Progress in microalgae cultivation photobioreactors and applications in wastewater treatment: A review

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Abstract: Using microalgae to treat wastewater has received growing attention in the world because it is regarded as a novel means for wastewater treatment. It is commonly recognized that large-scale cultivation and commercial application of microalgae are limited by the development of photobioreactor (PBR). Although there are a lot of PBRs for microalgae pure cultivation which used culture medium, specialized PBRs designed for wastewater treatment are rare. The composition of wastewater is quite complicated; this might cause a very different photosynthetic effect of microalgae compared to those grown in a pure cultivation medium. Therefore, PBRs for wastewater treatment need to be redesigned and improved based on the existing PBRs that are used for microalgae pure cultivation. In this review, different PBRs for microalgae cultivation and wastewater treatment are summarized. PBR configurations, PBR design parameters and types of wastewater are presented. In addition, the wastewater treatment efficiency and biomass productivity were also compared among each type of PBRs. Moreover, some other promising PBRs are introduced in this review, and a two-stage cultivation mode which combines both closed and open system is discussed as well. Ultimately, this article focuses on current problems and gives an outlook for this field, aiming at providing a primary reference for microalgae cultivation by using wastewater.

Keywords: microalgae cultivation, wastewater treatment, photobioreactor (PBR), review

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1 Introduction

Microalgae are one group of photosynthetic microorganisms which widely exist in nature, and which can grow in autotrophic, heterotrophic or mixotrophic manner. Microalgae cultivation has two main goals in recent scientific studies. One purpose is to generate high-value products. The increasing of renewable energy demands in the world and serious environmental pollution problems make the search for sustainable sources extremely urgent. Microalgae as the feedstock for the next generation of bio-energy and an effective approach for environmental remediation are receiving growing attention. In addition, microalgae can utilize photons to convert CO_2 and nutrients into biomass which include high-value added products such as lipids, proteins, carbohydrates, polyunsaturated fatty acids and natural pigments through the photosynthetic effect. These bio-products can be used for the production of healthy human foods, animal feeds, aquatic feeds, cosmetics and bio-pharmaceutical[1-4]. The other purpose is environmental

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purification. Photosynthesis also gives microalgae the ability of CO_2 fixation and nutrient assimilation, which makes microalgae cultivation not only for biomass production, but also for CO_2 sequestration and pollutant removal[5]. For these two goals, cultivating microalgae and generating algal biomass through bioreactors should be achieved first.

Photobioreactor (PBR) is defined as an open, closed or semi-closed vessel, made by transparent and waterproof materials, and able to provide an ideal growing environment for photosynthetic microorganisms. Microalgae cultivation needs a suitable PBR for different purposes. For generating high-value added products, axenic cultivation of microalgae is needed. Until now, different types of PBRs have been invented and produced for algae cultivation during the past decades and some of them have achieved large scale commercial production[6-8]. In general, microalgae cultivation is divided into two different systems: open system and closed system. The open system is the most common and popular form for the commercial application of microalgae cultivation in large-scale and environmental modification, because of its low economic cost, simple building construction and convenient operational method. However, the open system still has problems such as relatively low yield of biomass, easy contamination, uncontrollable environmental parameters and limited alternative algae species. In order to improve the above issues, a closed system for microalgae cultivation was developed and different types of PBRs have been studied in practical applications and scientific researches. Multi-forms of PBRs have their own characteristics and are appropriate for different culture locations, conditions, microalgae species, economic requirements and purposes.

As a supplementation of wastewater treatment technology, microalgae wastewater treatment technology has been developed recently and it has become more and more popular. The present substantial researches show that microalgae have a considerable potential for dealing with different types of wastewater treatment, such as municipal wastewater, livestock effluents and industrial wastewater. Removal of nitrogen and phosphorus from the microalgae cultivation environment is deeply associated with the biomass accumulation and affected by the growing conditions. Unlike the conventional approach in wastewater treatment (for example the activated sludge process), using microalgae for wastewater purification has a relatively long hydraulic retention time (HRT) and some important factors such as the supplementation of light and CO_2 are necessary[9]. In general the accumulation of biomass and the assimilation of nutrients, which are mainly determined by the PBRs, highly depend on the photosynthetic effect[10]. Appropriate configurations of PBRs lead to effective photosynthesis, a higher biomass yield and an optimal removal efficiency of nitrogen and phosphorus[11]. However, the types of PBRs that are used in algae pure cultivation do not always work well in wastewater treatment. As the components of wastewater is complicated, therefore, it is necessary to investigate the performance of PBRs in different types of wastewater cultivation situations and optimize the cultivation parameters. Finally, redesigning PBRs for microalgae wastewater treatment is the significant achievement and it is obviously good for commercial popularizing.

In this paper, the familiar forms of microalgae pure cultivation systems, containing open systems and closed systems are firstly presented. Based on the traditional microalgae cultivation systems, the systems that are used in wastewater treatment are all summarized from previous literatures and compared including the configurations of the PBRs, the significant design and operation factors for different types of PBRs. This paper provides the design principles for the PBR which can be promisingly used for microalgae wastewater treatment in the future.

2 PBRs for microalgae pure cultivation

Microalgae cultivation has been studied for over 70 years. The appropriate conditions, such as sufficient illumination, applicable algae strains, ideal climate condition, overall year-round production and minimum land use, are still challenges and have a long way to go in the future. Those are also the common concerns of scholars and industrialists in this field. Recently, large scale algae cultivation for commercial purpose has become a hot topic, in order to reduce culture cost, enhance biomass productivity and avoid contamination from other bacteria or fungi as much as possible, it is necessary to pay more efforts on the cultivation process.

Large-scale microalgae cultivation was firstly raised by the research of Carnegie Institute in 1952[12]. The Carnegie Institution of Washington sponsored construction of a pilot plant and supplemental laboratory studies. This work is summarized in a report which serves as a valuable source of information even today for algae cultivation. In the 1960s, a Japanese research group developed an "open circulation system", which used a shallow open pond to cultivate algae and make it circulated via a series of moving pipes equipped with jets for the injection of fresh culture fluid[13]. After that, the Japan Nutrition Association developed a 20 m diameter pilot plant to investigate the industrial cultivation of algae further. As time went on, Americans, Japanese, Europeans and Israel all made advances on algae commercial cultivation, either for human health products, animal and aquaculture feeds, fine chemicals or other human consumption products[7].

According to these previous academic works and commercial projects, microalgae cultivation patterns are generally divided into two typical systems: the open system and the closed system. Open system specially for outdoor culture, which takes full advantage of natural sunlight, while a closed system can either be located outdoors for free sunlight or indoor for an artificially controlled environment. The bioreactors used for microalgae cultivation are listed in the following sections.

2.1 Open system

There are mainly three types of open system: unstirred open system, circular pond and raceway pond.

2.1.1 Unstirred open system

Most of the natural water systems are without a stirred unit. This leads to poor mixing, but lower cost for commercial scale culture. The lakes, lagoons and ponds are the common PBRs. These culture systems provide an economic, simple and convenient way for operating and monitoring the culture process. The natural pond is usually less than half a meter in depth in order to ensure the light penetrates the water and is absorbed by the algal cells. A previous report claimed that plastic films can also be used by covering the surface of water for a better temperature control. Some algae species, for example *Dunaliella salina*[14], can be cultured in these kinds of open systems for commercial purposes.

2.1.2 Circular pond

A circular pond is mainly used for culturing *Chlorella* sp. in Asia[15]. The idea of using such a rounded pond with a long rotating arm was inspired by the circular reactor in wastewater treatment, thus a circular pond is very similar to the wastewater treatment pond. This type of pond is always 20-30 cm in depth and 40-50 m in diameter. The long rotating arm is set in the center of the pond which acts like a clock dial and performs a paddlewheel function which is familiar in the structure to that of a raceway pond. It is obvious that mixing of culture media and algae cells are more efficient than that in an unstirred pond, but as the algae is exposed to the surroundings, the contamination is inescapable. According to the research literature, the productivities in circular pond range between 8.5 $g/(m^2 \cdot d)$ and 21 $g/(m^2 \cdot d)$[16].

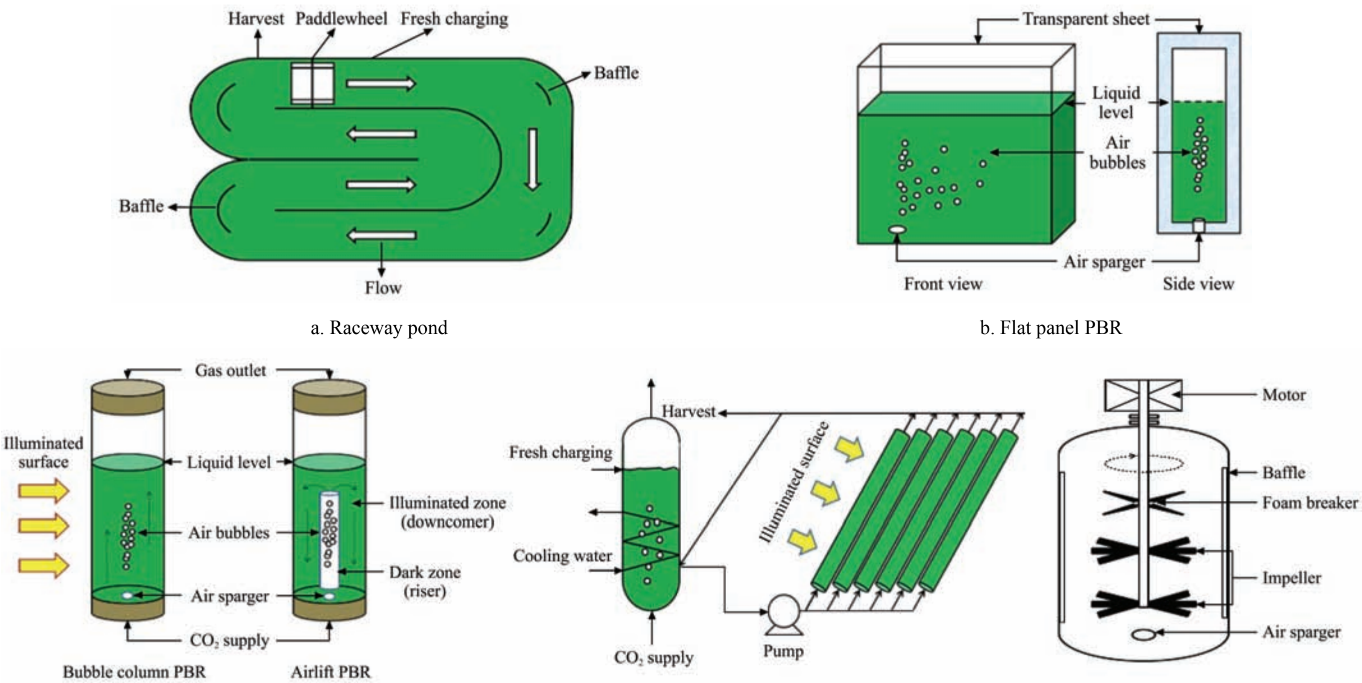
2.1.3 Raceway pond

During the past 40 years, the raceway pond (Figure 1a) has been the most popular and widely used open system reactor for the large-scale cultivation and commercial production of microalgae products. The raceway pond was first raised by Oswald in the 1960s and its appearance and structure have not been changed more compared with 40 years ago until today[17]. It was reported that *Chlorella*, *Spirulina*, *Dunaliella* and *Haematococcus* are the most common species of algae that can be cultivated in a raceway pond, and the culture mode can either be pure culture or wastewater culture, it can even integrate CO_2 capture technology in some power plants.

The materials for building a raceway pond can be concrete or plastic. The pond can be located on the ground or simply dug into the ground and lined with a wall to prevent the permeating of liquid from pond to soil. The configurations of the raceway pond are various, including a single channel and groups of channels. The depth of the culture medium in a raceway pond usually ranges from 15 cm to 50 cm. Most of the raceway ponds consist of paddlewheel, baffle and channels. Paddlewheels drive the liquid flow, ensures the algal cell is suspended in the culture medium and avoids sedimentation. Baffles rule the direction of flow and avoids dead zone of flow where cells will settle. In this way, algae cells will be sufficiently mixed and keep a continuous flow to receive sunlight and CO_2 from atmosphere. The fresh culture liquid can be easily added, while the high-density algae can be harvested through an outlet. The length to width ratio is a significant parameter while larger width may lead to a weak current speed and larger length may result in greater land use.

Compared with other open systems, the whole production process of the raceway pond is highly efficient and convenient, therefore, it is the first-choice reactor for the outdoor large-scale cultivation of microalgae. In a raceway pond, the biomass productivities can achieve 60-100 mg/(L·d)[18].

Although the open system has proven to be the most economical and sustainable for large-scale commercial cultivation of microalgae during the past decades, the open system still presents many problems, which make unsuitable for producing high value-added microalgae products such as fine chemicals and natural pigments. However, considering the economy and expansibility of the open system, it will still be the main form of bioreactor for large-scale microalgae cultivation, especially outside the laboratory. In addition, contamination by other microorganisms (undesirable algae species, bacteria, fungus or virus) is the most urgent issue which should be focused on seriously. In the future, a sequence of tasks, for example how to enhance light penetration, how to further decrease evaporation of water and how to improve mixing in an open unstirred pond or in a raceway pond, are worthy of further research by scientists and industrialists.



a. Raceway pond b. Flat panel PBR

c. Vertical tube PBR, left is bubble column, right is airlift d. Horizontal tube PBR e. Stirred tank

Figure 1 Schematics of different types of PBRs for microalgae pure cultivation

2.2 Closed system

In order to solve the problems which are generated in an open system and to achieve a better yield of microalgae biomass, the closed or mostly closed vessel has been developed, which does not allow direct mass transfer between culture media and the atmosphere and is able to provide a controllable environment (light, CO_2, temperature and nutrients)[19,20]. The cost of a closed system is often expensive while the air tightness, the control process and the mass transfer supplementary approaches will greatly increase the cost. In addition, in order to obtain the optimal illumination conditions, a large amount of energy is consumed, which seems uneconomical. Therefore, closed PBRs are generally considered to be difficult to promote to the pilot scale or large scale. The closed PBRs or system is of great value in high value-added products production and the status of these fine chemicals (bio-pharmaceuticals, top grade cosmetics, human health foods and biofuels) which are produced from microalgae became more and more important, therefore, the development of suitable and sustainable closed PBRs has great potential. The current common closed PBRs generally include flat panel, vertical tube (bubble column and airlift), horizontal tube, stirred tank and their modified configurations.

2.2.1 Flat panel

Flat panel (Figure 1b) is a kind of common PBR with a rectangular box appearance which is used either in algae pure cultivation or algae wastewater cultivation. It can be located either indoors exposed to artificial light sources or outdoor exposed to sunlight. Flat panels are made by transparent or semi-transparent materials (glass, plexiglass, polycarbonate and plastic bags etc.). Flat panels have a very-short light path which allows light to easily penetrate the culture liquid. Mixing is mainly driven by air bubbles which are generated from the air sparger. A pump is usually used to supplement air bubbles through the air sparger, circulating the algae cell suspension. The exhaust gas emission occurs at the gas and liquid junction. The reactor is inclined at a certain angle to obtain the best intensity of incident light when the reactor located outdoors.

The main advantages of flat panels include: (a) High surface area to volume ratio; (b) Not-too-serious accumulation of dissolved oxygen; (c) Convenient to clean; (d) The unit is flexible and it is suit for scale-up. Meanwhile, the main limitations of flat panel include: (a) It is expensive to control the temperature; (b) Hydrodynamic stress which is generated by aeration; (c) Biofouling near the internal surface.

2.2.2 Vertical tube

There are two types of vertical tube PBRs, bubble column and airlift. Both of them have an attached air sparer at the bottom of the reactor, converting the spared gas into tiny bubbles to ensure algae cells suspension and to enhance the mass transfer, to contain CO_2 capture and O_2 emission. The bubble column reactor (Figure 1c, left), has no internal structure, thus the fluid flow is driven all by bubbles, which are released by the air sparer at the bottom. Normally these types of cylindrical vessels with a height greater than twice the diameter and receive illumination externally. Near the axial area is the dark zone while illumination weakens as the result of cells covering. Under this configuration, bubbles force the algae cells to travel from the external illuminated surface to the axial dark zone, forming a so-called “light flash effect”, to improve the photosynthetic efficiency and mixing of microalgae. In a bubble column reactor, the gas flow rate is the only parameter that should be considered during the operation because it will deeply influence the light and dark cycle. The bubble column reactor has the advantages of a low cost, high surface area to volume ratio, simple configuration, and satisfactory mass transfer.

The airlift reactor can be regarded as an improvement of the bubble column but it has two interconnecting zones inside. One is called riser and the other is called down comer (Figure 1c, right). The riser zone is set as a concentric tube, while air is spared inside, driving liquid upward and forming the dark zone. Air bubbles drag the liquid from the riser zone inside the concentric tube up to the outside zone where algae cells can be illuminated, while in this way causing the liquid to recycle downward externally, finally back to the riser zone, this is the process of “airlift”, and received the “light flash effect” to enhance photosynthetic efficiency as well. This kind of cycle is also named the internal loop. Another form of airlift reactor is named the external loop, whose riser or dark zone is set outside the vertical tube while short horizontal sections is set at the top and bottom to separate the riser and down comer, forcing the liquid cycle and causing the “light flash effect”. In most of the airlift reactors, residence time of gas is quite significant to mass transfer. A lot of studies on the airlift reactor claimed the advantages as follows: (a) High mass transfer efficiency; (b) Well mixing with low shear stress; (c) Low energy consumption; (d) Good for immobilization of algae on moving particles.

2.2.3 Horizontal tube

Horizontal tube PBRs, also described as a tubular reactor, has the largest surface to volume ratio, which is beneficial to maximize exposure of the microalgae to light. Horizontal tube PBRs are basically constituted of tubes arranged in multiple possible orientations, such as horizontal, inclined, spiral, helicoidal and their variations. All these types have the same working process. The diameter of the tubes ranges from 10 mm to 60 mm and the length can reach several hundred meters. Like most tubular reactor in chemical engineering, flow velocity is a significant parameter. Another special characteristic of tube reactors is the mixing efficiency in the radial direction. A typical horizontal tube PBR is shown in Figure 1d. Beyond the tubes, there is a gas exchange system, which helps to add fresh culture medium, cycle cooling water and realizing the supplementation of CO_2. In addition, exhaust gas is also separated in this individual system. The impetus that forces the cycle between gas exchange system and irradiated tubes is mainly due to a pump. The culture liquid cycles and moves among these two systems and the algae cells are prevented from settling. Finally, the algae biomass can be harvested, after traveling cyclically and the whole culture process is continuous.

In spite of the largest surface to volume ratio and the continuous culture process, horizontal tube PBRs have many limitations. First of all is the uneven mass transfer in radial directions. This limitation is very easy to cause the inhomogeneous distributions of temperature and CO_2 and lead to an accumulation of dissolved oxygen. The second limitation is that the PBR system is hard to scale-up outdoors, due to its high-level requirement of land use and due to the fact that it is uneconomic in commercial cultivation. Furthermore, the photo inhibition which is caused by surface biological deposition will lead to difficulty in cleaning the tubes, and there are two strategies to avoid fouling in horizontal tube PBRs: use of plastic beads and air bubbling into the tube. This last strategy works also as an inline degassing. But the maintenance cost will increase correspondingly. Although the above problems exist, horizontal tube PBRs still have ideal performance, while the microalgae cultivation needs an automation control process and an adequate illumination.

2.2.4 Stirred tank

In the field of biological engineering, fermentation tanks are a high-efficiency heterotrophic culture reactor. Inspired by the fermentation tank, the stirred tank reactor was invented for algae cultivation with an external light source (fluorescent lamps or optical fibers), as Figure 1e shows.

In a stirred tank reactor, agitation is particularly sufficient by the mechanical movement of impeller which is driven by the electric motor, thus the stirred tank reactor has the optimal heat and mass transfer and mixing. A vortex may occur when the liquid revolves under high-speed conditions. To reduce the vortex, baffles are set around the inside wall of the tank. CO_2 is aerated through the air sparger at the bottom. The function of the foam breaker is to break the bubbles and reapportion. Only 70%-80% of the volume of stirred reactors is filled with liquid so the remained headspace is convenient for gas exchange.

The stirred tank indeed has a nice performance with agitation, mixing and transfer in indoor algae biomass production, but also leads to high energy expenditure. Furthermore, the very low surface area to volume ratio leads to a non-ideal penetration of light, which seriously reduces the photosynthetic efficiency of microalgae.

In conclusion, closed PBRs are more efficient for microalgae cultivation because of the easier environmental controlling than the open pond system. However, problems still exist in closed systems, for example this type of system is uneconomic, complicated to operate, hard to clean and difficult to scale-up. These issues need to be addressed in the future. Meanwhile, different algae species and different culture mediums far from each other and that must be considered comprehensively when designing a new PBR.

3 Application of PBRs in microalgae wastewater cultivation

Most of the bioreactors used for wastewater treatment are similar to the pure cultivation system. The design principles are derived from the pure cultivation systems.

In wastewater treatment, because of the special characteristics of wastewater, many of the bioreactor designs took the reference of wastewater treatment PBRs, therefore, those PBRs for wastewater treatment by microalgae which are presented in this article can be divided into two main kinds, the suspended system and the fixed system[21]. In the suspended system, microalgae cells are in the cell-free status, which leads to free movement of pollutants in different dilution concentrations. It is the most commonly used bioreactor in microalgae wastewater treatment. The open pond system, the tubular system, flat bioreactor system, and plastic bag system belong to the suspended system. However, the separation of the microalgae cell from wastewater can be helped by other separation methods such as coagulation, membrane filtration, etc., which further increase the cost of the whole cultivation system. The fixed PBR contains a biofilm bioreactor, immobilized bioreactor and microalgae membrane bioreactor. Although these types of PBRs can improve separation (or harvest), there are some other disadvantages for wastewater treatment such as the measurement of the biomass. The comparison of the PBRs used for wastewater treatment is shown in Table 1.

Table 1 Comparison of different types of PBRs

Cell condition	PBR type	Wastewater type	Scale	Microalgae or bacteria	Design parameters	References
Suspended system	Open pond system	Municipal and agricultural wastewater	Lab & pilot scale	Microalgae	Operation mode, aeration and HRT	[22-25]
	Flat system	Industrial and municipal wastewater	Lab scale	Microalgae	Illumination, HRT and aeration	[26-37]
	Tubular system	Industrial and agricultural wastewater	Lab & pilot scale	Microalgae	Cultivation mode, illumination, HRT and aeration	[38-43]
	Plastic bag	Industrial and agricultural wastewater	Lab scale	Microalgae	Aeration and sizes	[44-46]
	Cylinder system	Industrial wastewater	Lab & pilot scale	Microalgae	Illumination, HRT and aeration	[38, 47-50]
	New bioreactor	Municipal and agricultural wastewater	Large & pilot scale	Microalgae	Operation mode, illumination, flow rate	[51-53]
Fixed system	Immobilized beads	Municipal wastewater	Lab scale	Microalgae	Illumination, aeration, beads concentration and microalgae concentration	[54-58]
	Biofilm	Municipal wastewater	Lab scale	Microalgae or microalgae & bacteria	Illumination, HRT, aeration	[59-62]
	Microalgae membrane bioreactor	Municipal wastewater	Lab scale	Microalgae or microalgae & bacteria	Operation mode, illumination, HRT, flow rate, and aeration	[63-67]

It seems that the suspended system was more appropriate for treating wastewater with a high organic loading rate. These PBRs include open pond, flat, tubular, cylinder, and some new bioreactors. For the fixed system, they are more applicable for the treatment of municipal wastewater which always contain low concentrations of COD, TN and TP. In addition, most of the PBRs contain microalgae strains, while for biofilms, the microalgae or microalgae-bacteria colony as the existed forms of microbiology always appear in the biofilm PBRs. That might be because the EPS of the bacteria are easier to adhere on the biofilm materials. In addition, the design parameters are very different among each PBR. For the suspended system, especially for the flat, tubular, plastic bag and cylinder system, the illumination is the most important because the light intensity that is applied onto these PBRs is easy to control. According to the photosynthesis effect, the configuration of the PBRs can be concluded. While for the fixed system such as biofilm and MMBR, some of the areas in the PBRs are always dark because the microalgae cell is all fixed on the materials, the operation mode and HRT are more important.

3.1 Suspended PBRs

3.1.1 Open pond system

Before 2011, most of the wastewater treatment methods used by microalgae are batch experiments and carried out in flasks in addition to the high rate algae pond. The open pond system used for wastewater treatment can be divided into three methods, the open pond without paddle, the raceway pond and the high-rate algae ponds. The characteristics of some open pond system are presented in Table 2.

The open pond without paddle can also realize high pollutants removal. Yadavalli et al.[22] found that in the open pond system without the paddle, the ammonia, TP and COD removal could reach 98.17%, 96.87% and 87.93%, which demonstrated that this kind of simple PBR had a high potential for wastewater treatment efficiency and biomass productivity.

Table 2 Characteristics of different raceway pond PBRs

PBR configuration	Wastewater type	Microalgae species	Operation mode and scale	Temperature /°C	Aeration /$L \cdot min^{-1}$	HRT/d	Influent /$mg \cdot L^{-1}$	Removal /%	Biomass productivity /$mg \cdot (L \cdot d)^{-1}$	Ref.
Raceway pond without paddle	Municipal wastewater	*Chlorella pyrenoidosa*	Batch, lab scale	-	1.5	-	COD: 2900; NH_4^+-N: 351; PO_4^{3-}-P: 30	COD: 87.93; NH_4^+-N: 98.17; PO_4^{3-}-P: 96.87	-	[22]
Raceway pond with paddle	Anaerobic digestion piggery wastewater	*Chlorella* sp., *Scenedesmus* sp.	Semi-continuous, lab scale	<25	-	-	NH_4^+-N: 893.03±17.0	NH_4^+-N: 25.9±8.6 $mg \cdot (L \cdot d)^{-1}$	24±0.13	[68]
Raceway pond with paddle	Municipal wastewater	*Scenedesmus* sp.	Semi-continuous, pilot scale	13-23	20	3.3±0.2	-	COD: 84±7; TN: 79±14; TP: 57±12	(4±0)–(17±1) $g \cdot (m^2 \cdot d)^{-1}$	[24]
Raceway pond with paddle	Piggery wastewater	*Scenedesmus* sp.	Semi-continuous, pilot-scale	11±1	Flue gas	10	-	COD: 56±31; NH_4^+-N: 98±1	-	[69]

In the open pond system (raceway pond and HRAP), the existence of the paddle can promote the gas and nutrients transfer between liquid and microalgae. Nwoba et al.[68] studied the ammonia removal and biomass production in an open pond system with a paddle (160 L) and a closed tubular bioreactor (20 L and 40 L, with air-lift and pump supplementation system, respectively). Results showed that the ammonia removal had no difference between these two kinds of bioreactors, but the biomass productivity in the tubular PBRs was 2.1-fold greater than that of the open pond system. However, the existence of a paddle will increase the energy cost. In addition, the paddle rotation speed must be kept in appropriate ranges to ensure a stable flow of culture liquid. What's more, the nutrients are variant for different kinds of wastewater, leading to different transfer coefficients. Therefore, designing a proper paddle rotation speed is a crucial and complex factor in microalgae wastewater treatment processing in open ponds.

Supplementing the CO_2 can obviously promote biomass production, but have no obvious effects on pollutant removal. However, the coverage of the open pond system can accelerate the absorption of CO_2. Posadas et al.[24] built up three open ponds (700 L, 800 L and 850 L) to treat the secondary effluent of municipal wastewater by using *Scenedesmus* sp. Results showed that the supplementation of CO_2 can obviously promote the TP removal. Meanwhile, the CO_2 can be used for regulating pH. De Godos et al.[69] treated the pig effluent wastewater in 465 L HRAP with 7% CO_2 and the HRT was 10 d. Results showed that the CO_2 flow rate has little influence on COD, ammonia and phosphate radical removal, but it made pH decrease and enhanced the nitrogen effect of ammonia. In addition, although the HRAP is a bacteria-microalgae system, the supplementation of CO_2 indeed promoted biomass production, which demonstrated that the productivity of the HRAP was largely contributed to the inorganic carbon.

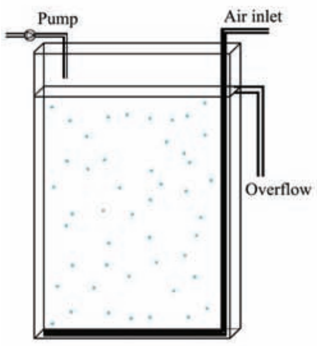
The open pond system is easy to realize at the pilot scale. However, it is easy to be contaminated by foreign species. In the future, special microalgae could be screened to fit the tolerance of the wastewater pollutants and decrease the risk of other microbiology condemnation.

3.1.2 Flat system

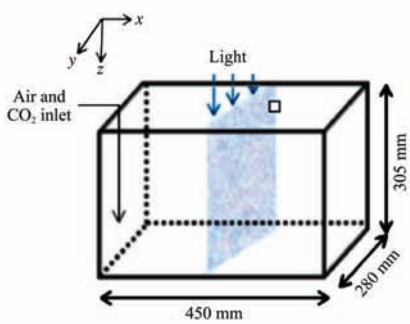
The flat bioreactor is relatively simple. It is mainly composed of two parts, the flat transparent bioreactors and the supplementation system. In previous studies, researchers used these types of bioreactor to treat wastewater are not always being signed based on the characteristics of wastewater but based on the utilization process. These studies mainly concentrated on the influence of operation conditions to treat wastewater. There are some factors that influence the wastewater treatment efficiency. Some of flat systems are presented in Table 3 and Figure 2.

Table 3 Characteristics of different flat panel systems

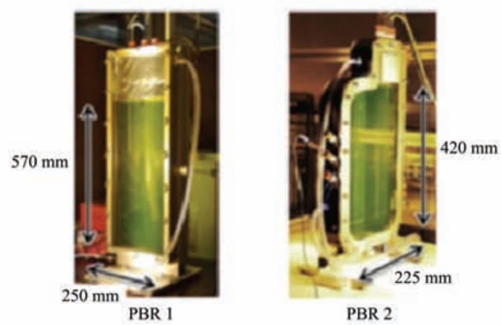
PBR	Wastewater type	Algae Species	Operation mode and scale	HRT /d	Culture conditions	Influent /mg·L^{-1}	Effluent /mg·L^{-1}	Removal /%	Biomass productivity /g·(L·d)$^{-1}$	Ref.
Two plat panels integrated PBR (0.6 L)	Fish processing wastewater	*Oocystis* sp., activated sludge	Batch, lab scale	5	(23±2)°C, (31±3)°C; 12 000 lx; 2%-15% CO_2	N: 46-50; TP: 2.7-10.7	-	N: ≥95; TP: 33.6-71.8	0.031-0.111	[27]
Glass tank CSTR mode (0.6 L)	Artificial wastewater	*Pseudomonas* MTl, *Chlorella vulgaris* MMl	Continuous, lab scale	2.7-4.0	(25±2)°C; 5000 lx; L:D=12:12	COD: 754-1451	-	N: 59-88	-	[70]
Transparent methacrylate flat panel PBR (4.5 L)	Urban wastewater	*S. obliquus* (SAG 276–10)	Batch, lab scale	0.5-4.3	(20±1)°C; flow rate: 2.8 L·min^{-1} (5% CO_2); 250 μmol·(m^2·s)$^{-1}$; L:D=14:10	N: 16.6-17.7; TP: 0.81-3.56	N: 1.7-16.6; TP: 0.08-2.0	N: 80.58-91.0; TP: 90.1-97.8	0.28-0.38 g·(m^2·d)$^{-1}$	[35]
Transparent methacrylate airlift photoautotrophic-heterotrophic PBR (890 L)	Digested starch processing wastewater	*C. pyrenoidosa* FACHB-9	Batch, lab scale	9-14	Culture period: 365 d; 7°C-45°C; flow rate: 15-20 L·min^{-1} (5%-9% CO_2)	N: 240.3-382.7; TP: 22.7-40.2	-	N: 3.8-84.4; TP: 36.0-97.55	0.11-0.63	[26]
Flat plate vertical PBR (0.25 L)	Urban wastewater	*Chlorella protothecoides*	Batch and continuous, lab scale	1.26	Culture period 7-9 d; 23°C 5% CO_2; 100 μE·(m^2·s)$^{-1}$; flow rate: 1 L·h^{-1};	N: 0.89-1144.05; TP: 1.02-53.7	N: 0.68-1.88; TP: 0.17-0.79	N: 4-97; TP: 50-71	0.4-0.6	[30]
Air lift flat panel PBR (10 L)	Urban wastewater	*Chlorella kessleri* UTEX2229, *Chlorella vulgaris* CCAP211/19, *N. oculata*	Batch, lab scale	-	25°C 100 μmol·(m^2·s)$^{-1}$	N: 130.0; TP: 5.76	N: 5, 10; TP: 0.04	N: 94-96; TP: 98-99	-	[29]
Semi open system PBR (1500 L)	Centrate wastewater	*Chlorella* sp.	Batch and semi-continuous, pilot scale	1-6	Culture period: 12 d; 25°C-26°C; 25 μmol·(m^2·s)$^{-1}$; flow rate: 38 L·min^{-1}	N: 275; TP: 392	-	N: 34.8-61.0; TP: 58.1-61.0	34.6 g·(m^2·d)$^{-1}$	[36]
Transparent polymethylmethacrylate optical panel PBR (37 L)	Domestic wastewater	*Chlorella vulgaris* FC-16	Batch, lab scale	-	Culture period: 15 d; (25±2)°C; L:D=8:16; 270-310 μE·(m^2·s)$^{-1}$; flow rate: 0.5 L·min^{-1} (CO_2 0.02 vvm)	N: 40.02; TP: 9.24	-	N: 78-97; TP: 20-80	0.40-2.97	[32]



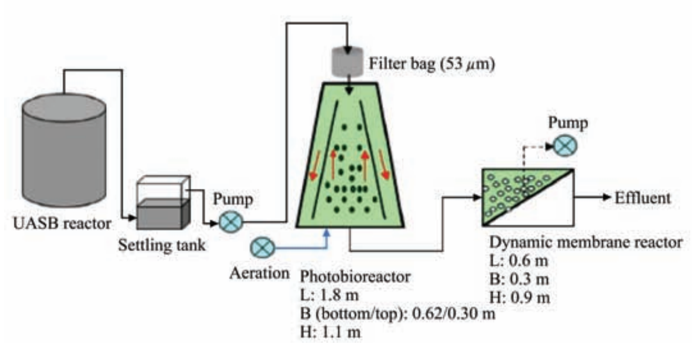
a. Flat panel[35]



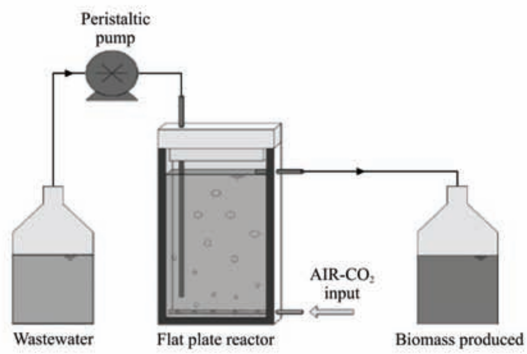
b. Optical panel PBR[33]



c. Short light-path flat panel[28]



d. Anaerobic process-microalgae cultivation/dynamic membrane filtration PBR[26]



e. Lab-scale continuous flow flat panel[30]

Figure 2 Flat panel systems

Flat PBRs are usually used for treating low organic load wastewater, such as urban wastewater, domestic wastewater, and fish processing wastewater, which have low solid contents and have better light transparent characteristics[27,29,32]. In addition, there is an example that is used for treating starch processing wastewater[26].

For the microalgae strain, *Chlorella* is the most commonly used microalgae strain. The bacteria-microalgae system is also used while the bacteria always play the role of activated sludge. There are seldom pilot scales for using flat panel PBRs to cultivate microalgae in wastewater. Most of the experiments are carried out in the lab-scale. Tan et al.[26] utilized an airlift circulation PBRs and dynamic membrane reactor for microalgae cultivation in combination with an up-flow anaerobic sludge bed (UASB) reactor for starch processing wastewater treatment in every season. The temperature is a very important reason for biomass production, nutrients removal, and cell composition for large scale operation in outdoor. The most useful operation mode is the batch mode, although there is the continuous mode.

For the design parameters, HRT is very important because high condensed growth microalgae will have the self-shading effect, which leads to a lower biomass production and nutrients absorption. The module equation will help to predict the adequate HRT. However, it is also very important to find the photosynthesis effect of the bioreactor according to different types of microalgae strains.

In the flat panel system, the light intensity decreases exponentially with distance from the reactor wall as the concentration of cell and product are increased. Especially when algae are cultivated in raw wastewater, this shading effect can be further aggravated by the high content of particulate matter. One of the choices to solve this problem is to increase the surface to volume ratio. Choi[33] designed an optical panel flat bioreactor that inserted the LED light source with different light pathways to increase the light intensity in the bioreactor and save energy provided by artificial light. In this PBR, although the light pathway is very important and chlorophyll content per cell and diffuse light intensity appeared in the shortest light pathway run, the biomass concentration and nutrients recovery efficiency were obtained in the longer light pathway run. He also investigated the residence time for the highest biomass production during continuous operation mode because the light self-shading effect can be avoided or relieved by diluting the microalgae cell density. After that, Choi and Lee[32] also investigated the effluence of N/P on biomass production and nutrient removal in this bioreactor.

The flat panel is relatively simple compared to the other PBRs. Avoiding the light-shade effect is the most important design factor. Meanwhile, the HRT and operation mode (batch or continuous mode) must be considered into the construction of this type of PBR when deciding the light pathway.

3.1.3 Tubular system

The tubular bioreactor is one of the most commonly used closed microalgae cultivation systems. The recent studies that this kind of bioreactor was used to treat wastewater are summarized in Table 4 and Figure 3. These systems are very similar to those used in microalgae pure cultivation.

It seems that the types of wastewater are more diverse and the pollutants concentration of wastewater is higher than the others. The wastewater mainly includes municipal wastewater, industry wastewater, and livestock and poultry breeding wastewater. The organic load of wastewater is very high. For example, the COD and TN concentration can reach as high as 893.03 mg/L and 773 mg/L, respectively[37,67].

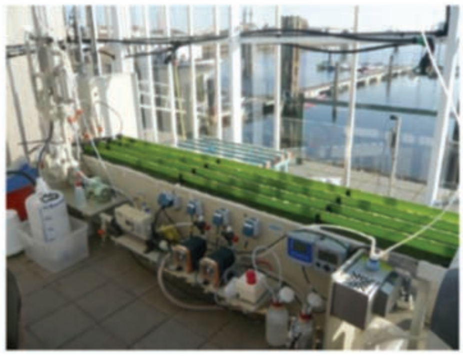
Tubular reactors are feasible for operation mode, such as batch, semi-batch, continuous and turbidostat. This kind of bioreactor has the most potential to realize large-scale production because it is easy to control. There are many studies that use tubular bioreactor to treat different kinds of wastewater at pilot scale[41-43]. For the pilot scale tubular PBR, the working volume is maintained at 8 L to 380 L. Di Termini et al.[42] investigated the horizontal tubular PBRs to treat tertiary from a municipal wastewater treatment plant under controlled or natural conditions. Results showed that nearly 100% of pollutants were removed from wastewater under controlled conditions, which was significantly higher than that in outdoor bioreactors without light, temperature or other conditions. Arbib et al.[43] and Nwoba et al.[68] also compared the pollutants removal and biomass production between the tubular PBR and high rate algae pond when treating tertiary and piggery effluent. Results showed that the photosynthetic activity in TPBR was during entire experiment higher than HRAP. TN and TP removal and biomass production were all higher in TPBR than that in HRAP. However, there are some disadvantages for TPBR that biofouling which attaches on the tubular inner wall will force the experiment to stop. In addition, the TPBR was more limited at low temperatures.

The operation mode influenced the pollutants removal and biomass production. Only one type of microalgae is used in the operation of tubular PBRs. Yang et al.[40] investigated cultivation of *Chlorella pyrenoidosa* in cassava ethanol fermentation wastewater with batch, fed-batch and continuous modes. Under the continuous mode, the biomass accumulation and daily productivity obtained the highest value. In addition, the continuous mode kept steady operation for 54 d. Michels et al.[41] also carried out a continuously operated tubular PBR to cultivate *Tetraselmis suecica* and treat the fish farm wastewater. This pilot scale experiments were carried out indoor turbidostat and lasted for 65 d. This type of PBR can also be used for high organic load wastewater treatment. Alexei Solovchenko et al.[38] explored a high density and semi-batch cultivation PBR to cultivate *Chlorella sorokiniana* by using alcohol distillery wastewater. The PBR was equipped with an online monitoring system and ring LED. The initial COD was as high as 20 g/L. The nitrate removal reached 95.0% after 3 to 4 days' treatment.

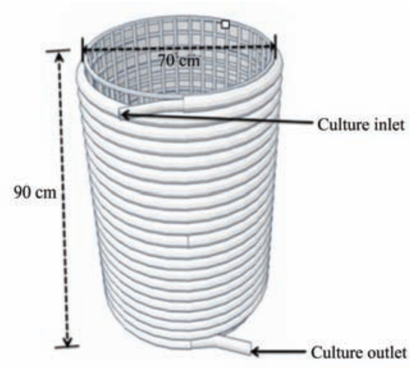
Table 4 Characteristics of different tubular PBRs

PBR	Wastewater type	Algae species	Operation mode and scale	HRT/h	Culture conditions	Influent /mg·L^{-1}		Effluent /mg·L^{-1}		Removal /%		Biomass productivity /g·(L·d)$^{-1}$	Ref.
						N	TP	N	TP	N	TP		
Cycle glass tubular PBR (0.2 L)	Cassava ethanol fermentation wastewater	*Chlorella pyrenoidosa*	Batch, fed-batch, continuous, lab scale	6-54	Culture period: 6 d (batch and fed batch), 54 d (continuous); 25°C-30°C, 35°C-40°C; 1000-5000 lx	-	-	-	-	-	-	0.58-0.74	[40]
Two plexiglass tubular PBRs (outdoor: 18.13 L, indoor: 8 L)	Tertiary treatment	*Scenedesmus*	Batch, pilot scale	-	Culture period: 7 d; 24°C-28°C; 80-1300 μE·(m^2·s)$^{-1}$	7.43-16.23	0.99-21.4	0.10-4.61	0.03-0.44	90-99.9	80-99.9	0.02-0.39	[42]
Transparent PMMA tubular airlift PBR (TPBR), fiberglass high rate algae pond (HRP) (HRAP: 533 L, TPBR: 17 L)	Urban wastewater	*Scenedesmus obliquus*	Batch and continuous, pilot scale	TPBR: 2-5; HRAP 7-10	Culture period: 157 d; HRAP flow velocity: 0.2-0.3 cm·s^{-1}	24.92-26.16	1.77-2.23	-	-	HRAP: 77.02; TPBR: 94.9	HRAP: 63.2; TPBR: 94.0	HRAP: 8.26 g·(m^2·d)$^{-1}$; TPBR: 21.76 g·(m^2·d)$^{-1}$	[43]
Tubular bubble column PBR (1.37 L)	Piggery wastewater	*Chlorella zofingiensis*	Batch, lab scale	-	(25±1)°C; 5%-6% CO_2; (230±20) μmol·(m^2·s)$^{-1}$	148	156	-	-	68.96-82.70	98.17-100	106.28-296.16 mg·(L·d)$^{-1}$	[39]
Tubular PBR (40 L)	Fish farm wastewater	*Tetraselmis suecica*	Continuous (turbidostat), pilot scale	15 d	Culture period: 65 d	40.7	4.96			95.7	99.7	0.35-0.52	[41]
Annular PBR (50 L)	Alcohol distillery wastewater	*Chlorella sorokiniana*	Semi-batch, lab scale	120	Culture period: 400 h; 27°C; 0.1 VVM; 5 L·min^{-1}; 180 μmol·(m^2·s)$^{-1}$	15	773	-	-	≥95.0	77.0	0.6	[38]
Clean vinyl tubing helical tubular PBR (40 L)	Piggery effluent	*Chlorella* sp., *Scenedesmus* sp., *pennate diatom*	Semi-continuous, pilot scale	-	Culture period: 99 d; 0-40°C; 0-400 W·m^{-2}	893.03	-	-	-	39.2	-	21.03 mg·(L·d)$^{-1}$	[68]

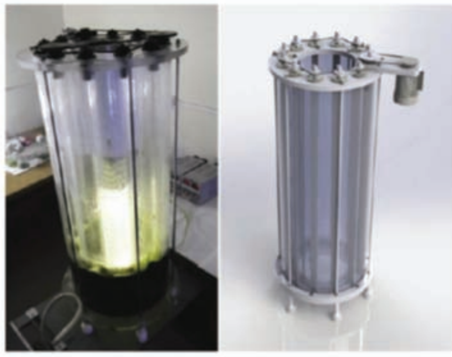
a. Airlift tubular PBR[43]



b. Tubular PBR[41]



c. Airlift tubular PBR[68]



d. Annular PBR[38]

Figure 3 Tubular PBRs for wastewater treatment

3.1.4 Plastic bag bioreactors

The plastic bag bioreactors belong to the closed system. It is composed by three parts: plastic bags, frame (supports the plastic bag), and aeration systems. The statement of same plastic bag PBRs used for wastewater treatment can be seen in Table 5 and Figure 4. In the wastewater treatment area, the plastic bags are the cheapest bioreactor and it has high potential in large scale wastewater treatment compared with the other PBRs. In recent studies using this type of PBR to treat wastewater, the size, materials, aeration types (insert the aeration tube or setting the aeration outlet at the bottom), mixing ways (air lift or mixing by sway) and structure of the frame are key design factors.

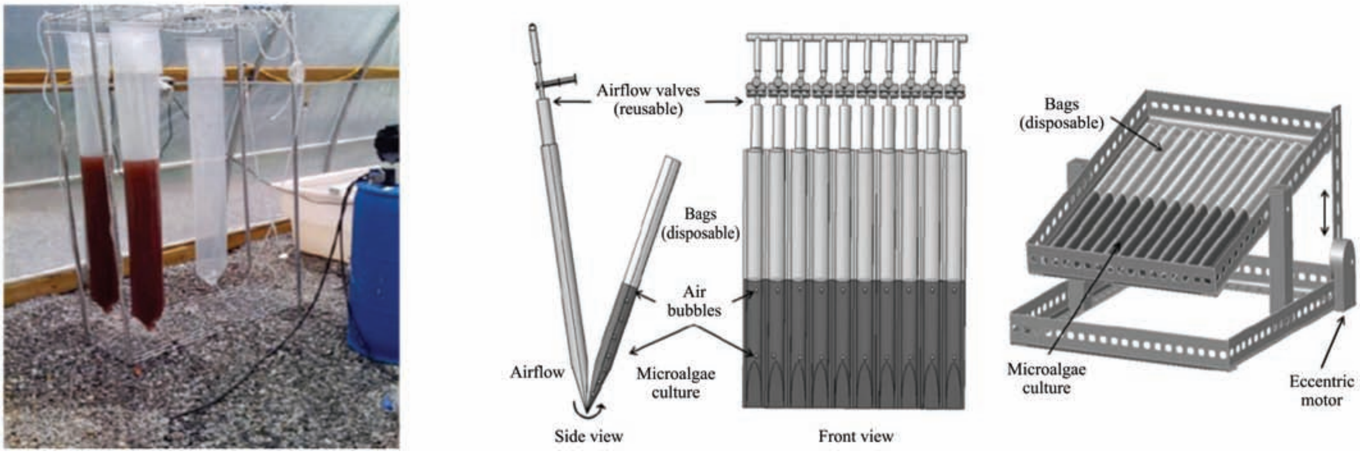
It has been proved that the plastic bags PBRs had very effective pollutants removal. Wang et al.[46] used *Spirulina platensis* to treat pig effluent in 20 L transparent plastic bags. With the aeration of 4.4 L/min, the ammonia nitrogen, nitrate and phosphorus removal reached 91.8%, 54.0% and 65.4%, respectively, and the COD and SS reached to the drainage standard.

The microalgae for wastewater treatment in plastic bag PBRs are always pure without bacteria. *Chlorella* is also the popularly used strain. Menke et al.[44] developed two types of plastic bags for screened microalgae cultivation (*D. salina*, *T. tetrathele* and *N. salina*) in high salt wastewater. One of bioreactor is vertical airlift bioreactor. The gas was supplemented by pumping into the bags (the flow rate is 13 L/h); the other is seesaw plastic bag. The plastic bags were put on the seesaw and mixing microalgae and liquid together. Results showed that *Tetrathele* and *N. salina* can tolerate hydraulic shear that is generated by air-lift gas. While *D. salina* was more suitable in a smoothly shaking seesaw PBR[44].

Until now, there are few studies about using the plastic bags to treat wastewater. The designs of this type of PBR are as follows: Chinnasamy et al.[45] used carpet manufacturing wastewater to cultivate *Chlamydomonas globosa* and *Chlorella minutissima* in a raceway pond (2000 L), a vertical tubular bioreactor (100 L) and a suspended plastic bag (20 L). Results showed that the highest biomass was generated in the plastic bags with the value of 21.1 g/(m^2·d). The economic analysis results showed that the biomass yield can reach up to 51-77 t/(hm^2·a) for the plastic bag PBR. However, the economics could be improved by decreasing the size to 10 m^2 for a large scale building[45]. Although it is a very low cost cultivation system, the scale-up of plastic bag PBR is not easy yet, while there are too much connection and structure units that must be installed and maintained.

Table 5 Characteristics of different plastic PBRs

PBR	Wastewater type	Microalgae species	Operation mode and scale	Culture conditions	Influent /mg·L^{-1}	Removal /%	Biomass productivity /g·(L·d)$^{-1}$	Ref.
Polybag	Carpet industry wastewater	*Chlamydomonas globosa, Chlorella minutissima*	Batch, lab scale	32.1°C; Aeration: 0.4-0.8 L·min^{-1}; Culture period: 8 d	NO_3-N: 0.009-0.327; NH_4^+-N: 0.020-45.668; PO_4^{3-}-P: 0.003-10.69	-	21.1 g·(m^2·d)$^{-1}$	[45]
Air bag	Hypersaline industrial wastewater	*Dunaliella salina*	Batch, lab scale	37 μmol·(m^2·s)$^{-1}$; 23°C; Aeration: 0.217 L·min^{-1}; Culture period: 35 d	Wastewater salt concentration: 30%	-	8	[44]
Transparent plastic tank	Swine farm wastewater	*Spirulina platensis*	Batch, lab scale	1500 lx; 20°C-29°C; Aeration: 4.4 L·min^{-1}; Culture period: 7 d	COD: 1073; NH_4^+-N: 82.1; PO_4^{3-}-P:32.6; NO_3-N:20.3	COD:85; NH_4^+-N: 91.8; PO_4^{3-}-P: 65.4; NO_3-N:54.0	1.2	[46]



a. Polybag PBR[45]

b. Two kinds of disposable bags PBR[44]

Figure 4 Configuration of plastic bag PBRs

3.1.5 Cylinder PBR

Cylinder PBRs contain two types of configurations, the bubbling and airlift style. In this type of PBR, the microalgae are in the suspended state. The statement of this type of PBR can be seen in Table 6 and Figure 5. The material of the bioreactors is one of the factors which influence the wastewater treatment efficiency. The transparent resin and borosilicate glass are the most popular materials which could use for constructing the main bioreactor.

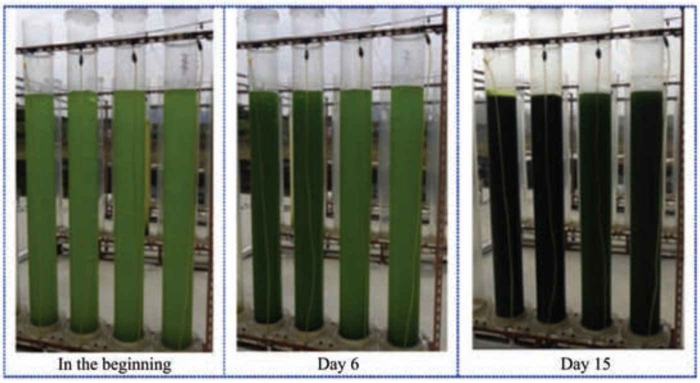
Zhu et al.[47] utilized the bubbling cylinder PBR to treat synthetic wastewater by *Chlorella zofingiensis*. The PBR was made by a transparent resin, included a bubbling outlet on the bottom. With the acetic acid as the pH regulation method, the TN and TP removal and biomass production reached up to 73.5%, 100% and 66.94 mg/(L·d). Maroneze et al.[50] designed a cylinder PBR which was made by boron silicate glass to treat cattle-slaughterhouse wastewater. There was no light provision. The PBR in fact was a fermentation tank. However, the COD, TN and TP removal reached 90%, 57% and 52%, respectively, which also showed high potential in high organic load wastewater treatment. However, the materials chosen must be carefully chosen to avoid the erosion of the wastewater and meanwhile keeping the high light optical transmission property.

In addition, the position of the air outlet and light source and the specific area and height/diameter ratio are also important. *Chlorella* and *Scenedesmus* are the most popular microalgae strains. For this type of PBR, the light provision and gas supplementation are two very important design factors and it is easier to realize at the pilot scale.

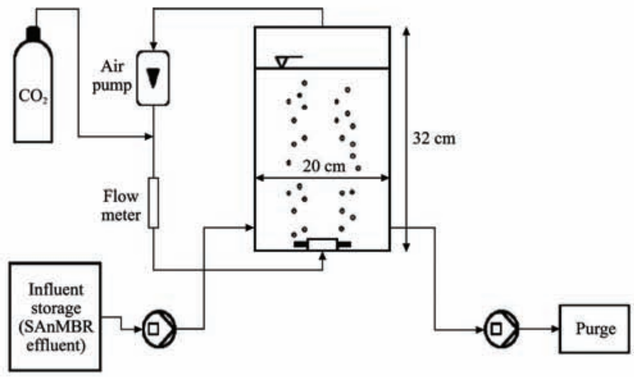
A cylinder PBR is often used in treating flue gas. Van Den Hende et al.[71] combined flue gas treatment technology and wastewater treatment technology to cultivate a bacteria-microalgae colony in a 5 L bubbling cylinder PBR. Finally, with an HRT of 0.67 d and gas flow rate of 0.6 L/h, the nitrogen, phosphorus, turbidity, pH and SO_X, NO_X in the flue gas reached the European drainage standard. Meanwhile, the biomass reached up to 0.18 g/(L·d) under low intensity light.

Table 6 Characteristics of different cylinder PBRs

PBR	Wastewater type	Microalgae species	Operation mode and scale	Culture conditions	Influent/mg·L^{-1}	Removal /%	Biomass productivity /mg·(L·d)$^{-1}$	Ref.
Cylindrical tube	Artificial wastewater	*Chlorella zofingiensis*	Batch, pilot scale	79-935 μmol·(m^2·s)$^{-1}$; 6.0°C-17.2°C; Air bubble; Culture period: 15 d	–	TN: 73.5; TP: 100	66.94	[47]
Cylindrical glass tube	NF membrane treated olive mill wastewater	*Scenedesmus dimorphus*	Batch, lab scale	90-80 μmol·(m^2·s)$^{-1}$; 28°C; Aeration: 130×10^{-3} Nm^3·h^{-1}; Culture period: 5d	COD: 8210; Polyphenols: 766; Phenol: 381	COD: 35; Polyphenols: 55; Phenol: 39	–	[48]
Bubble column	Cattle-slaughterhouse wastewater	*Phormidium* sp.	Batch, lab scale	20°C; Aeration: 1 VVM; Culture period: 168 h	COD: 7692.5 ± 5193.3; TN: 155.1 ± 80.1; TP: 23.0 ± 10.1	COD: 90; TN: 57; TP: 52	–	[50]
Bubble column	Anaerobic membrane bioreactor wastewater	*Chlorococcales*	Semi-continuous, lab scale	143-209 μE·(m^2·s)$^{-1}$; 28°C-32°C; Aeration: 0.8-1.0 L·min^{-1}; Culture period: 42 d	–	NH_4^+-N: 67.2; PO_4^{3-}-P: 97.8	234	[49]



a. Cylindrical tube PBR[47]



b. Lab-scale bubble column cylindrical PBR[49]

Figure 5 Configurations of some cylinder PBRs

3.2 Fixed system

3.2.1 Immobilized microalgae beads

One of the major and practical limitations in microalgae wastewater treatment processing is harvesting and separation of algae biomass form the treated wastewater. Therefore, immobilization as a fixed technology has been proposed for more flexibility and in the reactor design when compared with the traditional suspension systems. Most of the past studies concentrated on immobilization technologies such as how to choose suitable materials for microalgae beads production and the characterization of immobilization systems[72].

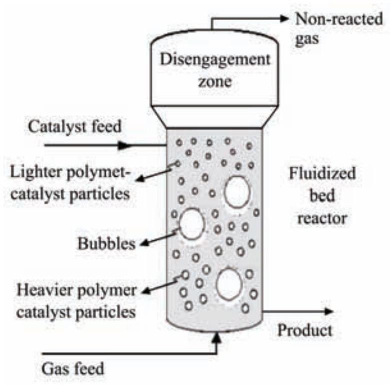
An immobilized cell is defined as a living microalgae cell that by natural or artificial means is prevented from moving independently of its neighbors to all parts of the aqueous phase of the system[73]. The immobilization of algae to treat wastewater has been studied for 40 years. The most common and effective method that is used for immobilizing microalgae is immobilization in polymers. This means that microalgae are fixed alive within the gel matrix. Several matrixes such as acrylamide, polyurethane, polyvinyl, resins, and natural polymer derivatives of algae polysaccharides (alginate, carrageenan, agar and agarose), and chitosan, an amino polysaccharide derived from chitin, has been experimentally used. Regardless of the polymers used, the material must be hydrophilic, allowing wastewater to diffuse into the bed. The most commonly used polymers are the natural polymers alginate and carrageenan. After the microalgae are immobilized by some matrixes, the bioreactors can be designed.

There are four kinds of immobilized bioreactors for the algae bead to treat wastewater. They include the fluidized bed bioreactor (FBR), packed bed bioreactor (PBR), parallel plate bioreactor (PPR), and airlift bioreactor (ALR). Fluidized-bed bioreactors are always used for relatively short residence time processing concepts. Small biocatalyst particles must be used to ensure fluidization, and they must be sufficiently stable to withstand significant shear forces for long periods of time. The typical design of fluidized-bed bioreactors can be seen in Table 7[75] and Figure 6a. PBRs are the most often studied bioreactors than the others. This system can perform well under long retention times and external biomass build-up is minimal. The configurations can be seen in Figure 6b. PBRs are by definition capacitive coupled, which are either bottom or top powered. Etching in a top powered reactor is referred to as 'plasma mode' etching and etching a bottom-powered reactor it is referred to as 'reactive ion etching'. This is a bit of inaccuracy, because both systems are generically 'plasma etchers'. The specific configuration can be seen in Figure 6d. The ALR is a commonly used PBR in microalgae pure cultivation. It is a homogeneous system and very suitable for laboratory optimization. The content in this system is stirred by air or gas, and the flow will depend on the geometry of the system. The configuration can be seen in Figure 6c.

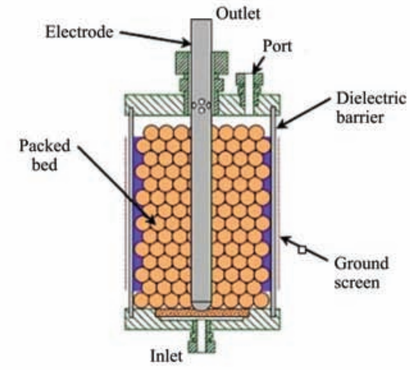
Compared with the cell free mode, the pollutants removal of immobilized microalgae is relatively lower in immobilized mode under the same environmental conditions (light intensity, photoperiod, pH, etc.). The analysis of protein and lipid content suggesting that the immobilized system does not represent an advantage over free-cell systems[57]. The microalgae cells near the bead surface have a very high activity due to the better contact with the light. While for the cells located inside, the self-shading effect leads to slow growth. But the pigment production does not decrease. They have a 100-1000 times pigment content than that of free cells. They can therefore compensate for the self-shading effect.

Table 7 Characteristics of different immobilized beads PBR

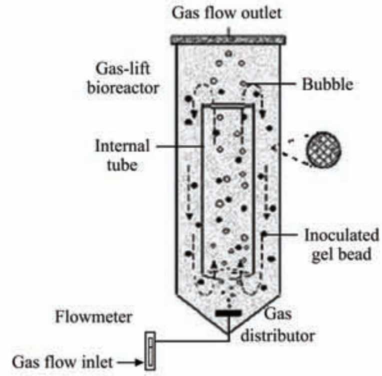
PBR	Wastewater type	Algae species	Operation mode and scale	HRT/h	Culture conditions	Influent/$mg\cdot L^{-1}$		Removal/%		Biomass productivity /$g\cdot(L\cdot d)^{-1}$	Ref.
						N	TP	N	TP		
Plexiglass immobilized PBR (1 L)	Sewage wastewater	*Chlorella kessleri* LARG/2, *Chlorella vulgaris SR/2*	Continuous, lab scale	8	Culture period: 6 months; (30±5)°C; L:D=13:11; 10.8 L/min (3% CO_2); Flow rate: 480 L/d; 40 $W\cdot m^{-2}$	50.7-64.2	6.85-9.2	7.1-72.9	58.6-70.6	-	[75]
Alginate chitosan Carrageenan Agar	Wastewater and heavy metal	*Anabaena doliolum*, *Chlorellu vulgaris* immobilized	-	-	Culture period: 15 d	-	-	70	-	25-60 $ug\cdot mL^{-1}$ (Chkiriohy II a)	[74]
Sodium alginate in plastic foams both polystyrene and polyurethane as cubes (1 L)	Sewage wastewater, cattle manure wastewater	*Chlorella vulgaris* SR/2, *Chlorella kessleri* LARG/2, *Scenedesmus quadricauda*	Continuous, lab scale	Hydraulic loadings: 2.5 $L\cdot d^{-1}$	Culture period: 5-10 weeks; L:D=13:11; 1-14 $MJ\cdot m^{-2}$	31/237	6/34	42.0-56.9	44.0-64.1	-	[79]
Transparent PVC immobilized PBR (51 L)	Domestic wastewater	*Chlorella vulgaris*	Continuous, lab scale	8	Culture period: 48 h; 35 $mL\cdot min^{-1}$ (air); (23±2)°C; 174 $\mu E\cdot(m^2\cdot s)^{-1}$; L:D=24:0	28-30	5.5	45.3-100	83.6-93.9	-	[76]
Polyethylene parallel-plate bioreactor (sodium alginate solution) (0.35 L)	Domestic secondary effluent	*Scenedesmus* sp.	Continuous, lab scale	48	Culture period: 21 d; (20±2)°C; 5000 ± 300 lx; L:D=13:11	14-22	0.2-1.7	100	100	400-1400 $ug\cdot mL^{-1}$ (chlorophyII a)	[58]
Sodium alginate solution (3 L)	Second effluent	*Scenedesmus obliquus*, *Chlorella vulgaris*	Batch, semi and continuous, lab scale	6 or 35	Culture period: 300 h; (25±1)°C; 135-200 $\mu E\cdot(m^2\cdot s)^{-1}$	32.5	2.5	60.1-100/ 10-97	53.3-85.1/ 18-82	0.110-0.401	[57]
Transparent cast acrylic tubes integrated 3 chambers of PBR (chamber volume: 2.5)	Sewage wastewater	*Chlorella minutissima*	Continuous, lab scale		Culture period: 48 h; 3 $L\cdot min^{-1}$ (air); (25±2)°C; 6480±300 lx; L:D=12:12	37	12.8	100	100	≤0.03	[56]
Alginic acid sodium salt opaque PVC PBR (4 L)	Tertiary wastewater	*C. vulgaris*	Batch and continuous, Lab scale and larger lab scale	6.5-12	Culture period: 24 h; 30°C;	7.1-10.4	0.08-1.78	100	60-90	0.18-1.55	[54]



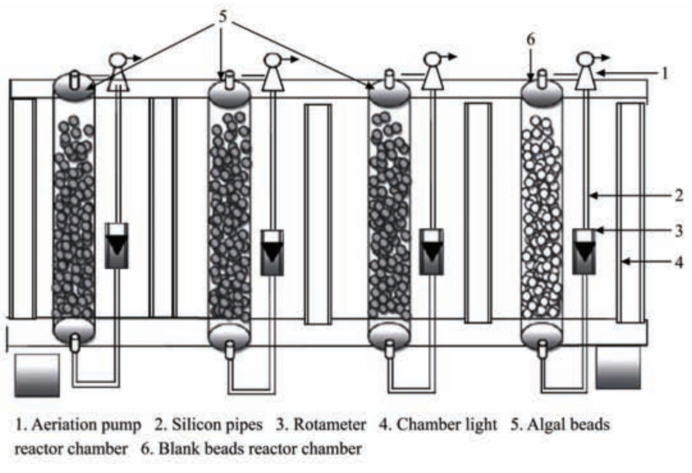
a. FBR system



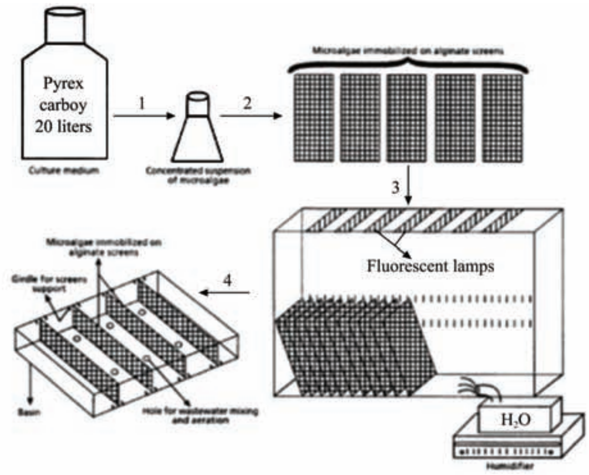
b. Packed bed filter immobilized PBR



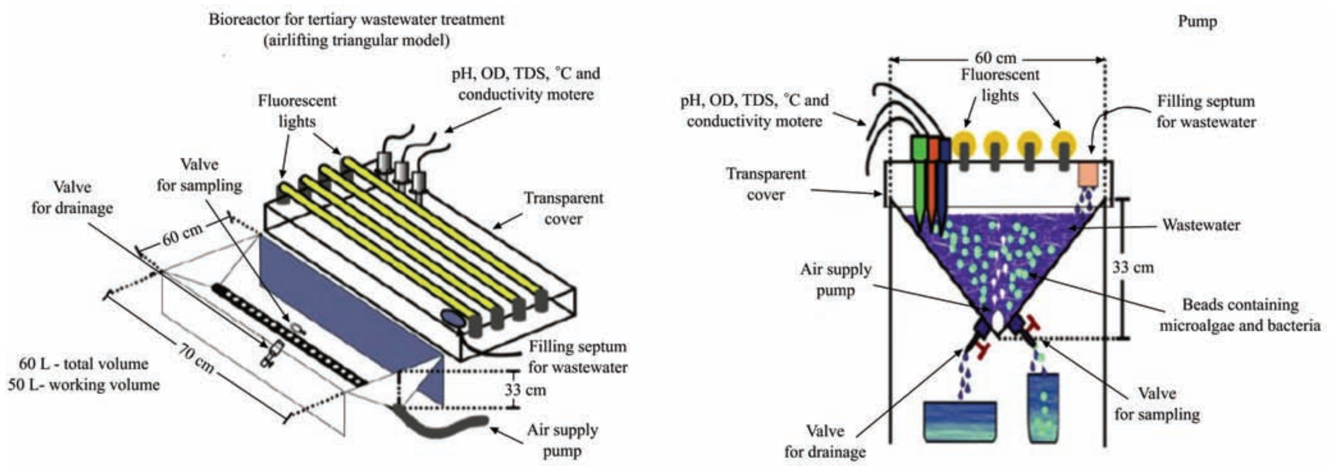
c. AFR system



d. Immobilized microalgae PBR[56]



e. Wastewater treatment by microalgae immobilized on screens and starved in air, saturated at 100% relative humidity[77]



f. 60 L, triangular, airlifting autotrophic immobilized microalgae PBR[78]

Figure 6 Different immobilized PBRs for wastewater treatment

The immobilized bioreactor design has two important influence factors, the intrinsic and extrinsic. The interaction refers to algae spices, wastewater components, environmental factors and the gel matrix, culture density, photon energy availability, temperature, pH and CO_2 concentration. The extrinsic condition refers to the advantages and constraints of each system with different aims.

For microalgae strains, it is obvious that in all previous works, *Chlorella* and *Scenedesmus* are the most commonly used microalgae strains for wastewater treatment in immobilization beads. The immobilized microalgae for wastewater treatment mainly focused on low concentration wastewater treatment. Most of them are municipal wastewater, sewage wastewater, domestic wastewater, and second effluent and tertiary wastewater[54-58]. The initial concentration of the wastewater always stayed nitrogen below 70 mg/L and

TP below 13 mg/L. There are also cattle manure wastewater treatment experiments by using this method with the initial TN and TP 237 mg/L and 34 mg/L, respectively[79].

The immobilized materials are important for the beads long staying in wastewater. Mallick and Rai[74] found that the chitosan was more effective at moving nutrients from wastewater than cell immobilized on agar, alginate, carrageenan or even free cell. The alginate and chitosan immobilization also got higher biomass yield.

Travieso et al.[75] found that the flow pattern of the reactor can influence the wastewater treatment efficiency. The study results showed that the fluidized-bed performance was better for COD removal than the packed bed mode. This might be caused by the greater area of contact between beads and dissolved nutrients and CO_2 in the wastewater. In addition, the gel thickness and cell density in the bead can influence the photosynthesis effect of microalgae, which finally affect the biomass production and nutrients removal. Zhang et al.[58] found that in a parallel-plate bioreactor, the cell density in the mixture of algae gel was more important than the thickness of the gel and the cell density of the reactor during pollutants removal. That might be caused by light limitation and substrate diffusion. There needs to be a balance between the cell density and the thickness of the beads. A high density and thickness of the beads will lead to the light shading effect, while a low density and thickness of the beads might lead to low nutrients absorption. In addition, Tam and Wong[76] found that algae beads concentration affects the ammonia removal, but the effect on phosphorus removal was less oblivious. Higher algae beads concentration leads to a higher ammonia removal efficiency.

The operation mode also influences wastewater treatment efficiency in the immobilized bioreactor. In a batch culture, the microalgae seem to suffer from nutrients depletion, while performed in semi-continuous mode, a higher biomass is obtained because of continuous nutrients supplementation.

It is very convenient for immobilized beads to separate the microalgae from wastewater; however, the microalgae need to be re-suspended in the liquid to acquire pure microalgae without other additional elements. In addition, the biomass is difficult to evaluate during the microalgae wastewater treatment processing because all the microalgae are packed in the matrix. Therefore, the beads must be broken up to count the microalgae cell or test the Chlorophyll content.

Although there are a lot of studies in wastewater treatment by using algae beads, there are some factors influencing the wastewater treatment efficiency which remain insufficiently studied, such as CO_2, light and temperature because the nutrients transfer between algae, the fixed materials and wastewater are very different from each other. There is a need to increase the understanding on the effect of immobilization on algae cell physiology and biochemistry.

3.2.2 Biofilm PBRs

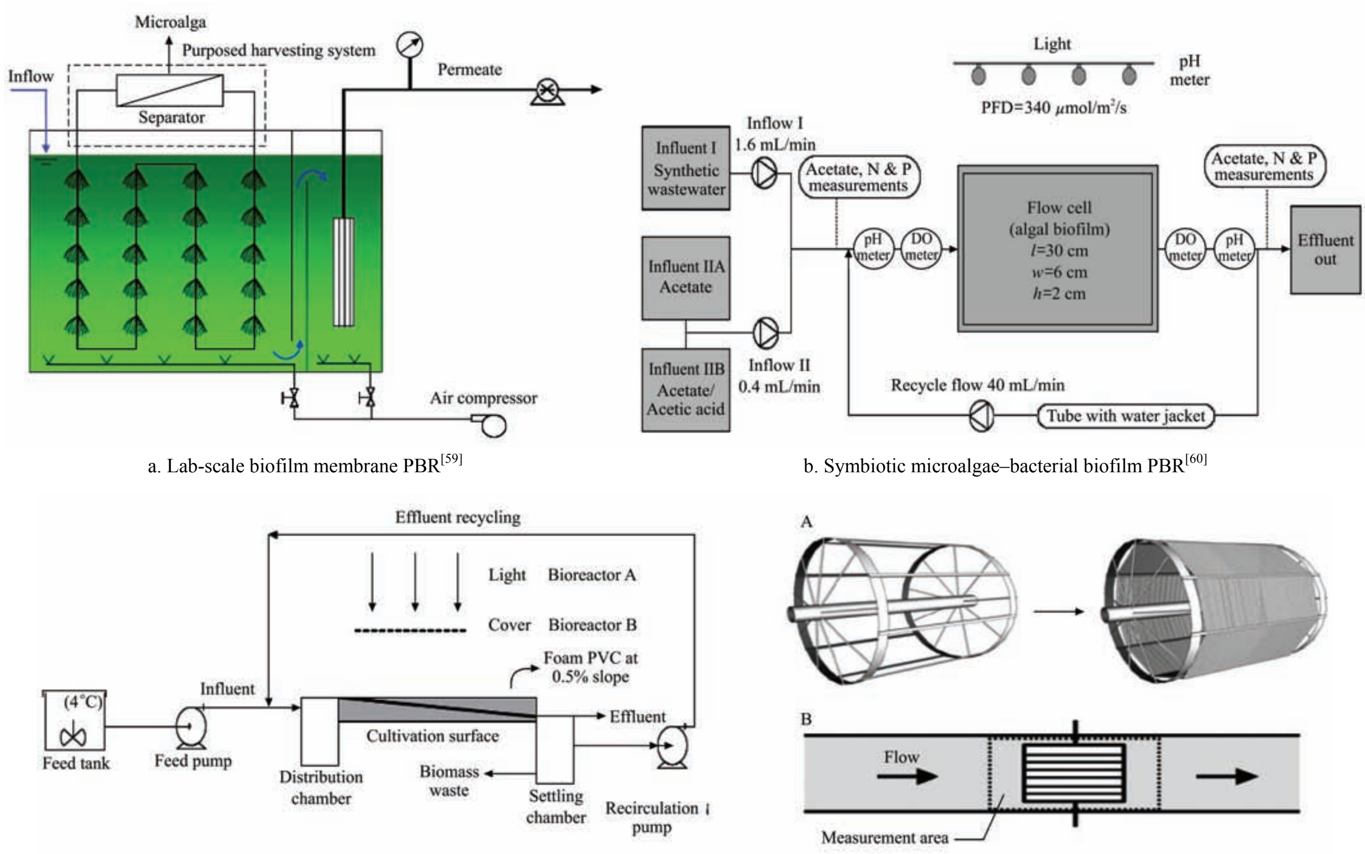
Microalgae can attach to the rough surface of the supporting materials to form a biofilm[80]. Most biofilms are composed of a bacteria-microalgae colony. It is very similar to the biofilm wastewater treatment technology[81]. In the biofilm PBR, the microalgae attaches to the supporting materials, and wastewater is poured down through the biofilm, which decrease the nutrients concentration. In addition, this kind of PBR facilitates microalgae collection has advantages compared to the suspended PBR. In this system, when the activity of bacteria and microalgae are stable, CO_2 and O_2 can realize the balance and does not need additional CO_2. The specific biofilm PBR wastewater treatment cases and configurations can be seen in Table 8 and Figure 7.

The supporting materials are very important for microalgae attachment. It can be divided into bio-materials and non-bio-materials. The rougher the surface of the materials is, the easier the microalgae will attach on to it. When choosing supporting materials in designing the biofilm PBR, the microalgae strain and the characteristics of wastewater must be both considered. The reaction between the materials and the substances in some types of wastewater must be avoided.

Gao et al.[59] found that the pollutants removal in the biofilm PBR was higher than that in the suspended microalgae membrane bioreactor. They used the secondary effluent from a municipal wastewater treatment plant to cultivate *Chlorella vulgaris*. The biomass productivity reached up to 0.072 g/(L·d), which was 1.44-fold greater than the suspended microalgae membrane bioreactor. In addition, 72.4% of the biomass was fixed on the biofilm, which further promoted nutrients absorption from the wastewater. There is 0.28 mg/L biomass retained in the suspended state, which promoted the recycled biomass quantity.

Table 8 Characteristics of different biofilm PBRs

PBR	Wastewater type	Microalgae species	Scale	Operation mode	Culture conditions	Influent /mg·L^{-1}	Removal / g·(m^2·d)$^{-1}$	Biomass productivity /g·(L·d)$^{-1}$	Ref.
Biofilm membrane	Municipal wastewater	*Chlorella vulgaris*	Lab-scale	Batch	Maximum 8000 lx; 25°C-28°C; Aeration: 2.0 L·min^{-1}; HRT: 48 h; Culture period: 20 d	NH_4^+-N: 5; PO_4^{3-}-P: 0.8; Total inorganic N: 15	NH_4^+-N: 96%; PO_4^{3-}-P: 85.9%; Total inorganic N: 82.5%	0.072	[59]
Microalgal–bacterial biofilm	Synthetic municipal wastewater	*Monoraphidium* sp., *Scenedes musacutus*	Lab-scale	Batch	340 μmol·(m^2·s)$^{-1}$; 23°C additional HCO^{3-} and aerobic bacteria; HRT: 4.5 h; Culture period: 26 d	NH_4^+-N: 50; PO_4^{3-}-P: 10; Acetate: 323	NH_4^+-N: 3.2; PO_4^{3-}-P: 0.41; Acetate: 43 g COD (m^2·d)$^{-1}$	-	[60]
Rotating algae biofilm	Municipal wastewater	-	Pilot-scale	Batch	208 μmol·(m^2·s)$^{-1}$; 11.8°C; HRT: 6 h; Culture period: 12 d	-	TN: 2.1; TP: 14.1	31	[62]



a. Lab-scale biofilm membrane PBR[59]

b. Symbiotic microalgae–bacterial biofilm PBR[60]

c. Lab-scale biofilm PBR[82]

d. Rotating algae biofilm PBR[62]

Figure 7 Configurations of different biofilm PBRs

The coordination effect between bacteria and microalgae led to a higher pollutants removal. Posadas et al.[82] built two open biofilm PBRs. One of them was filled with bacteria-microalgae colony, the other was filled with bacteria. Results showed that the performance of the former was better with regard to the pollutants removal than the latter. The COD, TN and TP removal reached 91%±3%, 70%±8% and 85%±9%. Boelee et al.[60] found that when using a bacteria-microalgae colony for treating municipal wastewater in a biofilm PBR, the supplementation of carbonate and light made the CO_2 demand of the microalgae and the O_2 demand of bacteria were in an equilibrium state. No extra CO_2 and O_2 are needed for this type of PBR.

When the carbonate and light were withdrawing, the growth of bacteria and microalgae were inhibited. In addition, the pollutants degradation rates obviously decreased. These results further proved the coordination between bacteria and microalgae in biofilm PBR.

In addition, novel biofilm PBRs for wastewater treatment is being explored. Christenson and Sims[62] invented the rotating biofilm PBRs for three scales (8 L, 535 L and 8000 L). These kinds of new PBR performed better on pollutants removal and biomass production than the suspended open pond system. The TN, TP removal rate and biomass productivity reached up to 2.1 g/(m^2·d), 14.1 g/(m^2·d) and 5.5-31 g/(m^2·d). Based on this kind of PBR, a new recycling method was invented and finally, microalgae lipid with 12%-16% content was obtained. The energy cost was only 6.3 W/m^2.

3.2.3 Microalgae membrane bioreactors

Membrane bioreactors (MBR) are the most popular and an effective wastewater treatment technology used in the water treatment area. After treated via a membrane, the pollutants in the effluent are relatively low. For microalgae wastewater treatment technology, one of the big problems is collection of microalgae. For the traditional PBR such as the flat bioreactor, microalgae can be easily washed out of the bioreactor. The membrane has well-known function of excellent micro-size particles separation. Hence, applying membranes combing microalgae to treat wastewater allow decoupling the dilution rate (related to HRT) and biomass retention time (MRT). Higher biomass concentrations and productivities may be obtained by this way. Without membranes, the attained biomass concentrations can be too low to be considered for recovery, as this would finally result in a very high harvesting cost. Therefore, MBR was applied in the microalgae wastewater treatment processing, called microalgae MBR (MMBR).

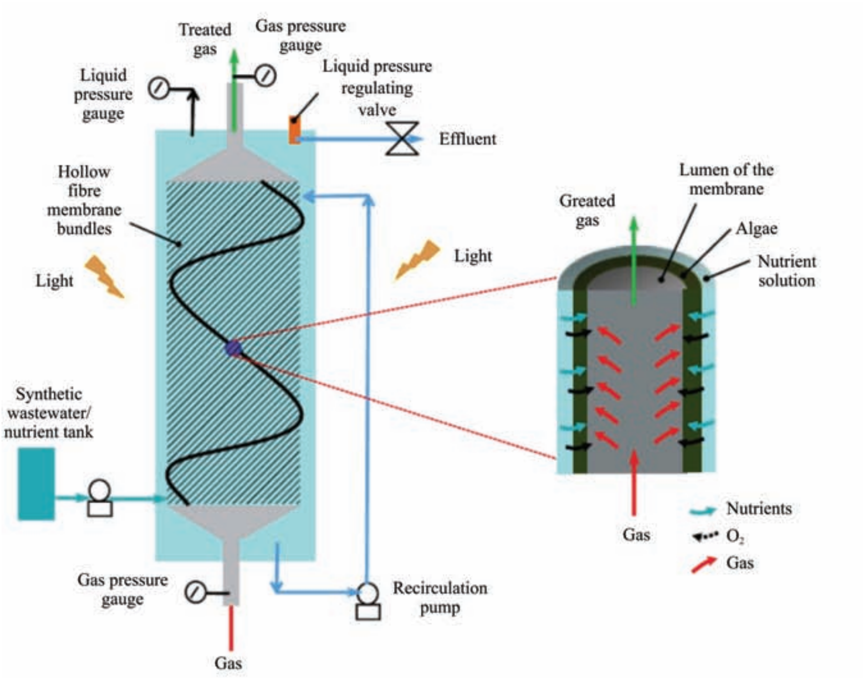
Usually, the MMBR are composed of four main parts: main reactor with cylinder or square-shaped, membrane module, light provision system and gas supplementation system. In addition, some of the MBR systems have a cycle tanker to ensure the recycling of the water from the bioreactor[83]. The microalgae, wastewater, and membrane are all posted in the main bioreactor. The surface of the bioreactor is transparent to allow the light to penetrate. The membrane module is always submerged into the wastewater to separate the liquid (wastewater) and solid (biomass). The MMBR has main types of bubble diffuser mode. The CO_2 and air mix gas was pumped into the MMBR from the bottom of bioreactor to provide a carbon source to microalgae or adjust pH of wastewater in the MMBR system. In addition, the mixed gas also can diffuse microalgae and wastewater to accelerate nutrients transformation. In order to promote the utilization efficiency of CO_2 by microalgae, Kumar et al.[84] explored another novel MMBR which supplies the CO_2 and air mixed gas through the lumen of a hollow fiber membrane in addition to the air supplemented into the bioreactor through the bubbling which located at the bottom of the reactor. The membranes were composed of composite laminated hollow fibers with a thin dense polyurethane layer sandwiched between two microporous polyolefine layers. This kind of MMBR can promote the contact area available for gas transfer. Compared with the traditional bubble diffuser reactors, MBR have a low overall mass coefficient, higher surface area of membrane per unit volume of fibers, leading to retaining more CO_2 in the bioreactor. The CO_2 removal during wastewater treatment processing can reach 85%.

The membrane materials are the traditional MBR membrane materials such as PVDF, PE, DHPE or ceramic. The pore size ranges from 0.1 μm to 0.45 μm. For the ceramic membrane, the pore size is 200 nm. It is very important to know whether the membrane is hydrophilic or hydrophobic, because different kinds of membrane materials will influence the mass transfer which is closely related to biomass production and nutrients removal.

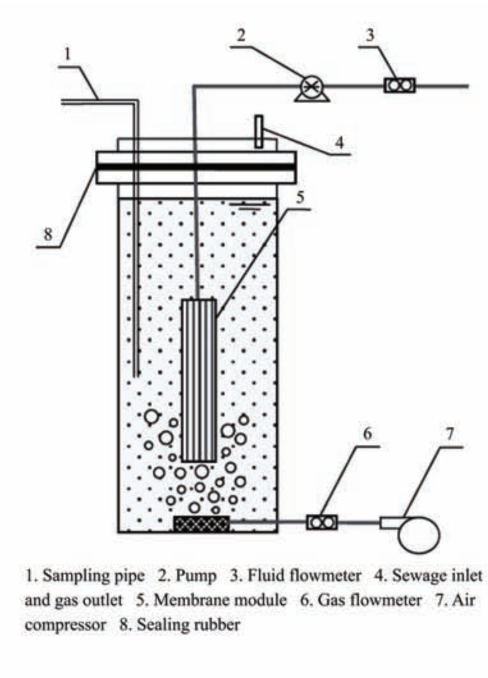
Although it has many advantages, the MMBR is now gradually applied in treating low concentration wastewater, such as MBR permeate, sewage wastewater, second effluent and aquaculture wastewater which the nitrogen and phosphorus concentration below 60 mg/L and 7 mg/L, respectively. Therefore, some of the design factors that are used in the traditional MBR factors must be considered in treating wastewater by using the microalgae MBR system. In this bioreactor, *Chlorella* and *Scenedesmus* are still the most dominated microalgae strains for wastewater treatment[83]. There is also other diversity microbiology such as microalgae and activated sludge combination as the inoculum. It seems that the bacteria-microalgae system performed better than that only microalgae system for the COD removal, however, the bacteria-microalgae system is hard to stay steady because of its complicated intraspecific relationship among different microbiology.

Table 9 Characteristics of MMBRs

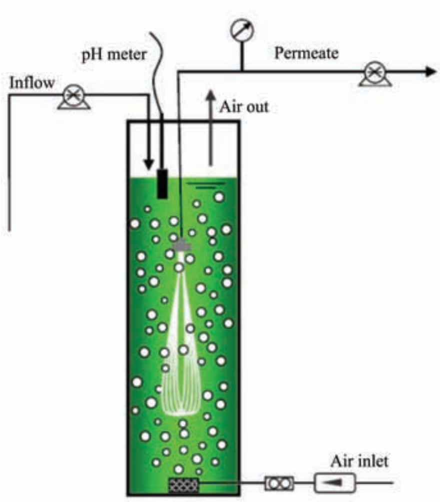
PBR	Wastewater type	Algae species	Operation mode and scale	HRT/d	Culture conditions	Influent/mg·L^{-1}		Removal/%		Biomass productivity /g·(L·d)$^{-1}$	Ref.
						N	TP	N	TP		
Glass MBR with hollow fiber membrane (0.5 L)	Synthetic wastewater	*Spirulina platensis*	Continous, lab scale	5	Culture period: 0-49 d; 9000 lx; L:D=24:0; 2%-3% CO_2	-	-	NO_3^-: 68-82	-	0.1968	[84]
Photo MBR (1300 L)	electronic device factory effluent	*Scenedesmus* sp. LX1	Batch and continuous, pilot scale	–	Culture period: 15 d; 22°C-28°C; 6000 lx	TN: 0.8	0.04	NO_3^-: 46	100	0.09 (batch); 0.02 (continuous)	[89]
Transparent glass tank-flat panel MMBR	MBR permeate	*Chlorella* sp., *Chlorella vulgaris, Scenedesmus quadricauda*, *Scenedesmus dimorphus*	Continuous, lab scale	1.6	Culture period: 23 d; (24±2)°C; 4000±300 lx; L:D=12:12	0.8-1.5	7-17	50	60	0.084×10^6 cell·L^{-1}	[90]
Dual MBR with polyethersulfone UF membrane (80 L)	Domestic wastewaters	*Chlorella vulgaris*	Continuous, pilot scale	-	Flow rate: 6.0 L·(m^2·s)$^{-1}$	≤5	-	-	-	-	[88]
Plexiglass tankMBR with immersed hollow-fiber membrane (10 L)	Sewage	*Chlorella vulgaris*	Batch, lab scale	2.5	(25±2)°C; 8000 lx	11.26	1.24	-	-	0.3993	[67]
Submerged ceramic MBR (7 L)	-	*C. vulgaris* CCAP 211/11B	Continuous, lab scale	6.5-72 h	Culture period: 60 d; 6% CO_2; 7500 lx; 26°C-32°C; Suction cycle: 8 min	NO_3-N: 55.45	-	-	-	0.1617-0.1762	[86]
PE MBR (25 L)	-	*Chlorella vulgaris*	Lab scale	1.5	Culture period: 6 months; L:D=24:0	N: 7.48-22.1	P: 1.69-2.17	-	-	-	[87]
Algae-based MBR with Hollow fiber membrane (7.2 L)	-	*Chlorella emersonii*	Continuous, lab scale	24 h	Culture period: 150 d; 80±5 μmol·(m^2·s)$^{-1}$; L:D=12:12	-	-	-	66	32.5	[85]
MBR with polymeric membrane (40 L)	Municipal wastewater from preliminary sedimentation	*Chlorella vulgaris*	Continuous, lab scale	9 h	Culture period: 150 d; (25±3)°C; 270-310 μE·(m^2·s)$^{-1}$; L:D=16:8	11.61	4.36	87.4	84.6	-	[91]
Double column-type MBR with hollow fiber microfiltration membrane (10 L)	Secondary sewage effluent	*Chlorella* sp. ADE4, *Chlorella vulgaris*	Continuous, lab scale	2	Culture period: 18d; (25±2)°C; 50 μmol·(m^2·s)$^{-1}$; L:D=14:10	18.1	1.01	66.5	94.5	0.055	[66]
Biofilm MBR (2 m^2 growth area)	Tertiary wastewater	*Cyanobacteria, Phormidium autumnale, Pseudanabaena* sp., *Chrococcus* sp., *Coccal green algae, Scenedesmus acutus, Monoraphidium contortum, Diatoms, Cymbella minuta*	Continuous, large scale	7 min	Culture period: 2 a; 19°C-24°C; 0-904 mmol photons·(m^2·s)$^{-1}$ L:D=24:0, 12:12	49.4	2.9	-	97 (L:D=24:0); 36-41 (L:D=12:12)	12.21±10 g·(m^2·d)$^{-1}$ (L:D=24:0); 5.6 g·(m^2·d)$^{-1}$ (L:D=12:12)	[65]
Plexiglass cylinderical MPBR with hollow-fiber microfiltra membrane (4 L)	Domestic second effluent	*Chlorella vulgaris*	Continuous, lab scale	2	Culture period: 35d; 25°C-30°C; 120.8 μmol·(m^2·s)$^{-1}$; 4% CO_2	14.12	0.78	87.70	76.70	0.05072	[64]
Commercial thin film composite MBR with osmotic membrane (5.5 L)	Tertiary wastewater	*Chlorella vulgaris* ATCC 13482	Continuous, lab scale	3-4	Culture period: 162 d; 5% CO_2; 1000-1500 lx	8.8-22.8	2.4-6.0	NH_3-N: 93	PO_4^{3-}: 89	0.03086	[63]
Transparent plexiglass cylindrical MPBR with hollow-fiber membrane (4-5 L)	Aquaculture wastewater	*Chlorella vulgaris*, *Scenedesmus obliquus*	Continuous, lab scale	1 d	16 d; 99.9% CO_2; (25±2)°C; 9000 lx	6.81	0.42	86.1	82.7	0.0426	[92]



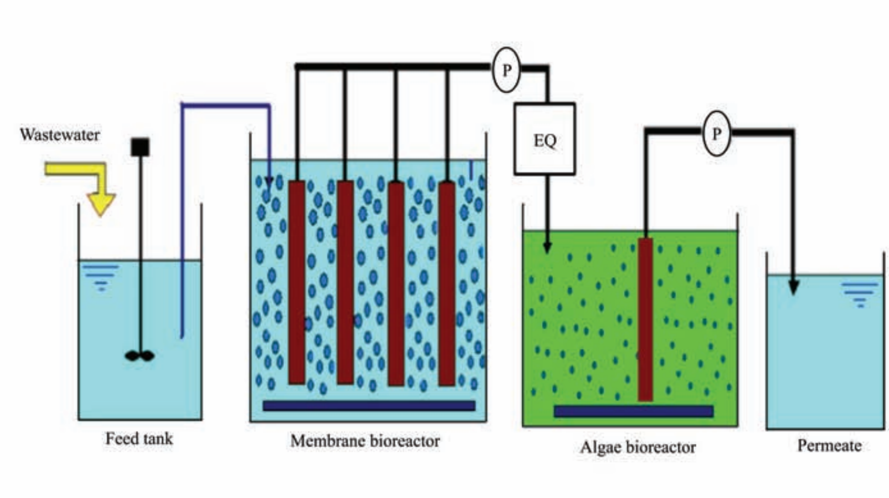
a. Bench-scale hollow fiber membrane PBR system[84]



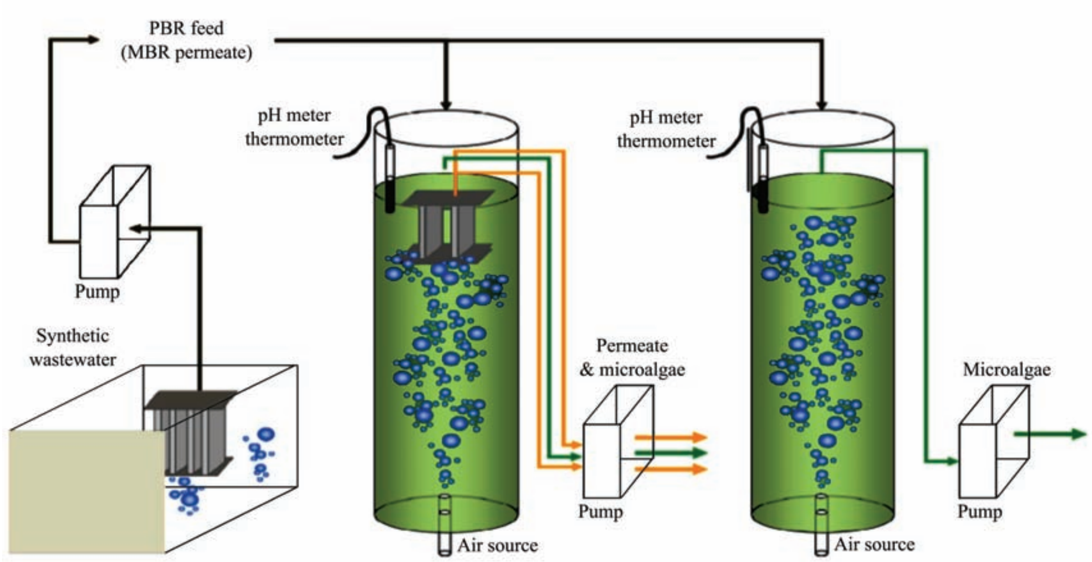
b. Lab-scale membrane PBR[67]



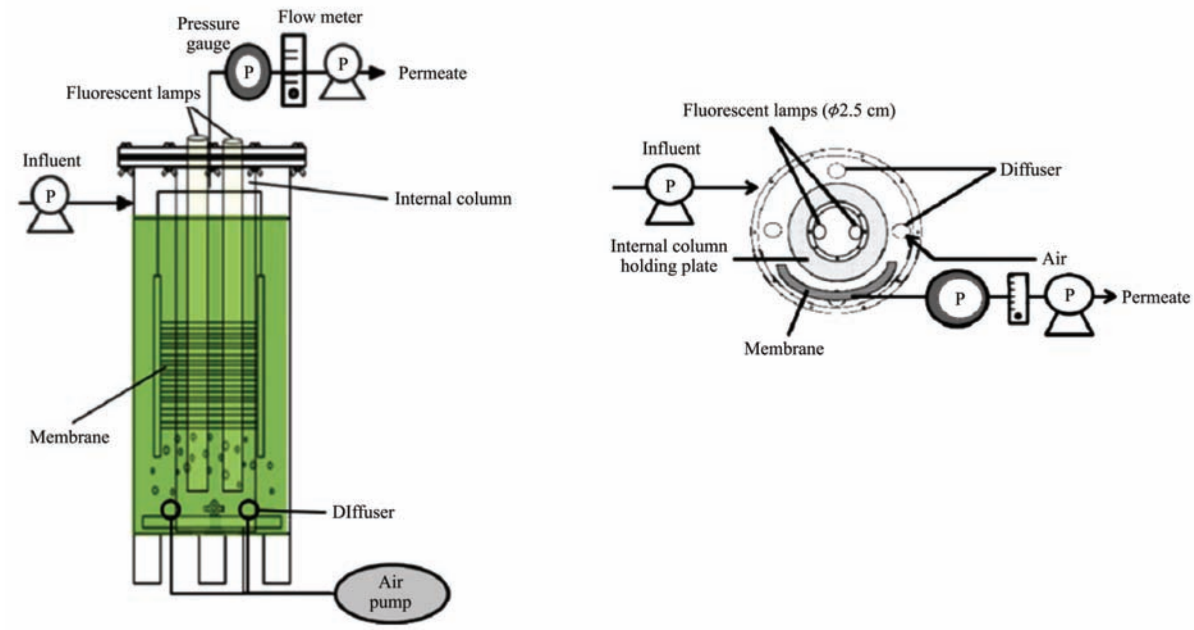
c. Lab-scale membrane PBR[92]



d. MBR–algae continuous bioreacto r[90]



e. Combined aerobic MBR[87]



f. Membrane PBR[66]

Figure 8 Configurations of MMBRs

The filtration effect of the membrane modules submerged in the reactor prevented the microalgae cells from being wasted out and enabled the reactor to operate at a high supply rate. Although Xu et al.[85] found that a high-density cultivation process could facilitate algae harvesting, the prolonged SRT led to self-shading. Liquid circulation could be used as a partial solution to the self-shading problem. Therefore, the dilution rate and HRT is important to the MMBR processing operation that make the final goal of high biomass production and nutrients removal, especially in the continuous operation mode. In addition, the MMBR also has another parameter that can be exploited, namely the volumetric concentration factor. Applying a high volumetric concentration will force the system to work at a very high biomass concentration, thus increasing the uptake rate and removal efficiency. However, under this condition, membrane fouling will probably be more severe because the membranes are exposed to a high biomass concentration.

Low et al.[86] found that the biomass production decreased with the increase of HRT, while the lipid productivity was a significant higher under the low HRT run. Marbelia et al.[87] compared the influence of the dilution rate on biomass productivity and nutrients absorption by using PBR and MMBR. Results showed that the MMBR was able to prevent wash-out by keeping the biomass concentration relatively higher in a wider range of dilution time. Finally, the biomass in MMBR was 3.5-fold compared to PBR was obtained by MMBR. This reduced the load of the harvesting step. Gao et al.[67] also got the same results. MMBR also acquired a high pollutants removal under higher dilution rate and lower HRT compared with the traditional PBR, which means it can reduce the volume of the bioreactor. The increased dilution rate in the MMBR is followed by an increase of the N and P supply rate (mg/(L·d)), which leads to the low removal of nutrients. Therefore, using a high dilution rate may provide benefits due to the high microalgae productivity, but the incomplete nutrient removal becomes a drawback in which a high concentration of N and P exist in the wastewater. Thus, it is important to still choose the optimum dilution rate in order to compromise between biomass concentration and productivity and the nutrient removal, and even the low membrane fouling propensity.

There are also different operation modes for MMBR. Gao et al.[64] proposed a new MMBR to completely separate the HRT and the biomass retention time of the MMBR. Compared with batch system, the MMBR has a higher organic load and flow rate. They use this kind of MMBR to treat aquaculture wastewater. They found that microalgae performance was better in continuous mode than that in batch mode, the biomass productivity was 5.8-fold larger than that achieved in batch cultivation in flasks. They can also remove most of the nutrients and nearly all the unionized ammonia from the aquaculture wastewater with a short HRT of only 1 day, which was well below than that used in some previous batch cultivation studies.

In addition, there are other factors affect HRT. Singh et al.[90] combined MBR and MMBR together to further purify the MBR permeation. The cell residence time (MCRT) and HRT to achieve the maximum biomass content or pollutants removal were variant for different microalgae strains and different types of wastewater. In order to solve this problem, Wang et al.[88] proposed the two stage MBR system. The first MBR was used for nitrification and bacteria filtration, the second MBR was used for nitrate absorption by using microalgae. Different nitrogen sources demand different MCRT. Nitrifying bacteria seems to compete with *Chlorella* in the bioreactor.

MBR improves the scale-up feasibility. It requires less space and is more feasible to be applied. For MMBR, although there are some pilot-sale experiments, most of them are at the lab scale. Some of the problems have not been solved such as the membrane fouling and integrity, the long-term algae population stability and biomass viability. In addition, this kind of bioreactor may not be suitable for treating high density biomass or solid-containing wastewater for the high membrane fouling problem. In fact, the membrane fouling of MMBR is not as serious as the traditional activated sludge MBR with the same operation cycle although the EPS contents of these two bioreactors are similar to one another. The potential fouling factors are complicated, such as the nature of the NOM, solution characteristics, membrane properties, and system operation conditions etc., and the combined effects of the factors result in more severe fouling problems. Boonchai and Seo[66] studied the influence of flux to separate biomass from wastewater in the MPBR system. Higher flux increased the degree of membrane fouling. The flux below 58.5 L/(m^2·h) was recommended for sustaining operations in MMBR. Praveen and Loh[63] found that the salt accumulation and a large quantity of sugars and chlorophyll on the membrane filtration might be the main reason causing membrane fouling. The filtration performance can be recovered through periodic backwashing of the membranes. Little research has been performed using real wastewater or with transient loads. The combined influence of varying process parameters such as temperature and light will also affect algae productivity.

Kumar et al.[84] estimated the cost for building the wastewater treatment plant using the hollow fiber MMBR. The price was twice that of a conventional nutrient removal plant. However, they did not consider other important influence factors such as biofuel production, potential carbon sequestration credits, geographical location, species growth rate and wastewater composition. Further investigation needs to be made according to the aim and the specific conditions.

3.3 New PBRs for wastewater treatment

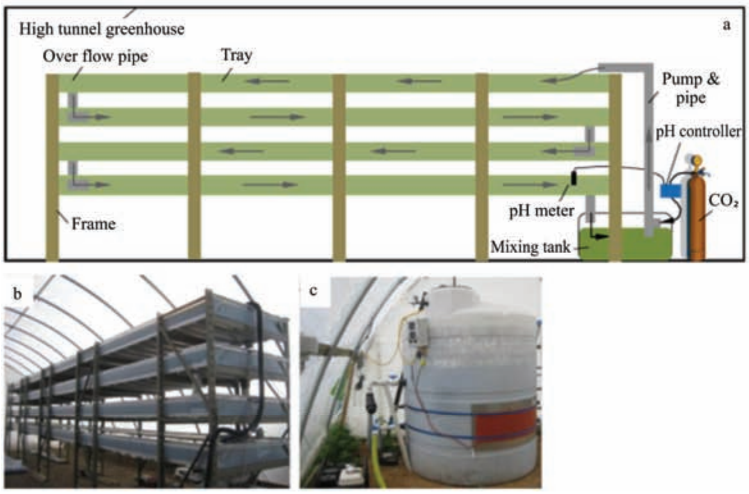
In addition to the traditional PBRs, there are some novel PBRs invented by scientists to treat wastewater. Min et al.[53] built up a pilot scale system of PBRs which contains multi-storey trays to treat anaerobic piggy digested effluent with a high organic load. This kind of PBR system can be put in the greenhouse to control temperature. This kind of PBR system also consists of a supporting frame, a stack of four trays, a mixing tank, a sump pump, a CO_2 tank, and a pH controller. Each PBR had an overall dimension of 4 ft (W)×32 ft (L)×8 ft (H) in which each tray can hold up to 6 inch of water equivalent to a volume of 1800 L. With the mixing tank and four trays, the total capacity of each PBR was 7500 L. The CO_2 was supplanted into the mix tank. This kind of PBR system has the advantages of a high mixotrophic growth rate, low operating cost, as well as reduced land footprint due to the stacked-tray bioreactor design used in the study. The biomass productivity, COD, TN and TP adsorption rate reached up to 19.15-23.19 g/(m^2·d), 7.2 g/(m^2·d), 2.65 g/(m^2·d), and 3.19 g/(m^2·d), respectively.

Shi et al.[51] developed a prototype-scale Twin-Layer PBR made by a porous sheet-shaped material, the nylon filter sheets and a reinforced glass fiber mesh as the framework, which was used for microalgae fixation. The Twin-Layer PBR was operated with three modules of

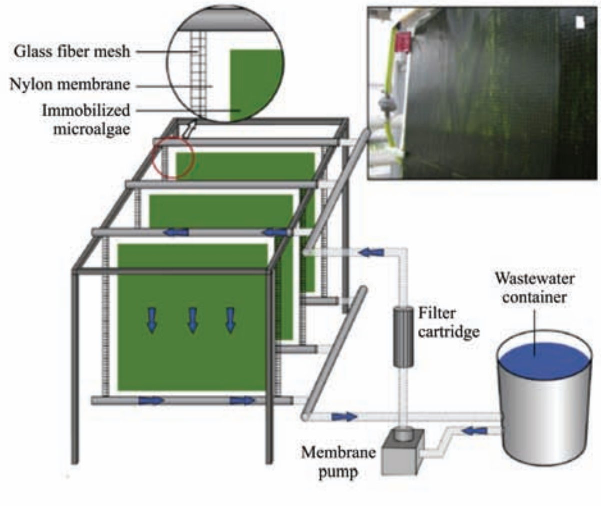
1.0×1.0 m area mounted vertically on a metal rack. The wastewater was poured from the top of the twin-layer and flowed through the whole surface of the twin-layer under the effect of gravity. Therefore, the substances in wastewater were homogeneous in the microalgae that fixed on the twin-layers. Compared to the traditional suspended system, this kind of PBR reduced the outflow of microalgae, and promoted the recycled microalgae cell. This kind of PBR was used for treating municipal wastewater by *Halochlorella rubescens* and the TN and TP removal reached 99.05% with a biomass productivity of 6.3 g/(m^2·d).

Table 10 Characteristics of different novel PBRs

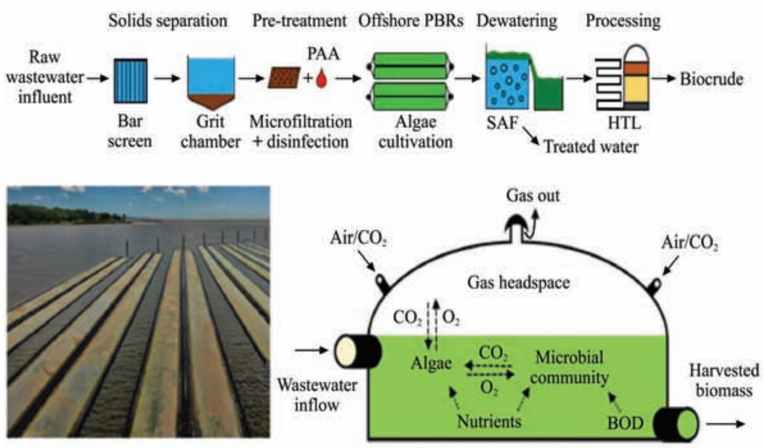
PBR	Wastewater type	Microalgae species	Operation mode and scale	Culture conditions	Influent /mg·L^{-1}	Removal /%	Biomass productivity /g·(m^2·d)$^{-1}$	Ref.
Greenhouse-based vertically arranged multilayer	Swine manure wastewater	*Chlorella* sp.	Batch, pilot scale	560.57 μmol·(m^2·s)$^{-1}$; 24.38°C; Culture period: 30 d	-	COD: 7.21 g·(m^2·d)$^{-1}$; NH_4^+-N: 2.65 g·(m^2·d)$^{-1}$; PO_4-P: 0.067 g·(m^2·d)$^{-1}$; TN: 3.19 g·(m^2·d)$^{-1}$	14.59	[53]
Twin-layer	Municipal wastewater	*Halochlorella rubescens*	Batch, pilot scale	22-220 μmol·(m^2·s)$^{-1}$; 18°C-32°C; Culture period: 32 d	NH_4^+-N: 11.10; PO_4^{3-}-P: 3.81	NH_4^+-N: 5.52 mg·(L·d)$^{-1}$; PO_4^{3-}-P: 1.53 mg·(L·d)$^{-1}$	6.3	[51]
Floating offshore	Municipal wastewater	*Chlorella*, *Cryptomonas*, *Scenedesmus*	Continuous, large scale	Culture period: 14 d	COD: 542; NH_4^+-N: 26.5; TN: 40.0; PO_4^{3-}-P: 2.98; TP: 4.22	COD: 84; NH_3-N: 80; TN:75; PO_4^{3-}-P: 95; TP: 93	3.5-22.7	[96]
Floating offshore	Municipal wastewater	*Desmodesmus* sp.	Continuous, large scale	Culture period: 23 d	-	NH_4^+-N: >90; CO_2-conversion: >50%	14.1-20.0	[97]



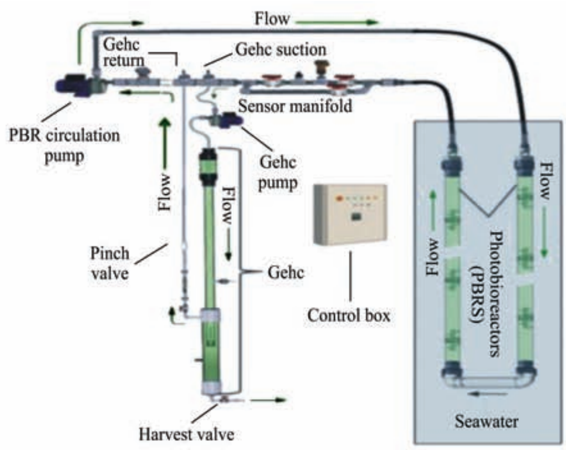
a. Greenhouse-based vertically arranged multilayer PBR[53]



b. Twin-layer PBR[51]



c. Offshore PBR field in Mobile Bay, AL.[96]



d. "OMEGA system" offshore PBR[97]

Figure 9 Configurations of new PBRs

In summary, the structures of the new PBRs are very complex. Some of them combined the characteristics of open system and closed system. The new PBRs enlarged the area affected by light. They also improved the light quality and enhanced the flash light effect[93, 94]. The supplementation of CO_2 is effective and very special while it can reduce the uneven mass transfer. Some of the PBRs also resolved the microalgae separation problems and meanwhile lowered the overall cost. Some of the PBRs are even equipped with automatic control functions or on-line monitoring of water quality functions[95].

4 Conclusions

Although there are a lot of PBRs for microalgae pure cultivation, the PBRs that are used for wastewater treatment by microalgae have not been maturely developed. There are not too many studies concerning the bioreactors. In addition, most of the PBRs for wastewater treatment are adopted directly, there is no legitimate design for wastewater treatment. In fact, the traditional wastewater treatment technologies can be applied in microalgae wastewater treatment processing. Therefore, some of the characteristics and treatment theories can be used in the microalgae wastewater treatment processing and PBR design. In addition to the regular parameters (such as HRT, operation mode, air flow, etc.) must be taken into account, the photosynthetic effect of the microalgae must be considered into the PBR design and wastewater treatment because nutrient removal is highly dependent on CO_2 assimilation which is carried out by the photosynthetic effect. Therefore, when designing a PBR, the characteristics of wastewater, microalgae strains, and basic theory of wastewater treatment must be combined.

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