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Nitrogen and phosphorus removal efficiency and algae viability in an immobilized algae and bacteria symbiosis system with pink luminescent filler

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ABSTRACT

In this study, an immobilized algae and bacteria symbiotic biofilm reactor (ABSBR) with pink luminescent filler (PLF) was constructed. The effects of PLF addition in the construction of an algae and bacteria symbiotic biofilm system on the nitrogen and phosphorus removal efficiencies and algae viability were evaluated. Our results showed that for influent TN and TP concentrations of 40 ± 5 and 5 ± 0.8 mg/L, respectively, the pollutant removal rates (PRRs) of TN and TP by the ABSBR can reach up to 74.74% and 88.36%, respectively. The chlorophyll-a (chl-a) concentration on the PLF reaches approximately 5,500 μg/L with a specific oxygen generation rate (SOGR) of 65.48 $\mu molO_2$ mg^{-1}Chl-a h^{-1}. These results indicate that the adding PLF into algae and bacteria symbiosis systems can effectively improve the nitrogen and phosphorus removal efficiencies of the sewage as well as increase biomass and viability of the algae in the system.

Key words: algae viability, immobilized algae and bacteria symbiosis system, nitrogen and phosphorus removal, pink luminescent filler

HIGHLIGHTS

- *Chlorella* can be densely attached to the pink luminescent fillers.
- The immobilized algae and bacteria symbiosis biofilm reactor with pink luminescent fillers can effectively remove nitrogen and phosphorus pollutants in sewage.
- The addition of pink luminescent fillers can effectively control the loss of algae and improve algae viability.

INTRODUCTION

In recent years, the amount of urban sewage discharge has sharply increased (Lu 2016). When parts of the sewage, not adhering to discharge standards, enters a water body, the excessive nitrogen and phosphorus contents have been reported to cause algae and plankton proliferation, which severely disrupts the ecosystem balance and aggravates water pollution (Paerl *et al.* 2014; Wurtsbaugh *et al.* 2019). However, traditional sewage treatment methods such as activated sludge systems and anaerobic digestion processes have various problems such as low pollutant removal efficiencies, complex control of process conditions, and excessive energy consumption (Kong *et al.* 2019; Xu *et al.* 2019). Therefore, there is an urgent need to develop a new wastewater treatment technology with high efficiency, stable nitrogen and phosphorus removals and low energy consumption to enhance urban sewage treatment and ensure the sustainability of water resources.

As a new wastewater treatment technology, the algae and bacteria symbiosis system has highly efficient nitrogen and phosphorus removals, low energy consumption and recyclable biomass resources; therefore, it has been extensively studied by scholars (Whalen *et al.* 2002; Zhang *et al.* 2012; Saravanan *et al.* 2021). Chen *et al.* (2019) constructed a *Chlorella* and activated sludge symbiosis system to remove nutrients from artificial municipal wastewater. Their results showed that the NO_3^--N in the wastewater was completely absorbed; the PO_4^{3-}-P and NH_4^+-N were significantly removed with removal rates of 99.82% and 87.13%, respectively. However, in the algae and bacteria symbiosis systems for sewage treatment, the algae are usually only 3–8 μm in size with the poor gravity sedimentation performance and the extracellular polymeric substances (EPS) secreted by the activated sludge are less likely to clump with the algae (Pena 2007). This leads to the easy loss of algae, causing

additional pollution, thereby significantly reducing the effect of the algae and bacteria symbiosis system on sewage treatment. Simultaneous light scattering by algae, light absorption by pigment, and light blocking by sludge flocs causes the light attenuation phenomenon, wherein the light intensity received by the algae inside the reactor is lower than the light compensation point. Owing to the lack of sufficient light, the amount of organic matter synthesized by algae photosynthesis is lower than the amount of organic matter consumed by respiration (Su *et al.* 2012), which limits the growth rate and viability of the algae (Tang *et al.* 2016, 2018), thereby affecting the nitrogen and phosphorus removal efficiencies of the algae and bacteria symbiosis system (Arcila & Buitron 2017; Cuellar-Bermudez *et al.* 2017). Therefore, effectively alleviating this easy loss of algae and the light attenuation phenomenon is essential to construct a stable algae and bacteria system that efficiently removes nitrogen and phosphorus with various practical future applications.

In this study, an activated sludge reactor (ASR) and an algae and bacteria symbiosis reactor (ABSR) were constructed as the 'blank' and 'control object', an immobilized algae and bacteria symbiosis biofilm reactor (ABSBR) with pink luminescent filler (PLF) was constructed as the 'research object'. Single-day pollutant concentrations (SPCs) and pollutant removal rates (PRRs) of total nitrogen (TN) and total phosphorus (TP) in the effluent, the chlorophyll-a (chl-a) concentrations in the devices and the effluents, and the specific oxygen generation rates (SOGRs) were measured for all devices during the 80-day operation period. We evaluated the nitrogen and phosphorus removal efficiencies and algae viability to understand the influence of PLF addition to construct an immobilized algae and bacteria symbiosis system.

EXPERIMENT METHOD

Algae selection and cultivation method

Su *et al.* (2012) found that the sedimentation performances of *Phormidiaceae*, *Chlorella* and *Scenedesmus* were prominent; of these, *Chlorella* exhibited the best nitrogen and phosphorus removal ability. Moreover, *Chlorella* is algae widely used in scientific research and practical engineering. Their selection as the 'experimental algae' in this study makes our research conclusions more representative and referenceable. The algae selected for this experiment was *Chlorella Pyrenoidosa*, which was cultured in BG11 medium.

The purchased *Chlorella* needs to be activated and domesticated. For activation, 10 mL of *Chlorella* solution was inoculated into 100 mL BG11 medium, placed in a 250-mL Erlenmeyer flask, and cultivated for 4–7 days at a temperature of 24–26 °C, light intensity of 2,000–3,000 lux, and a light-to-dark ratio of 12:12 h, while shaking the Erlenmeyer flask 3–4 times a day. Once the *Chlorella* solution color became darker, 1 mL of the solution was taken and counted using a hemocytometer. When the number of *Chlorella* reached 1×10^7/mL, then the *Chlorella* was considered domesticated; subsequently, they were expanded and re-cultured.

Chlorella domestication is performed in a culture solution comprising synthetic sewage and BG11 medium mixed in a ratio of 1:3. The domestication conditions were the same as those aforementioned for activation. Artificial synthetic sewage was gradually added to the culture solution, until finally the artificial synthetic sewage is completely used to cultivate *Chlorella*. The domesticated *Chlorella* was used as the experimental object and in the preparation of seed liquid.

The seed liquid was prepared by centrifuging 50 mL of domesticated *Chlorella* solution at 4,000 rpm for 10 min, and discarding the supernatant. Subsequently, synthetic sewage was added and the centrifugation was repeated 3–4 times. Finally, the obtained *Chlorella* mud was added to the activated sludge and mixed well. It was stored at −4 °C for subsequent experiments.

Sludge source and synthetic sewage

The sludge used in this study was obtained from the return sludge of the secondary settling tank in a sewage treatment plant in Nanchang City, Jiangxi Province, China. Activated sludge was domesticated and cultivated with artificial synthetic sewage to ensure that it maintains good activity. After 15 days, it was mixed with the domesticated *Chlorella* for the experiment. The composition of artificial synthetic sewage was shown in Table 1. The actual measured values of TN and TP concentrations of synthetic sewage in the experiment were 40 ± 5 mg/L and 5 ± 0.8 mg/L, respectively.

Table 1 | The composition of the artificial synthetic sewage

Composition	$NaHCO_3$	$C_6H_{12}O_6$	$(C_6H_{10}O_5)_n$	NH_4Cl	$MgSO_4{\cdot}7H_2O$	$K_2HPO_4{\cdot}3H_2O$	$CaCl_2{\cdot}2H_2O$	A5
Concentration (mg/L)	300	200	200	155	50	38	5	1

Luminescent filler preparation

Most scholars indicate that red light has the most significant increasing in the growth efficiency of *Chlorella* compared with other light qualities (Lee 2011; Yan *et al.* 2013; Umar *et al.* 2021). Therefore, red light was selected as the light quality in this study. The pink luminescent material was mixed with UV glue at a mass ratio of 3:5; and stirred with a rod to ensure a bubble-free liquid. Then, a woolen brush was used to evenly coat this liquid onto the surface of the ordinary filler, which was then cured using an ultraviolet lamp at 9 watts to prepare the final luminescent filler. The ordinary filler is hollow, spherical, polypropylene filler with a diameter of 25 mm and a specific surface area of 500 m^2/m^3. The main function of the ordinary filler is to act as a carrier for algae and bacteria to form biofilms. The UV glue is a transparent liquid that solidifies the luminescent material onto the filler surface. The luminescence mechanism of luminescent materials involves the substrate absorbing the energy transferred by the activation light, and then transferring it to the activator (rare earth element), thereby bringing it to an excited state. When it returns to the ground state, the energy is radiated in the form of light. The pink luminescent material is in powder form; its main material composition is $SrCO_3$, Al_2O_3: EU^{2+}, DY^{3+}, and the emission wavelength is 680 nm. The schematic diagrams of ordinary fillers and pink luminescent fillers are shown in Figure 1. During the entire experiment period, the luminous intensity and duration of the PLF remained stable due to the supply of external artificial lighting.

Device construction

The three experimental devices used in this study were made of polyvinyl chloride, having uncovered cylindrical structure with cross-sectional diameters of 15 cm, heights of 60 cm and effective volumes of 8 L. A water outlet hole with a diameter of approximately 1 cm was cut out in the middle of the cylinder, and a plastic hose of appropriate length was connected to facilitate the collection of water samples. The ASR only contained activated sludge as the blank group, and the ABSR only contained *Chlorella* as the control group on the basis of ASR, and the algae and bacteria were in a suspended state. In the ABSR, PLF were added to immobilize *Chlorella* thereby constructing an ABSBR as the experimental group. The schematic diagrams of the ABSR and ABSBR devices are shown in Figure 2.

The concentration of activated sludge in all devices was 1,600 mg/L, the concentration of *Chlorella* in the ABSR and ABSBR was 400 mg/L, and the filling ratio of the fillers in the ABSBR was approximately 20%. Artificial synthetic sewage was selected as the inlet solution; the sequencing batch reactor activated sludge process was adopted. In this process, 2 cycles are run each day, each cycle is 12 h long, and reactors were operated for 80 days. In each cycle, the solution inlet is for 30 min, aeration for 8 h, left to stand still for 3 h, and effluent removed for 30 min. LED light tapes were wound onto all the devices externally for artificial lighting with the light intensity reaching 4000 lux. Artificial lighting was used during the aeration phase of each operation cycle, and illuminated for 6 h. The aeration volume was set to 0.4 L/air•min, the inlet and outlet solution volumes were 4 L, which was 50% of the device volume, ensuring that the hydraulic residence time was 24 h. Each device was manually discharged and 100 mL of sludge was collected every day; the sludge age was 20 days.

Sample collection, measurement and evaluation

PVC buckets were used to collect the outlet samples from the three devices for each operation cycle; these samples were mixed with a stir bar and then transferred into a sample bottle; excess water samples were discarded. The sample bottle

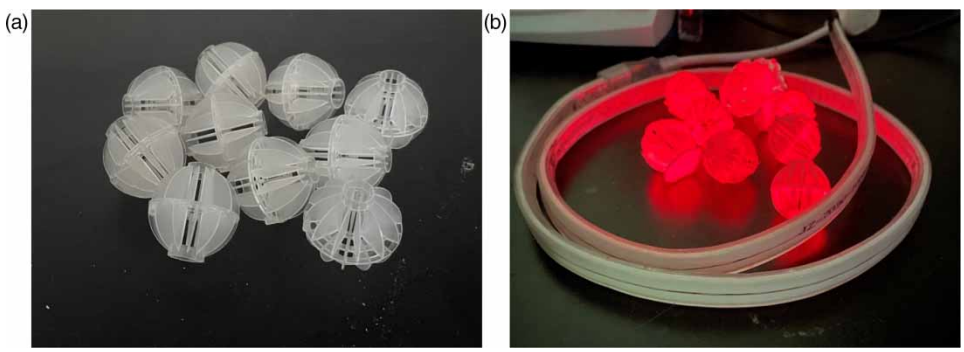

Figure 1 | Schematic diagrams of ordinary and pink luminescent fillers. (a). Ordinary fillers, (b) Pink luminescent fillers.

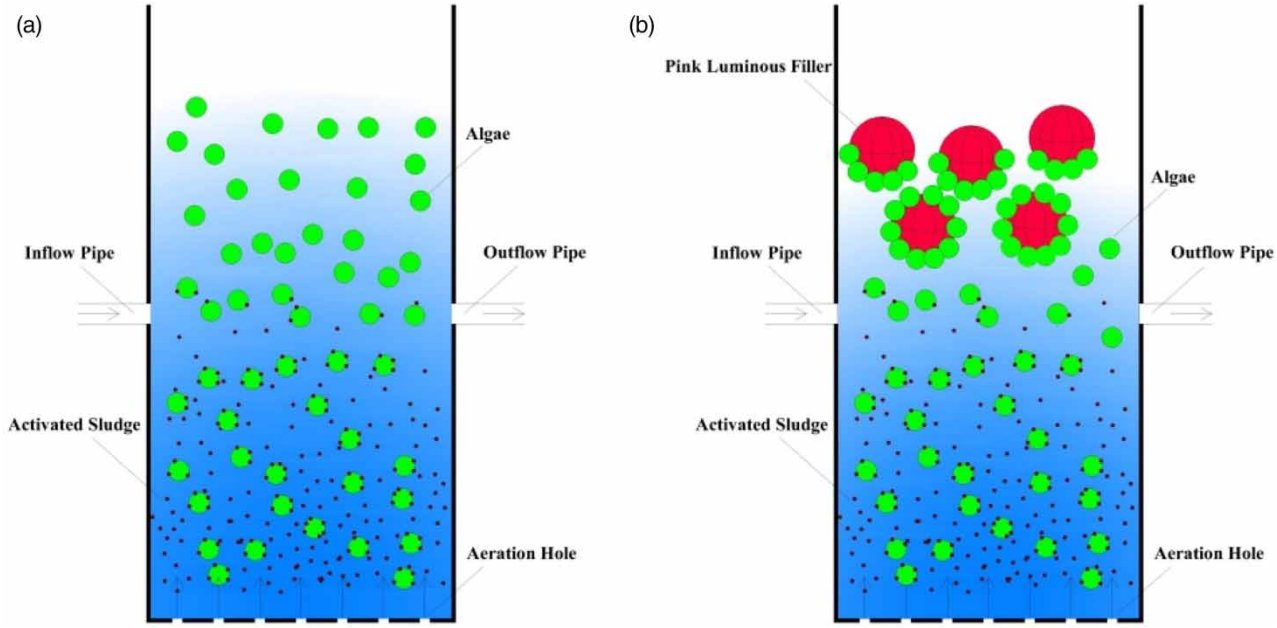

Figure 2 | Schematic diagrams of the ABSR and the ABSBR. (a) ABSR. (b) ABSBR.

was stored in a 4 °C freezer, and the TN and TP concentrations of the samples were measured within 24 h and recorded. Alkaline potassium persulfate digestion and UV spectrophotometry were used to determine the TN concentration; the ammonium molybdate spectrophotometric method was used to determine the TP concentration. In this study, the nitrogen and phosphorus removal efficiencies of the devices were evaluated by measuring SPCs and PRRs. The SPC was calculated using the following formula:

$$SPC_i = \frac{C_{2i\text{-}1} + C_{2i}}{2} \tag{1}$$

where SPC_i is the single-day pollutant concentration (mg/L); i is the number of days the device runs (days); $C_{2i\text{-}1}$ is the pollutant concentration of the outlet sample from the device in the 2i-1-th operation cycle (mg/L); C_{2i} is the pollutant concentration of the outlet sample from the device in the 2i-th operation cycle (mg/L).

The PRR was calculated using the following formula:

$$PRR = \frac{C_s - SPC_i}{C_s} \times 100\% \tag{2}$$

where PRR is the pollutant removal rate (%); C_s is the pollutant concentration of the inlet water (mg/L); SPC_i is the single-day pollutant concentration (mg/L).

In this study, the chl-a concentration was used to characterize the growth and reproduction of algae in the devices. The chl-a concentrations were determined in the ABSR, ABSBR and PLF, and the effluents of ABSR and ABSBR.

In this study, the concentration of chl-a was determined by removing 5 mL of sample from the devices into a 10-mL centrifuge tube every 5 days, centrifuging at 12,000 rpm for 5 min, and then aspirating the supernatant. The centrifuged algae were resuspended in 80% acetone. The 10-mL centrifuge tube was completely wrapped with foil, placed in a dark place, and heated to 55 °C in a water bath for 30 min, then centrifuged again at 12,000 rpm for 5 min. The supernatant aspirated and the pellet was diluted in 5 mL of 80% acetone. Finally, an ultraviolet spectrophotometer was used to measure the optical density (OD) at 663 nm. The chl-a concentrations in the centrifuge tube were determined and then the chl-a concentrations in

the devices or effluents were calculated using the following formula:

$$C_a = \frac{C_A \times V_1}{V_2} \tag{3}$$

where C_a is the chl-a concentration in the devices or effluents; C_A is the result of the OD calculation, $C_A = OD_{663\ nm}/82$; $OD_{663\ nm}$ is the optical density at 663 nm; V_1 is the total amount of sample (L); V_2 is the influent or effluent volume of the devices (L).

This study used SOGR to characterize the rate of oxygen generation per chl-a in algae cells, which was used to evaluate the activities and photosynthesis capacities of algae.

In this study, the light and dark bottle technique was used to determine the SOGR. Two 50 mL suspensions were taken from the devices every 40 days, centrifuged at 4,000 rpm for 5 min. After discarding the supernatant, the sediment was resuspended in the original volume with ionized water. After mixing again, centrifugation was continued at 4,000 rpm for 5 min; this step was repeated 3–4 times. The sediment was placed in two 300-mL dissolved oxygen bottles, and synthetic sewage is processed in the bottles. One of the dissolved oxygen bottles is wrapped with black cloth or foil to ensure that it is completely opaque and was called the 'black bottle'. The other dissolved oxygen bottle was surrounded by a light source and called the 'white bottle'. Magnetic stirrers were put into the two dissolved oxygen bottles to mix the sediment and the artificial sewage completely and evenly. Then, the dissolved oxygen (DO) meter was used to record the DO change in the two dissolved oxygen bottles over time. The DO recorded for the black bottle is the oxygen consumption of microorganisms in the sewage, and the DO recorded for the white bottle is the difference between the oxygen production of algae photosynthesis and the oxygen consumption of microorganisms in the sewage. The sum of both is the oxygen production of algae photosynthesis. The SOGR can be calculated using the following formula:

$$SOGR = \frac{\Delta DO \times 1{,}000}{32 \times C_a \times t} \tag{4}$$

where SOGR is the specific oxygen generation rate ($\mu molO_2\ mg^{-1}$Chl-a h^{-1}); ΔDO is the oxygen production of algae photosynthesis (mg/L); C_a is the chl-a concentration in the devices or effluents (mg/L); t is the time interval between two dissolved oxygen concentration determinations (h).

The method for determining the SOGR of the algae on the fillers is the same as the method used for the suspension in the devices described above. The samples were selecting 20 PLFs at the end of aeration and dividing them into two equal parts. The sludge on the surface of the PLFs was gently rinsed with deionized water and then the fillers were soaked in deionized water for 5 min. The soaked luminescent fillers were poured into a 300-mL dissolved oxygen bottle and the SOGR was measured for the algae on the fillers based on the light and dark bottle technique described above. The SOGR of the algae on the fillers can be calculated using the following formula:

$$SOGR = \frac{\Delta DO \times 1{,}000 \times n}{10 \times 32 \times C_a \times t} \tag{5}$$

where SOGR is the specific oxygen generate rate ($\mu molO_2\ mg^{-1}$Chl-a h^{-1}); ΔDO is the oxygen production of algae photosynthesis (mg/L); C_a is the chl-a concentration in the devices or effluents (mg/L); t is the time interval between two dissolved oxygen concentration determinations (h); n is the total number of fillers in the devices.

RESULT AND DISCUSSION

The removal efficiencies of TN from artificially synthesized sewage for the three devices are shown in Figure 3. As shown in Figure 3(b), the TN concentrations in the effluents of the three devices were relatively high during the early stage of the experiment. The TN concentrations in effluent of ASR, ABSR and ABSBR in the 0–10 d were 24.12–26.34 mg/L, 18.92–21.00 mg/L and 12.58–16.36 mg/L, respectively. And the TN concentrations in effluent of ASR, ABSR and ABSBR gradually decreased with the continuous operation of the devices, which were 20.18–23.76 mg/L, 14.19–17.48 mg/L and 8.18–10.85 mg/L in the last 10 d. This is because the assimilation and absorption of microorganisms and algae in the three devices as well as nitrification/denitrification can all effectively remove TN from the sewage (Cai *et al.* 2013; Karya *et al.* 2013). Of all reactors,

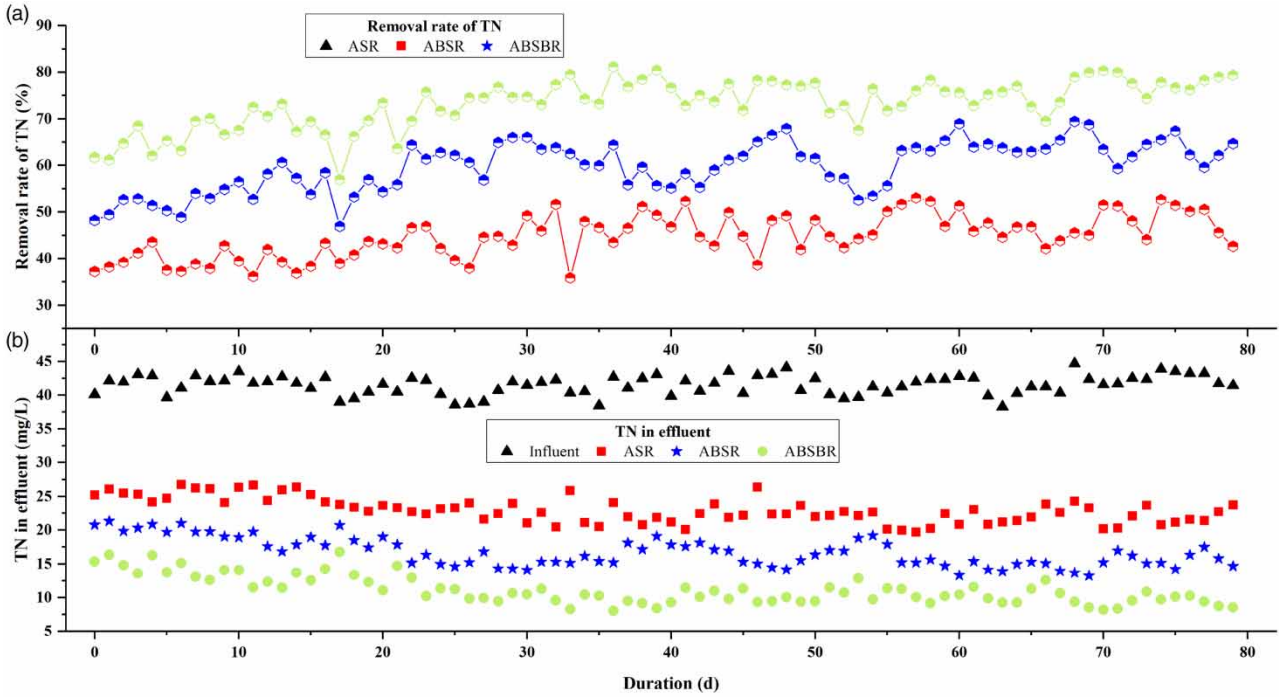

Figure 3 | Removal efficiencies of TN in the artificially synthesized sewage by the three devices.

ASR had the worst TN removal efficiencies throughout the entire experimental period. With TN in the influent at 40 ± 5 mg/L, the average SPC of TN in the effluent of the ASR during the entire experimental period was 22.29 mg/L, and the PRR of TN was 46.12%. This is because the removal of TN in the ASR only relies on nitrification/denitrification. In an aerobic environment, ammonia nitrogen is converted into nitrate nitrogen by nitrification; and nitrate nitrogen is converted into nitrogen by denitrification in an anaerobic environment. The synergistic effect of algae and bacteria in the ABSR on TN removal from the sewage causes the concentration of TN in the effluent to gradually decrease with certain fluctuations. The average SPC of TN in the ABSR effluent during the entire experiment period was 15.97 mg/L, and the PRR of TN was 61.36%. Compared to the ASR, the ABSR improved the removal efficiency of TN in the sewage. This may because in addition to the nitrification/denitrification in the system, the assimilation of *Chlorella* itself directly absorbs the nitrogen in the sewage and converts it into amino acids needed in the body (Molinuevo-Salces *et al.* 2019). Except due to the light attenuation phenomenon in ABSR, the internal *Chlorella* do not receive enough light for photosynthesis, thereby the growth of *Chlorella* is probably inhibited. Simultaneously, *Chlorella* is easy to be partly lost in the effluent from the devices, failing to form a relatively stable ABSR, resulting in fluctuating TN concentrations in the effluent of the ABSR. The algae and bacteria in the ABSBR gradually adhere to the luminescent fillers to form a biofilm with a stable community structure (Zhang *et al.* 2020). The ABSBR showed a significant and stable removal of TN from sewage. The SPC of TN in the ABSBR effluent stabilized at 10.43 mg/L during the entire experimental period, and the PRR of TN was 74.74%. This is because the PLF is added to the ABSBR to immobilize the algae and bacteria in the device, thereby reducing the loss of *Chlorella*. Furthermore, the PLF can supplement the light for the internal *Chlorella*, reducing the light attenuation effect and enabling the *Chlorella* to grow and reproduce normally, thereby improving the overall TN removal efficiency of ABSBR.

The removal efficiencies of TP from artificially synthesized sewage for the three devices is shown in Figure 4. As shown in Figure 4, all three devices showed a certain effect in removing TP from the sewage. During the entire experimental operation period, comparing the TP removal capacity in the sewage of the three devices revealed the order ABSBR > ABSR > ASR. For an influent TP concentration of 5 ± 0.8 mg/L, the average SPC of TP in the ASR effluent during the whole experimental period was 2.36 mg/L, and the PRR of TP was 49.62%. This is because of the assimilation of microorganisms in the ASR and the excessive phosphorus uptake of phosphorous accumulating organisms to remove TP in sewage. The assimilation of phosphorus by microorganisms means that phosphorus is an indispensable element when microorganisms synthesize nucleic acids, ATP, and certain sugar metabolism intermediates. Soluble organic phosphorus compounds in sewage can be

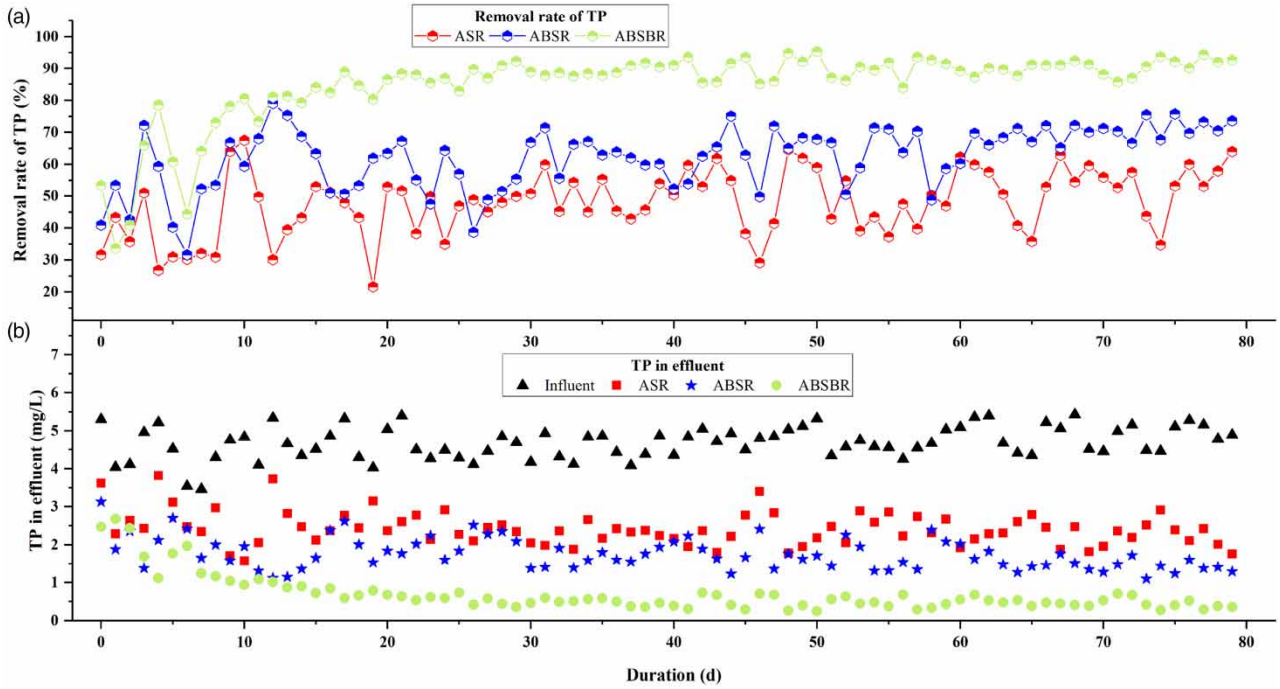

Figure 4 | Removal efficiencies of TP from the artificially synthesized sewage in the three devices.

assimilated and absorbed by microorganisms, eventually becoming structural components of the microorganisms (Ma *et al.* 2014). Phosphorus accumulating organisms exist in activated sludge and can reverse the concentration gradient to absorb excess phosphorus to synthesize polyphosphate particles and store them in the body (Solovchenko *et al.* 2016). ABSR adds a certain amount of *Chlorella*, which promotes the overall TP removal efficiency in the sewage. The average SPC of TP in the ABSR effluent during the entire experimental period was 1.69 mg/L, and the PRR of TP was 63.88%. This is because the activated sludge microorganisms and *Chlorella* in ABSR can synergistically remove TP in the sewage. Some studies (Jordan *et al.* 2016) have shown that algae cells have a stronger assimilation effect on phosphorus than bacteria. Algae and phosphorus accumulating organisms also have excessive phosphorus absorption capacity, and can show stronger phosphorus removal efficiency relative to phosphorus accumulating organisms (Lee *et al.* 2015). However, because suspended *Chlorella* in ASBR is easy to lose in the effluent and *Chlorella* cannot grow and reproduce normally under the phenomenon of light attenuation, the change trend for the TP concentration in the effluent of the ABSR during the entire experimental period is similar to that for TN, revealing an unstable treatment effect. The ABSBR with PLFs added showed a significantly better removal effect and system stability for TP removal from sewage than that by ABSR only added with *Chlorella*, which further improves the removal efficiency of TP in sewage through the algae and bacteria symbiotic system. The average SPC of TP in the ABSBR effluent during the entire experimental period was 0.54 mg/L and the PRR of TP was 88.36%. This is mainly because the addition of the PLFs in the ABSBR provides a stable growth environment for algae and bacteria, increases the biomass of algae and bacteria in the device, thereby improving the assimilation and excessive phosphorus absorption capacity of the algae and bacteria, and further improved the TP removal efficiency from sewage by the algae and bacteria symbiotic system (Su *et al.* 2016).

The concentration of chl-a in the ABSR and ABSBR are shown in Figure 5. As shown in Figure 5, we can find that at the beginning of the experiment, the chl-a concentration in the ABSR and ABSBR decreased rapidly from the initial 3,108.23 μg/L and 3,169.55 μg/L to 2,638.71 μg/L and 2,549.06 μg/L, respectively. This is because the growth of *Chlorella* in the initial stage of the devices is not stable, and the *Chlorella* loss in the effluent from the devices is serious. Due to the phototaxis properties of algae, a large number of *Chlorella* gradually adhere to the PLFs added in the ABSBR and immobilized to form a biofilm; the concentration of chl-a on the PLF also gradually increased. With the continuous progress of the experiment, the concentration of chl-a in the ABSR gradually increased and stabilized at approximately 4,900 μg/L. The concentration of chl-a in the ABSBR is continuously decreasing and tends to be stable, about 2,100 μg/L. At the same time, it can be observed that at 30

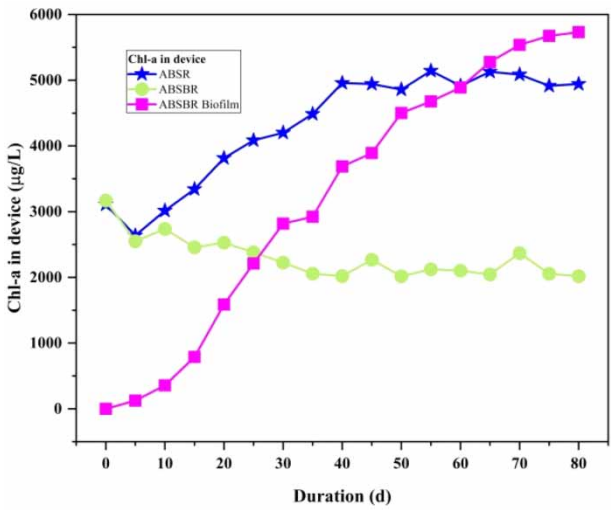

Figure 5 | The concentration of chl-a in ABSR and ABSBR.

days, the chl-a concentration on the PLF begins to exceed the ABSBR, and finally stabilizes at about 5,500 μg/L, which is about 2.83 times the chl-a concentration in the ABSBR and higher than the stable concentration of chl-a in the ABSR. This shows that in the ABSBR, *Chlorella* is easier to adhere to the fillers. Liu *et al.* (2017b) used aerobic granules to rely on EPS bridge function as the immobilized material of Chlorella. Under similar conditions, the reactor could only remove TN of 59.9% and TP of 35.0% in the influent, while the chl-a concentration in the reactor was relatively low. Adding PLF in algae and bacteria symbiosis system can significantly increase the chl-a concentration and pollutants removal efficiencies of reactor compared with aerobic granules. At the same time, the addition of PLFs enhances the stability of the algae and bacteria symbiotic system, greatly slows down the loss of *Chlorella* due to hydraulic disturbance, and keeps the biomass of *Chlorella* in the device stable (Mallick 2006). The light source emitted by the pink luminescent filler can increase the optical energy received by the *Chlorella* on the fillers, reduce the negative effect of optical attenuation, and effectively promote the growth and reproduction of *Chlorella* in the ABSBR. Pink luminescent filler as the light source also improves the biomass productivity and growth of the ABSBR, and this leads to the same as Prates *et al.* (2018) research conclusion. The good growth and enrichment state of algae is more beneficial to the algae's ability to absorb and degrade nitrogen and phosphorus in sewage (Su *et al.* 2011; Tang *et al.* 2012), which indirectly explains the reason why continuous concentration of TN and TP in the effluent decreased and stabilized after the addition of PLFs in ABSBR.

The concentration of chl-a in the effluent of ABSR and ABSBR is shown in Figure 6. When the devices were initially running, the chl-a concentration in the effluent of the ABSR was 459.84 μg/L, and the chl-a concentration in the effluent of the ABSBR was 400.15 μg/L. This is due to the small size of *Chlorella*, which is easy to be lost in the effluent process. At the same time, the immobilized structure of the algae and fillers in the ABSBR is not formed, so the chl-a concentration in the effluent of ABSR and ABSBR is relatively high. During the subsequent operation period of the devices, the chl-a concentration in the effluent of ABSR and ABSBR gradually decreased, and finally stabilized at about 110 μg/L and 46 μg/L, respectively. This is because the EPS secreted between the algae and bacteria can stick the algae and the sludge together, which greatly reduces the loss of algae during the effluent process of ABSR and ABSBR (Salama *et al.* 2016). Compared with ABSR, ABSBR with PLFs has a lower chl-a concentration in the effluent. This also proved the addition of PLFs can make *Chlorella* more stably enriched and grow on the fillers. The chl-a concentration in the ABSBR suspension is relatively low, resulting in a decreasing in the chl-a concentration in the effluent of ABSBR. Therefore, the addition of PLFs can effectively reduce the algae loss in the algae and bacteria symbiotic system.

The SOGR of *Chlorella* in ABSR and ABSBR is shown in Figure 7. In the initial state, the SOGR of ABSR and ABSBR were 36.33 and 34.28 $\mu molO_2\ mg^{-1}$Chl-a h^{-1}, respectively. The SOGR of PLF is 0 because there is no *Chlorella* attached when the device is just running. It may be because the addition of fillers in ABSBR can block light to a certain extent, which is not conducive to photosynthesis of *Chlorella*. The ability of *Chlorella* to absorb optical energy in the ABSBR is lower than that in the ABSR, resulting in poorer algae viability (Valigore *et al.* 2012; Tricolici *et al.* 2014). With the process of the devices

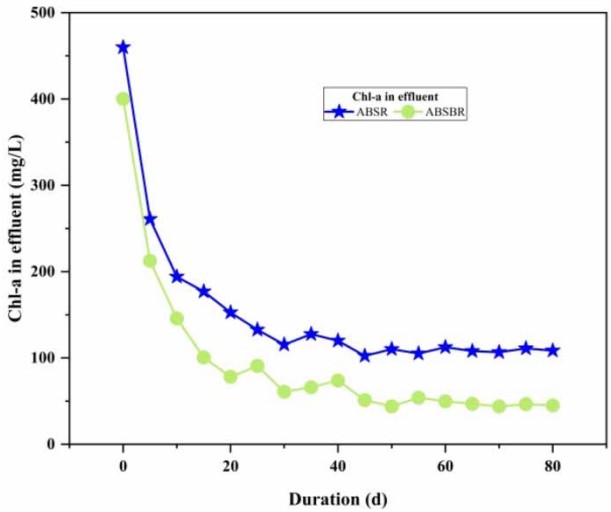

Figure 6 | The concentration of chl-a in the effluent of ABSR and ABSBR.

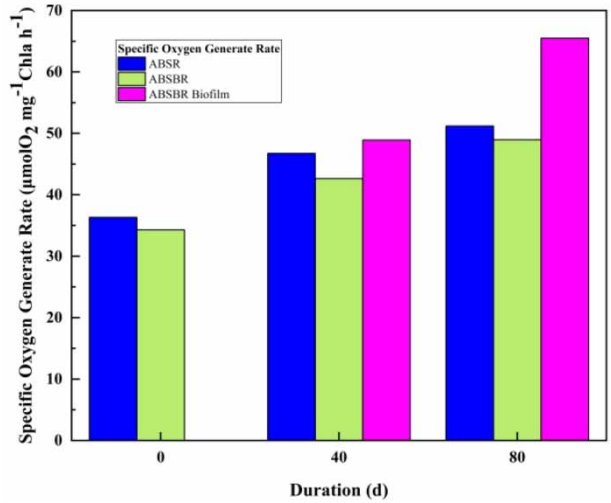

Figure 7 | The specific oxygen generate rate of *Chlorella* in ABSR and ABSBR.

operation period, the SOGR of *Chlorella* on the 40th day of ABSR, ABSBR and PLF were 46.72, 42.65, 48.92 $\mu molO_2 mg^{-1}$ $Chla h^{-1}$. The SOGR of *Chlorella* on the 80th day of ABSR, ABSBR and PLF were 51.24, 48.95, and 65.48 $\mu molO_2\ mg^{-1}$Chl-a h^{-1}. According to the experimental results, it can be found that the SOGR of the *Chlorella* in the ABSR, ABSBR, and PLF showed a certain increase compared to that in the initial state of the devices. This is because the activated sludge in the devices can promote algal viability and photosynthesis efficiency. The SOGR of *Chlorella* on PLF is relatively the highest. On one hand, the PLF supplements optical energy to the *Chlorella* attached to the filler, which improves the photosynthesis capacity of the *Chlorella*. On the other hand, immobilization improves the anabolic activity of the *Chlorella*, delaying the senescence of the *Chlorella*, thereby reducing the catabolic activity of the *Chlorella* to a certain extent (Liu *et al.* 2017a).

CONCLUSION

1. Within 80 days of the continuous operation period, the ABSBR has the best nitrogen and phosphorus removal efficiency. The removal rates of TN and TP in the effluent from the ABSBR were 74.74% and 88.36%, respectively, which were increased by 13.38% and 24.48% relative to ABSR, and 28.62% and 38.74% relative to ASR. Adding PLFs to construct

an algae and bacteria symbiotic biofilm system can significantly improve the nitrogen and phosphorus removal efficiencies in sewage treatment.

2. In the late stage of the operation period, the chl-a concentration in the ABSR reached 4,900 μg/L, the chl-a concentration in the ABSBR stabilized at 2,100 μg/L, and the chl-a concentration on the PLFs reached approximately 5,500 μg/L. The chl-a concentration in the effluent of the ABSR and ABSBR were stabilized at 110 and 46 μg/L, respectively. This shows that the addition of luminescent fillers can effectively solve the problem of easy *Chlorella* loss in the algae and bacteria symbiosis system and increase the *Chlorella* biomass in reactor to ensure the efficient and stable system operation.
3. In the ABSBR, *Chlorella* gradually attaches to the biofilm. In the later stage of the operation period, the SOGR of the *Chlorella* on the PLF was 65.48 $\mu molO_2\ mg^{-1}$Chl-a h^{-1}, which was higher than the SOGR of the *Chlorella* in the ABSR and ABSBR. This shows that the addition of PLFs can alleviate the optical attenuation phenomenon, thereby promoting the growth and reproduction of *Chlorella* and enhancing algae viability.
4. The addition of PLF in algae and bacteria symbiosis system is a new technology that effectively improves the nitrogen and phosphorus removal efficiency and algae viability. Next more relevant studies should be carried out immediately, such as the technology mechanism, the transportation and transformation of nitrogen and phosphorus in all forms and the contribution analysis of each factor.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

Not applicable.

CONSENT FOR PUBLICATION

Not applicable.

AVAILABILITY OF DATA AND MATERIALS

Not applicable.

COMPETING INTERESTS

The authors declare no financial/commercial conflicts of interest.

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AUTHORS' CONTRIBUTIONS

All authors contributed to the study conception and design. Material preparation, data collection and analysis, tables and figures drawing were performed by Chen Xu, Liupeng Wang and Zaohong Liu. The first draft of the manuscript was written by Chen Xu and all authors commented on previous versions of the manuscript. Guanjun Cai, Jian Zhan revised the manuscript critically for important intellectual content. All authors read and approved the final manuscript.

DATA AVAILABILITY STATEMENT

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

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