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Feasibility study of underexplored algal strains for nutrients removal from municipal wastewater

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ABSTRACT

The paper aims to provide an initial assessment of the potential for phosphorus (P) and nitrogen (N) removal from wastewater by two underexplored algal strains, *Vischeria helvetica* ACUS 00025 and *Klebsormidium nitens* ACUS 00207. The experimental setup consisted of two 4.5 L open photo-sequencing batch reactors (PSBRs) operated at laboratory scale for three months using secondary effluent from a full-scale municipal wastewater treatment plant. The comparative analysis showed that *Klebsormidium nitens* strain had more consistent results for all parameters throughout the experimental period: i) higher biological phosphorus removal for *Klebsormidium nitens* (1.02 ± 1.66 $mgP \cdot d^{-1}$) than for *Vischeria helvetica* strain (0.25 ± 0.55 $mgP \cdot d^{-1}$); ii) higher nitrogen removal for *Klebsormidium nitens* (1.254 ± 1.84 $mgN \cdot d^{-1}$) than for *Vischeria helvetica* (0.66 ± 1.61 $mgN \cdot d^{-1}$); iii) *Klebsormidium nitens* showed superior stability in monoculture, with increased resistance to contamination by other algal strains and consumption by other heterotrophic organisms, a shortened wastewater adaptation period, and consistent settling properties via autoflocculation. Both strains maintained relatively universal conditions favourable to effluent and water quality after treatment. These conditions include an average pH of 8.2 and 8.63 and an average DO concentration of 8.48 and 8.85 $mgO_2 \cdot L^{-1}$ respectively. The results are particularly important given the paucity of data in the existing literature on the specific technological parameters required for algal bioreactors.

Keywords: algae-based technology, microalgae (Algae), wastewater treatment, Vischeria, Klebsormidium.

INTRODUCTION

The use of microalgae in wastewater treatment has gained substantial recognition in recent years. It has been demonstrated that microalgal treatment systems can effectively reduce the nutrient loads in effluents, producing nutrients-rich algal biomass that can be utilized in a range of industries, including the production of biofuels, animal feed and biofertilizers (Su et al., 2021; Ali et al., 2022; Khan et al., 2023). A further advantage is that microalgal systems can effectively reduce the concentration of emerging contaminants, including persistent organic pollutants, pesticides, heavy metals, etc., via bioaccumulation, adsorption, precipitation and other mechanisms (Liu et al., 2021; Goh et al, 2023; Sarma et al, 2024). Unlike conventional wastewater treatment methods, which often require significant energy input, algal-based systems are more energy and resource efficient (Huang et al, 2022; de Lima Barizão et al., 2023). Additionally, microalgae-based treatment can reduce greenhouse gas emissions by sequestering carbon dioxide during photosynthesis, further enhancing its environmental appeal (Hasnain et al., 2023).

The potential of microalgae in wastewater treatment has been recognized in strategic policies and environmental initiatives both within Europe and globally. For instance, the European Union's Circular Economy Action Plan emphasizes the need for innovative, sustainable technologies that reduce waste and repurpose resources, aligning with the principles of algal-based wastewater treatment (European Commission, 2020-a). Additionally, the European Green Deal, with its focus on reducing greenhouse gas emissions and promoting sustainable resource management, supports the advancement of algal-based technologies as a means of addressing wastewater management challenges in an eco-friendly manner (European Commission, 2020-b). Some countries in Europe, for example Germany and France, have established national bioeconomy strategies that prioritize sustainable bio-based innovations, focusing on algal-based technologies specifically, due to their applications in wastewater treatment and bioresource recovery. These local governmental documents highlight the technology as an example of how bioeconomy principles can contribute to environmental and economic sustainability (Ministère de l'Agriculture, 2017; Federal Ministry of Education and Research, 2020). Globally, organizations like the United Nations Environment Programme (UNEP) have highlighted the importance of resource recovery and sustainable wastewater management, encouraging the adoption of innovative approaches like algal-based treatments to help achieve the sustainable development goals (SDGs) (specifically SDG 6 on Clean Water and Sanitation, SDG 12 on Responsible Consumption and Production, and SDG 13 on Climate Action), particularly in areas of clean water and sustainable cities (United Nations, 2015).

Notwithstanding the advantages, the institutional and policy support, algal-based municipal wastewater treatment faces several challenges that hinder its broader implementation (Abdelfattah et al., 2023). Key limitations include the need for optimal environmental conditions (light, temperature, and nutrient availability) to sustain algal growth, the risk of contamination by other microorganisms, and the potential for system instability due to fluctuations in wastewater composition (Geremia et al., 2021; Valchev and Ribarova, 2022). Furthermore, large-scale applications require significant space and infrastructure, which can be economically and logistically challenging for urban settings (Geremia et al., 2021). Effective nutrient removal often demands high-density algal cultures, which can lead to issues with biomass harvesting, as separating microalgal cells from water remains a costly and energy-intensive process (Xu et al., 2020; de Morias et al., 2023). These technical and operational constraints underscore the need for continued research for the application of algal-based systems in urban wastewater treatment.

To address these challenges, a number of studies focus on identifying and engineering microalgal strains suitable for wastewater treatment (Radi et al., 2021; Liu et al., 2022; Wang et al., 2022). The selection and cultivation of robust, high-performance strains, capable of thriving in variable wastewater compositions and exhibiting high tolerance to pollutants, are essential to maximizing the efficiency and feasibility of these systems. The removal of nitrogen and phosphorus in algae-based technologies is achieved through two principal mechanisms: direct uptake and symbiotic interactions with bacteria (Oviedo et al., 2022, Díaz et al., 2022). Consequently, researchers are exploring metabolic (algal starvation, luxury uptake, etc.) and engineering (biomass immobilization, CO_2 aeration, etc.) approaches to enhance the resilience and productivity of the strains, thereby expanding the scope of algal-based wastewater treatment in diverse environmental settings (Lavrinovičs et al., 2020; Han et al., 2023; Nordio et al., 2023). Even though some algal genera like *Chlorella*, *Scenedesmus*, and *Spirulina* (*Arthrospira*) have been thoroughly investigated for wastewater treatment, no single strain has yet demonstrated the ideal balance of growth rate, nutrient uptake efficiency, and resilience needed for large-scale, consistent wastewater treatment without preliminary sterilization (Chawla et al., 2020; Abreu et al., 2023; Tan et al., 2023). While algae effectively assimilate nutrients, each algal strain has unique tolerances and metabolic characteristics that impact its performance under different wastewater conditions. For instance, variations in wastewater composition, such as fluctuations in nutrient concentrations, organic load, pH, temperature, light availability, and presence of toxic contaminants, can affect the viability and efficacy of certain microalgal strains (Li et al., 2019; Molinuevo-Salces et al., 2019; Mohsenpour et al., 2021). Additionally, the selected strain should produce a biomass that is easy to harvest and is potentially valuable for downstream applications, such as biofuels or bioproducts (de Morias et al., 2023, Ali et al., 2021). Many naturally occurring strains lack one or more of these traits, and those that perform well in laboratory settings may not translate to real-world conditions in large-scale wastewater facilities. This variability poses a challenge for selecting a strain that can maintain stable performance across diverse municipal wastewater types and treatment systems (Li et al., 2023; Ugwuanyi et al., 2024).

This research paper examines two underexplored algal strains from the species *Vischeria helvetica* and *Klebsormidium nitens*, with the objective of advancing our understanding of their potential for phosphorus (P) and nitrogen (N) removal from municipal wastewater treatment plant (WWTP) effluent. The study assesses the performance of the strains in three distinct aspects: 1) Their ability to overcome the main challenges of this specific technology, in particular, meeting the requirements for high phosphorus and nitrogen removal; 2) Their resistance to other contaminating algal strains and consumption by protozoa, rotifers, crustaceans, etc., and 3) Their favourable settling properties. The two strains are novel additions to the municipal wastewater treatment field. To the best of our knowledge, no other studies have reported on the use of *Vischeria* for municipal wastewater treatment, with the exception of those conducted by the authors of this study (Valchev et al., 2019). On the other hand, *Klebsormidium* has only recently emerged as a prominent genus in the wastewater treatment field, particularly in suspended and attached growth reactors (Valchev et al., 2021; Lawton et al., 2021; Novak et al., 2024; Sabatte et al., 2024).

MATERIALS AND METHODS

Experiment set up

Algal strains

The two following algal strains were used in the experiment:

1) *Vischeria helvetica* (Vischer and Pascher) Hibberd – the strain was isolated from Pirin Mountain (Bulgaria) and cultivated as *Vischeria helvetica* ACUS 00025 in the Collection of Living Algae ACUS of the Sofia University "St. Kliment Ohridski" on standard Bold-Basal medium (BBM) (Uzunov et al., 2012; Stoykova et al., 2019). The species is a unicellular coccal alga that belongs to the class Eustigmatophyceae from the phylum Ochrophyta and grows in different types of habitats with preference to the aeroterrestrial mode of life (Stoykova et al., 2019; Ettl and Gärtner, 2014; Stoyneva-Gärtner et al., 2019-a; Stoyneva-Gärtner et al., 2019-b).
2) *Klebsormidium nitens* (Kützing) Lokhorst – the strain was isolated from high-alpine soils of Rila National Park (Bulgaria) and cultivated as *Klebsormidium nitens* ACUS 00207 in the Collection of Living Algae ACUS of the Sofia University "St. Kliment Ohridski" on standard Bold-Basal medium (BBM) (Uzunov et al., 2012; Stoyneva-Gärtner et al., 2019-c). The strain belongs to a filamentous alga of the class Klebsormidiophyceae from the phylum Streptophyta which is preferably developing in aeroterrestrial habitats (Ettl and Gärtner, 2014; Stoyneva-Gärtner et al., 2019-c).

The two strains were preliminary cultivated as isolated monocultures in the sterile BBM water-nutrient medium in enclosed 100 mL laboratory flasks at ambient room temperature (22 to 25 °C) and natural sunlight illumination. Stirring was performed by laboratory shakers without stirrers in the flasks to avoid cell damage.

These specific strains were selected due to several factors. They are local for the region of the experiment (Bulgaria). This is an important element since they can rapidly acclimate to the specific environmental conditions of the area. Furthermore, despite their preferably aeroterrestrial character, both strains were able to quickly adapt to and strive in the real wastewater medium (Valchev et al., 2021). An additional benefit of *Vischeria helvetica* is that it can accumulate valuable substances like carotenoids, lipids, etc. in its biomass, making it more beneficial as a residual product with great reclamation potential (Stoyneva-Gärtner et al., 2019-a; Stoyneva-Gärtner et al., 2019-b). On the other hand, *Klebsormidium nitens* that belongs to a genus of a great biotechnological potential demonstrated resistance to consumption by other heterotrophic organisms and monoculture contamination in our previous study, which is a significant advantage since these are major problems in open algal reactors for wastewater treatment (Valchev and Ribarova, 2022; Valchev et al., 2021; Stoyneva-Gärtner et al., 2019-c).

Reactors

The experimental set-up consisted of two 4.5 L open photo-sequencing batch reactors (PSBRs) at laboratory scale (see Figure 1) (Wang et al., 2021; Arias et al., 2017; Jia et al., 2018; Ye et al., 2018).

The reactors use suspended-growth algal systems, operating with natural sunlight without any artificial light source and without additional aeration. The reactor body was a glass cylinder with D = 150 mm and H = 150 mm. Each reactor was equipped with a Heidolph (RZR 2021) electric stirrer set to 35 rpm. The propeller size was B

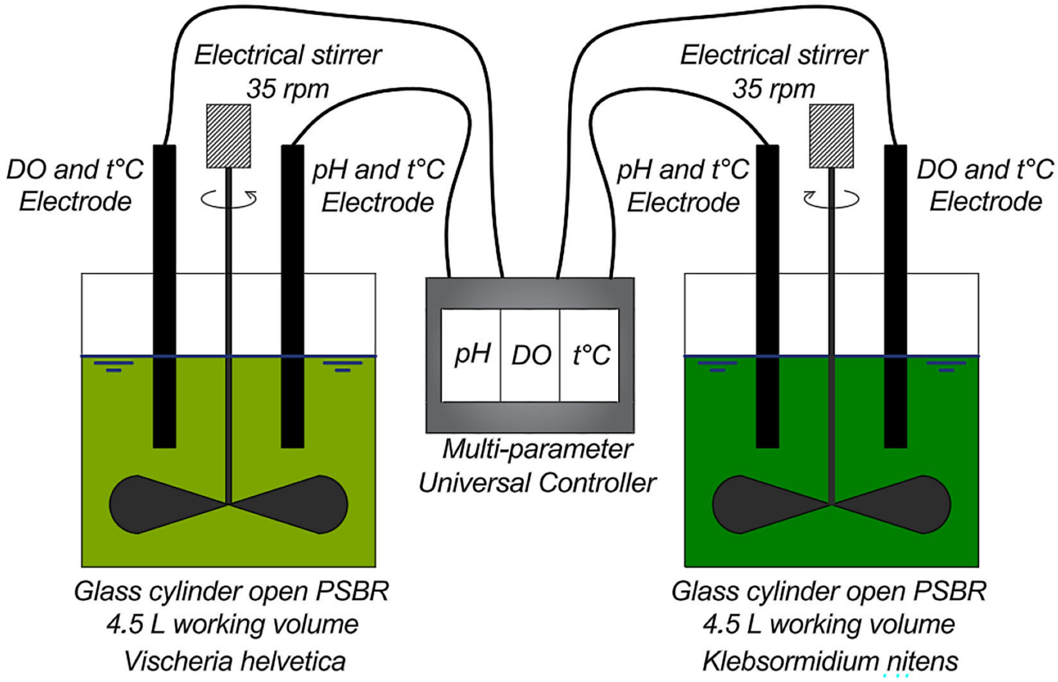

Figure 1. Scheme of the laboratory scale open photo-sequencing batch reactors (PSBRs)

= 120 mm and H = 50 mm). A Multi-parameter Universal Controller Display SC1000 (HACH Lange) with a Luminescent Dissolved Oxygen (LDO) Sensor, a 1200-S pH Sensor and a temperature sensor was used to measure DO, pH and temperature in real time and record the readings once every hour.

Wastewater

Unsterilized wastewater taken from the municipal WWTP of Sofia (1 300 000 p.e.), located in Kubratovo village, Bulgaria was used in the experiments. The treatment process of the WWTP consists of coarse screens, aerated grit chambers, primary clarifiers, activated sludge reactors, and secondary clarifiers. The P removal process is currently achieved through chemical precipitation (using $FeCl_3$ in the biological step). Biologically treated municipal wastewater was collected after the secondary settling tank and the experimental set-up was loaded with it. The treated wastewater that was taken at the outlet effluent channel of the plants and stored at 4°C in a refrigerator.

The used wastewater parameters' concentrations varied in the following ranges: TP = 0.25–1.5 mgP·L^{-1}; TN = 5.7–10 mgN·L^{-1}; Total Organic Carbon = 10–12 mgC·L^{-1}; Chemical Oxygen Demand = 17.5–21.5 mgO_2·L^{-1}; pH = 7.5–9. At the beginning of each cycle the used wastewater was additionally spiked with KH_2PO_4 and $NaNO_3$ (0.1% laboratory prepared stock solution for each) in order to increase the initial TP and TN concentrations. The goal was to simulate effluent wastewater quality as closely as possible to a scenario in which the wastewater treatment process does not include targeted TP and TN removal. The proposed technology considers the biological removal of organic pollutants in a conventional reactor, followed by an algal-based reactor for the recovery of nutrients (subject of the study).

Reactor operation

Once the requisite biomass had been achieved in the preliminary cultivation stage, each strain was introduced to the corresponding reactor containing the biologically treated and clarified wastewater from the Kubratovo WWTP. The adaptation period to the new medium conditions, including the wastewater type, temperature, photoperiod, working mode, and so forth, was complete once the results from each reactor began to show consistency in a stable monoculture system. Following this period, the actual experimental cycles of the PSBR were initiated. The PSBR algae treatment was used as an additional fourth stage of the municipal wastewater treatment for the reduction of the biogenic compounds.

The reactor was subsequently operated in four distinct phases, inherent to the SBR mode:

filling, reaction, settling (spontaneous auto-flocculation) and decanting. Each reactor operated for approximately three months - the *Vischeria helvetica* PSBR from early January to late March and the *Klebsormidium nitens* PSBR from early June to late August. The ambient temperature in the laboratory and the temperature of the water medium of the reactor were kept constant at 22 to 25 °C. The length of each cycle varied between 1 day and 1 week depending on the needed correlation.

Chemical and biological analysis

All samples were filtered using a glass fiber filter with a pore size of 0.45 µm. Subsequently, the filtrate was analyzed for total TP and TN using HACH Lange cuvette tests and spectrophotometric method (approved by ISO 15705).

The unfiltered samples were investigated on a light microscope (LM) at regular intervals. The examination was performed utilizing non-permanent slides with a Motic BA400 microscope. Microphotographs were acquired using a Moticam 2 camera and processed with the Image Plus software on the same microscope. The images presented in this paper were captured at a 40x objective magnification to depict the quantity and relative abundance of the observed algal specimens.

Data processing

TP and TN removal rates

The primary data processing of the TP and TN results included: 1) the concentrations from the chemical analysis were transformed into mass load based on the volume of the reactor at the certain moment, 2) The mass dynamics were monitored over each experimental cycle, and the data were represented graphically, and 3) The TP and TN removal rates were estimated through regression analysis for the reduction rates of phosphorus and nitrogen per cycle, expressed in $mgP·h^{-1}$ or $mgP·d^{-1}$, and $mgP·h^{-1}$ or $mgN·d^{-1}$, respectively.

pH level and DO concentration variations

Hourly pH and dissolved oxygen (DO) concentration data were analyzed to assess the influence of natural sunlight intensity (photoperiod) on algal activity and reactor conditions. Variations in both parameters were tracked over 24-hour cycles, with each hourly measurement represented as a point within the daily profile. Daily profiles were overlaid across multiple days to identify patterns and correlations in parameter dynamics.

RESULTS

Phosphorus removal

Phosphorus removal mechanisms in algal-based systems

Phosphorus removal in algal systems primarily occurs through two mechanisms: assimilation and chemical precipitation (Vaz et al., 2023; Zahmatkesh et al., 2023). During assimilation, microalgae incorporate phosphorus into essential cellular processes such as ATP production, phospholipid synthesis, and nucleic acid formation (Wu et al., 2021; Bossa et al., 2024). Algae preferentially absorb inorganic phosphorus species ($H_2PO_4^-$ and HPO_4^{2-}) via active transport, utilizing them through oxidative and photophosphorylation pathways (Lin et al., 2016; Gonçalves et al., 2017). When phosphorus is present in organic forms, algae release phosphatases to convert these compounds into orthophosphates for uptake (Carrillo et al, 2020). Certain microalgae can store excess phosphorus as polyphosphates in volutin bodies, enabling sustained growth during periods of phosphorus scarcity (Cuellar-Bermudez et al., 2017). Following nutrient limitation, uptake rates may increase when conditions improve (Larsdotter et al, 2006).

Chemical precipitation, or spontaneous auto-flocculation, occurs under intense photosynthetic activity, often driven by strong sunlight, without the need for chemical additives. As algae consume carbon, primarily in the form of CO_2 (and HCO_3^- when needed, with assistance from carbonic anhydrase), the resulting pH increase promotes the precipitation of phosphate ions (Moroney et al., 2001; Manusiwa et al., 2024). These ions form insoluble minerals such as calcium phosphate ($Ca_3(PO_4)_2$), magnesium ammonium phosphate (struvite), and other phosphate salts, depending on the wastewater's ionic composition. These precipitates contribute to auto-flocculation and sedimentation of algal biomass (Larsdotter et al, 2007; Munasinghe-Arachchige et al., 2020; Abeysiriwardana-Arachchige et al., 2021).

Phosphorus removal typically results from the combined action of assimilation and precipitation, with efficiencies ranging from 30% to 100%, depending on algal species, wastewater characteristics, and environmental conditions (Gonçalves et al., 2017; Ting et al., 2017).

Phosphorus removal rates (PRRs)

The variations in the average hourly TP concentration as a function of the average hourly pH dynamics in both reactors are presented in Figure 2 and Figure 3. The average hourly values are based on the data generated from all the measurements during the full operation period of the reactors.

The two systems exhibit chemical precipitation of P during periods of intense illumination, with subsequent recycling of P back into the water medium once the sunlight source reduces its potency. The peak pH values were observed to occur shortly after the hours with the highest amount of solar radiation. This resulted in a minimum concentration of TP in the medium of both reactors, which reached its lowest point in the *Vischeria* reactor earlier in the day – at 15:00 on average

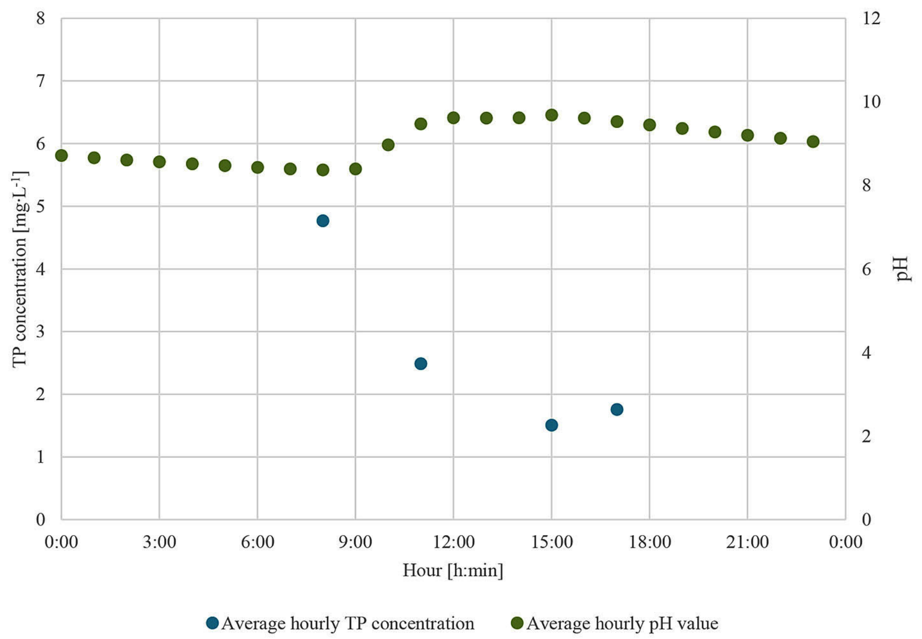

Figure 2. TP concentration and pH dynamics in the *Vischeria Helvetica* reactor

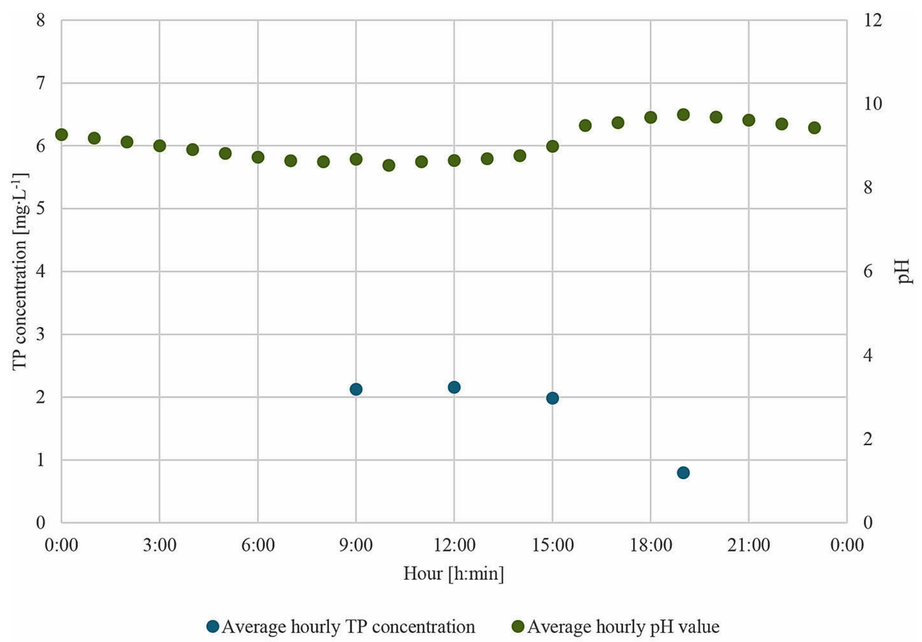

Figure 3. TP concentration and pH dynamics in the *Klebsormidium nitens* reactor

– while in the *Klebsormidium* reactor the lowest values were observed at 19:00 on average. Two factors contributed to this outcome. Firstly, the location of the reactor setup was at a northern window that received higher illumination during the second part of the day. Secondly, the *Vischeria* reactor was active during the first part of the year (January to late March) and the *Klebsormidium* reactor was active from early June to late August. This is a possible reason for the observed displacement of the peak illumination of the two reactors.

Similar results have been achieved in other studies in literature with strains such as *Chlorella vulgaris*, *Neochloris oleoabundans*, and *Tetradesmus obliguus* (Syn. *Scenedesmus obliquus*), where peak pH values coincided with periods of maximum photosynthetic activity (Alemu et al., 2018; AlMomani et al., 2020; Hussain et al., 2021). This phenomenon resulted in phosphorus precipitation in the form of calcium phosphate during the day, followed by a gradual release back into the aqueous phase as pH levels declined with reduced illumination. Studies have particularly highlighted the strong diurnal pattern of pH-driven phosphorus precipitation, with peak phosphorus removal efficiency observed during midday, as well as subsequent re-dissolution occurring toward the evening (Larsdotter, 2006).

These changes in the pH level and its correlation with the respective momentary TP concentration should be closely monitored when investigating a specific algal strain since each monoculture behaves differently with distinct wastewaters. This is an important technological parameter that could lead to a significant reduction in the hydraulic retention time in the reactor when algae are used for P removal. This is the mechanism that is responsible for the most rapid P removal in the algal-based wastewater treatment systems, while, at the same time, helping with the easier algal harvesting from the treated water. By aligning reactor operations with diurnal light cycles and selectively choosing algal strains that are resilient to daily pH fluctuations, it may be possible to enhance phosphorus retention within the biomass or precipitated form (Valchev et al., 2021). However, most of the P is recycled back into the water medium once the pH is back to neutral (Larsdotter, 2006; He et al., 2022). As this dynamic process is influenced by both environmental factors and strain-specific characteristics, ongoing research is essential to refine these systems for improved phosphorus management in wastewater treatment applications. The results in terms of the phosphorus removal rates from both mechanisms of P reduction (assimilation and precipitation) are discussed in the next section of the paper.

The range of the PRRs in both reactors, obtained from the initial data processing, are presented in the box plot graph in Figure 4.

Since the phosphorus removal mechanism plays a significant role in the algal-based wastewater treatment, the assessment of the performance of each used strain in the experiment is represented as the rate at which the algal monoculture takes up the phosphorus mainly biologically

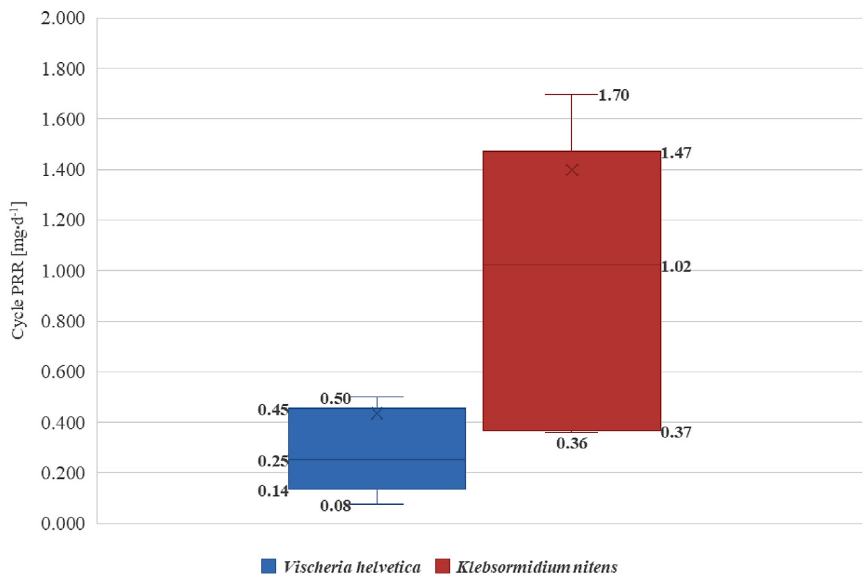

Figure 4. Full cycle PRRs in both reactors

(active transport, biosorption, bioaccumulation). This is a much slower process than the biologically induced chemical precipitation, and the graph includes the data from the full individual experimental cycles. This approach has been selected since the P removal due to precipitation (peak pH) on a daily basis may be more rapid, but after the end of the cycle, the new portion of the treated water in the SBR would reduce the pH of the medium and recycle the P back into dissolved state, making the reactor effluent quality unpredictable. After a few cycles the water will then reach a higher P concentration artificially that can only be reduced biologically. For this reason, the assessment of biological P removal in our study is based on a regression analysis of the initial data for the entire cycle. The measurement of the individual samples was performed before sunrise, at the pH minima values (peak values for TP) for the day and the P removal rate was measured in $mgP \cdot d^{-1}$.

A comparison analysis of the biologically induced P chemical precipitation through the increased pH levels during intensive photosynthetic activity was also done. However, the data is not presented in a graph since it could result in a misleading interpretation of the results and unrealistically performance of the strains. Since this is a chemical reaction and not an autotrophic/mixotrophic biological process, the PRR in this case is much higher compared to the other mechanism of P removal.

The graph in Figure 4 shows that in terms of the full cycle PRRs, the algal strain *Klebsormidium nitens* sustains much higher rates than *Vischeria helvetica* with median value that is approximately 5.88 times greater. Furthermore, *Klebsormidium nitens* reached higher P removal efficiency ranging from 49.74% to 80.64%, whereas *Vischeria helvetica* removed 28.24% to 67.25% of the initial P from the treated water. However, *Klebsormidium nitens* the PRRs vary in much larger range than *Vischeria Helvetica* making the operation of the process less predictable and more susceptible to meteorological changes. This may result in a more difficult reactor operation if the system depends mainly on the biological P uptake.

In terms of the daily biologically induced chemical P precipitation, *Vischeria* performs better than *Klebsormidium*. Its median rate is 2.36 times higher and its average rate with this mechanism is 0.52 ± 0.78 $mgP \cdot h^{-1}$, while *Klebsormidium*'s average rate is only 0.18 ± 0.09 $mgP \cdot h^{-1}$. The faster P removal through precipitation by *Vischeria helvetica* would result in a much smaller algal-based reactor footprint. However, such a system would be highly susceptible to delayed P release and higher risk of WWTP effluent deterioration. The results suggest that the P removal mechanism and the algal strain selection should be considered together at the designing phase of the reactor since this could lead to a dramatic change in the performance of the treatment technology.

The review paper by Nguyen et al. 2022 reports comparable P removal rates to those observed in our study (Figure 4), ranging from 0.59 to 0.9 $mgP \cdot d^{-1}$ with the monoculture of *Tetradesmus obliquus* (Syn. *Scenedesmus obliquus*) for the treatment of primary and secondary municipal effluents (Nguyen et al., 2022). The removal efficiencies reported in the paper fall within the range of 65% to 100%. Another study by Gonçalves et al. 2017 reports PRRs of 0.17 to 0.67 $mgP \cdot d^{-1}$ (calculated based on the removal efficiency and the reactor volumes) for the treatment of municipal wastewater with different microalgal consortia, which are primarily composed of algae from the green evolutionary line. However, the removal efficiencies in that study are considerably higher, at 93 to 99% (Gonçalves et al., 2017). This comparison with the literature demonstrates that the median PRR of *Klebsormidium nitens* (1.02 $mgP \cdot d^{-1}$) is within the upper range of the spectrum, while that of *Vischeria helvetica* (0.25 $mgP \cdot d^{-1}$) is within the lower range. This observation lends further support to the assertion that *Klebsormidium nitens* is the more efficacious species for P removal from municipal wastewater.

Nitrogen removal

Nitrogen removal mechanisms in algal-based systems

The N removal in algal-based wastewater treatment occurs mainly through two principal mechanisms. The first mechanism is the direct uptake of nitrogen by some algae for utilization in their biological processes (Ugwuanyi et al. 2024). The second mechanism is nitrogen stripping, which involves the volatilization of nitrogen compounds induced by increase in the pH levels (Alazaiza et al., 2023). The initial mechanism (N uptake) is fundamental to the metabolic processes of algae, as nitrogen is an essential nutrient for the synthesis of proteins, nucleic acids, and chlorophyll. Algae prefer to absorb dissolved nitrogen in the form of ammonium (NH_4^+) over other N species present in the water medium since it can be used directly for cellular structures (Sisman-Aydin et al., 2022). When ammonium is not available in the water medium, algae switches to other inorganic N sources such as nitrites (NO_2^-) and nitrates (NO_3^-) by reducing them first with nitrate reductase enzymes (Ugwuanyi et al. 2024). The algae then incorporate these nitrogen sources into the biomass that support growth and photosynthesis (Nguyen et al., 2022). The process effectively reduces nitrogen concentrations in wastewater, provided that the accumulated biomass is harvested on a regular basis to prevent the re-release of nutrients upon cell decay. The rate of nitrogen uptake is contingent upon a number of factors, including the species of algae involved, the intensity of light, and the availability of nutrients within the reactor (Ugwuanyi et al. 2024; Nguyen et al., 2022).

The second principal mechanism of nitrogen removal - nitrogen stripping, occurs through ammonia volatilization, which is closely associated with diurnal pH fluctuations within the algal treatment system (Cai et al., 2023). As algae raise the pH of the water medium during intense photosynthesis (under conditions of strong sunlight), and its value exceeds approximately 9.5, ammonium (NH_4^+) in the water shifts towards ammonia (NH_3), which is volatile in this state (Romero-Villegas et al., 2018). This gaseous form of ammonia readily escapes from the water into the atmosphere, effectively stripping nitrogen from the wastewater without relying on biomass assimilation. The efficiency of this volatilization process is influenced by the degree of sunlight exposure, pH levels, temperature, and the concentration of ammonium in the water. Furthermore, this process is amplified when aeration is added to the system that helps the formed ammonia to escape into the atmosphere (Romero-Villegas et al., 2018). For instance, Guštin, S., and Marinšek-Logar report in their study that a 92% stripping of the initial total ammonium is reached at pH levels above 9.5, at 70 °C, and with a continuous airflow that is fed to an open high-rate algal pond (Guštin and Marinšek-Logar, 2011).

Nitrogen removal rates (NRRs)

The used wastewater was taken after the secondary clarifier (and after an aeration chamber in the activated sludge reactor). The form of N in the effluent is almost fully NO_x^- (essentially no amounts of NH_4^+). Also, the laboratory set-up did not include any aeration of the reactors with air that is artificially enriched with CO_2. These preconditions show that the stripping nitrogen effect would be negligible, and it cannot be considered as a mechanism of N removal in this experiment. In this regard, the main process of N reduction in wastewater, with this construction of the two algal-based reactors, is through the biological uptake of N by algae. Since there are no drastically different N removal rates through the full experimental cycles and during each day of the individual cycle (only biological mechanism of removal), the graphs in Figure 5 include the full N removal data of the experiment.

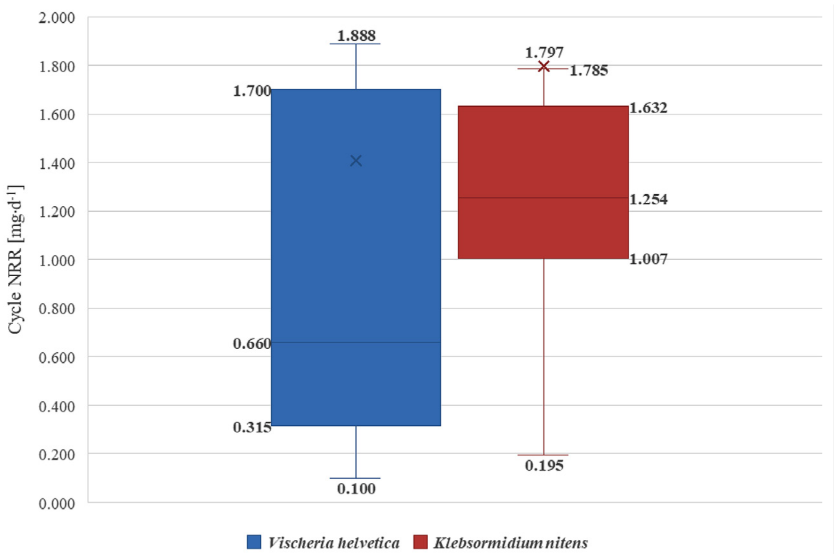

Figure 5. NRRs in both reactors for the entire experiment duration

Even though the median value of the *Klebsormidium nitens* (1.254 $mgN·d^{-1}$) is 1.9 times higher than that in the *Vischeria helvetica* reactor (0.66 $mgN·d^{-1}$) and the third quartile (75th percentile) limit value is 3.2 times greater, the range of the N removal rates is similar in both reactors. Still, the overall comparison of the algal strains, in terms of NRR, shows that the strain from the *Klebsormidium* genus performs better. This means that a future application of *Klebsormidium nitens* over *Vischeria helvetica* for N removal in a larger scale algal-based bioreactor for municipal wastewater treatment could lead to a reduction in the hydraulic retention time (HRT). The capital cost and the accumulated operational cost savings of a WWTP, connected to the HRT, and respectively the size of the built and maintained reactors, are major factors for the real applicability of the technology in urban and suburban areas.

These NRRs are in the lower spectrum in comparison to the reported data on the parameter in the scientific literature. For example, Ji et al., 2021 uses five algal monocultures and reports NRRs close to the ones achieved in our study, ranging from 0.84 to 0.97 $mgN·d^{-1}$ in a simulated domestic sewage with initial TN of 50 $mg·L^{-1}$ (Nezbrytska et al., 2022). On the other hand, in the Sisman-Aydin, 2022 experiment the NRRs with three algal monocultures and one algal consortium reached 6.1 to 8.3 $mgN·d^{-1}$. However, this study uses sterilized primary-treated municipal wastewater with high initial concentration of 60 $mg·L^{-1}$ while in our study the initial TN concentration was in the range of 9.91 to 38.5 $mg·L^{-1}$ with raw secondary treated wastewater that may contain residual amounts of grazers (Sisman-Aydin, 2022). The NRRs from both studies were calculated based on the removal efficiency and the reactor volumes used in each experiment.

Moreover, the N removal efficiency in the *Klebsormidium nitens* reactor was slightly higher reaching 15.59% to 66.45%, whereas in the *Vischeria Helvetica* reactor this parameter was between 16.56% and 57,04%. However, this removal efficiency is relatively low compared to the literature data. Tan et al., 2023 for example reports removal efficiency of 62.07% to 94.81% with four separate algal monocultures for primary domestic wastewater, but the main source of N in their medium was in the form of NH_4^+ (Tan et al., 2023). Alazaiza et al., 2023 also reports efficiency in the range of 80% to 95% for sewage water treatment using *Chlorella vulgaris*, however, the nitrogen was in the same form as in Tan et al., 2023 (ammonium-N) (Alazaiza et al., 2023). On the other hand, other studies like Nezbrytska et al., 2022 and Ji et al., 2021 also close to our low TN removal efficiency from wastewater in the range of 49 to 63% and 46% to 77.5% respectively (Nezbrytska et al., 2022; Ji et al., 2021). The lower N removal efficiency in our study was probably reached mainly due to the NO_x^- form of nitrogen in the feed water which is the less preferred source of the biogenous element for the algae than NH_4^+ (Sisman-Aydin, 2022). Also, there could be other factors like substate competition, suboptimal C:N:P ratio, toxic contamination due to regional industry, etc. that are specific to the local wastewater which remain beyond the scope of our study but need to be further examined in the future (Nezbrytska et al., 2022; Salgado et al., 2023).

pH and DO

Since the pH level and the DO concentration are both functions of light intensity (related to photosynthesis), their values vary significantly during the day. This is caused by the natural photoperiod of the algae, since the experimental set-up uses only solar radiation and no artificial sources of light. Peak DO values were observed in the reactor in the hour following the peaks in pH and vice versa in terms of the minimums. Comparison of the two strains in terms of maximum, minimum and average pH levels and DO concentrations is presented in Table 1.

With regard to pH levels, both strains of algae exhibited relatively consistent levels within the alkaline range, which is anticipated and attributed to the intense photosynthetic activity during the daylight hours. However, *Vischeria helvetica* reached lower maximum value below 10, mean value of 8.20 ± 1.10 and a median of 8.15. The strain also reaches higher minimum levels than *Klebsormidium nitens* leading to lower variation between the lowest and the highest values of the parameter. The mean value of the pH in the *Klebsormidium nitens* reactor was 8.63 ± 1.49 and the median was 8.16. Both strains exhibited higher minimum and lower maximum values than those reported in previous studies, despite *Klebsormidium nitens* approaching the highest pH levels documented in the literature (Whitton et al., 2015; Beltrán-Rocha et al., 2024; Yu et al., 2022).

In terms of DO concentration – the two strains perform closely in terms of the different

Table 1. pH level and DO concentration ranges in the reactors with the two algal strains compared to the highest and lowest values found in the literature

Parameter	pH in the *Vischeria* reactor	pH in the *Klebsormidium* reactor	pH in literature with other strains	Reference	DO in the *Vischeria* reactor	DO in the *Klebsormidium* reactor	DO in literature with other strains	Reference
Maximum	9.96	10.52	10.5–11.1	Whitton et al., 2015; Beltrán-Rocha et al., 2024	17.26 $mg \cdot L^{-1}$	17.76 $mg \cdot L^{-1}$	> 27 $mg \cdot L^{-1}$	Nordio et al., 2023
Minimum	7.38	6.83	< 4	Yu et al., 2022	5.92 $mg \cdot L^{-1}$	5.12 $mg \cdot L^{-1}$	< 5	Lage et al., 2021

value categories. Both *Vischeria* and *Klebsormidium* maintain very high DO concentrations in the reactor even at the lowest algal activity (always higher than 5 $mg \cdot L^{-1}$), at night, and extremely high of around 200% supersaturation (reactor temperature of 22–26 °C) at peak photosynthetic activity, during the part of the day with the highest amount of solar illumination. Algal reactors are sensitive to high DO concentration, since it could lead to inhibition of the vital processes of the strains in the system. In Nordio et al., 2023 for example, an open high-rate algal pond (HRAP) struggles to maintain relatively low DO concentration around 19 $mg \cdot L^{-1}$ with wastewater medium. A reduction to 14 mg·L-1 was successful with the use of a system for CO_2 injection for DO and pH control. One of their reactors reaches a maximum value of 27 $mg \cdot L^{-1}$ at peak illumination (without the control system) (Nordio et al., 2023). The DO concentration levels in both reactors of our study were below 18$mg \cdot L^{-1}$ without any control system and the mean values for *Vischeria* and *Klebsormidium* were 8.48 ± 0.41 $mg \cdot L^{-1}$ and 8.85 ± 0.50 $mg \cdot L^{-1}$ respectively. This means that both reactors perform very well in terms of maintaining DO concentration levels that do not inhibit the algal processes majorly.

In future applications of the algal-based wastewater treatment technologies, the two parameters and their timeline variations should be carefully considered, since they affect directly the ecosystem of the water body after WWTP's effluent discharge. Local legislations' permissions differ in their allowed values depending on the geographical area, the water body type and its' buffer capacity, the specific ecosystem, etc., but in general the pH values are considered safe in the range of pH = 6–9 for treated wastewater discharge (USEPA, 2010). In this regard, preliminary considerations should be made before scaling an algal-based reactor. The specific timing of the effluent release should be considered with respect to the photosynthetic activity in the reactor, the biologically induced precipitation of the algae and at what pH level does it occur with the specific strain, the HRT needed for the full treatment process to take place and finally the specifics of each strain.

In the case of this experimental research, if the monoculture strains are used in a reactor of a larger scale, a back-up reagent dosing system for pH level correction should be considered at the pilot scale/full scale WWTP and the effluent release timing should be connected to the achieved preliminary results with the specific strain (Figure 2 and Figure 3). The high DO concentration probably would not cause any harm to the receiving water body, since the dissolvability of the oxygen itself is very low, and the supersaturation values would get lower (to the point of saturation at the respective temperature) naturally after the removal of the algae from the treated wastewater. Furthermore, a high DO concentration in the effluent favors the ecosystem in the receiving water body and decreases the possibility of eutrophication and its' negative effects (oxygen depletion and anaerobic activity) and correspondingly decreases the "sensitivity level" of that water body (European Parliament and Council, 1991; European Parliament and Council, 2024; USEPA, 2024).

Changes in biomass, monoculture integrity and settling properties of the strains

Changes in biomass

Every specific strain of microalgae has its own natural development in their preferred habitat. Both strains used in this experiment showed their unique ways of adapting to the harsh environment of real wastewater. For the strain of *Vischeria helvetica* (Vischer and Pascher) Hibberd**.** In natural environment the cells have spherical or elliptical shape with size of 7 to 14 μm and an orange colored, carotenoid crystal is clearly visible in light microscope view. A light microscope photo of the initial moment when *Vischeria* is added to the new wastewater environment at the start of the experiment is provided in (Figure 6A). All the signs of natural growth are present, and the suspension has a prominent yellow-brown color (Figure 6B). On the photo, typical reproduction by cell division is visible.

After two weeks of staying in the experimental reactor with real wastewater and a few water changes of the cycles, the strain of *Vischeria helvetica* started to become much greener in color and a small floc formation occurred. Also, under a light microscope examination the cells appeared clustered together with smaller cell size and less visible carotenoid crystal in them (Figure 7). These symptoms of more intensive cell division, increased production of chlorophyll *a* (bringing the green color), increased photosynthesis and a less visible amount of carotenoids stored in the cell (less stock substances) show that the strain has adapted very well to the new conditions and that the wastewater medium is a suitable environment for its development.

The formation of the small flocs is not natural for *Vischeria* and this exception is probably due to the intensive photosynthesis, the increase in pH and respectively the activation of the alkaline, chemically induced precipitation (autoflocculation due to the decrease of free CO_2 and the formation of Ca^{2+} and Mg^{2+} phosphate salts enfolding the algal cells).

For the strain of *Klebsormidium nitens* (Kützing) Lokhorst. Since *Klebsormidium nitens* (Kützing) Lokhorst is a strain from the green algal evolutionary line, the green color and the synthesis of high amounts of chlorophyll *a* are natural for it. In its habitats, in nature, the strain is usually considered as a medium-sized filamentous algae. It usually forms algal mats, and it is examined as one of the algal land plant progenitors (Stoyneva-Gärtner et al., 2019-c).

In the experiment, the strain was kept in suspension. Due to the specific environment of the wastewater and its nutrient dense nature, the reactor adopted a light green color in the beginning of the experiment in a homogenous suspension and gradually progressed to a dark green suspension with visible flocs within two weeks. This was

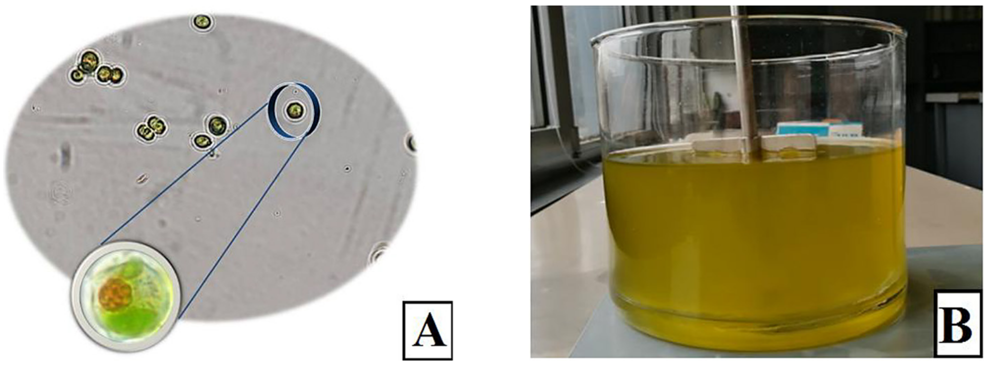

Figure 6. Light microscope photo of *Vischeria helvetica* (Vischer and Pascher) Hibberd (A) and a PSBR photo (B) right after the addition of it to the reactor

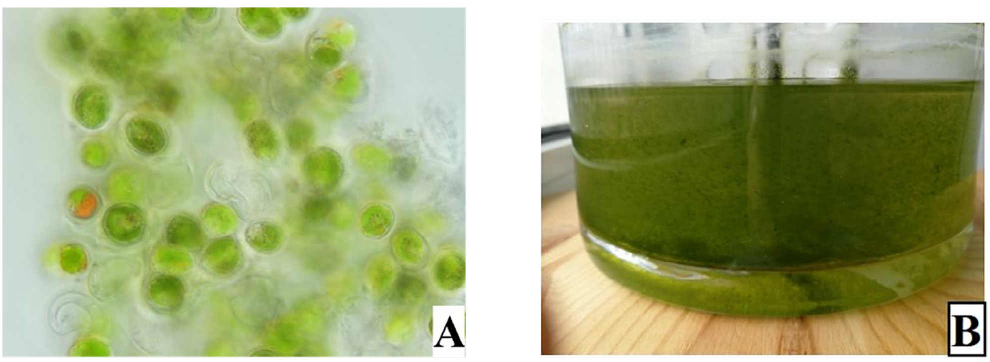

Figure 7. Light microscope photo of *Vischeria helvetica* (Vischer and Pascher) Hibberd (A) and a PSBR photo(B) after two weeks of development of the strain in the reactor

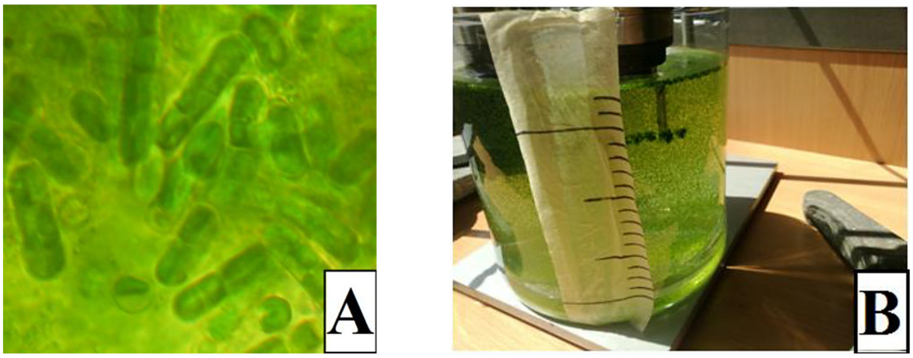

Figure 8. Light microscope photo of *Klebsormidium nitens* (Kützing) Lokhorst (A) and a PSBR photo (B) after two weeks of development of the strain in the reactor

probably caused by the same alkaline autoflocculation (Figure 8 B). Furthermore, the microphotos (Figure 8 A) from the reactor show that the strain divides faster, forms shorter filaments and grows thoroughly (typical for a well-adapted algae in a nutrient dense environment with favorable conditions).

In general, both strains adapted very well to the real wastewater with acute growth and intensive photosynthesis.

Monoculture integrity and strain contamination

During each of the experiments with the individual used monocultures, contamination with algae, native for the real wastewater, was noticed. The native algae etc., that inhabit flowing water streams (in this case the effluent channels of the studied WWTPs) caused the contamination observed in both reactors. However, it evolved differently with each used strain.

Both systems functioned in the same operation mode. In the reactor with the *Vischeria* strain, after a month and a half of regular wastewater change (each week) in the reactor for each new cycle, the strain started to lose dominance in the system. The native algae took over which led to a significant compromise in the wastewater treatment process - worse settling, reactor walls and bottom entwinement, less light penetration, color change in the suspension and fluctuation in the PRRs and NRRs (Figure 9 A). The reactor with the *Klebsormidium* strain contained relatively constant conditions even after a month and a half of operation and regular wastewater changes (each week). Even though some diatoms were noticed in some light microscopic photos of the reactor, the *Klebsormidium* strain remained dominant in the system (Figure 9 B) and the wastewater treatment process did not change significantly. The settling properties of the algae in the reactor were consistent, no significant color changes of the algae-wastewater suspension were visible, no major deviations from the standard variations in the pH levels and DO concentrations were observed and the PRRs and NRRs remained relatively the same.

Overall, *Klebsormidium nitens* maintained a more stable process compared to *Vischeria helvetica.* This displays a higher potential of *Klebsormidium* in terms of consistent results in

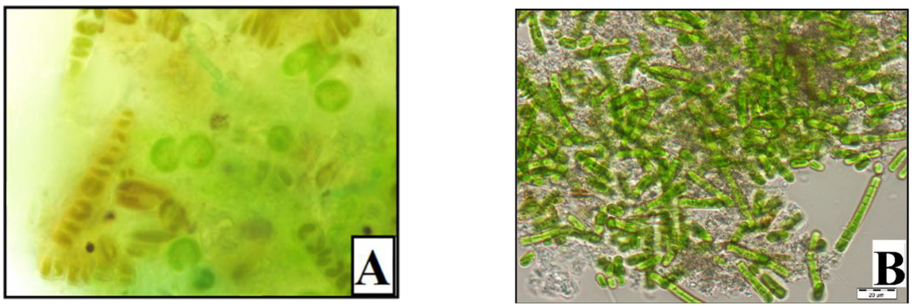

Figure 9. Light microscope photo of the two algal strains (A – *Vischeria helvetica* (Vischer and Pascher) Hibberd; B – *Klebsormidium nitens* (Kützing) Lokhorst)) in each of the PSBRs after a month and a half of reactor operation

a wastewater treatment process, compared to *Vischeria*. However, further research is needed to demonstrate whether this monoculture stability, the conditions and the technological parameters can be maintained in a larger scale system.

Settling properties

Both reactors with the individual monocultures showed a noticeable floc formation (autoflocculation) - size of 1–3 mm in diameter for each individual floc. The flocs became visible with unarmed eye, but in the *Vischeria* reactor they started to form at a slightly lower pH value than *Klebsormidium* – pH = 8–9 for *Vischeria* compared to pH = 8.5–9.5 for *Klebsormidium*. This allowed for a relatively easy harvesting of the biomass after the wastewater treatment process only through stoppage of the stirring unit and sedimentation of the algae (at the lowest possible pH level with visible flocs). No addition of any coagulants/flocculants to the system was needed. However, the process of easy algal harvesting through biomass settlement was possible through the whole experiment only with the strain of *Klebsormidium* (Figure 10). This was due to the remaining dominance of the strain in the reactor and the monoculture properties and conditions of the system that were maintained even after the constant weekly changes of the reactor's wastewater after every cycle. In the reactor containing the *Vischeria* algal strain, the settling properties of the biomass were also very good at the beginning of the experiment (Total Suspended Solids (TSS) around 25 mg·L^{-1} in the decant after 30 min of settling – Figure 11 A), but started to worsen with every wastewater change at the beginning of each new cycle. New native algal strains accumulated, which displaced the *Vischeria* strain and entwined the glass walls of the reactor. This led to problems with the harvesting in the *Vischeria* reactor throughout the later stages of the experiment (TSS around 100 mg·L^{-1} in the decant after 30 min of settling - Figure 11 B).

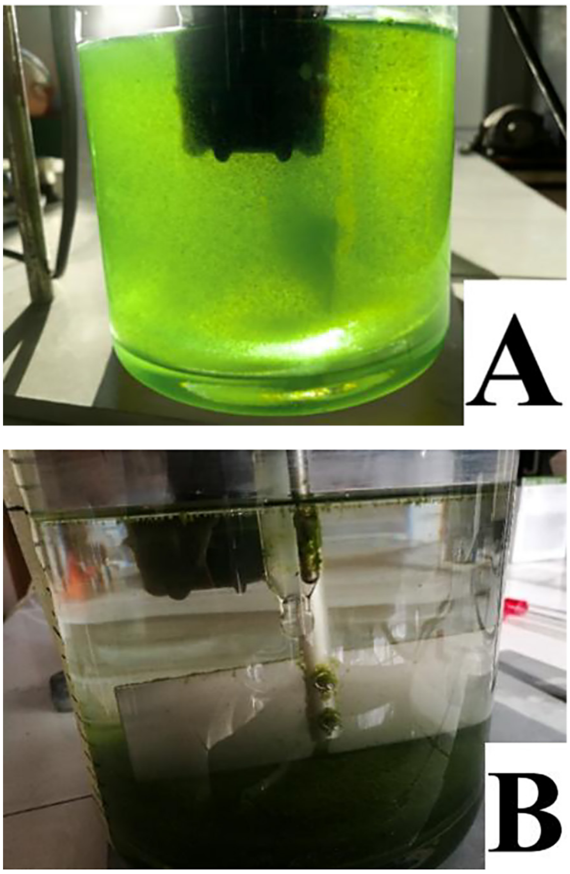

Figure 10. Photos of the PSBR with the strain of *Klebsormidium nitens* (A – with active mixing; B – at rest, after 30 minutes of settling)

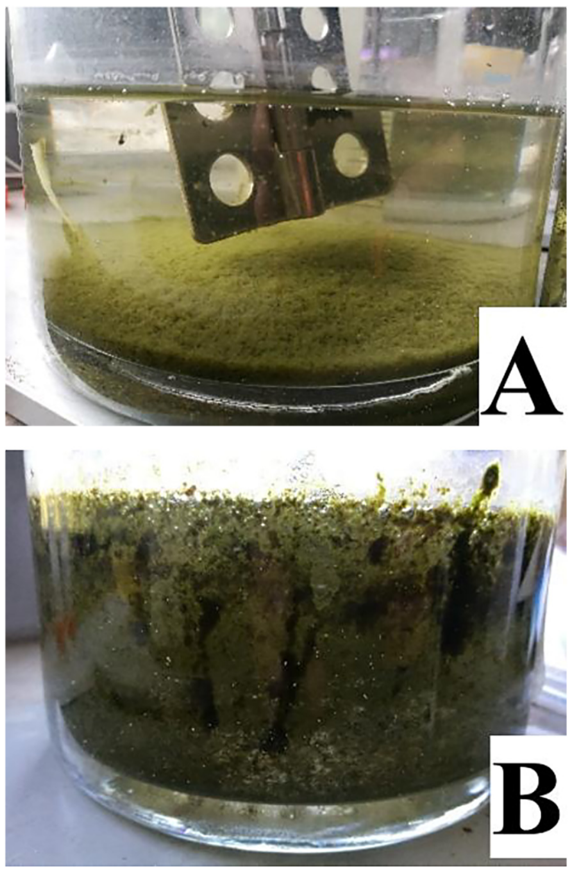

Figure 11. Photos of the PSBR with the strain of *Vischeria helvetica* at rest, after 30 minutes of settling (A – with dominant *Vischeria* monoculture; B – after contamination with native algal strains)

Table 2. Comparison analysis of the strains *Vischeria helvetica* and *Klebsormidium nitens*

Parameter	*Vischeria helvetica*	*Klebsormidium nitens*
Average PRR (biological removal of P)	0.25 ± 0.55 mgP·d^{-1}	1.02 ± 1.66 mgP·d^{-1}
Average PRR (biologically induced P precipitation)	0.52 ± 0.78 mgP·h^{-1}	0.18 ± 0.09 mgP·h^{-1}
Average NRR	0.66 ± 1.61 mgN·d^{-1}	1.254 ± 1.84 mgN·d^{-1}
P removal efficiency	49.74% to 80.64%	28.24% to 67.25%
N removal efficiency	16.56% to 57.04%	15.59% to 66.45%
Maximum reached pH	9.96	10.52
Average pH	8.20 ± 1.10	8.63 ± 1.49
Maximum DO concentration	17.26 mgO$_2$·L^{-1}	17.76 mgO$_2$·L^{-1}
Average DO concentration	8.48 ± 0.41 mgO$_2$·L^{-1}	8.85 ± 0.50 mgO$_2$·L^{-1}
Monoculture integrity maintenance	Unstable, severe contamination, drastic change in the reactor conditions and settling properties	Relatively stable, slight contamination, no change in reactor conditions and settling properties
Settling properties	Very good only at non-contaminated monoculture	Very good
Adaptation period to the wastewater	Slower adaptation (approx. one month)	Faster adaptation (less than two weeks)

Even though *Vischeria* manages to maintain slightly better reactor conditions during the autoflocculation phase (pH around 8), *Klebsormidium* is much more stable in terms of monoculture integrity and predation resistance. This is probably due to *Klebsormidium*'s filamentous nature (multicellular algae) that gives it an advantage over the unicellular strains (Valchev et al., 2021). These promising initial laboratory results will be examined and verified in future experiments to further validate the obtained results.

DISCUSSION ON THE COMPARATIVE ANALYSIS OF THE TWO ALGAL STRAINS

The results and conclusions from all experiments carried out in the 3-month long experiment with the two monoculture strains of algae, are gathered, generalized and compared in Table 2.

The comparison analysis brings a general view on the wastewater treatment performance of the two used algal strains and gives a perspective on the reactor conditions and technological parameters for future PSBR design and maintenance. Overall, both strains showed potential for wastewater treatment as a final P and N removal step of a WWTP at a laboratory scale. Even though the strain of *Vischeria helvetica* manages to maintain higher induced precipitation PRR and closer to neutral pH levels, the strain of *Klebsormidium nitens* outperforms the other strain in all other aspects (Table 2). The stable monoculture (relative resistance to culture contamination and consumption by other heterotrophic organisms), the faster adaptation period, the more rapid full biological P removal, the higher NRR and the constant conditions and parameters throughout the full period of the experiment make *Klebsormidium* the more applicable algal strain of the two for real wastewater treatment. Further research is needed to verify the results in the harsher environment of the pilot-scale or full-scale reactors but the gathered laboratory data from this experiment shows promising initial results and helps with the transition of the technology to a higher technological readiness level.

CONCLUSIONS

Two suspended growth PSBR systems with separate monocultures of underexplored algal strains, namely, *Vischeria helvetica* ACUS 00025 and *Klebsormidium nitens* ACUS 00207 were investigated for their feasibility in tertiary wastewater treatment for the removal of phosphorus (P) and nitrogen (N). The comparative analysis based on three months of operation in laboratory conditions revealed that:

- *Klebsormidium* maintained a 5.88 times higher mean value for biological PRRs and a third quartile (75th percentile) limit value for NRR that is 3.2 times greater than in the *Vischeria* reactor;
- *Klebsormidium nitens* reached higher P and N removal efficiencies than *Vischeria*

helvetica, however, both were in the lower spectrum of the efficiencies reported in the scientific literature;

- Both *Vischeria* and *Klebsormidium* maintain relatively universal conditions, favorable for the effluent quality and the water body after the treatment - average pH of 8.2 and 8.63, and average DO concentration of 8.48 and 8.85 $mgO_2 \cdot L^{-1}$);
- *Klebsormidium* maintained a more stable monoculture with higher resistance to contamination and consumption by other heterotrophic organisms, shorter wastewater adaptation period, and constant settling properties via autoflocculation;
- *Klebsormidium* produced more consistent results in all aspects throughout the whole period of the experiment.

The full-scale application, which has a more challenging environment, necessitates a more stable monoculture and more consistent parameters within the PSBR. The results of our feasibility study, conducted in laboratory conditions, indicated that the *Klebsormidium nitens* strain exhibited greater potential than *Vischeria helvetica*.

The research carried out in this experiment contributes to the advancement of our understanding of the applications of algal-based wastewater treatment technology with suspended biomass. This is particularly important considering the dearth of data in the existent literature on the specific technological parameters required for algae bioreactors and the continuous research efforts for finding an optimal strain or consortium of algae to achieve the highest possible performance in the field of wastewater treatment.

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REFERENCES

1. Abdelfattah, A., Ali, S. S., Ramadan, H., El-Aswar, E. I., Eltawab, R., Ho, S. H., Elsamahy, T., Li, S., El-Sheekh, M. M., Schagerl, M., Kornaros, M., Sun, J. (2023) Microalgae-based wastewater treatment: Mechanisms, challenges, recent advances, and future prospects. *Environmental Science and Ecotechnology. 13*. https://doi.org/10.1016/j.ese.2022.100205
2. Abeysiriwardana-Arachchige, I. S. A., Samarasingha, N., Rosalez, R., Munasinghe-Arachchige, S. P., Delanka-Pedige, H. M. K., Brewer, C. E., Nirmalakhandan, N. (2021) Maximizing phosphorus recovery as biofertilizer in an algal wastewater treatment system. *Resources, Conservation and Recycling, 170*. https://doi.org/10.1016/j.resconrec.2021.105552
3. Abreu, A. P., Martins, R., Nunes, J. (2023) Emerging applications of *Chlorella* sp. and *Spirulina* (Arthrospira) sp. bioengineering, 10(8). https://doi.org/10.3390/bioengineering10080955
4. Alazaiza, M. Y. D., He, S., Su, D., Abu Amr, S. S., Toh, P. Y., Bashir, M. J. K. (2023) Sewage water treatment using chlorella vulgaris microalgae for simultaneous nutrient separation and biomass production. *Separations, 10*(4). https://doi.org/10.3390/separations10040229
5. Alemu, K., Assefa, B., Kifle, D., Kloos, H. (2018) Nitrogen and phosphorous removal from municipal wastewater using high rate algae ponds. *INAE Letters, 3*(1), 21–32. https://doi.org/10.1007/s41403-018-0036-1
6. AlMomani, F., Örmeci, B. (2020) Assessment of algae-based wastewater treatment in hot climate region: Treatment performance and kinetics. *Process Safety and Environmental Protection, 141*, 140–149. https://doi.org/10.1016/j.psep.2020.03.031
7. Ali, M., Masood, A., Saleem, M. (2021) Microalgae cultivation in wastewater for simultaneous nutrients removal and biomass production. *International Journal of Energy and Environmental Engineering, 12*(3), 475–485. https://doi.org/10.1007/s40095-021-00383-3
8. Ali, S. S., El-Sheekh, M., Manni, A., Ruiz, H. A., Elsamahy, T., Sun, J., Schagerl, M. (2022) Microalgae-mediated wastewater treatment for biofuels production: A comprehensive review. *Microbiological Research, 265*. https://doi.org/10.1016/j.micres.2022.127187
9. Arias D., Uggetti E., García-Galán M., García J. (2017) Cultivation and selection of cyanobacteria in a closed photobioreactor used for secondary effluent and digestate treatment. *Science of the Total Environment*, 587–588, 157–167 https://doi.org/10.1016/j.scitotenv.2017.02.097
10. Beltrán-Rocha, J. C., Guajardo-Barbosa, C.,

Barceló-Quintal, I. D., Reyna-Martínez, G., Fariz-Salinas, E., Ramírez-Castillo, A., Rodríguez-Fuentes, H., López-Chuken, U. J. (2024) Effect of natural increase of pH and microalgae cyclical re-cultivation on biomass production and polishing of municipal secondary effluent. *Desalination and Water Treatment, 317*. https://doi.org/10.1016/j.dwt.2024.100103

11. Bossa, R., di Colandrea, M., Salbitani, G., Carfagna, S. (2024) Phosphorous utilization in microalgae: physiological aspects and applied implications. *Plants, 13*(15) https://doi.org/10.3390/plants13152127
12. Cai, T., Park, S. Y., Li, Y. (2013) Nutrient recovery from wastewater streams by microalgae: Status and prospects. *Renewable and Sustainable Energy Reviews, 19*, 360–369. https://doi.org/10.1016/j.rser.2012.11.030
13. Carrillo, V., Fuentes, B., Gómez, G., Vidal, G. (2020) Characterization and recovery of phosphorus from wastewater by combined technologies. *Reviews in Environmental Science and Biotechnology, 19*(2), 389–418 https://doi.org/10.1007/s11157-020-09533-1
14. Chawla, P., Malik, A., Sreekrishnan, T. R., Dalvi, V., Gola, D. (2020) Selection of optimum combination via comprehensive comparison of multiple algal cultures for treatment of diverse wastewaters. *Environmental Technology and Innovation, 18*. https://doi.org/10.1016/j.eti.2020.100758
15. Council Directive 91/271/EEC of 21 May (1991) Concerning urban waste-water treatment. Available from: http://data.europa.eu/eli/dir/1991/271/oj (Accessed on June 6th, 2025)
16. Cuellar-Bermudez, S., Aleman-Nava, G., Chandra, R., Garcia- Perez, S., Contreras-Angulo, J., Markou, G., Muylaert, K., Rittmann, B., Parra-Saldivar, R. (2017) Nutrients utilization and contaminants removal. A review of two approaches of algae and cyanobacteria in wastewater. *Algal Research, 24*(B), 438–449. https://doi.org/10.1016/j.algal.2016.08.018
17. Díaz, V., Leyva-Díaz, J. C., Almécija, M. C., Poyatos, J. M., del Mar Muñío, M., Martín-Pascual, J. (2022) Microalgae bioreactor for nutrient removal and resource recovery from wastewater in the paradigm of circular economy. *Bioresource Technology, 363*. https://doi.org/10.1016/j.biortech.2022.127968
18. de Lima Barizão, A. C., de Oliveira Gomes, L. E., Brandão, L. L., Sampaio, I. C. F., de Moura, I. V. L., Gonçalves, R. F., de Oliveira, J. P., Cassini, S. T. (2023) Microalgae as tertiary wastewater treatment: Energy production, carbon neutrality, and high-value products. Algal Research, 72. https://doi.org/10.1016/j.algal.2023.103113
19. de Morais, E. G., Sampaio, I. C. F., Gonzalez-Flo, Ferrer, I., Uggetti, E., García, J. (2023) Microalgae harvesting for wastewater treatment and resources recovery: A review. *New Biotechnology, 78*, 84–94. Elsevier B.V. https://doi.org/10.1016/j.nbt.2023.10.002
20. European Parliament and Council. *Directive concerning urban wastewater treatment (recast)*. PE-CONS 85/24, 2024. Retrieved from https://eur-lex.europa.eu/legal-content/EN/TXT/?uri=CELEX:01991L0271-20140101
21. European Commission (2020) *A new Circular Economy Action Plan: For a cleaner and more competitive Europe*. European Union. https://environment.ec.europa.eu/strategy/circular-economy-action-plan_en
22. European Commission (2020) *The European Green Deal*. European Union. https://commission.europa.eu/strategy-and-policy/priorities-2019-2024/european-green-deal_en
23. Ettl, H., Gärtner, G. (2014) *Syllabus der Boden-, Luft- und Flechtenalgen*. 2. Auflage. Springer Spektrum, Berlin. https://doi.org/10.1007/978-3-642-39462-1
24. Federal Ministry of Education and Research (BMBF) & Federal Ministry of Food and Agriculture (BMEL) (2020) Nationale Bioökonomiestrategie. *Federal Republic of Germany*. https://biooekonomie.de/themen/politikstrategie-deutschland
25. Geremia, E., Ripa, M., Catone, C. M., Ulgiati, S. (2021) A review about microalgae wastewater treatment for bioremediation and biomass production—a new challenge for Europe. *Environments, 8*(12). https://doi.org/10.3390/environments8120136
26. Goh, P. S., Lau, W. J., Ismail, A. F., Samawati, Z., Liang, Y. Y., Kanakaraju, D. (2023) Microalgae-Enabled Wastewater Treatment: A Sustainable Strategy for Bioremediation of Pesticides. *Water, 15*(1). https://doi.org/10.3390/w15010070
27. Gonçalves, A. L., Pires, J. C. M., Simões, M. (2017) A review on the use of microalgal consortia for wastewater treatment. *Algal Research, 24*, 403–415. https://doi.org/10.1016/j.algal.2016.11.008
28. Guštin, S., Marinšek-Logar, R. (2011) Effect of pH, temperature and air flow rate on the continuous ammonia stripping of the anaerobic digestion effluent. *Process Safety and Environmental Protection, 89*(1), 61–66. https://doi.org/10.1016/j.psep.2010.11.001
29. Han, M., Zhang, C., Ho, S. H. (2023) Immobilized microalgal system: An achievable idea for upgrading current microalgal wastewater treatment. *Environmental Science and Ecotechnology, 14*. https://doi.org/10.1016/j.ese.2022.100227
30. Hasnain, M., Zainab, R., Ali, F., Abideen, Z., Yong, J. W. H., El-Keblawy, A., Hashmi, S., Radicetti, E. (2023) Utilization of microalgal-bacterial energy nexus improves CO2 sequestration and remediation of wastewater pollutants for beneficial

environmental services. *Ecotoxicology and Environmental Safety, 267*. https://doi.org/10.1016/j.ecoenv.2023.115646

31. He, L., Chen, Y., Chen, S., Wu, X., Liu, J. (2022) Effects of Chlorella vulgaris on phosphorus release from ferric phosphate sediment by consecutive cultivations. *Royal Society Open Science, 9*(3). https://doi.org/10.1098/rsos.211391
32. Huang, H., Zhong, S., Wen, S., Luo, C., Long, T. (2022) Improving the efficiency of wastewater treatment and microalgae production for biofuels. *Resources, Conservation and Recycling, 178*. https://doi.org/10.1016/j.resconrec.2021.106094
33. Hussain, F., Shah, S. Z., Ahmad, H., Abubshait, S. A., Abubshait, H. A., Laref, A., Manikandan, A., Kusuma, H. S., Iqbal, M. (2021) Microalgae an ecofriendly and sustainable wastewater treatment option: Biomass application in biofuel and bio-fertilizer production. A review. *Renewable and Sustainable Energy Reviews, 137*. https://doi.org/10.1016/j.rser.2020.110603
34. Jia H., Yuan Q. (2018) Nitrogen removal in photo sequence batch reactor using algae-bacteria consortium. *Journal of Water Process Engineering, 26*, 108–115. https://doi.org/10.1016/j.jwpe.2018.10.003
35. Ji, L., Ge, Q., Li, Y., Gao, Y., Xie, S. (2021) A comparative study of the growth and nutrient removal effects of five green microalgae in simulated domestic sewage. *Water, 13*(24). https://doi.org/10.3390/w13243613
36. Khan, S., Thaher, M., Abdulquadir, M., Faisal, M., Mehariya, S., Al-Najjar, M. A. A., Al-Jabri, H., Das, P. (2023) Utilization of microalgae for urban wastewater treatment and valorization of treated wastewater and biomass for biofertilizer applications. *Sustainability, 15*(22). https://doi.org/10.3390/su152216019
37. Lage, S., Toffolo, A., Gentili, F. G. (2021) Microalgal growth, nitrogen uptake and storage, and dissolved oxygen production in a polyculture based-open pond fed with municipal wastewater in northern Sweden. *Chemosphere, 276*. https://doi.org/10.1016/j.chemosphere.2021.130122
38. Larsdotter K. (2006) *Microalgae for phosphorus removal from wastewater in a Nordic climate.* A doctoral thesis from the School of Biotechnology, Royal Institute of Technology, Stockholm, Sweden. https://www.researchgate.net/publication/279464505
39. Larsdotter, K., la Cour Jansen, J., Dalhammar, G. (2007) Biologically mediated phosphorus precipitation in wastewater treatment with microalgae. *Environmental Technology, 28*(9), 953–960. https://doi.org/10.1080/09593332808618855
40. Lavrinovičs, A., Mežule, L., Juhna, T. (2020) Microalgae starvation for enhanced phosphorus uptake from municipal wastewater. *Algal Research, 52*. https://doi.org/10.1016/j.algal.2020.102090
41. Lawton, R.J., Glasson, C.R.K., Novis, P.M. et al. (2021) Productivity and municipal wastewater nutrient bioremediation performance of new filamentous green macroalgal cultivars. *J Appl Phycol., 33*, 4137–4148. https://doi.org/10.1007/s10811-021-02595-w
42. Li, L., Gao, K., Yang, M., Zheng, Q., Zhang, M., Deng, X. (2023) Challenges and potential solutions of microalgae-based systems for wastewater treatment and resource recovery. In *Frontiers in Bioengineering and Biotechnology, 11*. https://doi.org/10.3389/fbioe.2023.1210228
43. Li, K., Liu, Q., Fang, F., Luo, R., Lu, Q., Zhou, W., Huo, S., Cheng, P., Liu, J., Addy, M., Chen, P., Chen, D., Ruan, R. (2019) Microalgae-based wastewater treatment for nutrients recovery: A review. *Bioresource Technology, 291*. https://doi.org/10.1016/j.biortech.2019.121934
44. Lin, S., Litaker, R. W., Sunda, W. G. (2016) Phosphorus physiological ecology and molecular mechanisms in marine phytoplankton. In *Journal of Phycology, 52*(1), 10–36. https://doi.org/10.1111/jpy.12365
45. Liu, R., Li, S., Tu, Y., Hao, X. (2021) Capabilities and mechanisms of microalgae on removing micropollutants from wastewater: A review. *Journal of Environmental Management, 285*. https://doi.org/10.1016/j.jenvman.2021.112149
46. Liu, X. Y., Hong, Y., Zhao, G. P., Zhang, H. K., Zhai, Q. Y., Wang, Q. (2022) Microalgae-based swine wastewater treatment: Strain screening, conditions optimization, physiological activity and biomass potential. *Science of the Total Environment, 807*. https://doi.org/10.1016/j.scitotenv.2021.151008
47. Manusiwa, B. C., Purwono, S., Nugroho, A. P. (2024) CO_2 Uptake and Domestic Wastewater Treatment by Chlorella vulgaris. *IOP Conference Series: Earth and Environmental Science, 1313*(1). https://doi.org/10.1088/1755-1315/1313/1/012007
48. Ministère de l'Agriculture et de l'Alimentation (2017) Stratégie Nationale Bioéconomie: Une stratégie bioéconomie pour la France. République Française. https://agriculture.gouv.fr/une-strategie-bioeconomie-pour-la-france-plan-daction-2018-2020
49. Mohsenpour, S. F., Hennige, S., Willoughby, N., Adeloye, A., Gutierrez, T. (2021) Integrating micro-algae into wastewater treatment: A review. *Science of the Total Environment, 752*. https://doi.org/10.1016/j.scitotenv.2020.142168
50. Molinuevo-Salces, B., Riaño, B., Hernández, D., García-González, M. C. (2019) Microalgae and wastewater treatment: Advantages and disadvantages. *Microalgae Biotechnology for Development of Biofuel and Wastewater Treatment,* 505–533.

https://doi.org/10.1007/978-981-13-2264-8_20

51. Moroney J., Bartlett S, Samuelsson G. (2001) Carbonic anhydrases in plants and algae. *Plant, Cell and Environment, 24*, 141–153. https://doi.org/10.1111/j.1365-3040.2001.00669.x
52. Munasinghe-Arachchige, S. P., Abeysiriwardana-Arachchige, I. S. A., Delanka-Pedige, H. M. K., & Nirmalakhandan, N. (2020) Algal pathway for nutrient recovery from urban sewage. *Algal Research, 51*. https://doi.org/10.1016/j.algal.2020.102023
53. Nezbrytska, I., Shamanskyi, S., Pavliukh, L., Kharchenko, G. (2022) Assessment of inorganic nitrogen and phosphorus compounds removal efficiency from different types of wastewater using microalgae cultures. *Oceanological and Hydrobiological Studies, 51*(1), 45–52. https://doi.org/10.26881/oahs-2022.1.05
54. Nguyen, L. N., Aditya, L., Vu, H. P., Johir, A. H., Bennar, L., Ralph, P., Hoang, N. B., Zdarta, J., Nghiem, L. D. (2022) Nutrient removal by algae-based wastewater treatment. *Current Pollution Reports, 8*(4), 369–383. https://doi.org/10.1007/s40726-022-00230-x
55. Nordio, R., Viviano, E., Sánchez-Zurano, A., Hernández, J. G., Rodríguez-Miranda, E., Guzmán, J. L., Acién, G. (2023) Influence of pH and dissolved oxygen control strategies on the performance of pilot-scale microalgae raceways using fertilizer or wastewater as the nutrient source. *Journal of Environmental Management, 345*. https://doi.org/10.1016/j.jenvman.2023.118899
56. Novak, I. N., Magnusson, M., Craggs, R. J., Lawton, R. J. (2024) Screening protocol for freshwater filamentous macroalgae bioremediation of primary municipal wastewater. *Journal of Applied Phycology*. https://doi.org/10.1007/s10811-024-03261-7
57. Oviedo, J. A., Muñoz, R., Donoso-Bravo, A., Bernard, O., Casagli, F., Jeison, D. (2022) A half-century of research on microalgae-bacteria for wastewater treatment. *Algal Research, 67*. https://doi.org/10.1016/j.algal.2022.102828
58. Rani, S., Gunjyal, N., Ojha, C. S. P., Singh, R. P. (2021) Review of challenges for algae-based wastewater treatment: strain selection, wastewater characteristics, abiotic, and biotic factors. *Journal of Hazardous, Toxic, and Radioactive Waste, 25*(2). https://doi.org/10.1061/(asce)hz.2153-5515.0000578
59. Romero-Villegas, G. I., Fiamengo, M., Acién-Fernández, F. G., Molina-Grima, E. (2018) Utilization of centrate for the outdoor production of marine microalgae at the pilot-scale in raceway photobioreactors. *Journal of Environmental Management, 228*, 506–516. https://doi.org/10.1016/j.jenvman.2018.08.020
60. Sabatte, F., Baring, R., Fallowfield, H. (2024) Suspended filamentous algal cultures for wastewater treatment: A review. *Journal of Applied Phycology, 36*(4), 1987–2004. https://doi.org/10.1007/s10811-024-03220-2
61. Salgado, E. M., Esteves, A. F., Gonçalves, A. L., Pires, J. C. M. (2023) Microalgal cultures for the remediation of wastewaters with different nitrogen to phosphorus ratios: Process modelling using artificial neural networks. *Environmental Research, 231*. https://doi.org/10.1016/j.envres.2023.116076
62. Sarma, U., Hoque, M. E., Thekkangil, A., Venkatarayappa, N., Rajagopal, S. (2024) Microalgae in removing heavy metals from wastewater – An advanced green technology for urban wastewater treatment. *Journal of Hazardous Materials Advances, 15*. https://doi.org/10.1016/j.hazadv.2024.100444
63. Sisman-Aydin, G. (2022) Comparative study on phycoremediation performance of three native microalgae for primary-treated municipal wastewater. *Environmental Technology and Innovation, 28*. https://doi.org/10.1016/j.eti.2022.102932
64. Stoykova, P., Stoyneva-Gärtner, M., Uzunov, B., Gärtner, G., Atanassov, I., Draganova, P., Borisova, C. (2019) Morphological characterization and phylogenetic analysis of aeroterrestrial Vischeria/Eustigmatos strains with industrial potential. *Biotechnology and Biotechnological Equipment, 33*(1), 231–242. https://doi.org/10.1080/13102818.2018.1561212
65. Stoyneva-Gärtner, M., Stoykova, P., Uzunov, B., Dincheva, I., Atanassov, I., Draganova, P., Borisova, C., Gärtner, G. (2019) Carotenoids in five aeroterrestrial strains from Vischeria/Eustigmatos group: updating the pigment patterns of Eustigmatophyceae. *Biotechnology & Biotechnological Equipment, 33*(1), 250-267 https://doi.org/10.1080/13102818.1562984
66. Stoyneva-Gärtner, M., Gärtner, G., Uzunov, B., Borisova, C., Draganova, P., Radkova, M., Stoykova, P., Atanassov, I. (2019) Current bioeconomical interest in stramenopilic Eustigmatophyceae: a review. *Biotechnology & Biotechnological Equipment, 33*(1), 302–314 (publ.online 7.2.2019, https://doi.org/10.1080/13102818.1573154
67. Stoyneva-Gärtner, M., Uzunov, B., Gärtner, G., Radkova, M., Atanassov, I., Atanasova, R., Borisova, C., Draganova, P., Stoykova, P. (2019) Review on the biotechnological and nanotechnological potential of the streptophyte genus Klebsormidium with pilot data on its phycoprospecting and polyphasic identification in Bulgaria. *Biotechnology and Biotechnological Equipment, 33*(1), 559–578. https://doi.org/10.1080/13102818.2019.1593887
68. Su, Y. (2021) Revisiting carbon, nitrogen, and phosphorus metabolisms in microalgae for wastewater treatment. *Science of the Total Environment, 762*. https://doi.org/10.1016/j.scitotenv.2020.144590
69. Tan, Y. H., Chai, M. K., Na, J. Y., Wong, L. S. (2023) Microalgal growth and nutrient removal

efficiency in non-sterilised primary domestic wastewater. *Sustainability, 15*(8). https://doi.org/10.3390/su15086601

70. Ting, H., Haifeng, L., Shanshan, M., Zhang, Y., Zhidan, L., Na, D. (2017) Progress in microalgae cultivation photobioreactors and applications in wastewater treatment: a review. *International Journal of Agricultural and Biological Engineering, 10*(1), 1–29. https://doi.org/10.3965/j.ijabe.20171001.2705
71. Ugwuanyi, E., Zamathula Queen Sikhakhane Nwokediegwu, Michael Ayorinde Dada, Michael Tega Majemite, Alexander Obaigbena. (2024) The role of algae-based wastewater treatment systems: A comprehensive review. *World Journal of Advanced Research and Reviews, 21*(2), 937–949. https://doi.org/10.30574/wjarr.2024.21.2.0521
72. United Nations Environment Programme (2015) *The Sustainable Development Goals (SDGs)*. https://www.unep.org/explore-topics/sustainable-development-goals
73. United States Environmental Protection Agency (2010) *National Pollutant Discharge Elimination System (NPDES) Permit Writers' Manual.* www.epa.gov/npdes - (Accessed on June 6th, 2025)
74. United States Environmental Protection Agency (2024) *Dissolved oxygen.* https://www.epa.gov/caddis/dissolved-oxygen#checklist (Accessed on June 6th, 2025)
75. Uzunov, B., Stoyneva, M., Mancheva, A., Gärtner, G. (2012) ACUS – the new collection of living aeroterrestrial algae of the University of Sofia "St Kliment Ohridski" – In: Petrova, A. (ed.), *Proc. VII Natl. Conf. Bot. 2012*, 29–30.09.2011, Bulg. Bot. Soc., Sofia, 271–274.
76. Valchev, D., Ribarova, I. (2022) A review on the reliability and the readiness level of microalgae-based nutrient recovery technologies for secondary treated effluent in municipal wastewater treatment plants. *Processes, 10*(2). https://doi.org/10.3390/pr10020399
77. Valchev, D., Ribarova, I., Uzunov, B., Stoyneva-Gärtner, M. (2021) Photo-sequencing batch reactor with Klebsormidium nitens: a promising microalgal biotechnology for sustainable phosphorus management in wastewater treatment plants. *Water Science and Technology, 83*(10), 2463–2476. https://doi.org/10.2166/wst.2021.149
78. Valchev, D., Ribarova, I., Uzunov, B., Stoyneva-Gärtner, M. (2019) *Comparison of two strains of microalgae used for wastewater post-treatment in a laboratory scale SBR*. Annual of the University of Architecture, Civil engineering and Geodesy, Sofia, 52(3), 819–832. https://www.researchgate.net/publication/338374509
79. Vaz, S. A., Badenes, S. M., Pinheiro, H. M., Martins, R. C. (2023) Recent reports on domestic wastewater treatment using microalgae cultivation: Towards a circular economy. *Environmental Technology and Innovation, 30*. https://doi.org/10.1016/j.eti.2023.103107
80. Wang, M., Yang, H., Ergas, S. J., van der Steen, P. (2015) A novel shortcut nitrogen removal process using an algal-bacterial consortium in a photo-sequencing batch reactor (PSBR). *Water Research, 87*, 38–48. https://doi.org/10.1016/j.watres.2015.09.016
81. Wang, Q., Wang, X., Hong, Y., Liu, X., Zhao, G., Zhang, H., Zhai, Q. (2022) Microalgae cultivation in domestic wastewater for wastewater treatment and high value-added production: Species selection and comparison. *Biochemical Engineering Journal, 185*. https://doi.org/10.1016/j.bej.2022.108493
82. Whitton, R., Ometto, F., Pidou, M., Jarvis, P., Villa, R., Jefferson, B. (2015) Microalgae for municipal wastewater nutrient remediation: mechanisms, reactors and outlook for tertiary treatment. *Environmental Technology Reviews, 4*(1), 133–148. https://doi.org/10.1080/21622515.2015.1105308
83. Wu, Q., Guo, L., Wang, Y., Zhao, Y., Jin, C., Gao, M., She, Z. (2021) Phosphorus uptake, distribution and transformation with *Chlorella vulgaris* under different trophic modes. *Chemosphere, 285*. https://doi.org/10.1016/j.chemosphere.2021.131366
84. Xu, Z., Wang, H., Cheng, P., Chang, T., Chen, P., Zhou, C., Ruan, R. (2020) Development of integrated culture systems and harvesting methods for improved algal biomass productivity and wastewater resource recovery – A review. *Science of the Total Environment, 746*. https://doi.org/10.1016/j.scitotenv.2020.141039
85. Ye, J., Liang, J., Wang, L., Markou, G., Jia, Q. (2018) Operation optimization of a photo-sequencing batch reactor for wastewater treatment: study on influencing factors and impact on symbiotic microbial ecology. *Bioresource Technology, 252*, 7–13. https://doi.org/10.1016/j.biortech.2017.12.086
86. Yu, H., Kim, J., Rhee, C., Shin, J., Shin, S. G., Lee, C. (2022) Effects of different pH control strategies on microalgae cultivation and nutrient removal from anaerobic digestion effluent. *Microorganisms, 10*(2). https://doi.org/10.3390/microorganisms10020357
87. Zahmatkesh, S., Karimian, M., Pourhanasa, R., Ghodrati, I., Hajiaghaei-Keshteli, M., Ismail, M. A. (2023) Wastewater treatment with algal based membrane bioreactor for the future: Removing emerging containments. *Chemosphere, 335*. https://doi.org/10.1016/j.chemosphere.2023.139134