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THE DOMESTIC WASTEWATER TREATMENT CAPACITY OF *SPIRULINA PLATENSIS* SP4 AND THE APPLICATION OF THE TREATED WASTEWATER IN STIMULATING RICE GERMINATION

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SUMMARY

Many scientists are interested in microalgae and cyanobacteria because of their richness in high-value bioactive metabolites (proteins, lipids, vitamins, plant growth regulators, and others). Microalgae and cyanobacteria can be used to produce valuable commodities such as dietary supplements, biofuel, animal feed, and agricultural fertilizer, etc. To improve the economic feasibility of the cyanobacterial product recovery process, it is possible to utilize domestic wastewater as a source of nutrients for their growth. This research focuses on assessing the ability of domestic wastewater treatment and treated domestic wastewater utilization to stimulate BC15 rice-grain sprouting by cyanobacteria *Spirulina platensis* SP4. Research results showed that *S. platensis* SP4 grown best in domestic wastewater at the initial density of 0.3 and the wastewater concentration of 100%. The incubation of *S. platensis* was performed in the laboratory under the condition of 16.8 g/L $NaHCO_3$; aeration during 8 h/day; light: dark cycle of 8:16; light intensity of 5000 lux. Under these conditions *S. platensis* SP4 could treat N-NH_4^+, T-N, P-PO_4^{3-}, T-P and COD well with the treatment efficiency of 96.37, 49.71, 67.05, 65.88 and 95.53%, respectively. The addition of treated domestic wastewater by *S. platensis* SP4 to BC15 rice grains stimulated the grouting by 99.54 ± 1.25%, which was 1.54 times higher than using the tap water and gave a germination efficiency equivalent to that of the experiments with adding cyanobacteria in standard medium with or without L-tryptophan addition for 48 h. The obtained research results have elucidated the feasibility of employing cyanobacteria in residential wastewater treatment as well as the reuse of treated wastewater for plant development.

Keywords: *Spirulina platensis*, biomass, microalgae, cyanobacteria, domestic wastewater.

INTRODUCTION

The global population is constantly increasing, and by 2050, around 66 percent of the world's population is expected to reside in cities. This will lead to an increase in demand for finite freshwater as well as the generation of a large amount of domestic wastewater, polluting the water environment (Elyssia *et al.*, 2018). Domestic wastewater arising from daily human activities is water that has been used and discharged from households, hospitals, hotels, schools, and amusement parks, containing waste in the daily life and sanitation of people (Ming-sheng *et al.*, 2016). In Vietnam, only 17% of urban wastewater and 4% of wastewater (out of total 21% of domestic wastewater) aresafely treated through centralized wastewater treatment plants, while the remainder (79%) is directly discharged into water bodies such as rivers, lakes, and canals (Tran *et al.*, 2020). The amount of untreated domestic wastewater discharged into the environment is rich in carbon, nitrogen, and phosphorus, which are factors that contribute to pollution as well as eutrophication in the water environment, negatively affecting living communities downstream (Conley *et al.*, 2009). As a result, sustainable wastewater treatment technologies are required to reduce the amount of nutrients and contaminants discharged into receiving sources.

On the other hand, chemical fertilizers are now commonly employed in agricultural production since they are inexpensive, do not require much effort, and can supply nutrients promptly. However, excessive use of chemical fertilizers leads to greenhouse gas emissions and can result in *in-situ* soil deterioration, nutrient contamination, and eutrophication (Wuang *et al.*, 2016). Several studies have been conducted in order to discover bio-based compounds that can replace chemicals and improve plant growth, yield and quality of crops while having no negative environmental effects. Bio-fertilizers and bio-stimulants are alternatives to chemical fertilizers. When administered at low concentrations to soil, seed, or crop, they can alter plant physiology in a variety of ways, such as stimulating plant growth, increasing nutrient uptake, resistance to abiotic stressors, and prolonging the shelf life of harvested goods. They are eco-friendly products that contain living microorganisms such as bacteria, actinomycetes, fungus, and algae in single or combination forms (Refaay *et al.*, 2021).

Microalgae and cyanobacteria have been employed in wastewater treatment in recent years. Microalgae and cyanobacteria require various nutrients to develop, and they are capable of successfully removing nutrients (mostly carbon, nitrogen, and phosphorus) from wastewater (Gonçalves *et al*., 2017; Lam *et al.*, 2017). Researches have mainly focused on the extraction of valuable products from their biomass after the wastewater treatment process (Zapata *et al.*, 2021). The biomass of microalgae and cyanobacteria is high in proteins, lipids, vitamins, and biological growth stimulants. In addition to its use in wastewater treatment, the biomass of microalgae and cyanobacteria after wastewater treatment may be used as a bio-fertilizer to boost crop output (Refaay *et al.*, 2021). *Spirulina platensis* is a nutrient-rich filamentous cyanobacteria, which is capable of treating domestic wastewater (Zhai *et al.*, 2017). *Spirulina* has also been proposed as a suitable alternative to artificial fertilizers and as a protein supplement in animal feed (Wuang *et al.*, 2016). Several studies have reported that *S. platensis* has the ability to produce intracellular and extracellular bio-stimulants when it is grown in dairy wastewater, brewery wastewater, piggery wastewater (Navarro-López *et al.*, 2020; Zapata *et al.*, 2021). In Vietnam, a number of studies have also demonstrated the possibility of using *S. platensis* in the treatment of cattle wastewater after biogas, catfish pond wastewater, and domestic wastewater to minimize environmental pollution, enhance water quality, and other activities (Oanh *et al.*, 2012; Viet *et al.*, 2017). *S. platensis* biomass has also been shown to stimulate plant growth, such as arousing the formation of multiple shoots and increasing the survival rate of *Paphiopedilum delenatii in vitro* and providing a high rate of shoot regeneration in cells of Hoang Thao Nghe Tam orchid (*Dendrobium loddigesii* Rolfe) and ginseng (*Curculigo orchioides* Gaertn) (Cuc *et al.*, 2014; Lai *et al.*, 2018 a; Lai *et al.*, 2018b). Previous studies have mainly focused on the utilization of cyanobacterial biomass as a biofertilizer, with little research on the reuse of treated wastewater to enhance plant growth. Therefore, the potential for domestic wastewater treatment of S. platensis SP4 as well as the prospective for boosting the germination of BC15 rice using the treated wastewater were evaluated in this study.

MATERIALS AND METHODS

Materials

S. platensis SP4 was obtained from the collection of Institute of Environmental Technology, Vietnam Academy of Science and Technology.

Domestic wastewater: Wastewater used in the experiment was collected at the sewer located on Nguyen Dinh Hoan street, Cau Giay district, Hanoi. The sampling position has coordinates of 20º 22' 27 N and 105º 48' 15 E. In this region, the domestic wastewater was not treated centrally and discharged directly into the To Lich river. Wastewater samples were taken after 7:30 a.m. since the effluent flow was high and the wastewater included distinctive components at that time.

Methods

Set up experiments

Experiment 1: Assessment of the effect of initial density on the growth of Spirulina platensis SP4

To determine the optimal density for *S. platensis* SP4 growth, this cyanobacterial strain was cultured in flasks of 250 mL with Zarrouk medium. Cyanobacteria initial densities in CT1, CT2, CT3, CT4, and CT5 formulas were 0.1, 0.15, 0.2, 0.3, and 0.4, respectively. The OD values were measured at 660 nm using UV-Vis 2450. Experiments were implemented with the standard Zarrouk medium 16.8 g/L $NaHCO_3$ (Zarrouk *et al.*, 1966), the temperature of 25ºC, lighting intensity of 5000 lux (the illumination intensity was measured with TENMARS TM-204), light: dark cycle of 8:16, and aerated for 8 h/day by an aquarium pump for 8 days. All the experiments were conducted in triplicate (n=3 for each treatment).

Experiment 2: Assessment of the domestic wastewater treatment efficiency of Spirulina platensis SP4 at the scale of 10 L

Domestic wastewater samples before being used for microalgae incubation were pretreated by settling for 30 minand pre-filtered by a vacuum filter using GF/A filter paper to remove suspended solids. The experiments were set up in the initial OD_{660nm} condition of 0.3 (the OD values were measured at 660 nm using UV-Vis 2450); 16.8 g/L $NaHCO_3$; domestic wastewater concentration of 100%; light: dark cycle of 8:16; light intensity of 5000 lux, and aerated for 8 h/day by an aquarium pump for 8 days. The control experiment was performed similarly but without the addition of cyanobacteria. All the experiments were conducted in triplicate (n=3 for each treatment).

Experiment 3: Evaluation of the ability to stimulate the germination of BC15 rice seeds

Some studies also reported that when supplementing with 0.5 L-tryptophan, some cyanobacteria strains were able to produce extracellular auxin (Duong *et al.*, 2021). To compare the germination efficiency of rice seeds in cyanobacterial cultures, the presence and absence of L-tryptophan in the culture medium were also investigated. The studies on germination rate of BC15 rice were conducted as follows: Each experiment used 100 rice seed in 50 mL solution without cyanobacterial biomass; the formulas of ĐC1 (tap water), ĐC2 (solution obtained after incubating *S. platensis* SP4 in Zarrouk medium containing 16.8 g/L $NaHCO_3$), TN1 (solution obtained after culturing *S. platensis* SP4 in standard Zarrouk medium containing 16.8 g/L $NaHCO_3$ + 0.5 g/L L-tryptophan), TN2 (treated domestic wastewater). BC15 rice-grains were soaked in the corresponding solution overnight before growing on paper. All the experiments were conducted in triplicate (n=3 for each treatment). The germination rate is the average number of seeds sprouted over a 2-day period.

Sampling domestic wastewater

The total volume of wastewater collected at each sampling was around 30–50 L, which was kept in 10 L PE containers. The wastewater samples were refrigerated and transported immediately to the laboratory.

Analyzing domestic wastewater sample

The parameters of pH, DO, salinity, temperature were measured with a rapid water meter U5000; The analyzed parameters include nitrogen from ammonium (N-NH_4^+), nitrogen from nitrite (N-NO_2^-), nitrogen from nitrate NO_3^-, total nitrogen (T-N), total phosphorus (T-P), phosphorus in the form of phosphate (P-PO_4^{3-}), chemical oxygen demand (COD) and total suspended solids (TSS). NH_4^+ analytical method was in accordance with 4500 NH_3-F, SMEWW, 1995; NO_2^- analytical method was in accordance with the improved Griss Satlman method; NO_3^- analytical method was photometric method using sulfosalicylic acid reagent according to TCVN 6180-1996; T-N was analyzed by Kjeldahl method; T-P, P-PO_4^{3-} were analyzed by spectrophotometric method using ammonium molybdate according to TCVN 6202:2008; COD was analyzed by $K_2Cr_2O_7$ according to TCVN 6491:1999; TSS analytical method was according to TCVN 6625:2000.

Treatment efficiency is calculated by the following formula:

$$H = \frac{C_0 - C}{C_0} . 100$$

In which, H: Treatment efficiency (%); C_o: Concentrations of parameters in input wastewater (mg/L); C: Concentrations of parameters in treated wastewater (mg/L).

Growth assessment of Spirulina platensis SP4

Determine the concentration of biomass in the medium, based on the optical absorbance of chlorophyll pigments in the cell. These pigments absorb mainly 2 wavelengths which are 445 nm and 660 nm, respectively. In this research, our team measured the OD at 660 nm using a UV-Vis 2450.

Data processing and analysis

All experiments were performed in triplicate and data were presented as mean ± standard deviation calculated on Excel, figures were shown on Origin 8.0.

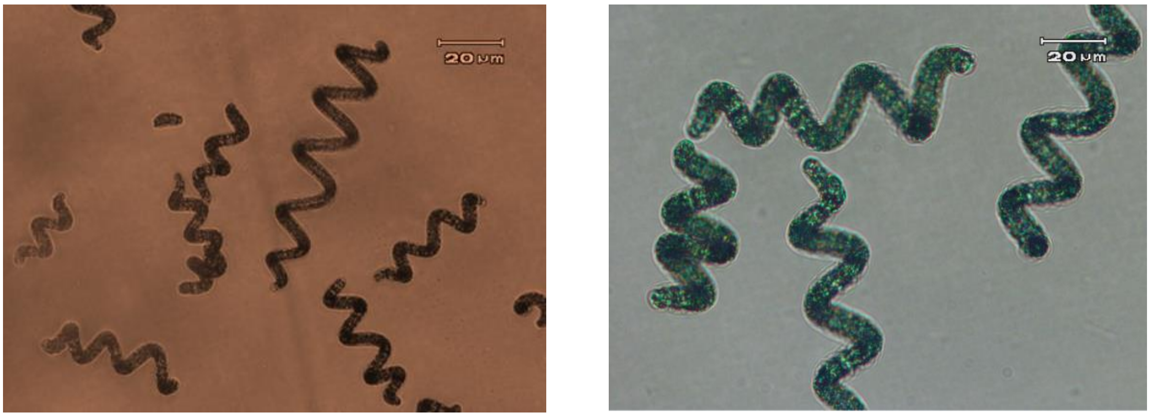
RESULTS AND DISCUSSION

Assessment of the growth ability of *Spirulina platensis* SP4

S. platensis SP4 is a cyanobacteria, helical, unbranched, non-heteromorphic, non-enveloped, cyanobacterial strain (Figure 1). Cyanobacterial filaments have 5–7 equal helices (diameter of about 35–50 µm, length of about 60 µm) that divide into cells with septa and can rotate around their axis. Depending on the vegetative and developmental cycle, their shapes can be twisted into the shapes of C, S, etc. These forms have different lengths, even in one form, the length of each strand is different. It is characterized by cylindrical, multicellular trichomes in an open helix.

For *S. platensis*, the initial seeding quantity has a significant effect on the growth process. Therefore, in this research, the rate of initial seed was investigated with an OD_{660nm} value of 0.10; 0.15; 0.20; 0.3; 0.4. Samples were periodically taken to determine the biomass. The results of the research are shown in Figure 2.

Experimental data showed that the initial cyanobacteria quantity has a significant effect on the growth rate of *S. platensis* SP4. In treatments of CT1, CT2 and CT3 with initial densities of OD_{660nm} = 0.1; 0.15 and 0.2, the growth rate of *S. platensis* was very slow. After 8 days of incubation, the OD_{660nm} value was only 0.441 ± 0.026; 0.572 ± 0.008 and 0.822 ± 0.048, respectively. This can be explained when the initial density is low and the biomass achieved is not high, leading to low biomass density. Research results of Vonshak *et al.* (1982) confirmed that low cultured algae density will inhibit photosynthesis because the light intensity reaching each algal cell is too high. In treatments of CT4 and CT5, when increasing the initial seeding density to OD_{660nm} = 0.3 and 0.4, the growth rate of *S. platensis* SP4 increased rapidly. After 8 days of incubation, the OD_{660nm} values reached 1.317 ± 0.025 and 1,214 ± 0.064, which were 2-3 times higher than those of other treatments. Cyanobacteria grew best at CT4, with an initial OD_{660nm} value of 0.3. The results of the above research showed that the initial density with OD_{660nm} = 0.3 is considered suitable for further studies.



a) b)

Figure 1. Morphology of cyanobacteria *S. platensis* under the microscope at a) 20X and b) 40X magnification. 20 µm scale.

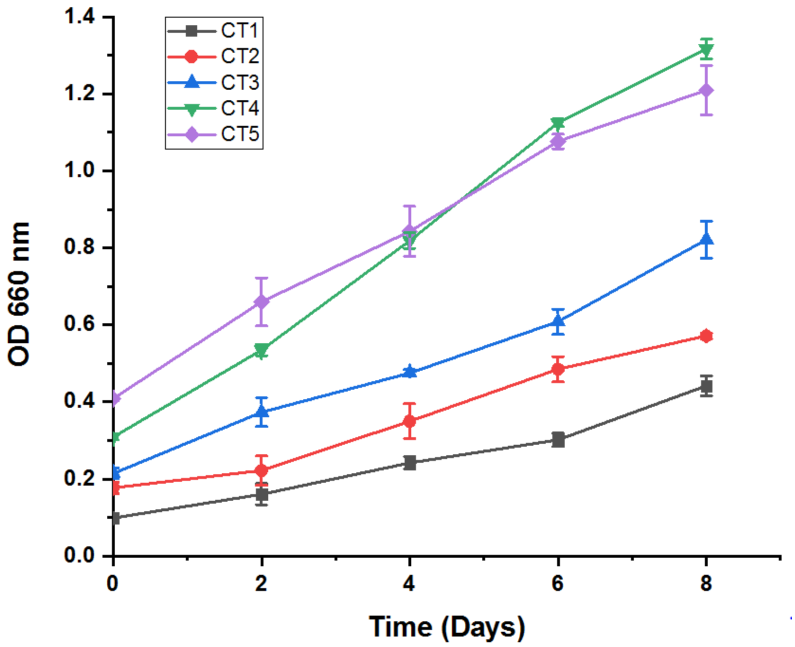


Figure 2. Growth of *S. platensis* SP4 at different input densities.

Components of domestic wastewater

Before implementing the experiment, domestic wastewater samples were analyzed for basic pollution parameters in order to assess their suitability for being put into the

Spirunila incubation model, and at the same time providing corrective measures if necessary. By sensory observation, the wastewater had a foul odor, contained many suspended solids and turbidity. Suspended solids will affect the ability of light diffusion into the wastewater, reducing the photosynthetic efficiency of cyanobacteria, therefore, domestic wastewater was settled for about 30 min and filtered to reduce suspended solids and turbidity before being put into the treatment tank.

Table 1. Concentrations of pollutants in domestic wastewater before the incubation of *S. platensis* SP4.

No.	Parameter	Unit	Value
1	pH	-	7.39 ± 0.25
2	Temperature	^oC	25.67 ± 0.62
3	DO	mg/L	5.43 ± 0.31
4	TSS	mg/L	0.063 ± 0.015
5	Salinity	mg/L	0.57 ± 0.02
6	$N\text{-}NH_4^+$	mg/L	28.08 ± 1.01
7	$N\text{-}NO_3^-$	mg/L	0.039 ± 0.012
8	$N\text{-}NO_2^-$	mg/L	0.01 ± 0.003
9	T-N	mg/L	30.53 ± 1.29
10	T-P	mg/L	7.18 ± 0.14
11	$P\text{-}PO_4^{3-}$	mg/L	5.21 ± 0.08
12	COD	mg/L	205.3 ± 2.20

The obtained analytical data shown in Table 1 indicated that the domestic wastewater filtered before being put into the treatment system had a neutral environment. COD ranged from 205.30 ± 2.20 mg/L. Domestic wastewater was rich in nitrogenous pollutants, in which $N\text{-}NH_4^+$ predominated with a concentration of 28.08 ± 1.01 mg/L. $N\text{-}NO_3^-$ and $N\text{-}NO_2^-$ were also detected at relatively small concentrations (less than 0.5 mg/L). $P\text{-}PO_4^{3-}$, a form of phosphorus compound was mainly found in domestic wastewater with concentration of 5.21 ± 0.08 mg/L. T-N and T-P recorded in this research were 30.53 ± 1.29 mg/L and 7.18 ± 0.14 mg/L, respectively. TSS measured after the filtration step was 0.063 ± 0.015 mg/L, which can be neglected in the measurement of biomass concentration during the cyanobacterial incubation. The analytical results showed that domestic wastewater contained elements necessary for the growth of cyanobacteria such as carbon, nitrogen, phosphorus compounds. However, the salinity and pH in domestic wastewater were relatively low compared with the incubation conditions of *S. platensis*. Therefore, it was necessary to add $NaHCO_3$ to increase pH as well as add carbon salts for the growth of cyanobacteria.

Assessment of ability of of *Spirulina platensis* SP4 in domestic wastewater treatment at the scale of 10L

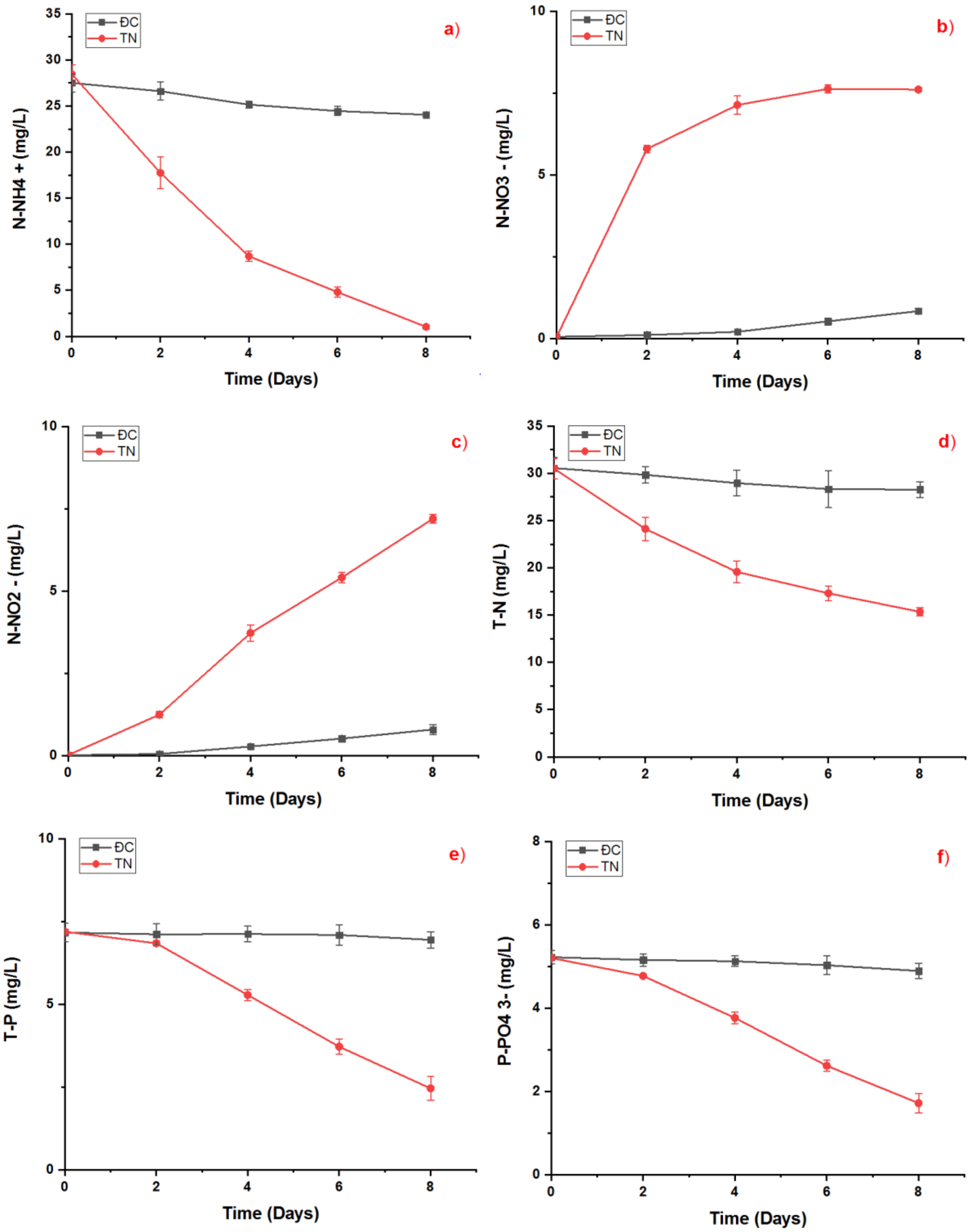
On the first day, after adding cyanobacteria and 16.8 g/L $NaHCO_3$ into domestic wastewater, the pH of the solution was about 8.0. After 8 days of experiment, the solution pH increased to 8.5, which is still within the allowable limit of the standard of column B of National Technical Regulation QCVN 14:2008/BTNMT.

The change in concentrations of N-NH_4^+, N-NO_3^-, N-NO_2^-, T-N, T-P, P-PO_4^{3-} and COD in the treatment system for 8 days of the batch cultivation was shown in Figure 3. In the ĐC formula (domestic wastewater without cyanobacteria), after 8 days of experiment, the concentrations of these substances decreased but not much, only about 12.54% for N-NH_4^+, 7.48% for T-N, 10.26 % for T-P, 6.32% for P-PO_4^{3-} and 25.73% for COD. Concentrations of N-NO_3^- and N-NO_2^- slightly increased compared to their initial values. This showed that, without adding microorganisms or cyanobacteria, self-treatment in wastewater still took place, but the treatment efficiency was low, and it was necessary to add microorganisms or microalgae/ cyanobacteria to increase treatment capacity.

For the TN formula with the addition of cyanobacteria in the wastewater, the changing trends of nitrogen species of N-NH_4^+, N-NO_3^-, N-NO_2^- and T-N are shown in Figure 3a–d. The concentration of N-NH_4^+ gradually decreased from 28.46 ± 1.01 to 1.03 ± 0.21 mg/L, the treatment efficiency reached 96.37%. The ability to remove N-NH_4^+ in domestic wastewater of *S. platensis* SP4 was twice as high as that of 10 mg/L reported for *Chlorella sorokiniana* UTEX 2805 (De-Bashan *et al*., 2008). These data indicate that *S. platensis* used in this research could survive in domestic wastewater with high concentration of N-NH_4^+. The removal of N-NH_4^+ from domestic wastewater by microalgae was due to two processes: (1) the direct use of N-NH_4^+ by cyanobacteria and (2) the separation of NH_3 (Mennaa *et al.*, 2015). The research of Tran *et al.* (2020) reported that the separation of NH_3 occurred only under conditions of alkaline, high temperature and abundant presence of urea in wastewater. In this research, the temperature values obtained did not exceed 27 °C and urea was not present in the wastewater, so the separation of NH_3 from the wastewater might be negligible and the loss of N-NH_4^+ could mainly be attributed to microalgae uptake (research data are shown in Figure 3a). Furthermore, the decrease in N-NH_4^+ concentration was attributed to biological nitrification that produced N-NO_3^- and N-NO_2^- and caused their concentrations to increase (Figure 3b, c). The N-NH_4^+ removal efficiency reached 96.37%, while the total nitrogen removal efficiency was determined to be 49.71% (Figure 3f), indicating that N-NH_4^+ was mainly consumed by *S. platensis* in this research. In fact, cyanobacteria can assimilate all nitrogen species and generate biomass at the same time (Wuang *et al*., 2016). Therefore, in this research, the assessment of the ability of *S. platensis* in removal of N-NO_3^- and N-NO_2^- still needs to be studied further.

After 8 incubating days, the concentrations of P-PO_4^{3-} and T-P in domestic wastewater decreased from 5.20 ± 0.05 mg/L to 1.71 ± 0.24 mg/L and from 7.19 ± 0.04 mg/L to 2.45 ± 0.36 mg/L, the average treatment efficiency was about 67.05% and 65.88% (Figure 3e, f). The ability to treat T-P of *S. platensis* SP4 in this research was lower than that for T-P of about 97.1–99.9% of *C. variabilis* TH03 grown in municipal wastewater (Tran *et al.*, 2020).

COD was significantly reduced from 206.40 ± 2.11 to 9.22 ± 0.27 mg/L after 8 incubating days (Figure 3g), resulting in a COD removal efficiency of 95.53%. The results imply that *S. platensis* SP4 used in this research could well consume various organic compounds as carbon sources besides $NaHCO_3$. This is similar to results reported by other researches that *S. platensis* can grow in hybrid manner (e.g. they can simultaneously use light and organic compounds as an energy source, assimilate CO_2 and glucose or simple acids as a carbon source (Zhai *et al.*, 2017). In general, the treated domestic wastewater meets the standard of column B of National Technical Regulation QCVN 14:2008/BTNMT. Therefore, after the biological treatment process, the treated wastewater was utilized to stimulate BC15 rice seed germination. Khan (2018) also proposed that wastewater be treated before being reused in agriculture to avoid the possibility of adverse impacts on human and animal health.


a)
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ĐC
TN
N-NH4 + (mg/L)
N-NO3 - (mg/L)
N-NO2 - (mg/L)
T-N (mg/L)
T-P (mg/L)
P-PO4 3- (mg/L)
Time (Days)

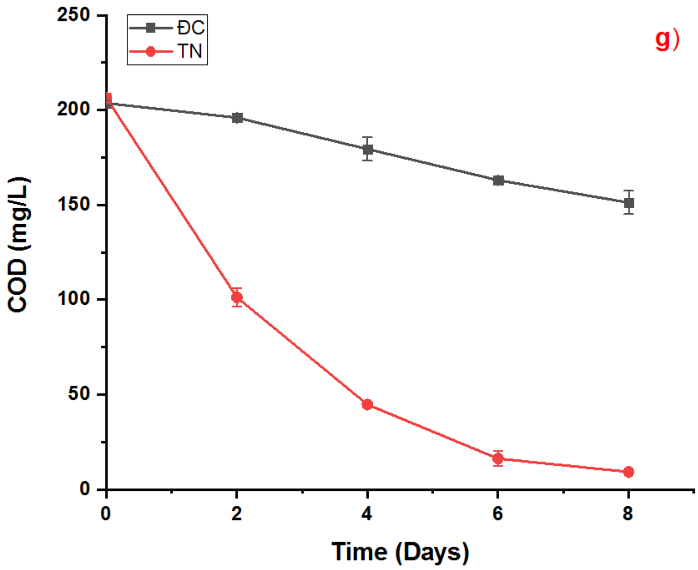

Figure 3. Domestic wastewater treatment ability of *S. platensis* SP4 at the scale of 10 L.

Initial evaluation of the efficacy of using treated wastewater on BC15 rice-grain germination

Many researches have reported that the biomass of cyanobacteria can be added in soil as a reproduction aid measure for rice. On average, these results have shown a 15-20% increase in rice yield in the field trials (Mishra *et al*., 2004). While previous researches focused on using the biomass of *S. platensis* as bio-fertilizer for rice, the impact of addition of treated domestic wastewater with *S. platensis* SP4 on BC15 rice-grain grouting were studied in this work. The grouting test determines the maximum viability of the rice-grains. The grouting rate of a rice-grain lot is an important indicator of its yield in the field.

Experimental results on Figure 4 showed that stimulating BC15 rice seedlings with cyanobacteria culture medium throughout the incubation phase yields positive outcome. In ĐC1 formula, BC15 rice-grains be soaked in tap water brought the lowest grouting efficiency of 64.65 ± 1.53% after 48 h. In the ĐC2, TN1 and TN2 formulas, the germination efficiency reached 87.46 ± 2.31%; 98.05 ± 2.32 % and 88.55 ± 1.53 % after 36 h of incubation, and reached 98.32 ± 1.17%; 99.61 ± 0.58 % and 99.54 ± 1.25 % after 48 h of incubation, respectively and significantly increased compared to ĐC1 ($P<0.05$). Using the extract solution containing L-tryptophan in TN1 formula gave the highest BC15 rice-grain grouting rate of 99.61 ± 0.58 % after 48 h of incubation. The results of this research initially show the effectiveness of the treated domestic wastewater utilization in stimulating BC15 rice-grain germination. The germination efficiency of BC15 rice-grain in TN2 formula (using domestic wastewater after treatment with cyanobacteria) was equivalent to that of ĐC2 and TN1 formulas, which used cultivation suspension of *S. platensis* SP4 in Zarrouk medium without L-tryptophan and with L-tryptophan. There was no statistically significant difference ($P>0.05$) in the germination of BC15 rice seeds in ĐC2, TN1 and TN2. The results of this research are also consistent with researches on soaking rice-grains in incubation medium to decrease losses from sulfate reduction processes and this is attributed to stimulate the grout and sapling's development (Shariatmadari *et al*., 2011). Pedrero et al. (2010) also found that the growth of plants irrigated with treated sewage water grew substantially faster, probably due to the positive impact of the nutrients in the treated sewage water.

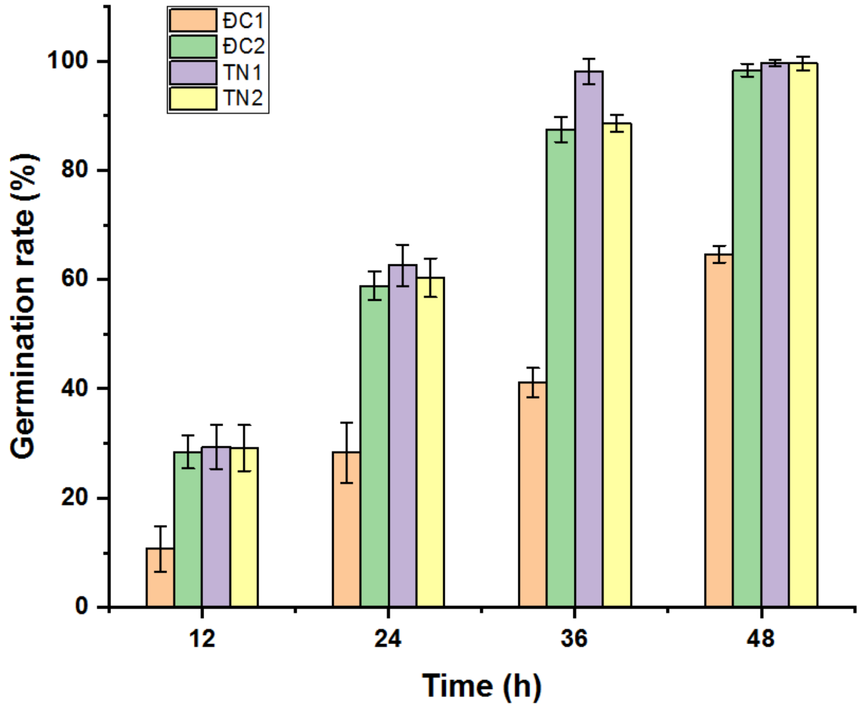

Figure 4. The germination rate of BC15 rice.

CONCLUSION

This research assessed the ability of domestic wastewater treatment and the effect of stimulating rice-grain sprouting of cyanobacteria *S. platensis* SP4. Cyanobacteria could well remove N- $N\text{-}NH_4^+$, T-N, $P\text{-}PO_4^{3-}$, T-P and COD in domestic wastewater with the efficiency of 96.37%, 49.71%, 67, 05 %, 65.88% and 95.53%, respectively. However, with 8 days of studying, *S. platensis* SP4 was not able to remove nitrite and nitrate in domestic wastewater and needed further research. The addition of treated domestic wastewater with *S. platensis* SP4 to BC15 rice grains stimulated the grouting by 99.54 ± 1.25%, which was 1.54 times higher than using the tap water and gave a grouting efficiency equivalent to that of the experiments with adding cyanobacteria in standard medium with or without L-tryptophan addition for 48 h. This work also initially demonstrated the usefulness of *S. platensis* SP4 in domestic wastewater treatment and the ability to utilize treated wastewater as a nutrient source in stimulating the BC15 rice-grain germination.

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