10: Effect of dilution rates on nutrient removal efficiencies

Removal efficiencies of zinc at different dilution rates (e.g. retention time) varied considerably. The removal efficiencies at the lowest dilution rates of 0.72 and 1.80 d^{-1} were about 30%.

The reported removal efficiencies for nitrogen and phosphorus are rather different depending on the microalgae species, waste stream, initial concentration and the type of the experiment. The results of our experiment are mostly in agreement with the previous experimental results, except for phosphorus where the removal rate was rather low, particularly at higher dilution rates.

The dilution rate 1.8 d^{-1} appeared to be optimal, both for the biomass production and for the removal of nitrogen and phosphorus from wastewaters (Figure 11). The higher removal efficiencies of the zinc were also observed at the lower dilution rates.

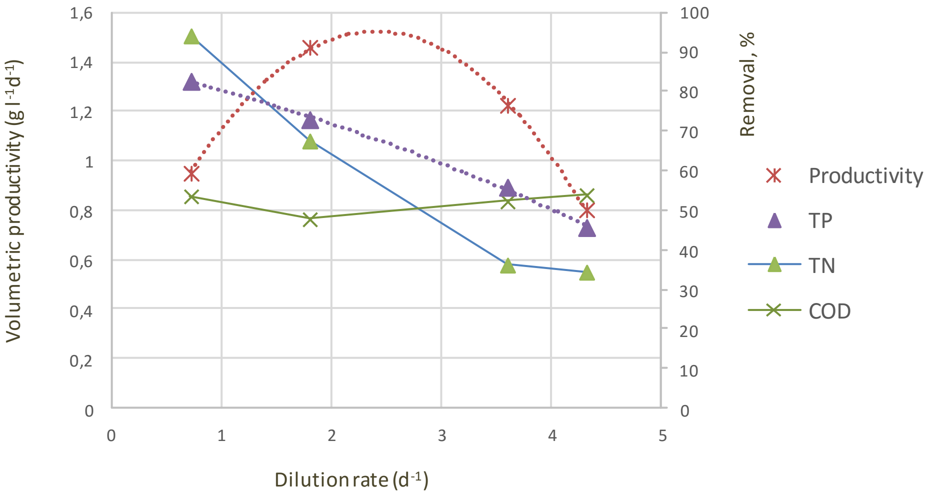

Figure 11: Effect of dilution rates on algae productivity and removal efficiencies of TP, TN and COD

The algae treatment at the lowest dilution rates performed rather similarly to the conventional removal of the total phosphorus. The removal of TN and COD did not perform that well and provided 65 to 42% weaker results, respectively, compared to the conventional treatment.

7. Conclusions

The treatment efficiency by using microalgae species largely depends on the type of wastewaters, applied parameters (e.g. light intensity and cycle, net biomass accumulation, etc.), the dilution rate and the specific algae species. Therefore, quite varying results on the removal of various compounds from the wastewater can be achieved. The microalgae species *C. sorokiniana* can well adapt to the wastewater chosen for this assessment and exhibits high biomass productivity. The algae performs rather well also when aiming to remove nutrients and other substances from the wastewater stream, although the removal of TN and COD did not perform as well as the conventional treatment at Kohtla-Järve WWTP and provided 65 to 42% weaker results, respectively. Achieving of both the high biomass yields and efficient wastewater treatment requires optimization of the conditions. The highest removal efficiency of nutrients and organic compounds was in most cases achieved with the lowest dilution rate. At the same time the lowest and the highest dilution rates provided lower biomass productivity and the highest biomass yields were detected with the mean dilution rate.

Further studies are needed to select cold tolerant species that are resistant to the poor light conditions during the long winter season in the Nordic climate. Probably, these species can be used in wastewater treatment processes, even though the productivity can be relatively low.

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