Chapter

# A New Insight of Phycoremdiation Study: Using Filamentous Algae for the Treatment of Tertiary Municipal Wastewater

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## Abstract

This book chapter demonstrated that the filamentous algae could be used as a promising phycoremediation approach to purify municipal tertiary wastewater. Initial screening of 25 algae strains across multiple genera revealed that *Spirogyra* sp. and *Klebsormidium* sp. were suitable to treat the tertiary effluent from a modern wastewater treatment plant (WWTP), and their co-culture was validated in three consecutive outdoor pilot tests. In the first two pilot tests, the nutrient concentrations of phosphorous and ammonium were depleted close to zero within 24 hours, whereas the pH value increased from 7 to 9 in the wastewater. Therefore, $CO_2$ was added for pH control in the 3rd batch, but the nutrient removal efficacy indicated that fresh algae inoculum was critical to maintain treatment efficiency. The biomass accumulated notable amounts of Ca, Mg, K, Fe, Al, and heavy metals from the effluent, while the algae production increased by two to three times over 7 days with an average algae biomass productivity of 1.68 $g\ m^2\ d^{-1}$. The derived biomass can be used for biogas production and biofertilizer applications based on the biochemical constituent. Given a great potential for further optimization and improvement, we provide a new insight to use phycoremediation approach to facilitate the green transition of wastewater treatment plants.

**Keywords:** filamentous algae, treated wastewater, phycoremediation, co-culture, *Klebsormidium*, *Spirogyra*

## 1. Introduction

Wastewater management has received increasing attention and interest in the context of circular economy. Different from the conventional perception, waste streams become emerging resource for valorisation instead of being treated as a problem [1]. This has gradually become a new consensus for the green transition of wastewater treatment plants (WWTPs), include the request to neutralize carbon emissions. Thereby, it is imperative to introduce new technologies and solutions to

WWTPs to alleviate their environment footprints as part of the green transition. Algae represent a promising tool to recover and recycle the residual nutrients from wastewater into bioproducts, coupled with significant carbon abatement. However, due to several constraints of scalable microalgae cultivation, it is still debatable whether the algae-based treatment technique (or phycoremediation) is a viable approach to facilitate the circular economy development of WWTPs. Therefore, this book chapter will provide a new insight to evaluate the phycoremediation for wastewater treatment.

Based on a case study, this book chapter will elaborate the potentials of using filamentous algae to treat the municipal tertiary effluent from a modern Norwegian WWTP. Both laboratory research and pilot scale tests were employed for the demonstration, since transferring the results from lab-scale R&D to pilot scale is a critical process for phycoremediation studies. Different from the conventional monoculture approach, the filamentous co-culture was exploited on purpose in order to enhance the resilience and viability of proposed phycoremediation strategy. High Rate Algal Pond (raceway) was used for the pilot study as it is an efficient system for algae—wastewater treatment [2]. The depletion of nutrients was monitored to indicate the treatment efficiency, while the productivity of algae biomass was detected in the pilot tests. Finally, the biochemical constituent and elemental content of the produced algae biomass were characterized with an attempt to assess the potential valorisation. It is anticipated that this study will shed light on how to effectively deploy the phycoremediation technology to facilitate the green transition of WWTPs.

## 2. Emerging interest on filamentous algae

Research in the application of microalgae in wastewater treatment was initiated in the 1950s. It has been highlighted that phycoremediation can remove up to 99% of nitrogen (N) and phosphorous (P) and reduce these nutrients concentration below 1 mg/L [3]. Moreover, the produced algal biomass can be valorised from niche markets of special materials (e.g. biopolymers and coatings) to the large-scale uses of fertilizer [4]. Phycoremediation has been demonstrated to be technically possible at lab scale, pilot scale, and industrial scale, but not economically viable. Although the cost for microalgae cultivation can be subsidized to a large extent by recovering nutrients from wastewater and sequestrating $CO_2$ released by WWTPs [5], harvesting microalgae cells is actually a premier obstacle to impede the popularity and scalability of phycoremediation [1]. It is an energy-intensive and expensive process. For an operational facility, this process can occupy over 90% of capital expenses [6] and above 20–30% of the overall production cost [7]. Meanwhile, it cannot be ignored that most fast-growing microalgae are vulnerable to the fluctuation of biotic and abiotic conditions in wastewater [1]. This is another inevitable challenge for those delicate microalgae cultivations, especially for the commonly proposed monoculture [4]. To address these inherent problems on phycoremediation, research attention has gradually shifted to filamentous algae in recent years, as they possess some unique traits that singular microalgae does not have.

Species from the genera *Oedogonium*, *Cladophora*, *Spirogyra*, *Klebsormidium*, and *Stigeoclonium* have been demonstrated as good candidates for wastewater treatment applications [4]. These filamentous algae have several impressive advantages for wastewater treatment, such as robust ability to uptake nutrients from wastewater to achieve about 60% increase in dry biomass per day [8], simplicity of harvesting, stronger resistance to a variety of aquatic grazers and competing organisms, as well as better adaptation to dynamic conditions [9]. Even in the environment with varying N:P ratios, the filamentous algae still can remove 99% of N and P simultaneously [10]. Moreover, these filamentous algae can naturally grow and bloom in a broad spectrum of waste streams including ash dam water [11]. Although filamentous algae have been highly recommended for wastewater treatment [4], there is little information to investigate their potentials for the contemporary requirement of WWTPs. With the declined interest of algae bioenergy and increased knowledge on pragmatic algae potentials, there is a growing consensus that the appropriate phycoremediation should be implemented and aligned with realistic demand and specific wastewater conditions.

## 3. New demand for treating municipal tertiary wastewater

In the past decades, there have been numerous attempts to employ phycoremediation technologies for the treatment of primary or secondary effluents, while less attention has been given to tertiary treated effluents. Although the advanced physical, chemical, and/or biological techniques used in modern WWTPs can remove the most nutrients, the tertiary effluents still contain considerable loads of N and P contributing to eutrophication. With more concerns of environmental eutrophication and rapid loss of nonrenewable resource of P, how to effectively eliminate the residual nutrients in the final discharge becomes an emerging request for most WWTPs. In Norway, for example, it was reported that more than 900 tons of phosphorus and about 15,000 tons of nitrogen were discharged into the ecosystem via WWTPs per annum [12]. These nutrients are equivalent to approx. 5% and 15% of agriculture P and N fertilizer consumptions as reported at a national level in 2017 [13]. Instead of releasing them to the aquatic environment as pollutants, it is apparently beneficial to recover these nutrients from the released wastewater.

Compared to primary and secondary wastewaters, tertiary treated wastewater has relatively stable pH and less turbidity, and these are acceptable conditions for algae to grow. Furthermore, along with the biological oxidation and denitrification process, tertiary wastewater contains much less organic macronutrients and the major dissolved nutrients are inorganic forms and less bioavailable organic compounds. In fact, they are the preferable medium conditions for algae proliferation. With the potential benefits of biomass valorisation and carbon sequestration, it is conceived that the application of filamentous algae to purify tertiary wastewater could represent a new win-win strategy for WWTPs. However, there is a suspicion on the algae productivity when the filamentous algae are exposed to the treated municipal wastewater. After all, the low concentration of those nutrient residuals (normally in a level of mg $L^{-1}$) seems not optimal for the algae growth. In order to address this concern, the case study introduced in this book chapter will provide more details to demonstrate the possibility of employing filamentous phycoremediation for a Norwegian WWTP.

## 4. Experimental validation of filamentous algae co-culture

In the case study, a total of 25 freshwater algae strains from 11 genera (**Table 1**) were selected from the Norwegian Culture Collection of Algae (NORCCA, www.norcca.no) aiming to test the suitability of phytoremediation to local climate condition. According to the record from a local WWTP (VEAS: VeasSelvkost AS, Slemmestad, Norway), the temperature of municipal discharge primarily ranges from 10 to 15°C over the year. This range was thereby used a criterion in the case study for

| No. | Phylum | Class | Species | Strain | Origin | Medium | Morphology |
|---|---|---|---|---|---|---|---|
| 1 | Chlorophyta | Chlorophyceae | Tetradesmusobliguus | NIVA-CHL6 | Lake Årungen, Akershus, Norway, 1946 | Z8 | Single cell |
| 2 | | | Coelastrum sp. | NIVA-CHL86 | Lake Malawi, Malawi, 1991 | Z8 | Single cell |
| 3 | | | Chlamydomonas reinhardtii | K-1016 | Amherst, Massachusetts, USA, unknown | CW15 | Single cell |
| 4 | | | C. reinhardtii | K-1017 | Amherst, Massachusetts, USA, unknown | CW15 | Single cell |
| 5 | | | Oedogonium vaucheri | K-0094 | Store Magleby, Amager, Denmark, unknown | NF2 | filamentous |
| 6 | | | Oedogonium cardiacum | K-1001 | Dry Drayton, England, unknown | 20% Z8 + vitamins + soil extract | filamentous |
| 7 | | | Oedogonium cardiacum | K-1002 | Dry Drayton, England, unknown | 20% Z8 + vitamins + soil extract | filamentous |
| 8 | | | Stigeoclonium sp. | K-0018 | Avernakø, Denmark, unknown | NF2 | filamentous |
| 9 | | | Stigeoclonium sp. | K-1030 | unknown | 20% Z8 + vitamins + soil extract | filamentous |
| 10 | | | Stigeoclonium sp. | K-1031 | unknown | 20% Z8 + vitamins + soil extract | filamentous |
| 11 | | | Stigeoclonium sp. | K-1032 | unknown | 20% Z8 + vitamins + soil extract | filamentous |
| 12 | | | Raphidocelissubcapitata | NIVA-CHL1 | River Nitelva, Akershus, Norway, 1959 | Z8 | filamentous |
| 13 | | Trebouxiophyceae | Chlorella vulgaris | K-1801 | Revo (TN), garden soil, Italy, unknown | Z8 | Single cell |
| 14 | | | Chlorella vulgaris | NIVA-CHL 108 | Germany, unknown | Z8 | Single cell |
| 15 | | | Chlorella sorokiniana | NIVA-CHL 176 | Austin, Texas, USA, 1953 | Z8 | Single cell |

| No. | Phylum | Class | Species | Strain | Origin | Medium | Morphology |
|---|---|---|---|---|---|---|---|
| 16 | | Conjugatophyceae | Spirogyra singularis | K-1019 | UtterslevMose, Denmark, unknown | 20% Z8 + vitamins + soil extract | filamentous |
| 17 | | | Spirogyra sp. | K-1454 | Samsø, Denmark, unknown | 20% Z8 + vitamins + soil extract | filamentous |
| 18 | | | Spirogyra sp. | CHL-189 | Pond, Kindrogan, Scotland, 2013 | 20% Z8 + vitamins + soil extract | filamentous |
| 19 | | Klebsormidiophyceae | Klebsormidium sp. | K-0148 | Little Island, Cork, Eire, unknown | NF2 | filamentous |
| 20 | | | Klebsormidium sp. | NIVA-CHL 142 | Dal, Akershun, Norway, 1993 | Z8 | filamentous |
| 21 | | | Klebsormidiumflaccidum | NIVA-CHL 80 | Spruce nursery, 1990 | Z8 | filamentous |
| 22 | Cyanobacteria | Cyanophyceae | Arthrospira platensis | NIVA-VYA 428 | Lake Lonar, Maharashtra, India, unknown | Z8 | filamentous |
| 23 | | | Anabaena subcylindrica | NIVA-CYA323 | Fuggdal, Rendalen, Hedmark, Norway, 1993 | Z8 | filamentous |
| 24 | | | Trichormus variabilis | NIVA-CYA 19 | Lake Mendota, Madison, Wisconsin, USA, 1948 | Z8 | filamentous |
| 25 | | | Trichormus variabilis | NIVA-CYA 410 | Mississippi, USA, 1964 | Z8 | filamentous |

**Table 1.**
*Algae selection for the experimental test. (unicellular species are included for a point of comparison).*

the algae screening and tests. Based on the previous knowledge on local rivers ecosystem [e.g., 14, 15], inclusion of filamentous algae selection was to avoid the potential risk of introducing invasive species to the local environment. The VEAS tertiary wastewater was freshly sampled and filtered (0.2 μm) on the day for the laboratory test below. All the selection process was conducted following the NORCCA's standard protocols. There were two selection criteria, (1) being filamentous and (2) at least 100% of increase in chlorophyll fluorescence. The filamentous strains with the highest growth rates were selected for co-culture combination studies.

Among these candidates, only K-1454 (*Spirogyra* sp.) and NIVA-CHL142 (*Klebsormidium* sp.) passed the criteria, and their growth rates (109–137%) were comparable to the level of unicellular strains (129–195%) (**Figure 1**). Although *Trichormus* sp. was selected as the third candidate for the subsequent co-culture tests, the growth of monoculture was only detected on *Spirogyra sp.* and *Klebsormidium sp.* when these three strains were exposed directly to the VEAS effluent, rather than on *Trichormus sp.* (**Figure 2**). In the following combination model that simulating the co-culture conditions, the results showed that the co-culture of *Spirogyra sp.* and *Klebsormidium sp.* could grow in the effluent, based on the detection of fluorescence increment in the end. Actually, the different sized algae cells have different uptake and scaled uptake affinities for the nutrient utilization [16], so the algae mixture in different cell sizes will be better for the purpose of nutrient recovery from wastewater reclamation. As the cell of *Spirogyra sp.* was much bigger than that of *Klebsormidium sp.* (approx. 10 time of difference), their co-culture was supposed a matched combination. Moreover, mixed culture would have a better resilience to the variable conditions and complex microbial community in the wastewater. Therefore, the co-culture of *Klebsormidium sp.* and *Spirogyra sp.* was selected as a model filamentous combination to treat the tertiary VEAS effluent.

At NIVA's Solbergstrand Algae R&D Facility, these two filamentous algae of *Klebsormidium* sp. and *Spirogyra* sp. were scaled up to 100 liters separately. All the algae cells were harvested by filtration (35 mm plankton net) when the inoculum biomass reached to the level of 2 g $L^{-1}$, and then washed by clean water, and weighed (after 10 min of air-drying) for the pilot test. A few grams of biomass were sampled from each species and lyophilised for a benchmark study, and the rest was used for

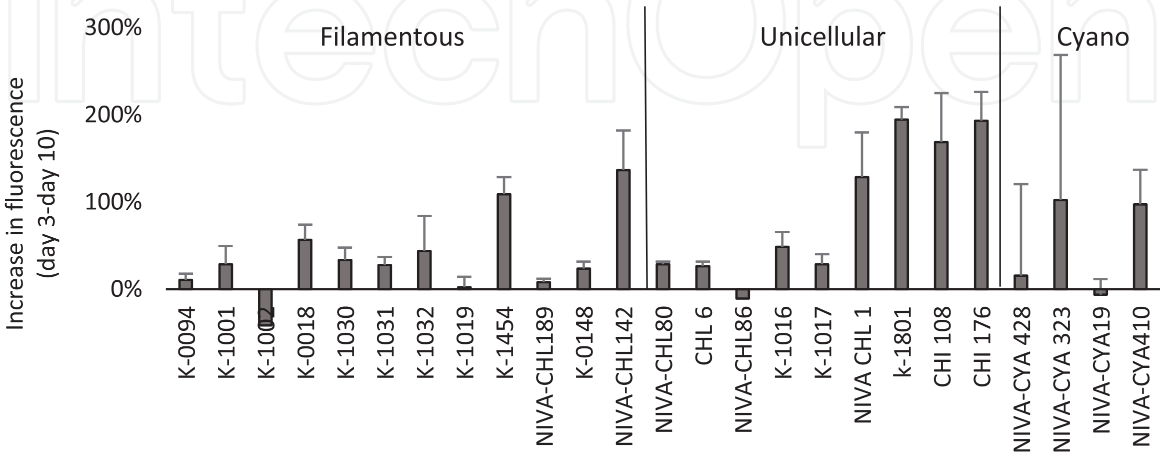

**Figure 1.**

*The comparison of fluorescence increments among different selected algae in the screening test (mean ± SD). (note: The first 3 days were omitted to allow inoculated cells for acclimation, and thereby the results were obtained between days 3 and 10 during their exponential growth phase. The chlorophyll fluorescent was measured at a wavelength of 685 nm with excited emission at 450-550 nm.)*

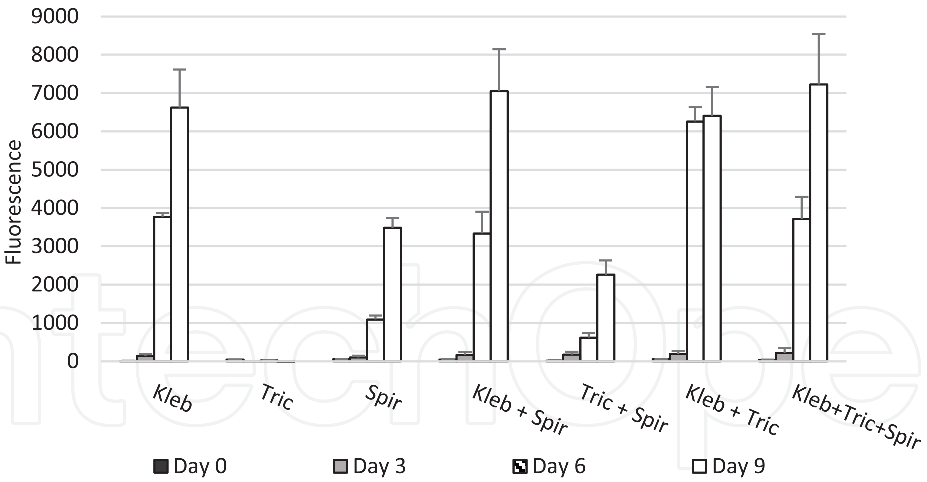

**Figure 2.**
*Monoculture and co-culture flask experiments (mean ± SD;* Kleb: *Klebsormidium sp.* Tric: *Trichormus sp.* Spir: *Spirogyra sp.)*

pilot tests. The pilot test was performed in three consecutive cycles with about 1500 L of municipal tertiary effluent (non-filtered, provided by VEAS) on each, which was conducted on 1:15 (v/v) with each indoor inoculum in a raceway. The 1st batch was inoculated with about 242 g wet *Klebsormidium* (equal to 22.3 g of dry weight, DW) and 547 g wet *Spirogyra* (about 39.9 g DW). With an attempt to continue algae cultivation, a similar amount (wet weight, WW) of co-culture was taken from the final produced biomass and subsequently used as the new inoculum for second batch, and so did on third cycle. The outdoor pilot test was carried out at 10–15°C (similar to the conditions at VEAS), with supplemental 24 hr. of LED light radiation (30–95 $\mu E\ m^{-2}\ s^{-1}$). The three batches were all monitored for 7 days, with 2 L of water sampling on each day. As the pH value in the raceway increased from 7 to 9 within 24 hr in the first 2 cycles (personal communication), the pH in the third batch was controlled at 7.5 with automatic $CO_2$ addition after day 2. In the end, the total co-culture biomass in the raceway was filtrated via a 80-mm plankton net, rinsed with tap water, and quantified for the yield measurement. The biomass was freeze-dried for various analytical analyses, including protein, starch, and lipids.

## 5. Nutrients depletion and biomass yield in the pilot test

This case study showed that the N and P residues (mg $L^{-1}$ range) in the tertiary treated wastewater could be effectively removed by the inoculated co-culture (**Figure 3**). In the 1st and 2nd batches, most of $NH_4^+$ and P were depleted within 1 day. However, the depletion rate became slower in the 3rd batch. Moreover, the nutrient depletion followed a similar pattern. The depletion of $NH_4^+$ preceded the other inorganic N nutrients. Then, nitrate and nitrite started to deplete once $NH_4^+$ was close to 0. This is consistent with the previous reports that algae have a preference on inorganic N: $NH_4^+$ over other sources of $NO_3^-$ [17, 18]. Regardless of the difference in 3rd batch, it seems that the total N could be reduced by 1.5 mg $L^{-1}$ in 3 days. As this rate was achieved by the experimental amount of inoculated co-culture, perhaps the nutrient depletion could be accelerated with more co-culture inoculum.

Albeit of a similar amount of algae inoculum used in each batch (**Table 2**), their production was different. As there was a low level of nutrients (especially P) in the

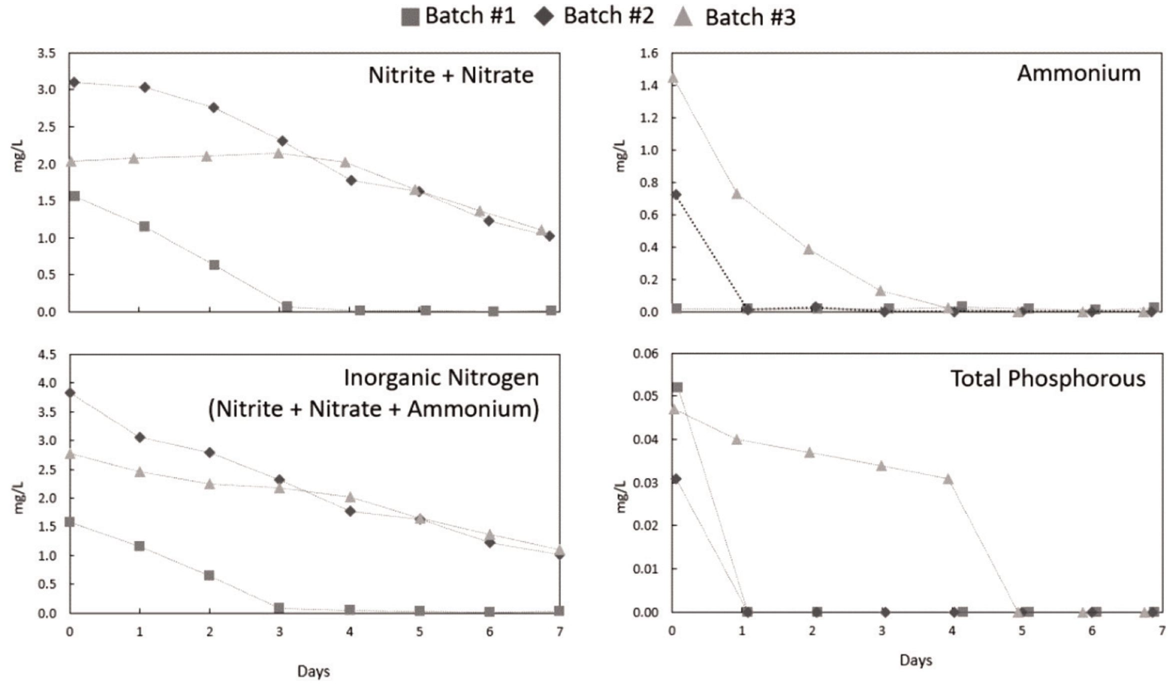

**Figure 3.**
*Macronutrients during WWTP effluent treatment with algal co-culture.*

| Batches | Batch #1 | Batch #2 | Batch #3 |
|---|---|---|---|
| Initial biomass inoculum (g $L^{-1}$, DW) | 0.04 | 0.04 | 0.05 |
| Harvested biomass on day 7 (g $L^{-1}$, DW) | 0.13 | 0.10 | 0.10 |
| Algae productivity over 7 days (DW g $m^2$ $d^{-1}$) | 2.27 | 1.51 | 1.26 |
| Biomass yield vs. total N consumed in 7 days (DW g $g^{-1}$) | 55.59 | 19.93 | 19.13 |
| Biomass yield vs. total P consumed in 7 days (DW g $g^{-1}$) | 1668 | 1801 | 641 |
| Initial N:P ratio in the wastewater | 30.38 | 123.29 | 58.94 |

**Table 2.**
*The summary of algae production and nutrient consumed in the pilot tests.*

treated wastewater, the exposed biomass was likely to confront prolonged P starvation with each subsequent batch. This is contradictive to findings that initial P-starvation can be implemented on microalgae to maximize the P uptake in wastewater [19]. This could happen in the 2nd pilot test but did not occurr in the 3rd batch. Although it was attempted to recycle the produced algal biomass for continuous effluent purification, the results support that the efficiency turned unsustainable after 14 days (duration of first two batches) even with $CO_2$ supplement. It is thereby deduced that the fresh algae inoculum is imperative to the rapid nutrient recovery in municipal tertiary wastewater.

Overall, the biomass of co-culture increased by 330, 250, and 200%, respectively, in three consecutive pilot tests (**Table 2**). The obtained algae productivity was consistent with the reported range of 0.8– 50.0 g $m^2$ $d^{-1}$ on filamentous algae [4]. The results showed that the algae grew slower after day 3 in the first two pilot tests, when the N and P became deficient afterward (**Figure 3**). It is indicated that the real productivity of co-culture would be above the average level over 7 days. The effective

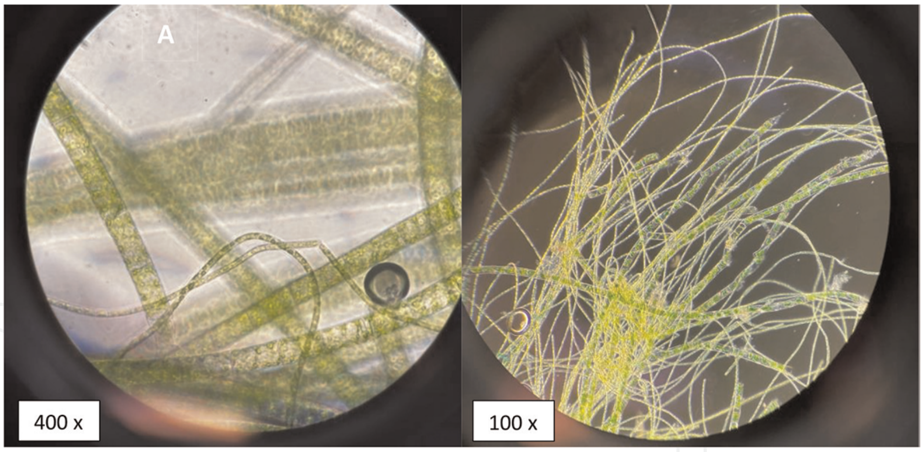

**Figure 4.**
*Microscopy picture of co-culture: A taken from the 1st batch pilot test on day 6. B taken from the 3rd batch pilot test on day 7. (*Spirogyra *sp.—Wider, spiral chlorophyll;* Klebsormidium *sp.—Thinner filaments; and air bubble—Empty round cycle).*

retention time for the wastewater reclamation could be less than 3 days as well. Obviously, the biomass yield can be elevated purposely by using an optimal amount of algae inoculum. Given the more biomass yield (vs both N and P consumed) in the first two batches (especially in the 1st cycle), this also underpins the above suggestion on fresh inoculum preparation for tertiary wastewater reclamation. Therefore, how to optimize the algae inoculum for the pilot treatment will be vital for further (semi-) continuous treatment process.

During the pilot test, both algae species grew well in the municipal treated wastewater. There was no sign of growth inhibition (dead cells or faded color) identified in the microscopy examinations (**Figure 4**). Interestingly, these two filamentous species clumped together. The co-culture formed numerous small algae colonies (about 1 cm) in the raceway, making biomass harvested much easier and quicker via a simple filtration on a 35 mm plankton net. Apparently, this can benefit to the practicability and scalability of selected co-culture cultivation for wastewater treatment. Only a few ciliates *Vorticella* were visualized to attach to the filamentous algae colonies in the 3rd batch, and they were possibly the "carryover" with the consecutive cultivation. However, they were not a predator to the co-culture as filamentous algae were too big to be the prey.

In this case study, big variations were obtained for algae growth measurements between sampling days (data not shown) by filtering 2 L of water samples. So, the biomass quantification was only based on the total algae production at the end of each pilot batch. The cell density of *Klebsormidium sp*. appeared gradually increase in the colonies along with the tests according to the microscope observation. In order to identify the change of combination ratio in the co-culture, the sensitive gene sequencing techniques (e.g. QPCR) or laser scanning confocal microscope (LSCM) with appropriate probes could be considered for future study.

## 6. Other discoveries in the phycoremediation pilot tests

Three consecutive pilot batches showed that the pH value increased from 7 to 9 within a day and stabilized at 9 if $CO_2$ was not supplied. At this point, it needs to clarify that the treated wastewater used in this study was collected after the denitrification treatment, and the water thereby could be $CO_2$ saturated. Apparently, the 3rd batch needed CO2 addition to maintain pH level. Albeit its algae growth was not as good as previous two batches (with the reasons mentioned above), it is believed that the fresh filamentous co-culture can rapidly deplete $CO_2$ in the treated wastewater. With this regard, a large amount of $CO_2$ supplement will be needed for the proposed filamentous co-culture in the process of wastewater purification. As a return, it is foreseen that this can significantly reduce the carbon footprint of WWTPs.

A total of 14 elements were measured in this case study, but only the mercury (Hg) was undetectable (**Table 3**). As a background study, these elements were also analysed on the indoor monocultures (inoculum), to exclude the influence of indoor cultivation medium. The differences between indoor inoculums of *Klebsormidium sp.* and *Spirogyra sp.* showed that they had variable update affinities to these elements. In contrast, the detected discrepancies from the outdoor co-cultures indicated the effectiveness of accumulation/absorption of these chemicals and heavy metals from the wastewater treatment. As discussed above, it was conceived that the biomass produced in pilot test could have less P. Interestingly, the indoor and outdoor biomass contained similar amount of Mg and K, while Ca content was more in the outdoor biomass. As showed in the results, these mineral chemicals in the municipal treated wastewater can be assimilated effectively by the tested co-culture to constitute the produced algae biomass at a level of g kg$^{-1}$.

It is worth noticing that there was a substantial accumulation of AI and Fe residues in the outdoor samples (g kg$^{-1}$). Although these two metal ions also existed in the algal cultivation medium, indoor algae samples contained much less than that of outdoor samples. One side, it is verified that there were still certain amounts of $AI^+$ and $Fe^+$ ions in the treated wastewater. It is believed that they were derived from the WWTP's chemical treatment process, as cationic coagulants/flocculants (e.g. ferric chloride,

| Parameter | Unit | Spirogyra[#] | Klebsormidium | Batch #1 | Batch #2 | Batch #3 |
|---|---|---|---|---|---|---|
| K | g kg$^{-1}$ | 6.10 | 11.2 ± 0.1 | 7.00 ± 0.09 | 6.50 ± 0.04 | 6.10 ± 0.05 |
| Mg | g kg$^{-1}$ | 1.45 | 2.35 ± 0.02 | 2.82 ± 0.04 | 3.21 ± 0.02 | 1.70 ± 0.01 |
| Ca | g kg$^{-1}$ | 9.14 | 2.74 ± 0.07 | 84 ± 1 | 77.3 ± 0.4 | 41.3 ± 0.6 |
| Tot-P | g kg$^{-1}$ | 3.24 | 8.31 ± 0.05 | 2.31 ± 0.03 | 1.46 ± 0.00 | 1.79 ± 0.01 |
| Fe | g kg$^{-1}$ | 0.13 | 0.34 ± 1.69 | 0.97 ± 0.04 | 1.29 ± 0.02 | 2.65 ± 0.03 |
| Al | g kg$^{-1}$ | <0.07 | <0.07 | 0.82 ± 0.00 | 1.34 ± 0.01 | 2.08 ± 0.03 |
| Cr | mg kg$^{-1}$ | <2 | <2 | 6.4 ± 0.2 | 5.14 ± 0.05 | 7.79 ± 0.02 |
| Cu | mg kg$^{-1}$ | <17 | 23.1 ± 1.69 | 19.2 ± 0.3 | 18.02 ± 0.09 | 45 ± 1 |
| Ni | mg kg$^{-1}$ | <2 | <2 | 5.1 ± 0.3 | 3.5 ± 0.1 | 3.1 ± 0.1 |
| Mn | mg kg$^{-1}$ | 187 | 34.0 ± 0.18 | 176 ± 4 | 334 ± 3 | 357 ± 5 |
| Pb | mg kg$^{-1}$ | <2 | <2 | 2.6 ± 0.3 | 2.50 ± 0.06 | 3.6 ± 0.1 |
| Zn | mg kg$^{-1}$ | 102 | 56.9 ± 0.06 | 153 ± 1 | 121.8 ± 0.4 | 194 ± 2 |
| Cd[*] | mg kg$^{-1}$ | <0.06 | <0.06 | 0.07 | 0.07 | 0.08 |
| Hg | mg kg$^{-1}$ | <0.04 | <0.04 | <0.04 | <0.04 | <0.04 |

[#] *Spirogyra is the mean value of 2 replicates because of an accident with one of the replicates.*

[*] *Cd is the result of only one replicate.*

**Table 3.**
*Chemical elements analysis in the experimental algae biomass (mean ± SE).*

aluminum chloride, and polymers) are usually used for P precipitation at the secondary treatment process of municipal wastewater. Although there is little information on their scavenge in water, using the filamentous algae can act a new approach to reduce these cationic residues from the municipal tertiary wastewater. Apparently, this can further diminish the environmental burden of the chemical treatment for the WWTP.

Heavy metals are hazardous substances and persistent pollutants in municipal wastewater [4]. Although they are close to undetectable levels at ug $L^{-1}$ or ng $L^{-1}$ [20], their appearance is a recalcitrant problem. As indicated in this case study, these trace elements were encapsulated in the algae biomass at a level of mg $kg^{-1}$ from tons of municipal wastewater. This process is normally accomplished through a robust combination of non-active biosorption and active metabolism-dependent mechanisms [21, 22] because of algae's high binding affinity, abundance of binding sites, and large surface area [23]. Numerous studies in recent years have approved the existence of heavy metals and emerging contaminants in aquatic system and pointed out wastewater discharge as one of the main pollution sources [24, 25]. Therefore, using filamentous algae co-culture can be an effective and pragmatic approach to purify the tertiary wastewater in an environmental-friendly manner.

## 7. Potential valorisations of produced biomass

Since Fourier-transform infrared spectroscopy (FTIR) represents a rapid, simple, and reproducible method to identify the different compositions in the different biomass [26], it was employed to firstly examine the quality of algae samples in this study. FTIR spectroscopy revealed different proximate biochemical composition (lipids, carbohydrates, phosphates, and proteins) of indoor and outdoor cultivated algae (**Figure 5A**). In comparison, *Klebsormidium* biomass had more lipid, protein, and phospholipid, but *Spirogyra* contained more carbohydrates (**Figure 5B**). For the co-culture, the biochemical profile was a bit consistent among three batches, and the proportion of those biochemicals seemed to between the levels of inoculums. In the principal component analysis (PCA) analysis (**Figure 5C**), it seemed that the *Klebsormidium* could take over *Spirogyra* during consecutive pilot tests. This result was coincident with the microscopy observation. This is probably because that *Spirogyra* prefers growing in warm temperature [27].

FTIR results were further validated by the following analytical analysis. About 50% of *Klebsormidium* biomass was protein, but *Spirogyra* biomass was only 21% (**Figure 6**). However, *Spirogyra* had more starch (7% of DW) than *Klebsormidium* (3.3%). In the outdoor pilot test, the protein content of co-culture was increased gradually from 1st to 3rd batch with 20–30% of DW. However, the starch content became less from 5–4%. Like the PCA analysis, this result also suggested that the proportion of *Klebsormidium* in the co-culture increased. The lipid content was detected below 8% of DW with a small variation across different samples. Despite microalgae could increase lipid content in a condition of nutrient starvation [6], this is not applicable to the biological response of experimental filamentous algae in the outdoor pilot tests. Maybe it is why that filamentous algae are not compelling to the research attention as did on most of microalgae for typical algae economy values (e.g. biofuel and omega-3 oil). Moreover, with the notable protein content, the potential impact on anaerobic digestion (AD) process shall be investigated if the biomass is used for AD biogas production.

Another consideration is the ash content in the produced biomass. It was high in the first two WWT batches (26.6% –23.3%), lower in 3rd batch (13.8%). However,

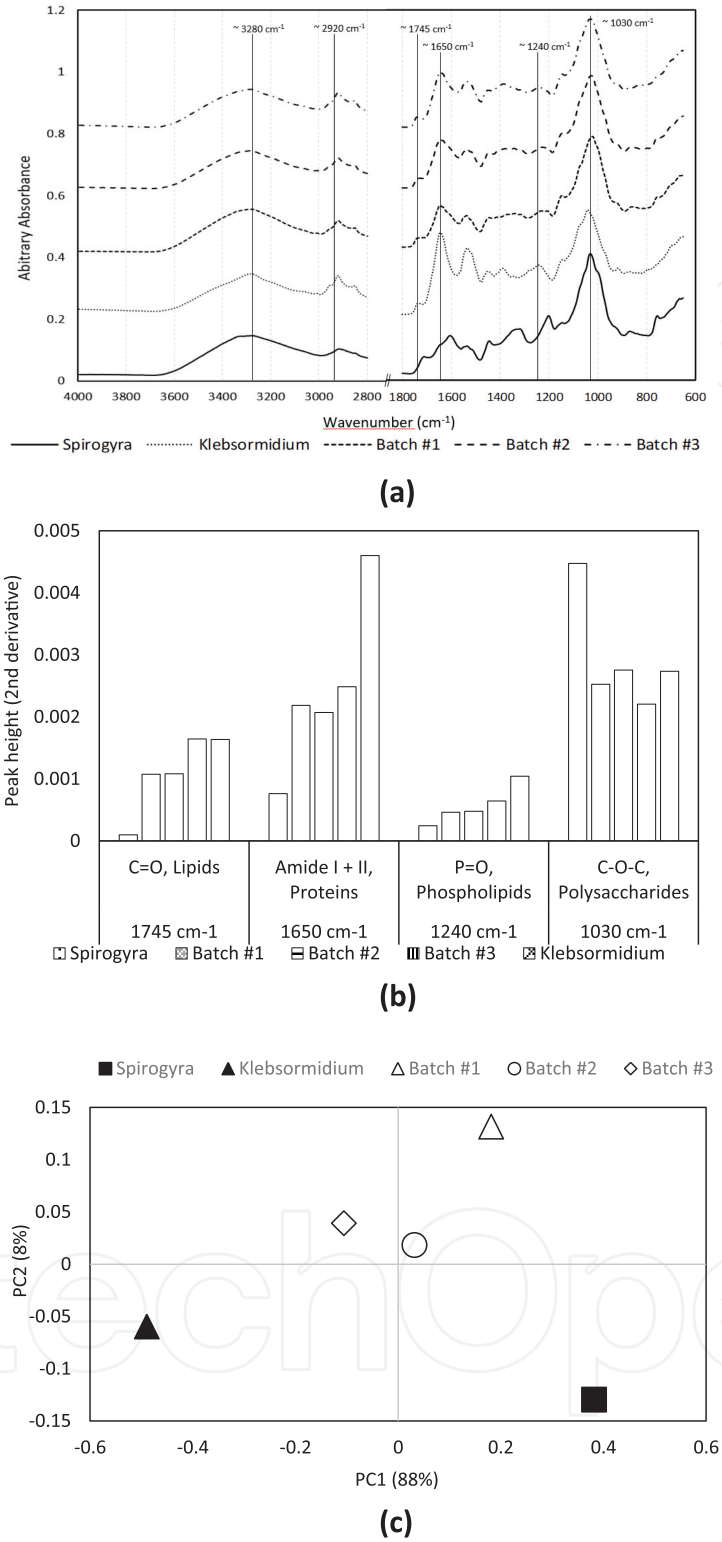

**Figure 5.**

*FTIR analysis of microalgae biomass. (A) FTIR spectra with characteristic bands noted (note: The moisture condition was similar between different algae samples (3400–3200 $cm^{-1}$). As the variation between 3000 and 2500 $cm^{-1}$ was not correlated to the changes in biochemical composition [31], the major differences between 1800 and 800 $cm^{-1}$ were used for assessment.), (B) peak height of characteristic bands, and (C) scores plot of principal component analysis (PCA).*

they were all more than the content in the indoor inoculums of *Klebsormidium* (6.4%) and *Spirogyra* (9.4%). Since the ash content is almost associated with the minerals content in biomass [28], those higher values of ash content in the pilot test also can

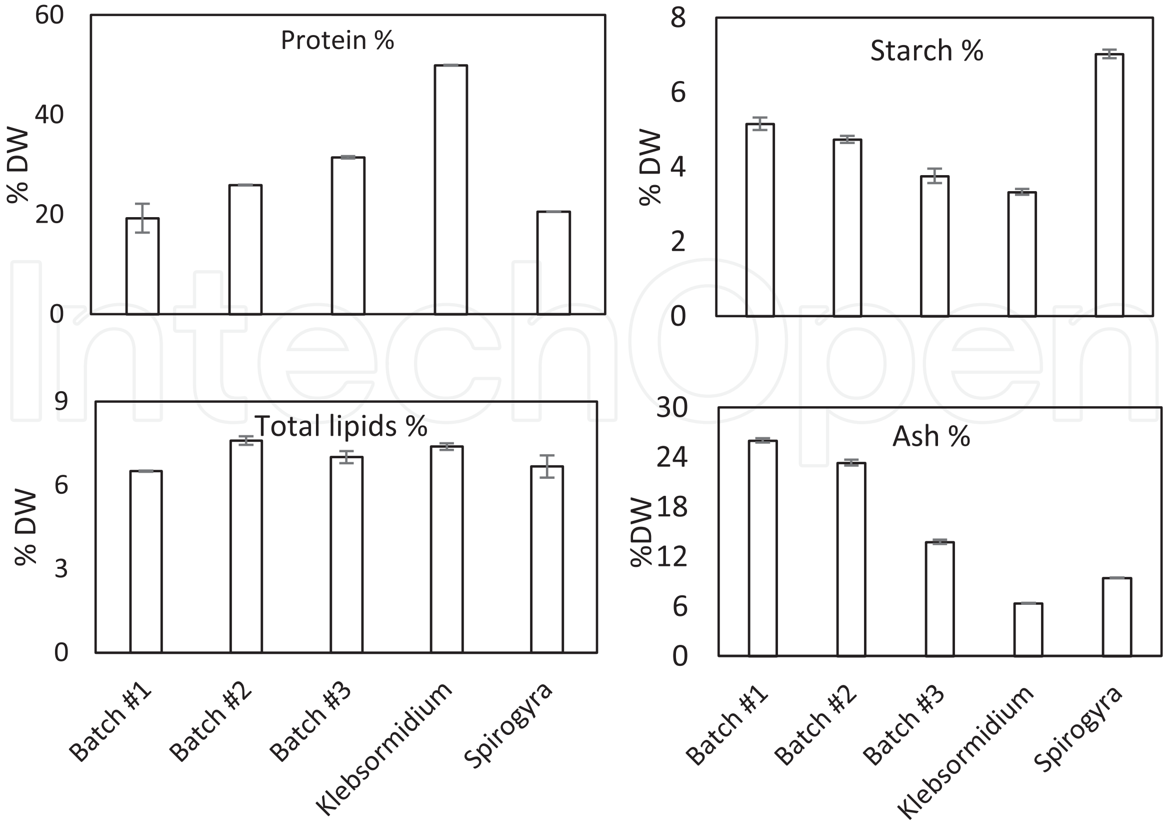

**Figure 6.**
*Proximate biochemical analyses of microalgae biomass from indoor monocultures and outdoor co-cultures on wastewater.*

evidence that filamentous algae removed certain amounts of mineral chemicals and heavy metals along with the treatment of tertiary wastewater in this case study. Regardless of heavy metals and/or other hazardous substances accumulated in the biomass, high ash content will also affect the algae inclusion level for food and feed utilization [29] and increase problems in combustion for energy conversion [30].

Given these concerns, there is still a great potential to utilize the produced biomass for biofertilizer, soil ameliorator, or new material development. For example, the heavy metals content in the produced biomass was below the maximum limit for permissible content in the organic fertilizers, according to the Norwegian regulations on organic fertilizers (FOR-2003-2007-04-951). There is no doubt that a comprehensive evaluation will be needed prior to this viable application, such as to match the restrictions of hygiene conditions, pesticides, and requirements for soil mixtures, as well as public perception. Apparently, it is going to be an inclusive question to evaluate the potential usage of produced biomass from this case study. However, it is undeniable that this represents a new value creation, as an authentic solution to facilitate the green transition of WWTP. Therefore, this case study provides a new paradigm for WWTPs to integrate the management of tertiary wastewater with emerging circular economy requirement.

## 8. Conclusions

Based on a realistic case study, this book chapter reveals that the mixed culture of filamentous algae *Klebsormidium* and *Spirogyra* can act as an effective tool to treat the municipal tertiary wastewater, with notable algae productivity. The co-culture can effectively recover macronutrients, mineral elements, and heavy metals from the wastewater, and their cultivation potentially can consume a considerable amount of CO2 for biomass production. Thereby, this phycoremediation process could significantly reduce or eliminate the environmental footprint of municipal tertiary wastewater in the ecosystem. Future research will focus on the remaining questions derived from the case study, which require proper optimization in areas of algae inoculum preparations, the nutrient depletion vs. carbon supplementation, hydraulic retention time reduction, and new cultivation strategy (e.g. two-stage) for continuous process. In order to improve the viability of proposed concept, the associated techno-economic analysis and environmental impact assessment will need to be deployed prior to the full-scale implementation.

Although the produced filamentous algae biomass will not be suitable for some typical algae economy purpose, their nutrient profiles and the easy scalable production can bring new hopes to other different value-added applications. As highlighted in the case study, the produced bulk biomass can become an optimal feedstock for new green fertilizer production. Although the case study was performed in pilot test, the outcomes endorse the feasibility of extrapolation to a full-scale wastewater purification or deployment. Thus, the proposed wastewater algae will represent a win-win strategy for WWTP and agriculture enterprise, as a typical model of circular economy. Apart from the contribution to the green transition of WWTP, this approach also can alleviate the pressure of soil deterioration and environment pollution due to the vast usage of chemical fertilizers. With a better understanding on the filamentous algae for municipal tertiary wastewater treatment, it is anticipated that this new phycoremediation approach can shape future investment plans of WWTPs or other new business consideration. Overall, this book chapter sheds lights on a new approach for the green transition of wastewater management and provides a new insight on the potentials of phycoremediation technology for WWTPs' sustainable development.

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## Competing interests

The authors declare that they have no competing interests.

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## References

[1] Liu R et al. Capabilities and mechanisms of microalgae on removing micropollutants from wastewater: A review. Journal of Environmental Management. 2021;**285**:112149

[2] Park JBK, Craggs RJ, Shilton AN. Wastewater treatment high rate algal ponds for biofuel production. Bioresource Technology. 2011;**102**(1): 35-42

[3] Silkina A et al. Large-scale waste bio-remediation using microalgae cultivation as a platform. Energies. 2019;**12**(14):2772

[4] Liu JJ et al. Wastewater treatment using filamentous algae—A review. Bioresource Technology. 2020;**298**: 122556

[5] Whitton R et al. Microalgae for municipal wastewater nutrient remediation: Mechanisms, reactors and outlook for tertiary treatment. Environmental Technology Reviews. 2015;**4**(1):133-148

[6] Amer L, Adhikari B, Pellegrino J. Technoeconomic analysis of five microalgae-to-biofuels processes of varying complexity. Bioresource Technology. 2011;**102**(20):9350-9359

[7] Rawat I et al. Dual role of microalgae: Phycoremediation of domestic wastewater and biomass production for sustainable biofuels production. Applied Energy. 2011;**88**(10):3411-3424

[8] Auer MT, Canale RP. Ecological studies and mathematical modeling of Cladophora in Lake Huron: 2. Phosphorus uptake kinetics. Journal of Great Lakes Research. 1982;**8**(1):84-92

[9] Grayburn WS et al. Harvesting, oil extraction, and conversion of local filamentous algae growing in wastewater into biodiesel. International Journal of Energy and Environmental Engineering. 2013;**4**:185-190

[10] Liu JZ, Vyverman W. Differences in nutrient uptake capacity of the benthic filamentous algae Cladophora sp., Klebsormidium sp. and Pseudanabaena sp. under varying N/P conditions. Bioresource Technology. 2015;**179**: 234-242

[11] Sternberg SPK, Dorn RW. Cadmium removal using Cladophora in batch, semi-batch and flow reactors. Bioresource Technology. 2002;**81**(3): 249-255

[12] Norskeutslipp. Wastewater Treatment Plant. 2022. Available from: https://www.norskeutslipp.no/no/Avlopsannlegg/?SectorID=100

[13] FAOSTAT. 2017. Available from: http://www.fao.org/faostat/en/#data/RFN

[14] Schneider SC, Lindstrøm E-A. The periphyton index of trophic status PIT: A new eutrophication metric based on non-diatomaceous benthic algae in Nordic rivers. Hydrobiologia. 2011; **665**(1):143-155

[15] Schneider S, Lindstrøm EA. Bioindication in Norwegian rivers using non-diatomaceous benthic algae: The acidification index periphyton (AIP). Ecological Indicators. 2009;**9**(6):1206-1211

[16] Edwards KF et al. Allometric scaling and taxonomic variation in nutrient utilization traits and maximum growth rate of phytoplankton. Limnology and Oceanography. 2012;**57**(2):554-566

[17] Van Den Hende S. Microalgal bacterial flocs for wastewater treatment :

From concept to pilot scale. In: Faculty of Bioscience Engineering. Ghent, Belgium: Ghent University; 2014. p. 324

[18] Lopez-Serna R et al. Removal of contaminants of emerging concern from urban wastewater in novel algal-bacterial photobioreactors. Science of the Total Environment. 2019;**662**: 32-40

[19] Solovchenko AE et al. Luxury phosphorus uptake in microalgae. Journal of Applied Phycology. 2019; **31**(5):2755-2770

[20] Kõrgmaa V et al. Removal of hazardous substances in municipal wastewater treatment plants. Water Science and Technology. 2020;**81**(9): 2011-2022

[21] Chekroun KB, Baghour M. The role of algae in phytoremediation of heavy metals: A review. Journal of Materials and Environmental Science. 2013;**4**(6): 873-880

[22] Matagi SV, Swai D, Mugabe R. A review of heavy metal removal mechanisms in wetlands. African Journal of Tropical Hydrobiology and Fisheries. 1998;**8**:23-25

[23] Cameron H, Mata MT, Riquelme C. The effect of heavy metals on the viability of Tetraselmis marina AC16-MESO and an evaluation of the potential use of this microalga in bioremediation. PeerJ. 2018;**6**:e5295

[24] Barra CA, Topp E, Grenni P. Pharmaceuticals in the environment: Biodegradation and effects on natural microbial communities. A review. Journal of Pharmaceutical and Biomedical Analysis. 2015;**106**:25-36

[25] Villar-Navarro E et al. Removal of pharmaceuticals in urban wastewater: High rate algae pond (HRAP) based technologies as an alternative to activated sludge based processes. Water Research. 2018;**139**:19-29

[26] Stehfest K, Toepel J, Wilhelm C. The application of micro-FTIR spectroscopy to analyze nutrient stress-related changes in biomass composition of phytoplankton algae. Plant Physiology and Biochemistry. 2005;**43**(7):717-726

[27] Graham JM et al. Physiological responses to temperature and irradiance in Spirogyra (Zygnematales, Charophyceae). Journal of Phycology. 1995;**31**(4):531-540

[28] Liu KS. Effects of sample size, dry ashing temperature and duration on determination of ash content in algae and other biomass. Algal Research. 2019; **40**:101486

[29] Austic RE et al. Potential and limitation of a new defatted diatom microalgal biomass in replacing soybean meal and corn in diets for broiler chickens. Journal of Agricultural and Food Chemistry. 2013;**61**(30):7341-7348

[30] Hupa M. Ash-related issues in fluidized-bed combustion of biomasses: Recent research highlights. Energy and Fuels. 2012;**26**(1):4-14

[31] Mayers JJ, Flynn KJ, Shields RJ. Rapid determination of bulk microalgal biochemical composition by Fourier-transform infrared spectroscopy. Bioresource Technology. 2013;**148**: 215-220