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Combined effects of flow rate and light on characteristics of biofilms grown on three-dimensional elastic carriers

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ABSTRACT

The combined effects of flow rate and light intensity on the characteristics of biofilm grown on three-dimensional elastic carriers were investigated in this study, to assess as well as to provide guidance for *in-situ* remediation of polluted ground water using biofilm method. Four identical biological reactors were used to cultivate biofilm on the carriers made from polyethylene terephthalate (PET), at varying flow rates (0.2, 0.4 and 0.8 $m^3 \cdot m^{-2} \cdot s^{-1}$) and under artificial lighting (approximately 130 μ mol photons$\cdot m^{-2} \cdot s^{-1}$) or dark conditions. The results showed that the characteristics of biofilm, including total biomass, extracellular polymeric substances (EPS), active biomass and microbial activity, were all significantly enhanced with increasing flow rate and under light conditions, indicating that the synergistic effect the high flow rates and stronger light on the improvement of biofilm characteristics.

Keywords: Biofilm; Combined effect; Flow rate; Light; Three-dimensional elastic carrier; EPS; Biomass; Microbial activity

1. Introduction

In recent years, *in-situ* biological contact oxidation technology, with application for the treatment of polluted water, has received increasing attention [1,2]. During the treatment process, a few kinds of artificial substrata are used to colonize many organisms on and form biofilm under certain hydraulic conditions [3]. The biofilm that settle on the surface of the substrata in a photolytic layer of water are complex communities composed mainly of photoautotrophic (algae) and heterotrophic microorganisms (bacteria, fungi, protozoa) [4]. Present in the unique interface between the substrata and water, biofilm play a fundamental role in the various biogeochemical cycles and dynamics of aquatic ecosystems [5,6].

When the biofilm method is used for *in-situ* remediation of polluted water, many factors, such as pH, temperature, pollutant concentration, oxygen distribution, substratum materials, microbial communities, and other characteristics can affect the formation and properties of biofilm [7–11]. Of all these factors, flow rate and light are particularly important.

Flow rate has been considered one of the most decisive factors for the formation of biofilm under certain hydrodynamic condition. Flow rate impacts the structure, biomass, metabolism, and kinetic behavior of biofilm [12]. There is evidence that a higher flow rate results in a thinner and denser biofilm [13,14]. Light is

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another important factor that affects the effect of light on biofilm formation. Ylla and co-workers incubated artificial biofilm in light (160–180 μ mol photons·m^{-2}·s^{-1}) under dark condition, and found that light enhances the growth of the both algae and bacteria as well as enhances extracellular enzyme activity [15]. Besides, Rier et al. [16] observed that the biomass of algae, fungi and microbes in biofilm colonized on glass-fiber filters and leaf litter was significantly influenced by the level of light and that biomass concentration was greater in high-light condition (≈150 μ mol photons·m^{-2}·s^{-1}) compared to low-light conditions (≈20 μ mol photons·m^{-2}·s^{-1}) during both sampling periods (8 and 23 d). Thus, flow rate or light alone can significantly influence biofilm formation. However, the combined effects of flow rate and light on biofilm settled on three-dimensional carriers are unclear.

Other points to note in biofilm formation include the selection of substrata. It is also critical when using the biofilm method for *in-situ* remediation of polluted water. Three-dimensional elastic carriers have drawn great interest for many years, as they can be easily assembled and managed used for *in-situ* treatment of polluted water [17], which have been mentioned to be used for imitating the real situation of river water flow pattern. They are usually made from polyester resin materials that have many excellent characteristics such as good flexibility, corrosion, high temperature resistance, high chemical stability, excellent antioxidation performance, and good blocking and knotting resistance. The biofilm settled on the carriers have many advantages, for example, ease of hanging and stripping, high heat shock loading, comparatively sufficient surface area, strong biological activity, and effective purification.

To investigate the combined effects of flow rate and light on the formation of biofilm colonized on three-dimensional elastic carriers made from PET material, four identical reactors were designed in this study to cultivate biofilm at various flow rates and under light or dark condition. The main characteristics of biofilm including total biomass, EPS, active biomass, and microbial activity were measured, and the combined effects of flow rate and light on the biofilm were discussed.

2. Materials and methods

2.1. Cultivation system set-up and operation

Four identical biological reactors labeled as R1, R2, R3 and R4, were used (Fig. 1a). The reactor was made of an organic glass cylinder with an internal diameter of 20 cm and a height of 180 cm, that is, a total volume of 0.05652 m^3; The flow rate was fixed to pump the medium from the feed tank to the cylinder; a circular perforated plate at the bottom of the

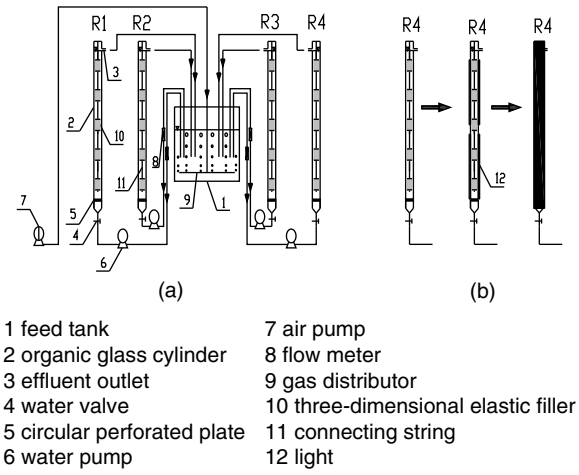

Fig. 1. Schematic diagram of experiment devices: (a) four identical biological reactors; (b) fixing and shading procedures of the light on the outer surface of cylinder of R4.

cylinder was set to homogenize water flow; an effluent outlet at the top of the cylinder was designed for the medium to return the feed bank. An aeration device was designed to provide air directly to feed tank through a gas distributor.

The three-dimensional elastic carriers used were made from PET material, which was produced by Lijing Filter manufacturer (Guangzhou, China). Each of elastic carriers was composed of hundreds of PET filaments that were twist clamped by thin stainless wires, with the size of 12 cm in length and 10 cm in diameter (Fig. 2). After pre-weighed, five carriers were connected by a string and submerged into medium. The depths of five elastic carriers were 20, 50, 80, 110, 140 cm, respectively, from the water level of effluent (Fig. 1a).

Fig. 2. A three-dimensional elastic carrier made from PET.

The cultivation of biofilm in R1, R2, R3 and R4 were operated at hydraulic loading rates 0.8, 0.4, 0.2, 0.2 $m^3 \cdot m^{-2} \cdot s^{-1}$, respectively. The medium used in this study was polluted river water from the campus river of Sun Yat-sen University in Higher Education Mega Center in Guangzhou, China. The characteristics of the polluted river water fed to the reactors were detailed in Table 1. Considering the Chemical Oxygen Demand (COD) in the polluted river water was low, to facilitate the biofilm formation glucose was addition to medium for increasing COD level of about 120 $mg \cdot l^{-1}$. The contaminated water was fed into feed tank, continuously pumped into the cylinders of the four reactors from the feed tank and then flowed back into the feed tank again through the effluent outlets of the reactors. To prevent or decrease biological toxicity, approximate 20% of the medium in feed tank was replaced every 3 d. The experiment was run at room temperature which was about 28°C.

Of the four biological reactors, only R4 was designed using artificial lighting, that is, R1-R3 were designed to use natural light, while R4 design as using artificial light. Four lights (30 W, Tao Xin Environmental Science and Technology Co., Ltd., Guangzhou) were fixed symmetrically the outer surface of the cylinder; together with the lights, the cylinder of R4 was sealed using non-transparent black plastic belts for preventing light transmission (Fig. 1b). The integrated light intensity in water at the center of the cylinder was approximate 130 μ mol photons$\cdot m^{-2} \cdot s^{-1}$, which was measured with a LI-1400 data logger (LiCor Biosciences GmbH, Bad Homburg, Germany). The cylinders of R1, R2 and R3 were also sealed as R4.

The duration of experiment was totally 18 d, from July 15 to August 2 in 2009.

During the experiment, the pH was maintained between 6 and 9 with additions of hydrochloric acid (1 M) and sodium hydroxide (1 M). The dissolved oxygen (DO) in the medium was controlled between 3.5 and 5.0 mg l^{-1}, which was achieved through intermittent aeration using a time switch (Hongjinda Electronics Technology Co. Ltd., Guangzhou). The pH and DO were monitored simultaneously using a SC100™ Universal Controller (Hach Company, America). All analyses were performed in accordance with standard methods [18].

Table 1
Characteristics of river water fed to the reactors

Parameters	Concentration ranges
COD_{Mn} ($mg \cdot l^{-1}$)	17.09–25.47
NH_3-N ($mg \cdot l^{-1}$)	2.27–1.19
Turbidity ($mg \cdot l^{-1}$)	107.5–158.6
TP ($mg \cdot l^{-1}$)	1.17–2.27
TN ($mg \cdot l^{-1}$)	4.83–6.43

2.2. Sampling method

At the end of the experiment, each of carriers was carefully taken from its cylinder. After cutting off the connecting string, each carrier was suspended in 150 ml phosphate buffered saline (PBS, pH 7.2), which contained 0.036 $g \cdot l^{-1}$ K_2HPO_4, 0.092 $g \cdot l^{-1}$ KH_2PO_4 and 0.493 $g \cdot l^{-1}$ NaCl, and then vigorously agitated the carriers with a glass rod to shed the biofilm off the carriers. The suspension was transferred into a 500 ml volumetric flask. The agitating and shedding operations were repeated triplicate using 150 ml PBS. Almost all biofilm attached on the carrier surface was removed into the suspension. The suspension was all transferred into the volumetric flask and prepared 500 ml sample with PBS for the following experiments. Five samples were taken for each depth for biofilm characteristics measurement.

2.3. Measurement of total biomass by dry weight (DW)

The total biomass of biofilm was measured in terms of DW [19]. After vigorous shaking, 50 ml homogeneous suspension sample was put into a crucible. After weighed, the crucible was placed in a GZX-9140MBE electric blast drying oven (Boxun Industrial Co., Ltd., Shanghai) and dried at 105°C, until constant weight was achieved. The crucible was then placed in a desiccator to cool to room temperature and weighed again. The DW of the biofilm sample was calculated in units of $g \cdot kg^{-1}$ of carrier. The measurement was repeated three times and the average was used as the final result.

2.4. Extraction and analysis of EPS production

The EPS were extracted using a cation exchange resin (CER) extraction method according to Frolund et al. [20]. The yields of EPS are represented by polysaccharides and protein since they are the primary components of EPS. CER (Tianjin Reagent Co., Ltd) at a dosage of 60 $g \cdot g^{-1}$ suspension solid (SS) was added to the prepared biofilm samples and mixed in a homogenizer for 1 h at 4°C, allowing the extraction of EPS from the biofilm. The residual solids were removed by a High-speed Refrigerated Centrifuge 5804R (Eppendorf, Germany) at 8000 rpm for 15 min. The supernatant was prepared for polysaccharides and protein analysis [21]. The phenol-sulfuric acid method was used to quantify polysaccharides [22], and the Coomassie procedure was used to measure protein [23]. The tests were in triplicate and took the average as the final result. Results are described as $mg \cdot g^{-1}$ carrier.

2.5. Active biomass quantification by phospholipids

Active biomass was measured using the phospholipids method [24]. Lipid-phosphate concentration has been revealed as a good method for active biomass estimation in biofilms [25]. 10 ml of suspension samples were used for the phospholipids test. A standard curve was established with K_2HPO_4 (Shanghai Reagent Co., Ltd) to calculate phospholipids content of each sample [21]. The tests were repeated three times and the average was used as the final result. Results was described as *n* mol P·g^{-1} carrier.

2.5. Development of microbial activity measurement with TTC

Microbial activity was measured using the 2,3,5-triphenyl tetrazolium chloride-dehydrogenase activity (TTC-DHA) method [19], which was sensitive and simple and performed basing on the method proposed by Mathew and Obbard [26]. The microbial activity was expressed as the quantity of TTC-formazan (TF), the resultant of TTC (Shanghai Reagent Co., Ltd, Shanghai) reduction. A calibration curve established with Na_2S as reductor was used to convert absorbance of each tube at 486 nm with a UV-250 spectrophotometer (SHIMADZU, Japanese) to μ g TF·g^{-1} carrier [21]. The tests were in triplicate and took the average as the final results.

3. Results and discussion

3.1. Observation of biofilm in the four biological reactors

After uncovering the nontransparent black plastic belts that shaded the four reactors, special care was taken with the observation of the structures and colors of the biofilm that settled on the three-dimensional elastic carriers (Fig. 3). The biofilm formed in the four reactors contained a significant proportion of filamentous organisms (bacteria and algae). At the same depths, the biofilm in R3, R2 and R1 were progressively thicker in proportion to increasing flow rates. The biofilm in R4 was thicker than that in R3 at the same depths. The color of the biofilm in R4 manifested as light or grey-green, while the color of the biofilm in the other three reactors were either gray or brown. As the artificial lighting was the primary difference between R4 and other reactors, we attributed the changes in color to light. The light or grey-green color was most likely due to the existence of abundant algae, whose proliferation is closely related to light [27].

At the end of cultivation, some sections of biofilm had been observed in the effluent, which was likely caused by partial sloughing of biofilm from the surface of the three-dimensional elastic carriers. Sloughing is an extreme form of cell shedding, and it is continuously

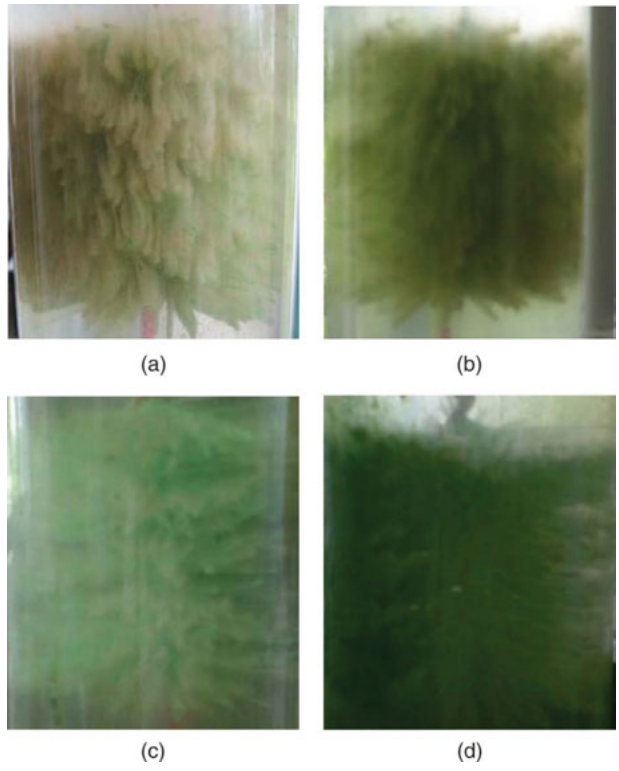

Fig. 3. Biofilm cultured in the four reactors at the depth of 110 cm. (a), (b), (c) and (d) showed the biofilm settled on the carriers at the same depths of 110 cm in cylinders of R1, R2, R3 and R4, respectively. Photographs were taken by Canon DIGITAL IXUS 55 before sampling. The attachment of biofilm on internal surface of cylinders of reactors made the photographs a bit obscure.

and simultaneously accompanied by cells attached to the biofilm. As the anaerobic bottom layer of the biofilm became aged, large batches of biofilm would slough from the surface of the carrier. To successfully evaluate the effects of flow rate and light on the characteristics of biofilm, sampling should be operated before the batch sloughing occurs. The biofilm attached to all carriers in the reactors was observed in Fig. 3.

3.2. Variations of biofilm characteristics with reactor depth

The combined effects of flow rate and light on biofilm were evaluated by measuring and analyzing several characteristics of biofilm in the four reactors. To better understand these factors, the difference of the characteristics (total amount, EPS, active biomass and microbial activity) of biofilm with respect to the depths of each reactor was identified and shown in Figs. 4–7, respectively. In each reactor, the four aforementioned characteristics all decreased as the depths decreased. This observation may be attributed to the decrease of the nutrient content level along the flow direction of the

medium in each reactor. When the medium in each reactor was fed through the carriers in series, the nutrients were absorbed by or utilized by biofilm and the content level decreased. In addition, the decrease of nutrient content, which could be verified by monitoring variations in COD along the flow direction [21], and thus prevented the growth of biofilm.

3.3. Effects of flow rate and light on total amount of biofilm

As shown in Fig. 4, there were significant combined effects of light and flow rate on the total amount of biofilm cultivated in the four reactors. The total amount of biofilm in DW ($g \cdot kg^{-1}$ carrier) increased with an increase in flow rate. The average DW of total amount of biofilm in R1, R2 and R4 were 2.38, 1.84 and 1.44 times that of R3, respectively, and the total amounts of biofilm in R4 was 25–55% higher than that in R3 at the same depths.

The primary reason for the increase in total amount of biofilm with greater flow rates was likely due to an enhanced supply of nutrients during the culture period. More nutrients facilitates the growth and reproduction of organisms colonized in the biofilm. A higher flow rate also resulted in a higher shear force and increased turbulence, which was helpful for diffusion of diffusion of nutrients into the biofilm [12]. The high flow rate can be also beneficial for the biofilm to secrete more EPS, which promotes cell cohesion of organisms and nutrient adsorption in the biofilm [28–30]. However, it should be noted that a higher flow rate also led to some unfavorable effects on the formation of biofilm, such as a decrease in mass transfer and a more compact and denser biofilm that hinders the diffusion of nutrients [12,31].

The reason of higher total amount of biofilm in R4 than in R3 at the same depths was probably due to the artificial lighting. The light made phototrophic organisms and algae photosynthesize efficiently, which provided the organisms in biofilm with nutrients and fueled processes and conversions in total biofilm community [32]. The light also spurred photosynthetic organisms and algae to secrete more EPS, which enhanced the attachment of biofilm community and increased the density of organisms within a given area of substratum [33,34]. As a result, the light effectively promoted the growth of biofilm.

3.4. Effects of flow rate and light on EPS production

A matrix of extracellular polymeric substances secreted by phototrophs and heterotrophs can enhance the attachment of biofilm and facilitate its formation [31]. Fig. 5 showed that EPS production increased as the flow rate increased with corresponding depths. The trend of EPS production ($mg \cdot g^{-1}$ carrier) with increasing flow rate was similar to the trend observed for the total amount of biofilm. The average EPS production in R1, R2, and R4 was approximately 1.96, 1.48, and 1.66 times that of R3 at an identical depth (Table 3), respectively. The production of EPS in R4 was significantly higher than that of R3 and was even higher than that of R2 above the depth of 100 cm. The effect of light on EPS production was apparent. Table 2 showed the contents of polysaccharide and protein, and the ratio of polysaccharide/protein (PS/PN) that there was much more polysaccharides than protein in the biofilm. The average ratio of PS/PN in R1, R2, R3, and R4 were 5.72, 4.71, 3.19, and 2.86, respectively; the ratio decreased as the flow rate decreased, and the average ratio in R4 was lower than that produced by R3.

As described before, a higher flow rate produced more total amount of biofilm ($g \cdot kg^{-1}$ carrier) that excreted more EPS proportionately. A higher shear force resulting from a higher flow rate might also have spurred microorganisms in the biofilm to secrete more EPS, as was observed by a shear force-associated phenomenon about biofilm [29,30]. More EPS absorbed more nutrients from the bulk medium, which promoted the reproduction

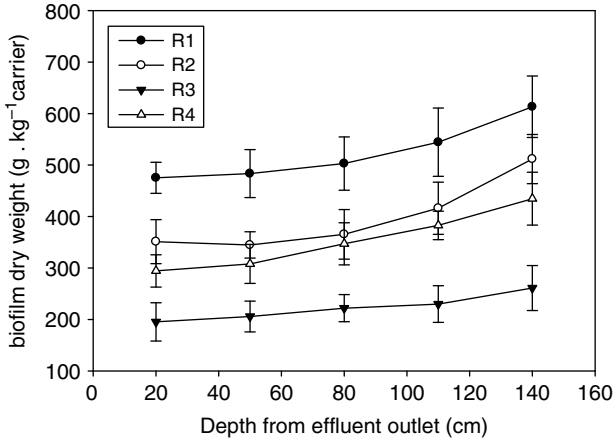

Fig. 4. Total amount of biofilm by DW.

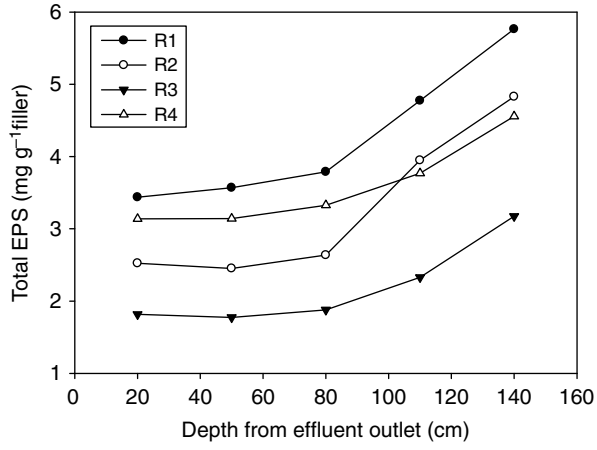

Fig. 5. EPS productions at different depths.

Table 2
Polysaccharides and protein contents in biofilm at different depths

Reactors	Depth from effluent outlet (cm)	Polysaccharides ($mg \cdot g^{-1}$ carrier)	Protein ($mg \cdot g^{-1}$ carrier)	PS/PN
R1	20	2.903 ± 0.387[a]	0.535 ± 0.087	5.43
	50	3.011 ± 0.584	0.558 ± 0.172	5.40
	80	3.202 ± 0.271	0.587 ± 0.118	5.45
	110	4.099 ± 0.406	0.676 ± 0.246	6.06
	140	4.970 ± 1.142	0.794 ± 0.169	6.26
R2	20	2.032 ± 0.372	0.491 ± 0.095	4.14
	50	1.968 ± 0.159	0.484 ± 0.252	4.07
	80	2.108 ± 0.496	0.529 ± 0.130	3.98
	110	3.359 ± 0.683	0.590 ± 0.243	5.69
	140	4.104 ± 1.354	0.726 + 0.194	5.65
R3	20	1.350 ± 0.164	0.466 ± 0.126	2.90
	50	1.329 ± 0.230	0.445 ± 0.230	2.99
	80	1.375 ± 0.554	0.504 ± 0.104	2.73
	110	1.829 ± 0.504	0.499 ± 0.148	3.67
	140	2.496 ± 0.435	0.678 ± 0.275	3.68
R4	20	2.352 ± 0.273	0.885 ± 0.096	2.66
	50	2.135 ± 0.512	0.907 ± 0.352	2.35
	80	2.401 ± 0.485	0.926 ± 0.164	2.59
	110	2.834 ± 0.835	0.934 ± 0.236	3.03
	140	3.576 ± 0.957	0.981 ± 0.151	3.65

[a]Results are presented as average plus standard deviation. For instance, the number 2.903 ± 0.387 denotes that the average is 2.903, and its standard deviation is 0.387.

and growth of microorganisms in biofilm, resulting in further increases in EPS production. From Table 2, it is apparent that polysaccharide to protein ratio in all four reactors increased upon elevation of the flow rate at the same depths, which is consistent with work conducted by Liu and Tay [12].

Light also had an important impact on EPS production in the biofilm. Light can facilitate algae and photoautotroph growth [16]. EPS production tightly correlates with photosynthesis and increases with additional light exposure up to a certain limit [35,36]. Light also promotes algae and other photoautotrophs to supply more nutrients to microorganisms in biofilm, which could effectively promote the microorganisms to excrete more EPS. The polysaccharides and protein content in R4 was higher than that of R3 at the same depth.

Furthermore, when comparing the ratios of PS/PN for R4 and R3 at the same depths, it can be concluded that light has a larger impact on the production of protein than on polysaccharide production (Table 2). This indicated that light provides a greater stimulus for microorganisms to produce protein than polysaccharides. However, the mechanisms behind this phenomenon remain elusive.

3.5. Effects of flow rate and light on active biomass

Phospholipids presented on bacterial membrane up to 90–98% do not form the cell reserves and are easily degradable during bacteria lysis and their measurement can be used to estimate the active biomass [37]. The profiles of active biomass (nmol $P \cdot g^{-1}$ carrier) from the four reactors illustrated that active biomass was strongly affected by flow rate and light (Fig. 6). The average amounts of active biomass in R1, R2, and R4 were 2.64, 1.95 and 1.73 times that of R3, respectively, which was consistent with the average increase of total biomass across reactors. A difference of describing the total amount of biofilm is that the value includes not only active biomass but also inactive mass such as EPS and substances absorbed on flocs and biofilm [38]. Measuring the active biomass is a more suitable parameter than the total amount of biofilm when evaluating the total biomass in biofilm.

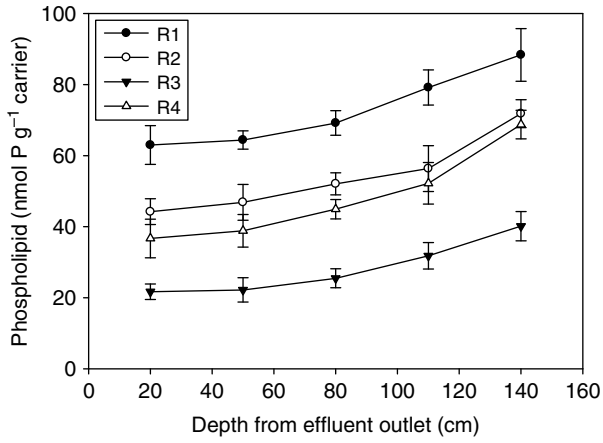

Fig. 6. Active biomass (phospholipids).

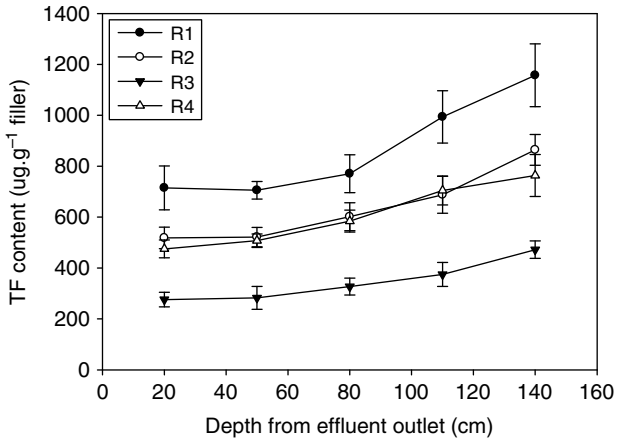

Fig. 7. Microbial activities by TF.

Because active biomass is one of the main components of total amount of biofilm and is proportional to this total amount, the effects of flow rate and light on total amount of biofilm are applicable to the active biomass.

3.6. Effects of flow rate and light on microbial activity

One potential method of readily monitoring the metabolic activity of biofilm is measuring dehydrogenase activity [39]. Fig. 7 shows the profiles of microbial activity by TF content (μg·g^{-1} dry carrier) across a series of depths for the four reactors. The microbial activity was enhanced by higher flow rates and the presence of light. The average TF contents in R1, R2, and R4 were found to be 2.51, 1.85, and 1.76 times that of R3, respectively. The microbial activity in R4 was significantly higher than that of R3 and approximated that the activity seen in R2 at the same depth. The microbial activity at a depth of 110 cm in R4 amounted to 704.87 μg·g^{-1} dry carrier, which was even higher than that at an identical depth in R2, which was 687.55 μg·g^{-1} dry carrier.

It has been reported that microbial activity typically correlates with microbial biomass [16]. Mei et al. [40] discovered that there is a significant correlation between the dehydrogenase activity and biomass. A correlation between biomass and microbial activity was also found in this study. As indicated in Table 3, the average ratios (R1/R3 and R2/R3) of total amount of biofilm and active biomass approached that of microbial activity.

Nevertheless, the average ratios (R4/R3) of total biomass and active biomass were lower than microbial activity (Table 3). This result was highly probably caused by the artificial lighting in R4. In the presence of light, algae and phototrophic microorganisms in biofilm may be encouraged to excrete more extracellular dehydrogenases [41], which was confirmed by Romaní et al. [42] that the biofilm formed in the presence of light have higher microbial activity than biofilm formed in the dark.

3.7. Combined effects of flow rate and light on the characteristics of biofilm

In-situ remediation of polluted water using the biofilm method requires an understanding of the combined effects of flow rate and light for the purposes of theoretical research and practical application. In this study, four

Table 3
Average ratios of characteristics of biofilms in four reactors

Ratio	Average ratio			
	Total amount of biofilm (DW) (g·kg^{-1} carrier)	EPS (mg·g^{-1} carrier)	Active biomass (Phospholipids) (nmol P·g^{-1} carrier)	Microbial activity (TF) (μg·g^{-1} carrier)
R1/R3	2.38[a]	1.96	2.64	2.51
R2/R3	1.84	1.48	1.95	1.85
R4/R3	1.44	1.66	1.73	1.76

[a]The average ratio of total amount of biofilm (DW) 2.38 denoted the average of ratios (R1/R3) of total amount of biofilm (g kg^{-1} carrier) at five depths of R1 and R3.

culture reactors were subjected to the identical conditions except for flow rate and light. As described above, the evidence indicated that the characteristics of biofilm, including the parameters of total amount of biofilm, EPS, active biomass and microbial activity, are enhanced by elevating the flow rate and the presence of light.

A higher flow rate may accelerate biofilm growth by encouraging more nutrients to flow through the carriers thus providing nourishment to organisms in the biofilm and accelerate their reproduction and growth. An increased amount of biofilm may result in additional EPS excretion that can absorb or adhere to many more nutrients from the bulk solution to supply to organisms in biofilm. Concomitantly, light can promote biofilm growth. Under illumination, the algae and photoautotrophs can excrete more extracellular substances such as EPS, which may absorb or adhere to more nutrients and ultimately promote the reproduction and growth of heterotrophic and autotrophic microorganisms. Thus, a combined effect of flow rate and light on biofilm characteristics was achieved. Considering the mutual promotion of flow rate and light on biofilm formation, it is easy to understand the combined effects of two conditions on the characteristics of biofilm.

It is significant to note that an increased flow rate and the presence of light will facilitate the formation and activity of biofilm within a certain ranges of the two treatments, but these manipulations will limit or even inhibit biofilm formation and its activity when applied beyond this range. Specifically, a lower flow rate would have a negative effect on the attachment of the biofilm to the carriers, whereas a higher flow rate would result in the detachment of the biofilm and a thinner or denser biofilm [11]. Similarly, a lower light intensity may not benefit the growth and excretion of biofilm, but an excessive light intensity may result in high temperatures that restrain the metabolism and reproduction of algae and photoautotrophs. Further research is needed to determine the correct ranges of flow rate and light intensity for the actual application of the biofilm method.

4. Conclusions

Four identical biological reactors using three-dimensional elastic materials as carriers were employed in this study to cultivate biofilm at a variety of flow rates and under light (photic) and dark (aphotic) conditions.

Under dark conditions, the total biomass, EPS, active biomass and microbial activity of biofilm subjected to flow rates of 0.4 and 0.8 $m^3 \cdot m^{-2} \cdot s^{-1}$ were, on average, 2.38 and 1.84, 1.96 and 1.48, 2.64 and 1.95, and 2.51 and 1.85 times that at the flow rate of 0.2 $m^3 \cdot m^{-2} \cdot s^{-1}$, respectively. At the identical flow rate of 0.2 $m^3 \cdot m^{-2} \cdot s^{-1}$, under illumination of about 130 μ mol photons$\cdot m^{-2} \cdot s^{-1}$, the four characteristics of biofilm were, on average, 1.44, 1.66, 1.73, and 1.76 times that under dark conditions, respectively. The characteristics of biofilm were enhanced as flow rates increased and in the presence of light. Considering the mutual improvement of biofilm characteristics, the combined effects will be enhanced by higher flow rates and stronger light intensity.

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