Bioresource Technology 241 (2017) 54–62

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Determination of the profile of DO and its mass transferring coefficient in a biofilm reactor packed with semi-suspended bio-carriers

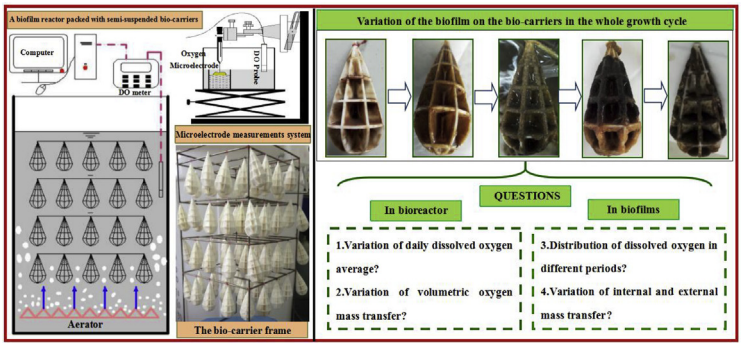
Bing Tang *, Haoliang Song, Liying Bin, Shaosong Huang, Wenxiang Zhang, Fenglian Fu, Yiliang Zhao, Qianyu Chen

School of Environmental Science and Engineering and Institute of Environmental Health and Pollution Control, Guangdong University of Technology, Guangzhou 510006, PR China

HIGHLIGHTS

- A novel semi-suspended bio-carrier was designed and used in a biofilm reactor.
- Profile of DO and its mass transfer coefficient in the reactor were determined.
- Biofilm is easier to attach and grow on the semi-suspended bio-carrier.
- The contents of EPS in the biofilm have a close relationship with the D_b of DO.

GRAPHICAL ABSTRACT



ARTICLE INFO

Article history:
Received 30 March 2017
Received in revised form 9 May 2017
Accepted 12 May 2017
Available online 16 May 2017

Keywords:
Semi-suspended bio-carriers
Biofilm
Dissolved oxygen
Mass transfer
Microelectrode

ABSTRACT

The work aims at illustrating the profile of DO and its mass transferring process in a biofilm reactor packed with a novel semi-suspended bio-carrier, and further revealing the main factors that influence the mass transferring coefficient of DO within the biofilm. Results showed that the biofilm was very easy to attach and grow on the semi-suspended bio-carrier, which obviously changed the DO profile inside and outside the biofilm. The semi-suspended bio-carrier caused three different mass transfer zones occurring in the bioreactor, including the zones of bulk solution, boundary layer and biofilm, in which, the boundary layer zone had an obvious higher mass transfer resistance. Increasing the aeration rate might improve the hydrodynamic conditions in the bioreactor and accelerate the mass transfer of DO, but it also detached the biofilm from the surface of bio-carrier, which reduced the consumption of DO, and accordingly, decreased the DO gradient in the bioreactor.

1. Introduction

As a commonly used bio-process, biofilm reactors have long been used to treat both industrial and domestic wastewater. Actually, the successful operation of a biofilm reactor totally depends on the formation of stable biofilms on the surface of bio-carriers (or bio-packings), and the basic factors to form sufficient biofilm include the suitable operational conditions and the habitat for the biofilm to attach on. The former are hydrodynamic conditions, oxygen and nutrients, which provide essential conditions for the survival of microorganisms, and the latter generally refers to bio-carriers, which provide necessary space for the microorganism to grow on as well as forming a well-defined microbial community with clear environmental function. In this regard, the bio-carrier is a crucial factor to determine the growth of microorganisms and further influence the performance of a biofilm process.

* Corresponding author at: No. 100, Waihuan Xi Road, Guangzhou Higher Education Mega Center, Guangzhou 510006, PR China.
E-mail addresses: renytang@163.com, tang@gdut.edu.cn (B. Tang).

http://dx.doi.org/10.1016/j.biortech.2017.05.071
0960-8524/

Traditional bio-carriers, so-called fixed biological packings, are generally fixed inside a reactor and have widely been used in the engineering fields of wastewater treatment. Though, many engineering applications have verified the success of fixed biological packing, its drawbacks are still obvious, including clogging and low mass transfer efficiency. For overcoming these drawbacks, a new kind of bio-carrier, suspended packing (or suspended bio-carrier), was widely investigated in recent years (Barwal and Chaudhary, 2014). Packed with such bio-carriers, a biofilm reactor, called moving bed biofilm reactor (MBBR), is composed, which has aroused lots of academic interests (Delnavaz et al., 2010; Piculell et al., 2016; Young et al., 2016). Previous investigation has proven the dominant merits of an MBBR including high biomass density, low head loss, and no clogging of bio-packing (Deng et al., 2016). However, for the hydrodynamic collision among bio-carriers, such disadvantages as the slow formation of biofilm on the surface and the abrasion among bio-carriers are still difficult to overcome during its start-up. Additionally, during the operation a biofilm reactor, the most essential factors include the supply of oxygen and nutrients as well as the removal of metabolite, which are heavily influenced by the mass transfer within or outside the formed biofilm. As it is well known, the formation and growth of biofilm are mainly restricted by the hydrodynamic conditions and the structure of the bio-carrier, and a reasonably structured bio-carrier is not only apt to be attached by the microorganisms for growing to a mature biofilm, but also can obviously improve the micro hydrodynamic conditions around a bio-carrier for stimulating the mass transfer. In this meaning, high-performance bio-carriers should be easier for the biofilm to attach on and are capable of improving the mass transfer, which are highly needed.

The value of dissolved oxygen (DO) is a limiting factor to determine the status of an aquatic environment, namely, an oxic, an anoxic, or an anaerobic status, and further influence the habitat of microorganisms and the biodiversity within an aquatic ecosystem. In each ecosystem, biodiversity is a crucial prerequisite to determine its ecological stability, and the mass and energy flow, thus, a multi-habitat environment is really needed to maintain the biodiversity no matter for a natural ecosystem, or a bioreactor with the purpose of treating the wastewater containing organic pollutants and nutrients (N and P) (Tang et al., 2014), but it is also influenced by the concentration of DO. In a biofilm reactor, the mass transfer of DO is influenced by many factors, including the concentration of biomass, aeration rate, hydrodynamic conditions, and characteristics of biofilm. For quantitatively describing the mass transfer of DO, two parameters, oxygen uptake rate (OUR) and oxygen mass transfer rate (OTR), are commonly used. The former refers to the rate of consuming DO by microorganisms, and the latter is generally expressed by the volumetric oxygen mass transfer coefficient (k_La) (Tang et al., 2015), which is very convenient to illustrate the difficulty of transporting DO in a bioreactor.

High-performance bio-carriers and suitable distribution of DO are two crucial factors for each biofilm process. Based on these analyses, it is necessary to make an improvement to overcome the shortcomings of traditional fixed bio-packing or suspended bio-carrier, therefore, a novel semi-suspended bio-carrier was designed in the laboratory. This kind of bio-carrier was designed to be a spindle shape, whose smaller end was fixed on a frame, and its larger end could be moved freely by the flowing of water and gas in a bioreactor. Such a configuration effectively avoids the collision and friction among bio-carriers, which is beneficial for the microorganism to attach on and grow, and also improves the mass transfer conditions within the bioreactor. However, due to its quite complex structure and shape, it cannot be accurately made with a traditional molding method, but can be easily made with a 3D printing technique. To the best knowledge, arranging bio-carriers in a semi-suspended status is a totally new idea, and this is the first case to fabricate a semi-suspended bio-carrier with a 3D printing technique.

Mass transfer and distribution of oxygen is of primary importance for any kind of biofilm reactors, in which bio-carriers play a crucial role to determine the hydrodynamic conditions and further influence the profile of DO. For testing the performance of this novel bio-carrier, the object of this work is to investigate the distribution and mass transfer of DO in a biofilm reactor packed with the semi-suspended bio-carrier, and further to reveal its main influencing factors, thus, two closing related aspects are mainly considered: (1) measuring the DO value inside the formed biofilm with a microelectrode and quantitatively describe its profile within the biofilm; (2) comparing the differences of the mass transfer of DO in three zones occurring in the bioreactor, including the bulking solution, the boundary layer and inside the biofilm, as well as exploring which factors are the most dominant factor to influence the mass transfer within the bioreactor.

2. Materials and methods

2.1. Experimental configuration and process

A semi-suspended bio-carrier with spindle-shape was designed, and made with a 3D printing technique. The details of the bio-carrier are listed in Table 1, and its real photo is shown in the Supporting Information (SI, Fig. S1(a)). With a volumetric ratio of 32% ($V_{bio\text{-}carrier}/V_{reactor}$), a bioreactor packed with this kind of bio-carrier was used to carry out all the experiments. The used bioreactor was a cylindrical shape with a working volume of 27.7 L (50 × 30 cm) (Fig. S1(b)). A metal frame (20 × 20 × 40.5 cm) was installed inside to fix the semi-suspended bio-carriers. For insuring a 32% volumetric ratio, the frame was arranged to be four layers in its vertical direction with each layer binding 25 bio-carriers (Fig. S1(c)). A circinate aeration trachea was evenly fixed under the frame to blow air bubbles for providing DO. During the start-up period (day 1–5), the bioreactor was operated in an intermittent aeration mode with a 24 h operational cycle. The whole cycle, which included 3 phases, namely, 15 min feeding, 23.5 h aeration, and 15 min drainage, was accurately controlled by an automatic time relay. After a stable biofilm has formed on the surface of bio-carrier, the aeration mode was changed to a continuous mode with 1.5 L/min aeration rate, and 56 days later, the aeration rate was increased to 2.5 L/min.

The original inoculation sludge was collected from the secondary sedimentation tank of a local municipal wastewater treatment plant (WWTP) (Lijiao, located at Haizhu district, Guangzhou, China), with the initial inoculation concentration being 1800 mg/L MLSS. The feed solution was synthetic wastewater, which was prepared by dissolving chemically pure glucose (187.36 mg/L), NH_4Cl (76.36 mg/L), KH_2PO_4 (8.75 mg/L) and other trace nutritive salts ($MnCl_2{\cdot}4H_2O$: 0.28 mg/L, $MgSO_4{\cdot}5H_2O$: 40.6 mg/L, $CuSO_4{\cdot}5H_2O$: 0.39 mg/l $CaCl_2$ 29.9 mg/L, $FeSO_4$ 2.49 mg/L) into tap water with

Table 1
Details of the used semi-suspended bio-carrier.

Material	Shape	Size	Surface area (m^2/m^3)	Packing fraction (n/m^3)	Density (g/cm^3)
Polyols: Isocyanate = 2: 1	Spindle-shape	Cone (4.3 cm height, 3.0 cm diameter) half sphere (3 cm diameter)	10.49	58139	1.06

Table 2
Aeration mode and rate at each operational stage.

Operational stage	Aeration mode	Aeration rate (L/min)
I (1–5 day)	Intermittent	1.5 L/min for 30 min, stop for 30 min
II (6–56 day)	Continuous	1.5
III (57–73 day)	Continuous	2.5

the ratio of COD: N: P setting at 100: 10: 1. After aeration for 24 h, the reactor was fed with the prepared wastewater. On the 6th day, an obvious layer of biofilm formed and attached onto the surface of bio-carrier, then, the excessive floc sludge was discharged from the reactor. The detailed parameters during the whole experimental period are shown in Table 2.

2.2. Analysis methods

Regular indexes of water quality, including COD_{Cr}, NH_3-N, NO_2^--N, NO_3^--N, TN and volatile solids (VSS), were all measured according to the standard methods (APHA et al., 2005). The procedure to measure the total solids (total biomass) on the surface of bio-carrier was illustrated in detail in SI. The value of DO in the bulk solution of reactor was automatically measured every 10 s by a DO probe (JPSJ-605F, INESA, China) installed above the aerator of 40 cm, and the obtained DO data were processed and stored by a computer. The values of pH were measured by a pH meter (pH2-S, INESA, China).

2.3. Extracellular polymeric substances (EPS) extraction and analysis

Biofilm samples, after measuring the DO profile, were stripped from one bio-carrier, and accurately weighed, then put into a tube to form 50 mL suspension by adding deionized water. After thoroughly mixing, 15 mL suspension was used to measure the content of VSS, and the rest 35 mL was used for EPS extraction. The procedure of extracting EPS was performed by citing a published reference (Nath and Chand, 1996) with the following procedures: (1) the suspension was first treated by an ultrasonic reactor at 20 kHz and 40 W for 30 s, then centrifugated at 4 °C and 2000×*g* for 15 min, the obtained supernatant was termed as soluble EPS (S-EPS); (2) the pellet in the tube was re-suspended into deionized water to 35 mL, then, 0.06 mL formamide (37%) was added into the suspension at −4 °C and the suspension was horizontally vibrated in an orbital incubator (20–30 rpm) for 1 h, after being centrifuged at 5000×*g*, 15 min, 4 °C, the supernatant was collected as loosely-bound EPS (LB-EPS); (3) the residual solids in the tube were re-suspended again with phosphate buffer to 35 mL, and then they were dealt with ultrasound (20 kHz, 120 W) for 6 min, finally, the tightly-bound ESP (TB-EPS) could be obtained by centrifuging at 10,000×*g* for 15 min. All the suspensions were filtered through 0.22 μm filters to collect the soluble fractions. The extracted samples were stored at −20 °C until the analysis was performed. The concentrations of EPS in each extract were analyzed with the anthrone method (Gaudy and Gaudy, 1962) with a glucose as standard, the modified Lowry method (Frølund et al., 1995) with a bovine serum albumin and humic acid as the standard, and the diphenylamine citric method (Sun et al., 1999) with a calf thymus DNA as the standard, respectively.

2.4. Determination of the volumetric oxygen transfer coefficient in the bioreactor

Rational distribution and rapid transfer of DO are key factors to each aerobic process. Generally, the volumetric mass transfer coefficient, a combined parameter of the gas-liquid mass transfer coefficient (k_L) and gas-liquid contact area (a), is used to quantitatively describe the oxygen mass transferring ability in the process. Considering the consumption of DO by microorganisms, a mass balance equation of DO in water can be expressed with the following equation:

$$\frac{dc}{dt} = k_L a_{(T)}(C^* - C_L) - x \cdot q_{O_2} \quad (1)$$

where $k_L a_{(T)}(C^* - C_L)$ and $x \cdot q_{O2}$ are the volumetric OTR and OUR, respectively. The volumetric mass transfer coefficient, was determined by the dynamic method described in a published reference (Garcia-Ochoa and Gomez, 2009). During the period of starting-up from the 2nd to 5th day, a sequencing batch operation was adapted (alternatively aerating and stopping for every 30 min). At about 8: 30 of each morning, the aeration was stopped, and after 5 min, drained the supernatant, then pumped fresh feed solution, at 9: 00, started to aerate and recorded the DO concentration continuously. On the 6th day, floc sludge was discharged from the reactor, and the operation mode was changed from a sequencing batch to a continuous operation, then DO in the reactor could be considered to be only consumed by the attached biofilm. At 8: 30 of each morning from the 7th to 73rd day, stopped the aeration till DO concentration declined to 0.5 mg/L, then re-started the aeration, during the period, the DO concentration was continuously recorded. With the above procedure, DO profile was obtained during this period, and the volumetric mass transfer coefficient was calculated with the following method.

After the air is turned off, the value of DO gradually declines, and Eq. (1) can be simplified to:

$$\frac{dc}{dt} = -x \cdot q_{O_2} \quad (2)$$

Plotting the measured data DO vs. time, and obtained a regression line, with which the value of OUR can be calculated from its slope. When the air inlet is reopened, Eq. (1) can be integrated from the starting time point of aeration ($t = t_1$, so $C_x = C_1$) to the second time point ($t = t_2$, so $C_x = C_2$), then, obtaining Eq. (3):

$$x \cdot q_{o_2} \cdot \Delta t + C_2 - C_1 = k_L a_{(T)} \cdot \int_{t_1}^{t_2} (C_0 - C_x) \cdot dt \quad (3)$$

The volumetric oxygen mass transfer coefficient can be obtained by solving Eq. (3) for each datum of the measured DO vs. the operational time. The operating temperature (T) of the reactor was measured every morning, and for the convenience of comparison, the obtained values of $k_L a_{(T)}$ were all transformed into the volumetric mass transfer coefficient at 20 °C with the following empirical equation (Stenstrom and Gilbert, 1981).

$$k_L a = k_L a_{(T)} \cdot 1.024^{(20-T)} \quad (4)$$

where $k_L a$ (min^{-1}) is the volumetric mass transfer coefficient at 20 °C, and $k_L a_{(T)}$ is the oxygen mass transfer coefficient at temperature T.

2.5. Determination of the effective diffusion coefficient DO in biofilm

DO profiles in biofilm obtained by the microelectrode system were used to calculate the boundary of biofilm, whose details were explained in SI. In the bulk solution, the continuously blowing air and the flowing water created a totally mixing status, which provided quite a suitable condition for the mass transferring of DO, thus, in the bulk solution, DO could be regarded as evenly distributed at the same height in the bioreactor. However, for the limitation of mass transfer and the rapid consumption of DO by the microorganism within biofilm, the DO concentration might decline very quickly, and the turning point in the DO curve could be taken

as the cutoff point of different zone of mass transfer within the biofilm (the boundary of a mass transfer area).

(1) Internal effective diffusion coefficient

Based on the Fick's diffusion law and the mass balance of DO within a biofilm, a mass transfer equation (Eq. (5)) can be obtained to express the internal effective diffusion coefficient in biofilm:

$$\partial C(z,t)/\partial_t = D_b \cdot \partial^2 C(z,t)/\partial z^2 - R(z) \quad (5)$$

where $C(z, t)$ is the concentration of DO ($mg{\cdot}L^{-1}$) at time t (s) and depth z (m) in a biofilm, D_b ($cm^2{\cdot}s^{-1}$) is the effective diffusion coefficient in the biofilm, and $R(z)$ denotes the net specific metabolic rate at depth z. After having reached a steady-state, the distance interval for measuring the DO micro-profile in the biofilm was adjusted for slightly longer (20 μm or more) (Hou et al., 2014), and thus, $R(z)$ can be considered to be a constant (R) and calculated by Eq. (2). So Eq. (5) can be simplified to the following equation:

$$D_b \frac{d^2C}{dz^2} = R \quad (6)$$

The boundary conditions for the solution of Eq. (6) are:

$$z = l_1, C_1 = C_{ob} \quad (7)$$

$$z = l_2, dC/dx = 0 \quad (8)$$

where C_{ob} is the DO concentration (mg/L) at the interface of the bulk solution/biofilm, and l_2 is the biofilm depth (m) where DO concentration is zero. Therefore, D_b can be obtained by fitting the curve of DO profile measured by the microelectrode.

After the DO effective diffusion coefficient in the biofilm was determined by the obtained DO profiles as mentioned above, and the effective diffusivity was transformed to be a dimensionless parameter D_r with the following equation:

$$D_r = D_b/D_w \quad (9)$$

where D_r is the dimensionless effective diffusivity; D_b ($cm^2{\cdot}s^{-1}$) is the effective diffusivity of DO in biofilm; D_w is the molecular diffusion coefficient of DO in water (2.09×10^{-6} cm^2/s for O_2 at 20 °C) (Hou et al., 2014).

(2) External effective diffusion coefficient in the boundary layer

On the surface of the biofilm, the occurrence of boundary layer also creates a dominant mass transfer resistance on DO from the bulk solution to the biofilm. Based on the two-film theory and the mass balance analysis, the rate of DO mass transfer in boundary layers can be expressed by the following equation:

$$\frac{dc}{dt} = k_L a_i (C_b - C_s) = \frac{D_i}{Lc} \cdot a_m (C_b - C_s) \quad (10)$$

where k_La_i (min^{-1}) is the volumetric mass transfer coefficient in boundary layer; C_b ($mg{\cdot}L^{-1}$) is the DO value in bulk solution; C_s ($mg{\cdot}L^{-1}$) is the DO value at the surface of biofilm; D_i ($cm^2{\cdot}s^{-1}$) is the effective diffusion coefficient in boundary layer; L_c (m) is the thickness of boundary layer (calculated with the method introduced in SI); a_m (cm^2) is the external surface area for mass transfer and can be calculated according to a cited reference (Nath and Chand, 1996).

3. Results and discussion

3.1. Formation, growth of the biofilm on the bio-carrier

Commonly-used suspended bio-carriers, such as round polythene carriers and cylindrical Kaldne series, are the main media employed in traditional MBBRs for wastewater treatment. For the drastic disturbance aroused by the aeration and the flowing water, the friction among bio-carriers obviously increases, which leads to a great difficulty in forming a stable biofilm on bio-carriers (Tang et al., 2016). The used bio-carrier in the present investigation is a semi-suspended bio-carrier with a spindle-shape (Fig. S1(a)), only one end is fixed on the frame, but the other end can float freely in water. In such a status, a layer of biofilm quickly formed on the surface of the bio-carrier. At a certain time interval, the bio-carriers were taken out from the reactor to observe the formed film with the results shown in Fig. S2.

Comparing with traditional moving bio-carriers, the specific structure and the semi-suspended status (Fig. S2(a)) of the used bio-carrier made it easier for the inoculated activated sludge to attach on and grow. Operating for only 6 days, a thin layer of biofilm with brown color successfully attached and grew on the surface of the bio-carriers (Fig. S2(b)). After 15 days, the thickness of the biofilm grew into 350 μm and the color became from maroon to black (Fig. S2(c)). Continuously operating the bioreactor for 45 days (Fig. S2(d)), the color of the biofilm totally turned into dark black. The apparent change in color of the biofilm signified the variation of microbial community in its amounts and species. On the 72nd day (Fig. S2(e)), some of the biofilm detached from the bio-carrier, which indicated the biofilm gradually became aging, which lost its adhesive strength to attach on the bio-carrier.

3.2. Performance of removing oxygen-consuming substances by the bioreactor

Oxygen-consuming substances, including some organics and N-containing substances, generally have an important effect on the distribution of DO in a bioreactor. For detecting their variation in the concentration along with the operational time, these substances were measured everyday with the results shown in Fig. 1.

On the 6th day, a layer of biofilm attached on the bio-carrier and grew very rapidly, then the excessive floated sludge was discharged from the bioreactor. After that day, these oxygen consuming substances can be considered to be only consumed by the microorganism in the biofilm. From the obtained results shown in Fig. 1(a), the COD removal reached a high percentage (>80%) after a very short time of fluctuation during the first 10 days, and then, it increased to more than 90% in no more than 10 days and kept stable for another 44 days, only at the last stage (the 65th–73rd day), the COD removal gradually declined to around 60%. COD removal is a rough parameter to reflect the bioactivity of the microorganisms in the bioreactor, and the decline in the COD removal implies the microbial communities in the bioreactor fall into a decaying growth phase.

Ammonia is a common kind of oxygen-consuming substances, which can be easily oxidized to nitrite first and then quickly to nitrate under an aerobic condition. In Fig. 1(b), the concentration of ammonia was at around 2–5 mg/L from the start-up to the end of the experiment, but the concentration of nitrite always kept at a rather low value, which demonstrated that the nitrifying bacteria accumulated very quickly in the reactor to satisfy the requirement of converting ammonia and nitrite. Nitrate is very stable in an aerobic environment, which needs an alternative aerobic-anaerobic condition to be totally converted to nitrogen gas by denitrifying bacteria. However, denitrifying bacteria grow so slowly and need a long generation cycle to accumulate to a sufficient concentration for totally removing nitrate. In Fig. 1(c), the removal of TN increased constantly and stably from the initial stage to the 55th day, and then decreased slowly, which coincided with the growing trend of the biofilm. Under the presented experimental conditions, the total removal of TN implied a combining aerobic-anaerobic condition had formed within the bioreactor.

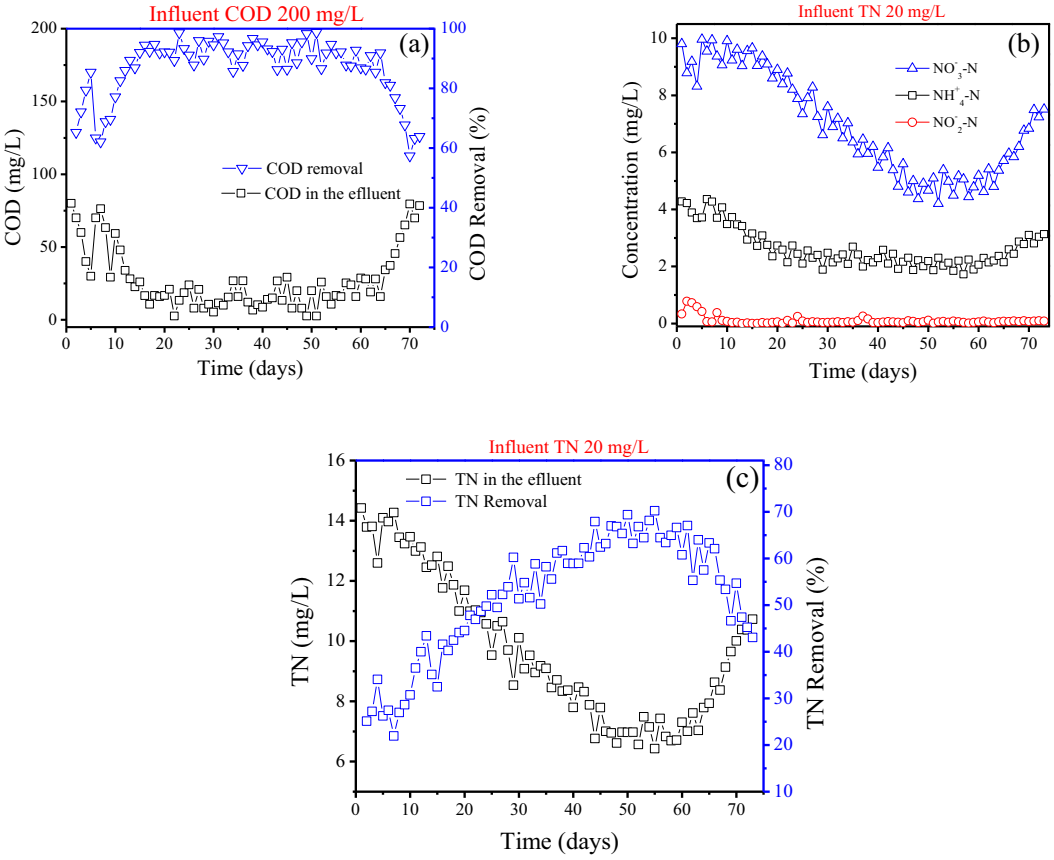


Fig. 1. Variation of oxygen-consuming substances: (a) the concentration of organics in the effluent and its removal efficiency; (b) the concentration of NH_3-N, NO_2^--N and NO_3^--N in the effluent; (c) the concentration of TN in the effluent and its removal efficiency.

3.3. Variation of EPS in the biofilm during the entire operational period

EPS have a quite complex composition and structure, they are secreted by the microorganisms during their metabolic activities, and always play an important role in maintaining the structure, strength and filterability of biofilms (Chen et al., 2013; Salama et al., 2015). Based on the relationship with the wrapped microbial communities, EPS can be roughly classified into three categories, including TB-ESP, LB-EPS and S-EPS, and the main components contained in them include proteins, carbohydrates, DNA, and humic acids. For assessing the variation of ESP along with the operational time, the above three categories and four main components were measured at a pre-set time interval after a stable and thick biofilm has formed on the surface of the bio-carriers, and the results are shown in Fig. 2.

After 15 days, a thick layer of biofilm formed on the surface of the bio-carriers, then the biofilm samples were collected to measure the contained EPS and their main components. As shown in Fig. 2(a), the total EPS include TB-EPS, LB-EPS and S-EPS, which increased to a peak (on the 55th day) and then declined. Among all kinds of EPS, TB-EPS accounted for the largest ratio and it basically determined the varying trend of total EPS; S-EPS occupied the second largest ratio, whose content in the biofilm increased gradually along with the operational time; LB-EPS was the least category of ESP, which almost kept unchanged during the whole period.

Proteins, carbohydrates, DNA, and humic acids are the main components in biofilms, but proteins and carbohydrates generally take up for more than 85% ratio (Baroutian et al., 2013). The results in Fig. 2(b) show that proteins are the highest content component, their ratio plus that of the contained carbohydrates almost exceed 90% in the measurement. DNA and humic acids are contained in a very small percentage in EPS, so the sum of proteins and carbohydrates determines essentially the profile of total EPS in the biofilm. In this meaning, TB-EPS, proteins and carbohydrates may be the main factors to determine the basic characteristics of biofilm, such as mass transferring.

3.4. Variation of DO concentration in the bioreactor over time

In an aerobic bioreactor, a detailed description about the DO distribution is very important for evaluating its performance. In the experiment, the DO concentration was continuously measured during the whole operational period, and for evaluating the impact of aeration rate and mode on the DO concentration, the aeration mode was adjusted on the 6th day from an intermittent to a continuous mode, and from the 57th day to the end of the experiment (the 73rd day), the aeration rate was increased from 1.5 L/min to 2.5 L/min (Table 2). The results are shown in Fig. 3.

The DO concentration in the bulk solution was automatically measured by a DO probe installed at the center of the bioreactor, and simultaneously, representative bio-carriers were taken out from the bioreactor every 5 days to measure the amount of the attached biofilm. The results in Fig. 3 show that the aeration rate and mode heavily influence the DO concentration. On the 6th day, the aeration mode was changed from an intermittent to a continuous mode, which caused the DO concentration increased instantly from 1.95 mg/L to 4.88 mg/L, while, on the 57th day, the aeration rate was improved from 1.5 L/min to 2.5 L/min, which also led to the DO concentration increasing from 2.21 mg/L to 3.22 mg/L. In addition, the amount of biomass on the bio-carrier is also an important factor to influence the DO concentration,

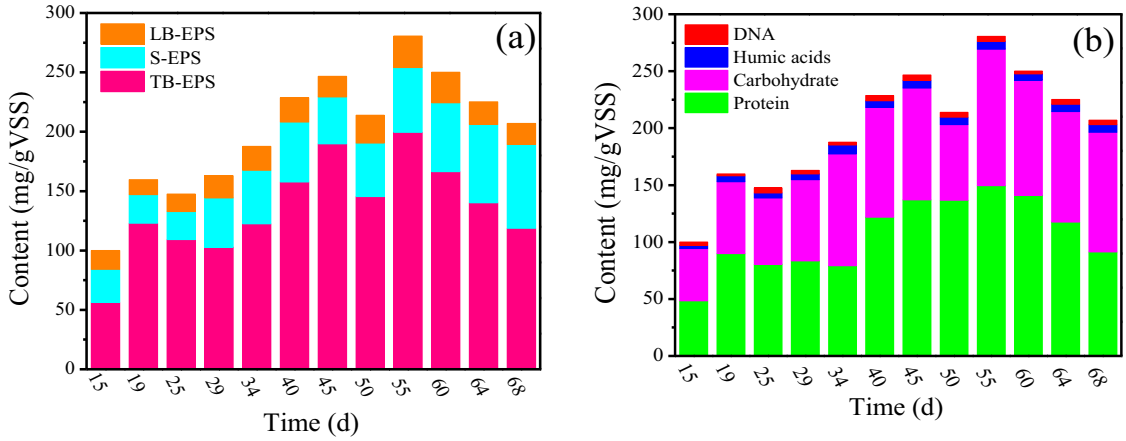


Fig. 2. Variation of EPS during the operating time: (a) Three categories of EPS in the biofilm; (b) Four main components of EPS in the biofilm.

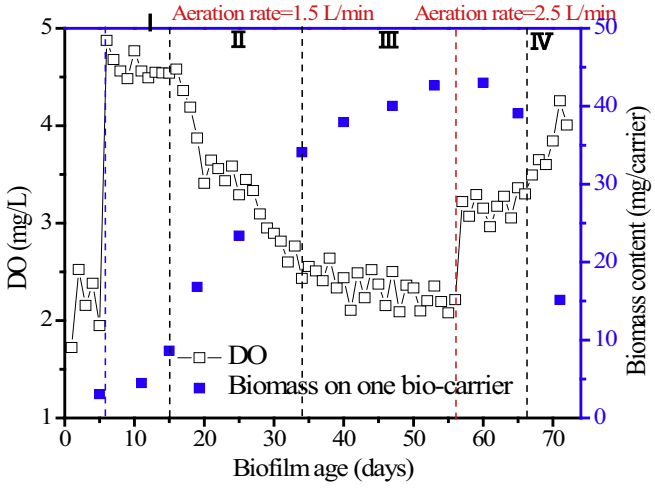


Fig. 3. Variation of DO value as the growth of biofilms in the bioreactor.

Fig. 3 shows the DO concentration decreased with the accumulation of biomass on the bio-carrier. However, after the 57th day, the biofilm gradually detached and resulted in the decreasing of the amount of biomass, and thus, the DO concentration increased accordingly.

3.5. DO profile within the biofilm

Biofilm is an aggregate of lots of microorganisms, which consumes most of DO in a biofilm reactor. However, due to the heterogeneous properties of the biofilm, the mass transferring resistance may exhibit a diversified influence on the profile of DO in the biofilm. This section aims at illustrating the DO profile within the biofilm at different time points. The DO profile within the biofilm was plotted by continuously measuring the DO concentration with a microelectrode system introduced in Section 2.4. At a pre-set time interval, a bio-carrier sample was taken out to measure the DO profile with the results shown in Fig. 4.

By setting the measuring step at 10 μm, the microelectrode system is capable of giving an accurate *in-situ* description of the DO profile within the biofilm. For making a comparison, on each sample, three points with 100 μm distance to each other was arranged to be an isosceles triangle (Fig. 4(a)) and selected to be the position for measuring DO concentration. In the early period (Fig. 4 (a) and (b)), a thin layer of biofilm with no more than 500 μm thickness was formed on the surface of bio-carrier, its relative homogeneous property did not cause any differences in the mass transfer of DO at the three points, thus, the curves at the three points were almost overlapped, and in such a thickness, DO could penetrate to the bottom of the biofilm to attain the concentration of around 1.5 mg/L. After 25 days, the thickness of biofilm grew thicker to more than 1000 μm, which gradually caused an obvious difference in influencing the mass transfer of DO. Fig. 4(c)–(i) show the DO profiles during the growing phase at the three positions, whose gradual deviation in the distance of the DO profiles indicates the heterogeneity in the characteristics of biofilm and may be the essential reason leading to an obvious difference in the mass transfer of DO (de la Rosa and Yu, 2005; Zhou et al., 2013).

After 55 days (Fig. 4(j)–(l)), some of the biofilms detached from the surface of bio-carrier, and the biofilm that still adhered to the surface exhibited a loose structure with lots of pores and channels (Ning et al., 2014), and the obviously heterogeneous properties caused a larger difference in the mass transfer. Nevertheless, an obvious DO gradient in the biofilm actually formed even the biofilm was still very thin on the surface, and under the presented experimental conditions, the maximum depth that DO could penetrate into the depth was no more than 900 μm, but the maximum thickness of the stable biofilm might reach to nearly 3 mm. In this meaning, a combining aerobic-anaerobic biofilm could be created on this novel semi-suspended bio-carrier, which was the fundamental reason to achieve a simultaneous nitrification-denitrification effect in a single bioreactor.

3.6. Variation of the mass transfer coefficient of DO

For evaluating the mass transferring performance of a bioreactor, a quantitative parameter is very necessary. k_La is a commonly used parameter to evaluate the difficulty of DO mass transferring, it also can be used to reflect the mass transferring performance of a bioreactor, and generally is an essential standard for comparing different bioreactors. During the experimental period, k_La was calculated everyday with Eq. (4) based on the measured data of DO. The results are shown in Fig. 5.

During the initial 5 days, the values of k_La as shown in Fig. 5 are very low. Such a low value indicated a poor mass transferring condition in this period. However, from the 6th day, the floc inoculated sludge was discharged from the bioreactor and the aeration rate was changed from an intermittent to a continuous pattern, which led to a sharply increasing in the value of k_La. Interestingly, as the biofilm grew to thicker (day 6–56) and the OUR increased to a larger value, k_La almost increased constantly, which was totally

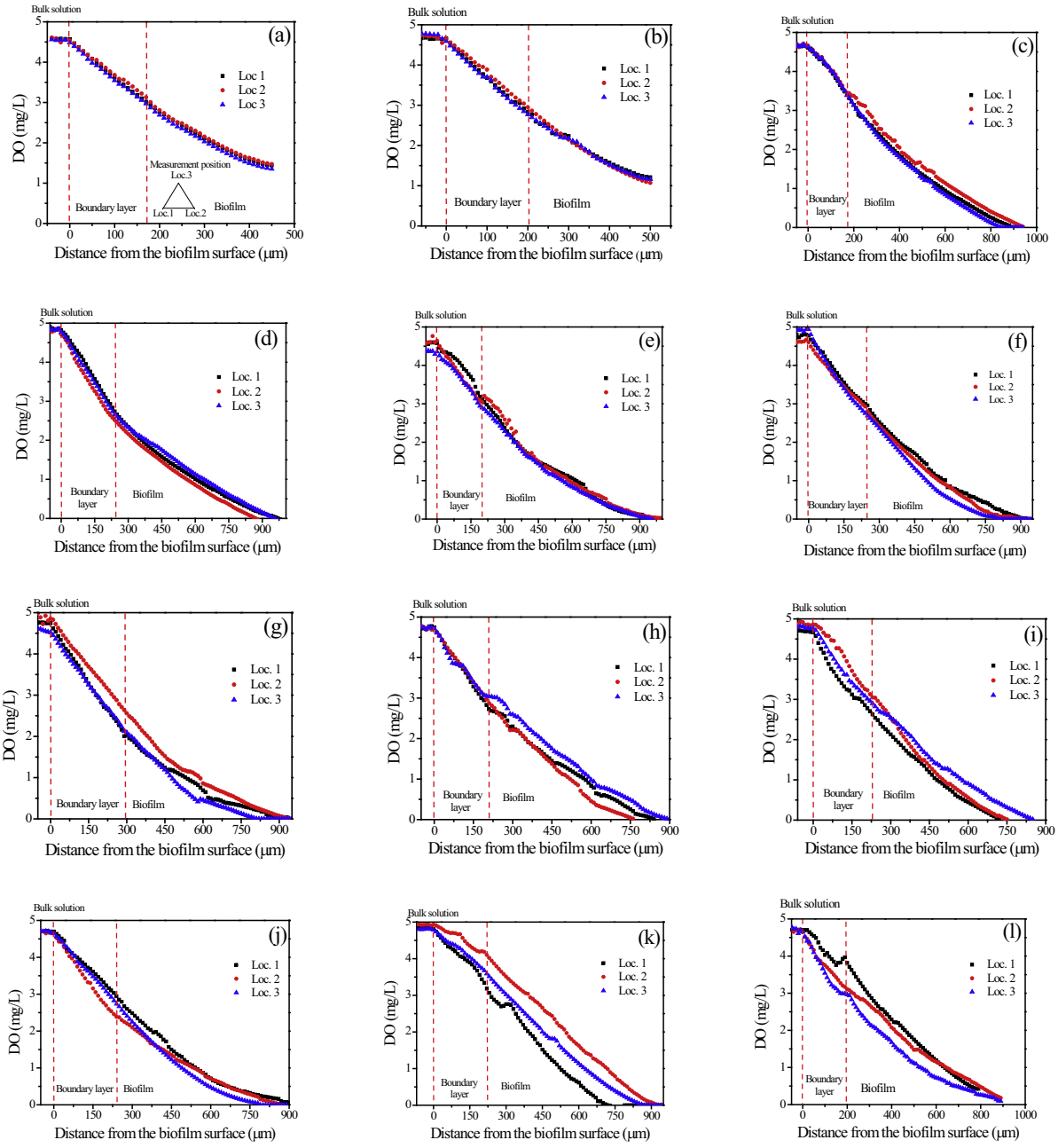


Fig. 4. DO profile within the biofilm at different time points: (a) 15 days; (b) 19 days; (c) 25 days; (d) 29 days; (e) 34 days; (f) 40 days; (g) 44 days; (h) 50 days; (i) 55 days; (j) 60 days; (k) 64 days; (l) 68 days.

opposite to that of a conventional activated sludge process and a membrane bioreactor (Germain et al., 2007; Tang et al., 2015). It is supposed that this special phenomenon may be caused by the following reasons: (1) the biomass grows mainly on the surface of bio-carrier, larger amount of biomasses consume more DO (as shown in Fig. 5, OUR increases constantly during this period), which creates a higher DO gradient between the surface of biofilm and the bulk solution, and promotes the mass transfer and improves k_La; (2) this novel semi-suspended bio-carrier moves freely and maximumly diminishes the suspended sludge, which effectively decreases the mass transferring resistance in the bioreactor.

From the 57th day, the aeration rate was adjusted to 2.5 L/min, k_La increased for a short time (no more than 5 days), then it decreased gradually. The increasing of aeration rate improved the hydrodynamic conditions in the bioreactor, and was one of the reasons to enhance the value of k_La. However, after the 60th day, some of the biofilm detached from the surface of bio-carrier (shown in Fig. 5), OUR begun to decrease, which implied the activity of biomass declined and decreased the consumption of DO. Less consumption of DO also reduced the DO gradient, and caused k_La decreasing accordingly. Actually, many factors, including the hydrodynamic conditions, sludge age, concentration of biomass and suspended sludge, temperature, and liquid viscosity (Jimenez et al., 2014), have different influences on k_La, but their combined effects may only be reflected by the DO gradient between the surface of biofilm and the bulk solution.

3.7. Mass transfer of DO in the biofilm and boundary layer

In a biofilm reactor, its whole mass transfer process includes the mass transferring in the bulk solution and in the biofilm,

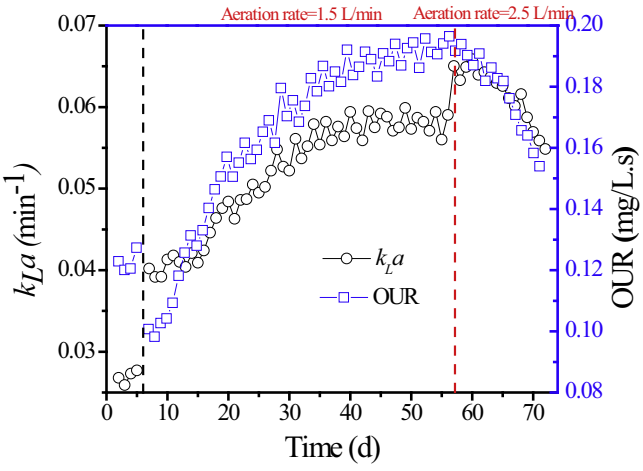


Fig. 5. Variation of k_La and OUR in the bulk solution.

respectively. In bulk solution, the main pattern of mass transfer is a convective way, in which the hydrodynamic conditions play an important role, while in the biofilm, the mass transfer pattern changes to a diffusive approach, and the diffusion process is determined by the basic characteristics of biofilm, including its density, porosity, pore size, thickness and chemical compositions. Additionally, a boundary layer, generally existing in a laminar flowing status, may occur between the bulk solution and the biofilm, which produces main resistances to limit the mass transferring. Therefore, the resistances in biofilms and their boundary layer should be considered as the main obstacle of mass transferring in the related process. With the method introduced in Section 2 and SI, the values of D_r at different periods were obtained and are shown in Fig. 6.

Comparing with the diffusivity in the bulk solution, DO is difficult to diffuse in biofilm for its higher mass transfer resistance, which always exhibits a lower value during the experimental period. From the 1st to 55th day, the diffusivity of DO in biofilm gradually declined to the lowest value, after that, the diffusivity of DO seemed to recover slowly. In contrast to the results of Fig. 2, the results in Fig. 6(a) imply that there is a contrary relationship between the DO diffusivity in biofilm and some characteristics of the biofilm. Therefore, for further revealing their relationship, the Pearson correlation method was used to correlate the relationship between the effective diffusion coefficient of DO in biofilm and the chemical components of the biofilm, and the results are shown in Table 3.

As shown in Table 3, different types of EPS and the chemical components in EPS have quite a different influence on the mass transfer of DO in biofilm. In biofilm, mass transferring is determined to a large extent by the mobility of water molecules. EPS generally surround living microbial cells to give them a protection and also act as food sources in a starvation period (Mori et al., 2006). Among all the factors in EPS, bound EPS and total EPS are the first two most important factors that have a negative influence on the mass transferring. In a published literature (Kapellos et al., 2007), a theoretical calculation indicated that the hydrogel in EPS acted as a diffusive barrier to the solute molecules approaching or leaving the surface of microbial cells, which created an actual mass transfer resistance in biofilm. As in bulk solution, the free water in biofilm is an important medium to promote the mass transfer of DO, but the hydrophilic groups in EPS bind the free water and heavily restrain the mobility of water molecules, which significantly decrease the diffusivity of DO in biofilm (Henkel et al., 2009). The difference in the content of hydrophilic groups and the density of biomass creates quite a different barrier to moving the free water in a biofilm, which shows an obvious negative influence on the diffusion coefficient of DO in biofilm. The values of D_i can be calculated by Eq. (10), and k_La_i is obtained by the method described in Section 2.

As shown in Fig. 6(b), the values of D_i keep stable around the value of D_w, which implies that the oxygen molecular diffusion coefficient in the boundary layer is rather close to that in water. Compared with that in the biofilm (Fig. 6(a)), stable diffusion (D_i) seems to occur in the boundary layer, and during the whole operational period, the diffusivity in the biofilm obviously declines till the aeration rate having been increased.

In the mass transferring process in bulk solution, the hydrodynamic conditions are the most important factors to affect the external mass transfer., while in the boundary layer, due to the laminar status, k_La_i is only determined by the molecular diffusion (Nogueira et al., 2015), and the values seem fluctuate in the range of 5–7 $\times$ 10^{-4} min^{-1}, which are two orders of magnitude smaller than that of in the bulk solution. Comparing with a traditional fixed biofilm reactor, the DO concentration seems have a similar profile (Guimerà et al., 2016), but have quite different values. Such results clearly signify that a major resistance still exists in the boundary layer of the biofilm attached on the used semi-suspended biocarrier.

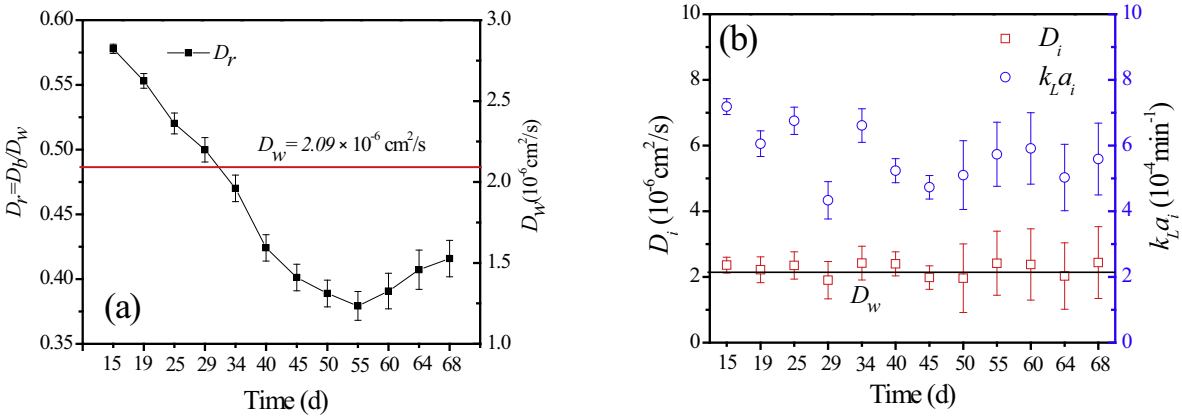


Fig. 6. Mass transfer of DO in the biofilm and boundary layer: (a) Variation of D_r in the biofilm; (b) Variation of D_i and k_La_i in the boundary layer.

Table 3
Correlation analysis of the diffusion coefficient with the different components in biofilm.

	Bound EPS	Total EPS	TB-EPS	LB-EPS	S-EPS	PS	PN	DNA	Humic acids
r	−0.846[a]	−0.803[a]	−0.796[a]	−0.734[a]	−0.387	−0.787[a]	−0.746[a]	−0.307	−0.264
p	0.001	0.002	0.002	0.007	0.198	0.002	0.005	0.298	0.328

Where r is the Pearson correlation; p is the significance of correlation.
[a] At 0.01 level (2-tailed) signification correlation.

4. Conclusions

A novel semi-suspended bio-carrier was designed and used in a biofilm reactor. By operating the reactor for 73 days, the mass transfer and distribution of DO inside and outside of the biofilm were investigated. Results indicated that different EPS showed quite a distinctive influence on the mass transfer in the biofilm, but the bound and total EPS were the two factors that had the most dominant negative effects on the mass transfer of DO. The semi-suspended bio-carrier led to the occurrence of three mass transfer zones, and the mass transfer varied with the properties and growing phase of the biofilm.

Acknowledgements

This work was supported by the National Natural Science Foundation of China [Grant Number 21476050] and the Foundation of High-level Talents of Guangdong Province [Grant Number 20140009].

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.biortech.2017.05.071.

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