Photo-sequencing batch reactor with *Klebsormidium nitens*: a promising microalgal biotechnology for sustainable phosphorus management in wastewater treatment plants

Dobril Valchev, Irina Riba, Blagoy Uzunov and Maya Stoyneva-Gärtner

**ABSTRACT**

This study aims at improving the existing algal-based wastewater treatment technologies by overcoming some of the major drawbacks of these systems such as large required land area, culture contamination, and energy-intensive algal harvesting. The experiments were carried out in an open photo-sequencing batch reactor at a laboratory-scale for nearly 2 months. A specific strain ACUS00207 of the aeroterrestrial green microalga *Klebsormidium nitens* (Kützing) Lokhorst was used. The strain is native to Bulgaria and belongs to a species that has never been used before in suspended growth systems for wastewater treatment for phosphorus removal. The culture of *K. nitens* showed promising results: phosphorus removal rates ranging from 0.4 to 1 mg total phosphorus L\(^{-1}\) d\(^{-1}\), efficient settling properties, and resistance to culture contamination with native microalgae. On the basis of the observed phosphorus removal mechanism of biologically mediated chemical precipitation/phosphorus precipitation, an innovative working mode of the sequencing batch reactor is suggested for reducing the hydraulic retention time and the required land area.

**Key words** | algae-based technology, circular economy, microalgae, phosphorus recovery, phosphorus removal

**HIGHLIGHTS**

- *Klebsormidium nitens* used for the first time in suspended growth wastewater treatment.
- Phosphorus removal rates ranging from 0.4 to 1 mg total phosphorus L\(^{-1}\) d\(^{-1}\).
- Efficient settling properties and resistance to culture contamination.
- Potential for reduction of the hydraulic retention time and the required land area.

**ABBREVIATIONS**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Meaning</th>
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<tr>
<td>COD</td>
<td>Chemical oxygen demand</td>
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<tr>
<td>DO</td>
<td>Dissolved oxygen</td>
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<tr>
<td>HRT</td>
<td>Hydraulic retention time</td>
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<td>LDO</td>
<td>Luminescent dissolved oxygen</td>
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<td>LM</td>
<td>Light microscopy</td>
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<tr>
<td>p.e.</td>
<td>People equivalent</td>
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<tr>
<td>PSBR</td>
<td>Photo-sequencing batch reactor sequencing batch reactor</td>
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<tr>
<td>PRR</td>
<td>Phosphorus removal rate</td>
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Phosphorus recovery is pertinent to the recent European policies for a circular economies as well as the green deal (COM 2019, 2020). These two documents stimulate the reduction of the yield of natural resources and encourage the search for methods, means, and partnerships that enable phosphorus recovery from waste. Biological technologies for wastewater treatment that allow direct recovery of the biogenic elements back in the natural cycle with minimum expenses of energy, materials, and capital are highly advocated.

Conventional widespread wastewater treatment technologies for phosphorus removal are based on two main linear methods developed to make the effluent wastewater quality of the WWTP compliant with the requirements of the Council Directive 91/271/EEC (1991): (1) chemical phosphorus removal with metal salts and (2) enhanced biological phosphorus removal using phosphorus accumulating organisms. These technologies either generate huge amounts of chemical sludge, mixed with the biological sludge (chemical methods), or the results from the treatment processes are not consistent enough (biological methods). This results in a very complicated and expensive subsequent phosphorus recovery and complex sludge management (Cuellar-Bermudez et al. 2017).

A promising opportunity for simultaneous phosphorus removal from wastewater and its transformation into a form for relatively easy recovery (closing of the cycle in line with the principles of the circular economy) is provided by the algal-based wastewater treatment technologies (Cuellar-Bermudez et al. 2017; Fernández et al. 2018). These eco-friendly cost-effective technologies, which result in non-toxic products, gain higher global popularity. Due to the current awareness, these technologies are included in one of the main documents of the European Commission for the future plans for wastewater treatment from 2020 – ‘A new Circular Economy Action Plan for a cleaner and more competitive Europe’ (COM 2020).

Algal-based wastewater treatment technologies are currently developed at a laboratory or pilot scale. The algal-based technologies might be grouped into three major clusters – reactors with suspended, immobilized, and attached biomass of algae (Gonçalves et al. 2016; Ting et al. 2017). The most extensively studied systems for wastewater application are the suspended growth microalgal systems and, more specifically, open suspended growth microalgal systems such as high rate algal ponds and raceway reactors (Gonçalves et al. 2016; Ting et al. 2017). These types of systems are preferred for municipal wastewater treatment, with phosphorus removal efficiencies varying between 50 and 99%, depending on the specifics of the system such as microalgal strains/consortia used, temperature, geographic region of the experiment, natural/synthetic sunlight source, photoperiod, etc. (Gonçalves et al. 2016; Ting et al. 2017). The use of the microalgal systems not only reduces the phosphorus in wastewater and transforms it into a form suitable for its recovery, but also improves the general quality of the effluent of the wastewater treatment plant (WWTP) by increasing the dissolved oxygen (DO) concentration and decreasing the total nitrogen (TN) concentration, pathogenic organisms, micro-pollutants, and heavy metals (Cuellar-Bermudez et al. 2017; Salama et al. 2019).

Algal-based technologies are prospective because of the abilities of algae to utilize sunlight to produce oxygen and to take-up nutrients from the water (Lavrinović & Juha, 2017; Bansal et al. 2018). However, major setbacks for these technologies are that (1) there is no strain or consortia of microalgae widely agreed as optimal for wastewater treatment (Gonçalves et al. 2016); (2) there are no well-set technological parameters of these systems (Christenson & Sims 2017; Shandilya & James 2018); (3) huge land area is needed for the reactors due to necessary extended hydraulic retention time (HRT) (Christenson & Sims 2017); (4) cultivated specific algal strain monocultures are very difficult to be kept from being contaminated with native wastewater algae (Christenson & Sims 2017; Gonçalves et al. 2016); and (5) algal biomass is troublesome with the harvesting processes after wastewater treatment (Lavrinović & Juha, 2017; Shandilya & James 2018).

The aim of this study is to overcome some of the drawbacks mentioned above by testing the application of the green microalga Klebsormidium nitens (Kützing) Lokhorst at a laboratory scale. This filamentous aeroterrrestrial alga belongs to the medium-sized genus Klebsormidium, which has previously received less attention but is now highlighted for its biotechnological potential (Stoyneva-Gärtner et al. 2014).
The species *K. nitens* in particular has never been used before in suspended growth systems for wastewater treatment for phosphorus removal despite the fact that different unidentified species of the genus *Klebsormidium* have been used in some experiments, with controversial results like attached growth system using Algae Turf Scrubber in natural conditions with horticultural wastewater (Liu et al. 2016; Stoyneva-Gärtner et al. 2019). Another innovative outcome of our study is the suggestion of a modification of the classical technological treatment scheme with a polishing step for phosphorus removal with microalgae.

**MATERIALS AND METHODS**

**Experiment set-up**

**Algal strain**

The algal strain used in the experiment belongs to the streptophyte algal species *K. nitens* (Kützing) Lokhorst. *K. nitens* is a green non-branched filamentous alga which grows in different types of habitats with a preference for the aeroterrestrial mode of life (Ettl & Gärtner 2014). The used strain was isolated from high-alpine soils of Rila National Park (Bulgaria) and was cultivated as ACUS00207 in the Collection of Living Algae ACUS of the Sofia University St. Kliment Ohridski on standard Bold-Basal medium (Uzunov et al. 2012; Stoyneva-Gärtner et al. 2019).

This specific strain was chosen because (1) the strain is local for Bulgaria and it is initially adapted to the environmental conditions of the latitude of the country of the experiment (phytocorpspecting) (Stoyneva-Gärtner et al. 2019); (2) the algal strain belongs to the filamentous multicellular species, which makes it more resistant to predation and monoculture contamination compared to the unicellular algae (Stoyneva-Gärtner et al. 2019); (3) the strain belongs to the Green evolutionary line of algae which is widely used for phosphorus removal from wastewater and has potential for relatively high phosphorus removal rates (Gonçalves et al. 2016); and (4) in the preliminary experiments, the strain showed fast adaptation to real wastewater and intensive growth in harsh conditions.

**Reactor**

The experiments were carried out in a laboratory scale open photo-sequencing batch reactor (PSBR) with suspended algal growth. This is one of the most preferred types of bioreactors for laboratory scale algal research in the literature due to its high illuminated surface to volume ratio, enhanced flocculation of the biomass, and simplified design that allows easy sample gathering (Ye et al. 2018).

The reactor is shown in Figure 1 and consists of: (1) a colorless, transparent cylinder glass body with D = 150 mm and H = 150 mm; (2) an electric stirrer (Heidolph, model RZR 2021) with a propeller size of B = 120 mm and H = 50 mm. The stirring frequency was preliminary set to 30–40 rpm, which corresponds to approximately 0.3 m s\(^{-1}\) tangential flow velocity. This velocity allows least amount of deposits from settlement of the suspended algae and also least amount of shear stress on the algal cells; (3) a HACH Lange SC1000 Multi-parameter Universal Controller Display, equipped with a Luminescent Dissolved Oxygen (LDO) Sensor and a 1,200-S pH Sensor that also measures the temperature of the water. The controller recorded all the values of the three parameters every hour.

The reactor was placed near a window and depended entirely on natural sun illumination. No artificial source of light was added to the laboratory set-up. This enables algae to grow in the closest to their natural habitat conditions and photoperiod. The transparency of the reactor provided a high illuminated surface to volume ratio which intensifies the process.

**Wastewater**

Wastewater from the WWTP of Sofia, located in Kubratovo village, Bulgaria, was used. The plant has capacity of
1,300,000 p.e. The technological water treatment steps of the WWTP include coarse screens, aerated grit chamber, primary clarifier, activated sludge reactor, and secondary clarifier. Phosphorus removal is performed by dosing of iron(III) chloride (FeCl₃) in the activated sludge reactor. The influent wastewater characteristics in the sampling date were: TP = 3.75 mgP L⁻¹; TN = 39.00 mgN L⁻¹; total organic carbon (TOC) = 94.50 mgC L⁻¹; chemical oxygen demand (COD) = 352.00 mgO₂ L⁻¹; pH = 7.5–8. The used wastewater for the experiment was taken after the secondary clarifier and stored at 4°C in a refrigerator. The same wastewater was used for all of the cycles of the current study, and its parameters’ concentrations were: TP = 0.26 mgP L⁻¹; TN = 9.78 mgN L⁻¹; TOC = 10.05 mgC L⁻¹; COD = 17.86 mgO₂ L⁻¹; pH = 7.5–8. To increase the starting TP concentration and to simulate a scheme without chemical phosphorus removal, monopotassium phosphate (KH₂PO₄) was added to the wastewater at the beginning of every cycle.

**Working mode of the PSBR**

Cultivated in a nutrient medium, the algal strain ACUS00207 was adapted to the new conditions in the laboratory set-up – wastewater from Kubratovo WWTP, temperature, working mode, etc. for a period of 11 days. After this adaptation period, four cycles were performed (Table 1).

Each cycle consisted of the following steps (Figure 2). (1) Filling – wastewater with a specific starting concentration of TP was added to the cultivated algal biomass from the strain ACUS 00207 of the species *K. nitens*. (2) Mixing and reaction – the added wastewater was mixed with the algal biomass into a homogenous suspension with a vertical stirrer. Immediately after the start of the stirrer, a starting sample was taken from the reactor. Samples were taken through certain amounts of time to establish the needed technological parameters of the system. (3) Settling – after step 2 was finished and the correlation was established, when the sunlight was intense enough and pH levels were about 8–9, the stirrer was stopped and the auto-flocculation processes of the algae were triggered. After 30 min of settling, the algal biomass was separated from the treated wastewater (decant) by the forces of gravity. (4) Decanting – the upper layer of treated water was decanted to a level at which no biomass was sucked out of the decanting pipe. (5) Next filling (beginning of the next cycle in Table 1) – after removing the decant, a new portion of wastewater was added to the reactor. (6) Mixing and reaction – after the addition of the new portion of wastewater, the stirrer was switched on again, a sample with the new starting concentration was taken, and the new cycle begun.

**Chemical analysis**

Every sample was filtered through a glass fiber filter with a non-organic binder with pore size 0.45 μm. The filtrate was then analyzed for the chemical parameters TP and TN, using HACH Lange cuvette tests and spectrophotometric method analysis.

**Light microscopy**

During the experiment, water samples were checked regularly on a light microscope (LM). The work was done on non-permanent slides using a Motic BA400 microscope and the microphotographs were taken on the same microscope using a Moticam 2 camera and the Image Plus Program. The microphotos provided in the paper are taken at objective ×40 to demonstrate the amount of the observed algae and their relative abundance.

**Data processing**

The collected data from the chemical analysis was processed using Microsoft Office Excel. The resulting concentrations from each chemical analysis for TP (measured once a day at approximately the same hour to ensure the reactor received the same light intensity) in relation to the respective day of the sampling were processed and presented in graphs, similar to those in Figures 3–6.

Each graph demonstrates the rate at which phosphorus is taken up by the algae. The estimation was achieved through a regression-based analyses in which each coefficient in front of the *x* variable indicates the phosphorus reduction rate in mgP L⁻¹ d⁻¹.
In addition, for the last cycle, a correlation between the pH levels in the reactor and the momentary phosphorus concentration was established. It was presented by comparing all the hourly pH value measurements of the SC1000 Controller and their corresponding TP concentrations with respect to the extended time of the cycle of the PSBR, again using Microsoft Office Excel (Figure 7).

**RESULTS AND DISCUSSION**

**Phosphorus removal rate**

Data processing for each cycle is shown in Figures 3–6.

The graphs show that there is a steady phosphorus reduction in each of the cycles with a very high coefficient.
Figure 4 | PRRs in Cycle 2.

Figure 5 | PRRs in Cycle 3.

Figure 6 | PRR in Cycle 4.
of determination ($R^2$) close to 1 with two exceptions – in Cycle 1 there is one $R^2$ value of 0.6152, and in Cycle 4 the $R^2$ value was 0.8757. Cycle 1 is the first cycle after the adaptation period. The lower $R^2$ value is due to the initial spike with phosphorus reagent (leading to a sharp increase in the phosphorus concentration) and the adaptation of the strain to these new conditions and working mode.

The absolute total phosphorus removal rate (PRR) varied between 0.4 mg L$^{-1}$ day$^{-1}$ and 1 mg L$^{-1}$ day$^{-1}$, calculated on the basis of the initial and final concentration of the TP and the total HRT of each cycle, respectively: 1.043 mg L$^{-1}$ day$^{-1}$ (Cycle 1); 0.376 mg L$^{-1}$ day$^{-1}$ (Cycle 2); 0.894 mg L$^{-1}$ day$^{-1}$ (Cycle 3); and 0.567 mg L$^{-1}$ day$^{-1}$ (Cycle 4).

The range of the PRR inside each cycle is higher (as shown in the graphs Figures 3–6) – from 0.203 to 2.9693 mgP L$^{-1}$ day$^{-1}$. This wide range is probably due to the phenomenon known as luxury uptake by the microalgae, which occurs when the microalgae uptakes more phosphorus than required for growth without a prior starvation stage. However, knowledge on this phenomenon, known for more than 50 years, is still quite limited (Solovchenko et al. 2019). Usually, higher initial concentrations of the phosphorus substrate in wastewater leads to its faster consumption and phosphorus storage in the algal cells (Solovchenko et al. 2019). Figures 3–6, with the ACUS00207 strain of K. nitens, show faster PRRs at the beginning of each cycle, a steady decrease in the reduction rates as the cycle progresses, and a total lowering in the concentration of phosphorus.

On the other hand, the very high initial TP concentration (21.7 mg L$^{-1}$) in Cycle 1 most probably induced luxury uptake in the algae cells and increased the phosphorus storage (resulting in a high removal rate in that cycle). This led to a decreased PRR in Cycle 2 due to the already available phosphates in the cells from the previous cycle. After the depletion of the phosphate stores in the cells, the algae increased their phosphorus uptake rate in Cycle 3 and stored more phosphorus in the cells.

Similar PRRs are reported by other authors using similar open suspended systems but different algal strains, as summarized in Table 2.

K. nitens shows average PRRs in the mid to high range of the spectrum compared to the results in the reported data (taking into consideration the initial TP concentration of the experiment). Since the experiment is performed in a laboratory environment, further research is needed to confirm the stability of the absolute PRRs in harsher conditions and that the strain K. nitens is appropriate for wastewater treatment with suspended algal cultures from the PRR perspective.

Since all the samples were taken at approximately the same time of day (relatively the same solar illumination and pH levels in the reactor for each sample taking), these results are considered representative of the mechanism of biological phosphorus removal, i.e. active transport of orthophosphates through the cell walls and membranes for phosphorylation and other vital processes of algae (Gonçalves et al. 2016). To reach a final TP concentration lower than 2 mg L$^{-1}$, the suggested technology with this specific algal strain requires HRT between 3 d and 15 d, depending on the initial phosphorus concentration in wastewater. Longer HRTs in the reactor with algae requires a
Table 2 | PRRs according to the literature data

<table>
<thead>
<tr>
<th>Algal strain/consortia</th>
<th>Wastewater used</th>
<th>Mode/volume</th>
<th>Starting TP</th>
<th>HRT</th>
<th>PRR</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Microcystis aeruginosa</em>, <em>Scenedesmus quadricauda</em>, <em>Chlorella vulgaris</em> and <em>Euglena viridis</em></td>
<td>Primary-treated municipal wastewater</td>
<td>Batch V = 60 L</td>
<td>4.6 mg·L⁻¹</td>
<td>5 d</td>
<td>0.300 mg·L⁻¹ d</td>
<td>Gonçalves et al. (2016); Tripathi &amp; Shukla (1993)</td>
</tr>
<tr>
<td>Microalgal consortium from a high rate algal pond treating domestic wastewater and activated sludge native bacteria</td>
<td>Domestic wastewater</td>
<td>Continuous V = 8,000 L</td>
<td>7.0 mg·L⁻¹</td>
<td>4 d</td>
<td>1.275 mg·L⁻¹ d</td>
<td>Gonçalves et al. (2016); Park &amp; Craggs (2011)</td>
</tr>
<tr>
<td>Municipal wastewater native microalgae and activated sludge native bacteria</td>
<td>Primary-treated municipal wastewater</td>
<td>Batch V = 14 L</td>
<td>8.8 mg·L⁻¹</td>
<td>14 d</td>
<td>0.588 mg·L⁻¹ d</td>
<td>Gonçalves et al. (2016); Su et al. (2012)</td>
</tr>
<tr>
<td>Municipal wastewater native microalgae and activated sludge native bacteria</td>
<td>Primary-treated municipal wastewater</td>
<td>Batch V = 14 L</td>
<td>8.8 mg·L⁻¹</td>
<td>8 d</td>
<td>0.704 mg·L⁻¹ d</td>
<td>Gonçalves et al. (2016); Delgadillo-Mirquez et al. (2016)</td>
</tr>
<tr>
<td>Mixed microalgae and bacteria culture</td>
<td>Primary-treated municipal wastewater</td>
<td>Batch, V = 200 mL</td>
<td>3.1–4.4 mg·L⁻¹</td>
<td>1.2 d</td>
<td>0.576 mg·L⁻¹ d</td>
<td>Delgadillo-Mirquez et al. (2016)</td>
</tr>
<tr>
<td><em>Chlorella vulgaris</em></td>
<td>Synthetic wastewater</td>
<td>Batch, V = 1,000 mL</td>
<td>From 7.7 to 199 mg·L⁻¹</td>
<td>–</td>
<td>Max. 2.000 mg·L⁻¹ d</td>
<td>Aslan &amp; Kapdan (2006)</td>
</tr>
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</table>
larger reactor size, thus larger land area requirements, and increased operation costs at a larger scale WWTP due to
the need for light penetration to all parts of the reactor
(high illuminated surface to volume ratio – Si/V) to achieve
the best photosynthetic effect (Huang et al. 2017).

Possible reduction of the HRT/needed land area

The biologically mediated chemical precipitation/phos-
phorus precipitation mechanism has already been well
discussed and documented (Larsdotter et al. 2010;
Gonçalves et al. 2016). It is induced when intensive photo-
synthesis takes place during the hours with the highest
amount of solar illumination on the reactor. This way the
remaining CO₂ in wastewater is almost depleted, which
leads to an increase in the pH levels (sometimes even
above pH 10). This highly alkaline water environment
and the calcium ions in the water lead to a spontaneous
formation of calcium–phosphate salts and algal cells. The
process promotes better algal harvesting and a higher rate
of phosphorus removal, which reduces the required HRT
of the reactor (Larsdotter et al. 2010; Gonçalves et al. 2016).

In the last cycle with the algal strain ACUS002027 of
K. nitens, the day–night pH level and TP concentration
dynamics were studied (Figure 7) to establish the effects
of the biologically mediated phosphorus precipitation
mechanism in the examined PSBR.

The results show (Figure 7) that as the day progresses
and the natural sun illumination intensifies, the pH levels increase
as the TP concentration decreases and vice versa. A minimum
in the TP concentration is reached when there is a peak in pH,
and a maximum in the TP concentration is reached when pH
is at its lowest. Each sequential TP concentration peak is lower
than the previous one due to the biological phosphorus
removal mechanism (active transport through the cell walls
and membranes) described in the previous sections of the
article. This leads to a steady assimilation of phosphorus by
the algae and continuous TP reduction from wastewater as
the time in each cycle progresses.

Another observed phenomenon is demonstrated in
Figure 7. The starting concentration at the beginning of
Cycle 4 is 3.02 mg L⁻¹ (point 1 on the graph). This concentra-
tion steadily decreases throughout the mornings of the
whole cycle until it reaches a final concentration for the
cycle of 1.27 mg L⁻¹ (point 3 on the graph) at the beginning
of day 4. But if we isolate the first day of the cycle, the
final concentration at peak illumination on that day is
0.98 mg L⁻¹ (point 2 on the graph), which is lower than
the concentration at the end of the whole cycle after 3 d of
HRT. This means that if a stable operation mode is estab-
lished, there is no need for extension of the HRT from 1 d
to 3 d.

These results evoked the idea of lowering the HRT of
the PSBR using the K. nitens strain to 1 d, and applying the
mechanism of biologically mediated phosphorus precipi-
tation. Each cycle (for a technology that uses this
microalgal strain) in the summer months, would consist of
the following steps (similar to Figure 2):

- Filling – at dusk when the sun starts to settle
- Mixing and reaction – approximately 1 d
- Settling – starts after 1 d of reaction when a peak in pH is
  reached and flocs are formed in the PSBR. The settling is
  for 30 min to an hour with this specific algae strain
- Decanting
- Secondary filling

Forthcoming experiments will verify the applicability of
this specific working mode of the reactor. Since Sofia is
supplied mainly by surface water with low Ca²⁺ and Mg²⁺
concentrations, wastewater influent also has low Ca²⁺ and
Mg²⁺ content. On the sampling day, the concentrations
were CCa²⁺ = 29.70 mg L⁻¹ and CMg²⁺ = 9.20 mg L⁻¹ which
results in total hardness of the water of 2.24 meq L⁻¹. This
soft wastewater actually gives the most unfavorable conditions
of this working mode due to the lower availability of cations
for the formation of calcium and magnesium salts that lead
to the alkaline flocculation of algae. Nevertheless, future
experiments would also include more comprehensive and
regular measurements of the Ca²⁺ and Mg²⁺ concentrations
in the used wastewater to establish a better understanding
of the process.

Settling properties

Algal harvesting after the wastewater treatment process is
one of the main challenges standing in the way of the applica-
tion of the algal-based wastewater treatment technologies
on a larger scale (Christenson & Sims 2011; Hwang et al.
2016). They usually include membrane coagulation/flocula-
tion with metal salts and flocculants, membrane filtration,
dissolved air flotation, centrifugation, electrophoresis, ultrasound,
and autoflocculation. These are either too expensive
or too unreliable for practical utilization (Hwang et al. 2016;
Branyikova et al. 2018). However, it is important to remem-
ber that filamentous algae, like the applied by us K. nitens,
have advantages in wastewater treatment over unicellular
microalgae, including the ease in harvesting and resistance
to predation (for details, see Stoyneva-Gärtner et al. 2019).
In the laboratory scale PSBR with the applied strain of *K. nitens* a noticeable floc formation (floc size – 2–3 mm in diameter) was observed at pH 8.5–9.5. This process significantly helped with the algal harvesting at the end of each cycle, since the only approach needed for this harvesting was an interruption of the mixing process and enough time for settlement of the formed flocs when the water was at rest. Figure 8(b) shows that the algal biomass is completely separated from the treated wastewater. Even after two months of reactor operation, no compromise in the supernatant quality was visible. This is possible probably due to the alkaline-induced autoflocculation processes of the algal biomass (increase in the settling and floc formation properties of the algal culture due to the reduction in CO₂ in the water medium and an increase in pH) in the reactor. This occurred at relatively low pH with no need for the addition of other reagents (coagulants, floculants, alkalizing agents, etc.), when the used ACUS00207 strain was applied.

These observations allow us to suppose that the strain ACUS00207 from *K. nitens* could be easily harvested with free settlement at pH 8–9 after the processes of wastewater treatment. This could lead to higher effluent and settled biomass quality and lower operation costs of the future application of the reactor in a real WWTP. Further research can show the exact total suspended solids (TSS) supernatant concentration, amount of light needed, environmental and reactor conditions, etc. to confirm the possibility of the practical application of this harvesting method using *K. nitens*, and its ACUS00207 strain in particular.

**Algal monoculture integrity**

One of the major drawbacks of the microalgal-based wastewater treatment technologies is the difficult preservation of the algal culture integrity. These systems are vulnerable to contamination with specific native algal strains or other algal consortia growing in each unique wastewater environment, which often leads to a displacement of the designed culture and a total shift in the technological and operational parameters of the reactor. This shift could affect directly the PRR, settling properties of the algae, biomass, and effluent quality, etc. Such displacement of the designed culture is not only highly undesirable, but also very difficult to prevent (Christenson & Sims 2011; Young et al. 2017).

The experiments, carried out for nearly two months with the algal strain ACU00207, showed that this specific monoculture manages to keep almost full integrity in the laboratory conditions with real wastewater. The strain clearly remained dominant in the reactor even after five wastewater changes (at the beginning of each cycle, Figure 9).
Only some diatoms were noticed in a few of the samples (mainly from the bottom of the reactor, Figure 10).

Despite the presence of some diatoms from the real wastewater, the overall parameters of the system remained unchanged – 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 PRR remained in the range of 0.4–1 mg L\(^{-1}\) d\(^{-1}\). This maintenance of a stable system and preserved dominance of the used strain of *K. nitens* also indicate the big potential of this specific monoculture for wastewater treatment. A full-scale reactor in an open or semi-closed site would be more vulnerable to single strain contamination with native algae from wastewater itself, and further studies will prove if these promising reactor and algal culture properties from this initial laboratory scale experiment can be maintained at a larger scale in a pilot reactor or in a full-scale WWTP.

### FUTURE PROSPECTS

The results obtained during this study allowed us to propose a modified and simplified technological scheme, fully based on biological processes (Figure 11). The organic substances, the bulk of the TN, some phosphorus, and the majority of the TSS are removed in the conventional biological treatment process (activated sludge reactor with a secondary clarifier). This effluent is still high in TP, carbon, and some amount of TN, and therefore it is a favorable medium for algal growth. The suggested sequencing batch reactor (SBR) with microalgae is the final polishing step, which lowers the TP and TN and increases the DO concentration in a way that significantly improves the final effluent quality. In addition, the SBR technology requires no further equipment construction (e.g. another clarifier) as all the processes take place in the same installation.

The laboratory experiments have shown that wise operation mode could reduce the HRT, and thus lead to a reduction of the required land area at the location of the bioreactors. This possible optimization of the process is mainly based on the use of the biologically mediated
chemical precipitation/phosphorus precipitation mechanism for phosphorus removal with microalgae. This mechanism allows a much higher phosphorus reduction rate than the full biological removal (active transport, biosorption, and bioaccumulation) because it depends on instant (spontaneous) alkaline chemical reactions. A possible combination of the two mechanisms (biologically mediated chemical precipitation and biological assimilation) could result in an HRT reduction from 3–15 d (as in Figures 3–6) to only 1 d (as in Figure 7). In real conditions, this HRT reduction corresponds directly to a required land area reduction as the respective wastewater storage volumes decrease, thus the number of reactors or equalizing tanks lowers. In addition to the significant reduction of the required HRT, such operation mode would contribute to better settling properties of the biomass due to the alkaline induced autoflocculation at a relatively low pH level (pH 8.5–9.5). Such a mode in the studied laboratory conditions should include the operation steps described earlier because the moment of mixing interruption should be chosen based on the minimum pH value at which the autoflocculation takes place in the specific particularity.

Since the results in this study were achieved under highly controlled laboratory conditions, further research is needed to verify the prospects and challenges of this specific working mode of the suggested PSBR in the pilot and full-scale implementations. Such a system is easy to maintain at a laboratory level, but a real SBR operating in an open environment involves a complicated operation (sensors, piping, multiple reactors and their specific combination, monoculture integrity management in the long term, etc.) and requires highly technically qualified staff to maintain the processes. This could lead to difficult practical implementation of the system, which should be considered in the forthcoming investigations of the technology and its operation modes.

Further research is also needed to explore the most suitable way of using the harvested biomass, which is rich in phosphorus and nitrogen and is appropriate for agricultural application (for details see Stoyneva-Gärtner et al. 2019). Such use will close the phosphorus cycle: fertilizers–plants–(animals)–humans–fertilizers.

CONCLUSIONS

A PSBR technology with suspended biomass of the algal strain ACUS00207 of K. nitens for removal of phosphorus from secondary treated wastewater was studied under laboratory conditions for nearly two months. The results indicate that the suggested technology has potential to overcome some of the drawbacks of the algal-based treatment technologies, namely the large area requirement, the energy-intensive separation of the biomass, and the poor monoculture integrity caused by the secondary contamination of the biomass with native algae.

The studied algal strain has not been used for wastewater treatment with suspended growth algal systems. Under laboratory conditions, it showed three positive characteristics:

- good PRR (0.4–1 mg TP L⁻¹ d⁻¹);
- effective settling properties; and
- high resistance to other unwanted microalgal strains.

These promising characteristics were observed in the specific laboratory, environmental, and wastewater conditions in the short period of two months in which the operation of the PSBR took place. The study is an encouraging first step to future research on the long-term applicability of the algal strain ACUS00207 of K. nitens for phosphorus removal in the wastewater treatment processes.

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DECLARATION OF COMPETING INTEREST

The authors declare that there are no conflicts of interest.
DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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