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Minireview

MINIREVIEW – Biotechnology & Synthetic Biology

Biofilm-based photobioreactors: their design and improving productivity through efficient supply of dissolved inorganic carbon

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One sentence summary: This research summaries current knowledge on biofilm-based photobioreactors, especially in respect of dissolved inorganic carbon supply.

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ABSTRACT

The potential of biofilm-based photobioreactors (PBRs) for various applications has long been recognized, and various types of biofilm-based PBRs have been developed for different applications. Compared to suspension-based PBR reactors, biofilm-based systems offer several advantages, including a significantly higher biomass concentration. However, due to the immobilization of the cells, in contrast to suspension-based systems, dissolved inorganic carbon (DIC) has to be transferred into the biofilm for consumption. Thus, to ensure efficient operation of these systems under a given lighting scheme (e.g. depending on geographical location), availability of DIC should be optimized. To achieve this, the dynamics of DIC inside the various biofilm-based PBRs, as well as the operational principles of these PBRs, need to be understood. The mini-review summarizes the designs of existing biofilm-based PBRs and reviews previous studies on DIC dynamics in various biofilms. Strategies to enhance DIC availability for the immobilized cells in biofilm-based PBRs are also discussed.

Keywords: photobioreactor; dissolved inorganic carbon; phototrophic biofilm; microalgal biotechnology; microalgal biofilm; CO_2

BACKGROUND

Biofilm-based photobioreactors

Photobioreactor (PBRs) are bioreactors for cultivating microalgae. These systems have been used for various applications, including the production of microalgal biomass and/or products derived from microalgae, waste water treatment and CO_2 sequestration (Chen *et al.* 2011; Abdel-Raouf, Al-Homaidan and Ibraheem 2012; Singh and Sharma 2012; Slade and Bauen 2013; Olivieri, Salatino and Marzocchella 2014; Gupta, Lee and Choi 2015). Biofilm-based PBRs utilize microalgal biomass immobilized on a substrate, and thus separate most of the biomass from the bulk culture medium (Olivieri, Salatino and Marzocchella 2014; Gross, Jarboe and Wen 2015; Katarzyna, Sai and Singh 2015; Kesaano *et al.* 2015b; Hoh, Watson and Kan 2016; Podola, Li and Melkonian 2017). Compared to suspension-based PBRs, biofilm-based systems offer several advantages. Most importantly, high dry biomass density of the biofilm enables

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a more energy-efficient operation and harvesting, because the high biomass concentration of the biofilm requires little or no dewatering before downstream processing (Olivieri, Salatino and Marzocchella 2014; Gross, Jarboe and Wen 2015; Podola, Li and Melkonian 2017). Also, the concentrated biomass in the biofilm leads to a significant reduction in reactor and/or medium volume required for cultivating the same amount of biomass (Olivieri, Salatino and Marzocchella 2014; Gross, Jarboe and Wen 2015; Podola, Li and Melkonian 2017).

Generally, biofilm-based PBRs can be further divided into submerged systems and porous substrate photobioreactors (PSBRs) (Podola, Li and Melkonian 2017). In submerged-biofilm PBRs, the biofilms are usually immobilized/attached onto impermeable substrates, which are either constantly or periodically submerged in the culture medium (Olivieri, Salatino and Marzocchella 2014; Gross, Jarboe and Wen 2015; Podola, Li and Melkonian 2017). In PSBR, the biofilm is attached to a microporous substrate, which separates the biofilm from the medium. Because the pore size of the substrate is smaller than the micro-algal cells comprising the biofilm, the biomass is confined to one side of the substrate, and thus separated physically from the flow of the medium (Nowack, Podola and Melkonian 2005; Shi, Podola and Melkonian 2007; Naumann *et al.* 2013). Table 1 provides an overview of the existing biofilm-based PBR technologies.

Biofilm-based PBRs have been used for production of microalgal biomass, production of microalgal-derived products (e.g. lipids, pigments), wastewater treatment and CO_2 sequestration (Olivieri, Salatino and Marzocchella 2014; Gross, Jarboe and Wen 2015; Katarzyna, Sai and Singh 2015; Kesaano *et al.* 2015b; Hoh, Watson and Kan 2016; Podola, Li and Melkonian 2017). All of these applications rely on rapid microalgal growth (i.e. high productivity), requiring high fixation rate of inorganic carbon. Unlike in suspension-based PBRs, the homogenous mixing of the biomass and the culture medium does not occur in biofilms. Due to the immobilization of the cells, mass transfer inside dense biofilms relies largely on diffusion (Siegrist and Gujer 1985; Liehr, Wayland Eheart and Suidan 1988; Liehr, Suidan and Eheart 1989, 1990; Murphy and Berberoglu 2014; Li, Podola and Melkonian 2016b). Compared to advection (i.e. solute transport by liquid flow), mass transfer through diffusion is much slower above micrometer scale (Siegrist and Gujer 1985; Brenn 2017). As a result, gradients of dissolved species, dissolved inorganic carbon (DIC) concentration among others, form inside the biofilms. Due to dense biomass packing, light gradient is also expected in phototrophic biofilms (Siegrist and Gujer 1985; Liehr,

Table 1 Schematic representations of different biofilm-based photobioreactors (PBRs).

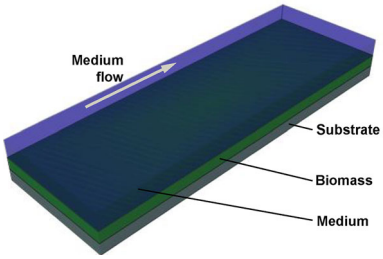
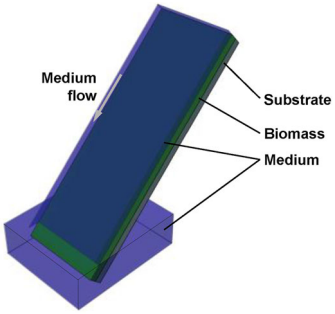
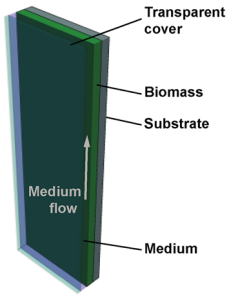
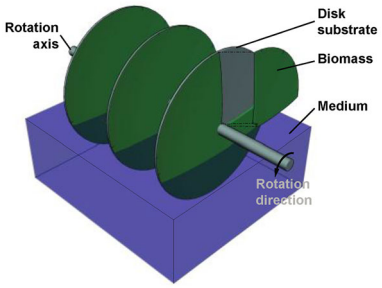
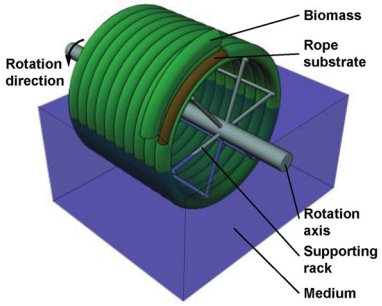
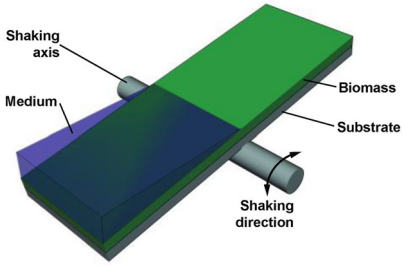
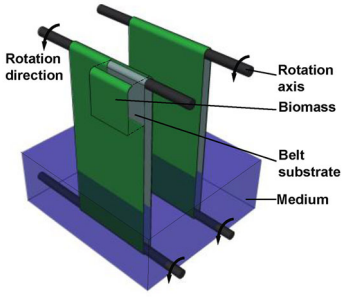
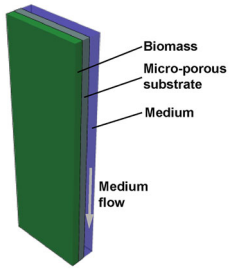
Type		Schematic	[a]Example
Permanently submerged	**Flat plate biofilm PBR** Horizontal orientation; medium can flow in any direction, or can be stagnant.		Guzzon *et al.* (2008); Cao *et al.* (2009); Boelee *et al.* (2014)
	Turf scrubber Inclined or vertical orientations; medium flows downward.		Setlik, Sust and Malek (1970); Adey, Luckett and Jensen (1993); Doucha and Livansky (1995)
	Closed biofilm PBR Inclined, horizontal or vertical orientations; medium flow direction can be down/up/side-wards.		Sharp *et al.* (2017)

Table 1. – *Continued*

Type		Schematic	[a]Example
Periodically submerged	**Rotating disk PBR** Orientation of the rotation axis parallel to the ground; medium can flow in any direction, or can be stagnant.		Torpey *et al.* (1971); Przytocka-Jusiak *et al.* (1984); Blanken *et al.* (2014)
	Rotating drum PBR Orientation of the rotation axis parallel to the ground; medium can flow in any direction, or can be stagnant.		Christenson and Sims (2012); Bernstein *et al.* (2014)
	Oscillating PBR Orientation of the shaking axis parallel to the ground; medium can flow parallel to the rotation axis, or can be stagnant.		Johnson and Wen (2010)
	Revolving Belt PBR Orientation of the rotation axis parallel to the ground; medium can flow in any direction, or can be stagnant.		Gross and Wen (2014)
Non-submerged	**Porous substrate PBR** Inclined, horizontal or vertical orientations; medium flow direction can be down/up/side-wards.		Shi, Podola and Melkonian (2007); Naumann *et al.* (2013); Murphy and Berberoglu (2014)

[a]Not all studies are presented.

Wayland Eheart and Suidan 1988; Liehr, Suidan and Eheart 1989, 1990; Wolf, Picioreanu and van Loosdrecht 2007; Murphy and Berberoglu 2014; Wang, Liu and Liu 2015; Li *et al.* 2016a; Li, Podola and Melkonian 2016b).

Factors affecting productivity of biofilm-based PBRs

Independent of the design, light, nutrients and DIC are the three major factors affecting productivity in PBRs (Schnurr and Allen 2015). For suspension-based PBRs, light gradients inside the reactor vessel can affect the productivity of the system (Quinn, Turner and Bradley 2012; Brindley *et al.* 2016). Optimization of PBR design, combined with mixing, can reduce the negative impact caused by light attenuation along the light path inside a suspension PBR (Quinn, Turner and Bradley 2012; Brindley *et al.* 2016). This also minimizes or even eliminates gradients of DIC and nutrients (Zhang, Kurano and Miyachi 2002; Guo, Yao and Huang 2015). In biofilm-based PBRs, the formation of gradients inside the biofilm is inevitable. For example, light is known to attenuate rapidly inside dense phototrophic biofilms (Murphy and Berberoglu 2014; Wang, Liu and Liu 2015; Li *et al.* 2016a). In one previous study, more than 95% of the photosynthetically active radiation applied to the surface of a very dense biofilm (ca. 250 g DW per L biofilm volume) was attenuated in less than 350 μm depth (Li *et al.* 2016a). Thus, part of the biofilm may receive insufficient light and this is can lead to a decrease in the overall productivity of the biofilm due to dark respiration (Wang, Liu and Liu 2015; Li *et al.* 2016a; Li, Podola and Melkonian 2016b). This problem can in theory be overcame by regular harvesting, thus ensuring that the thickness of the biofilm in the PBR is always less than the depth of the productive part of the photic zone. So far, no study has been performed to measure nutrient (i.e. N, P) gradients directly in biofilms inside biofilm-based PBRs. Although several modeling studies suggest that a nutrient gradient perpendicular to the biofilm surface may occur in the biofilm. As long as nutrients are supplied in excess in the bulk medium, their concentrations should still be sufficiently high in the biofilm as not to impair growth (Murphy and Berberoglu 2014; Li, Podola and Melkonian 2016b).

On the other hand, due to a considerably higher consumption rate, and/or an already insufficient supply at the surface of the biofilm, the gradients of DIC in biofilm-based PBRs can be significant. Steep DIC gradients are often expected even in the thin photic layer of a biofilm (Liehr, Wayland Eheart and Suidan 1988; Liehr, Suidan and Eheart 1989, 1990; Wolf, Picioreanu and van Loosdrecht 2007; Li, Podola and Melkonian 2016b). This can lead to a depletion of available DIC inside the photic zone of the biofilm, and subsequently, to excessive photorespiration (Lloyd, Canvin and Culver 1977; Spalding 1989) and to a loss of light utilization efficiency, resulting in sub-optimal biomass productivity. By increasing the supply of DIC to the cells in the photic zone, photorespiration can be reduced, as a result, light utilization efficiency is increased. However, maximizing productivity does not necessarily lead to an optimization in efficiency: as illustrated in Fig. 1, the gross income generated by the extra DIC supplied initially increases faster than the associated cost (e.g. energy). As the amount of additional DIC increases, saturation in biomass productivity leads to a flattening of gross income, although the associated cost still rises. Thus, the best strategy is to find the optimal ratio between the increase in cost caused by DIC addition and the gross income resulting from the extra biomass or product produced. Such an effective optimization requires the knowledge of the dynamics of DIC inside PBR biofilms.

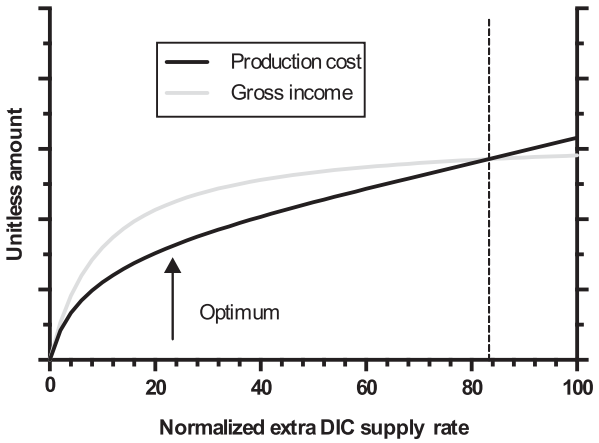


Figure 1. Change in production cost and gross income of biofilm-based photobioreactors (PBRs) when external inorganic carbon source is provided. X-axis gives the normalized amount of extra (i.e. external) inorganic carbon supplied. Y-axis represent the dimensionless amount of production cost (solid black line) and gross income (solid gray line). The arrow indicates where the profit is maximized (i.e. 'optimum point'); and the dashed line indicates where the cost due to extra inorganic carbon supply reduces profit to zero.

Dynamics of DIC in biofilm-based PBRs

DIC can exist as free dissolved CO_2 (i.e. H_2CO_3), bicarbonate ions (HCO_3^-) and/or as carbonate ions (CO_3^{2-}), depending on the pH of the environment. The total amount of DIC and the amounts of different DIC species in PBR biofilms will be affected by the total and/or local supply and uptake rates of DIC, pH, type of microalgae, light intensity, nutrient availability, etc. (Liehr, Wayland Eheart and Suidan 1988; Liehr, Suidan and Eheart 1989; Spalding 1989; Liehr, Suidan and Eheart 1990; Wolf, Picioreanu and van Loosdrecht 2007; Murphy and Berberoglu 2014; Li *et al.* 2016a). Spatial-temporal measurements and/or modeling are required to understand dynamic DIC partitioning and gradients. Furthermore, even though CO_2 is the final substrate in photosynthesis, some phototrophs can utilize bicarbonate for photosynthesis (Merrett, Nimer and Dong 1996; Amoroso *et al.* 1998; Huertas *et al.* 2000). Bicarbonate is either converted to CO_2 before uptake, or transformed to CO_2 intracellularly after uptake (Findenegg 1979, 1980). Due to differences in DIC uptake mechanisms among organisms and/or biofilms, the behavior of DIC in different biofilms subjected to the same conditions can be different. Therefore, results can only be extrapolated with caution. Nevertheless, experimental and/or modeling approaches can be adapted to different PBR biofilms with relative ease.

Investigation of dynamic interactions in dense and highly active phototrophic biofilms is not a recent topic, at least for submerged biofilm systems. Already in the 1980s, differences in light attenuation and the DIC consumption rate between biofilm-based and suspension systems were recognized (Novak and Brune 1985) and models describing dynamic interactions in submerged algal biofilms have been developed. However, until recently, only few studies have focused on the dynamics of DIC in PBR biofilms. Liehr, Wayland Eheart and Suidan (1988) and Liehr, Suidan and Eheart (1989, 1990) developed comprehensive mathematical models for microalgal biofilms. The proposed models utilize mass balances, charge balance and Fick's law of diffusion in conjunction with the chemical reactions of the carbonate system to describe the dynamics of algal biofilms. The models predicted a steep rise in pH inside the biofilms compared to the bulk medium, due to the uptake of dissolved CO_2.

Consequently, the remaining DIC may no longer be directly available (e.g. as CO_3^{2-}) for photosynthesis. Flora *et al.* (1995) improved the model by differentiating the diffusion coefficient of the different chemical species and by adding a phosphate buffer system. Their models predicted similar trends as in previous studies. Also, the model predicted that the presence of a phosphate buffer can significantly increase the DIC flux into the biofilm. With the buffer, the biofilm pH remained lower and consumption of DIC increased because more DIC was present as CO_2 and less as carbonate. Wolf, Picioreanu and van Loosdrecht (2007) developed the 'PHOBIA' model. Among other improvements, this model recognizes bicarbonate as a DIC species available for uptake, although the model does not distinguish between different DIC uptake mechanisms (e.g. extracellular conversion to CO_2 or direct bicarbonate uptake). The PHOBIA model differentiates growth using CO_2 or bicarbonate, assuming higher CO_2 concentrations inhibit bicarbonate uptake (i.e. bicarbonate-based growth). The PHOBIA model predicted, similar to previous studies, that in the absence of a buffer, a steep pH gradient would form inside the biofilm. The slope of this gradient was dependent on the biomass density of the phototrophs in the biofilm.

Microsensors have previously been applied to investigate gradients inside naturally occurring biofilms (e.g. Kühl 1993; Grötzschel and de Beer 2002; Al-Najjar *et al.* 2012). In several recent studies, microsensors have also been used to investigate PBR biofilms. For submerged biofilms, Pringault and Garcia-Pichel (2000) measured oxygen and pH profiles inside a mono-strain cyanobacterial biofilm grown in a benthic chamber. DIC profiles were not directly measured. However, their results did show that increasing irradiance at the biofilm surface led to higher biofilm dissolved oxygen concentrations (DO) and gross photosynthetic productivities. This was accompanied by an increase in pH inside the biofilm. This result verified the link between DIC uptake and pH in dense phototrophic biofilms. Bernstein *et al.* (2014) used oxygen microsensors to probe a rotating biofilm PBR. The measured DO profiles were consistent with model predictions by Wolf, Picioreanu and van Loosdrecht (2007).

For PSBRs, several studies have addressed dynamic interactions inside their biofilms. Murphy and Berberoglu (2014) proposed a dynamic model for a PSBR system. In their study, individual dissolved species (e.g. DO, DICs) were modeled as state variables (i.e. time/location-dependent variables). Both carbonate buffering and phosphate buffering were taken into consideration. The proposed model described a very thin (30 μm) biofilm with a relatively long (5 cm) medium flow path parallel to the biofilm surface. As a result, the model predicted no significant DIC gradient inside the biofilm perpendicular to the surface of the biofilm, and the predicted DO hardly increased with biofilm depth. The growth rate gradient along the depth direction was observed to be the result of decreasing light intensity. The results also showed that the growth of biomass should be limited by nutrient availability (in their case, P), because of a very low medium flow rate (0.12 $\mu m \cdot s^{-1}$) along the parallel plane of the biofilm surface. This led to the prediction that total DIC should actually increase along the medium flow direction, caused by continuous transfer of CO_2 across the biofilm surface combined with decreasing CO_2 uptake because of nutrient limitation.

Li, Podola and Melkonian (2016b) proposed a model for a similar PSBR. In their study, a 1D (along the depth direction of the biofilm) model was applied to predict the distribution of light, DO, pH, DIC and productivity of a PSBR system. The model predicted that both DO and pH increased with increasing depth in the photic zone and decreased with depth in the dark/weakly illuminated region of the biofilm. Also, the DIC uptake was shown to be controlled more by the local pH rather than the local total DIC concentration. When exposed to high surface irradiance and low gas phase CO_2 concentrations, the growth was DIC limited. However, similar to the prediction for submerged biofilms, this was not caused by a complete depletion of DIC. Instead, high local pH partitioned most of the DIC into the carbonate pool, which was unavailable for uptake. On the other hand, contrary to the prediction for the submerged biofilm, the model suggested that the addition of buffers into PSBR system exposed to high light and low CO_2 could actually decrease the flux of DIC into the biofilm. This was caused by a lower pH near the biofilm surface, leading to a lower CO_2 transfer from the gas into the biofilm. A model based on dynamic interactions was also proposed recently for a PSBR system by Ji *et al.* (2017). This model simulated pH and DIC dynamics in the medium reservoir rather than inside the biofilm. Li *et al.* (2016a) also performed comprehensive microsensor measurements on a PSBR. The microsensor profiles of DO and pH fitted well to those predicted by the model (Li, Podola and Melkonian 2016b).

Most studies consider the biofilms to be of homogeneous composition. This assumption should be addressed in future studies. Gradients or stochastic biological processes could lead to the occurrence of micro-niches inside biofilm. This could promote growth of specific organisms in multi-species biofilms (Davey and O'Toole 2000; Nadell, Drescher and Foster 2016). Even in single species biofilms the phenotype may change due to exposure to different local conditions (Kiperstok *et al.* 2017). As a result, the effective diffusion coefficients of dissolved species can vary within the biofilm (Wieland *et al.* 2001), also, the kinetic parameters may vary with depth. Similarly, DIC consumption and/or production rates may not be constant but may be a function of time and space. Consequently, results from previous studies assuming a homogeneous biofilm should be evaluated with caution. To better understand the effect of biofilm inhomogeneity on the DIC dynamics in PBR biofilms, future dynamic studies should incorporate investigations of the structure and/or the structural composition of the biofilm (e.g. individual-based modeling and 3D imaging of PBR biofilms) (Wieland *et al.* 2001; Murphy and Berberoglu 2014; Thomas *et al.* 2014).

Figure 2 provides an overview of the DIC dynamics in different biofilm-based PBR systems. To summarize, in biofilm-based PBRs DIC is supplied to the biomass at the interface of medium/air and the biofilm (e.g. surface of the biofilm). The relatively low fluxes (i.e. diffusion) and/or rapid consumption of DIC inside the biofilm can lead to steep DIC gradients. A complete depletion of DIC is usually not encountered. As discussed, low local DIC concentrations in combination with high local pH can make most of the remaining DIC unavailable for uptake. If in addition not enough external DIC is supplied, the result is a suboptimal light utilization throughout the photic zone of the biofilm due to photorespiration. For example, Li *et al.* (2016a) observed that when PSBR biofilms were subjected to a surface irradiance of 1000 μmol photons $m^{-2} \cdot s^{-1}$, even a higher CO_2 concentration in the gas phase appeared to be insufficient to saturate the O_2 productivity of the system. In this case, biofilms supplied with lower CO_2 concentrations very likely suffer from photorespiration and non-optimal biomass productivity. When comparing submerged and non-submerged biofilms, it is obvious that when inorganic carbon is supplied as CO_2 gas, permanently non-submerged systems have the clear advantage. By removing the layer of medium on top of the biofilm, a DIC mass transfer barrier has been removed. Thus, the CO_2 can directly enter the biofilm, instead of being first dissolved in the

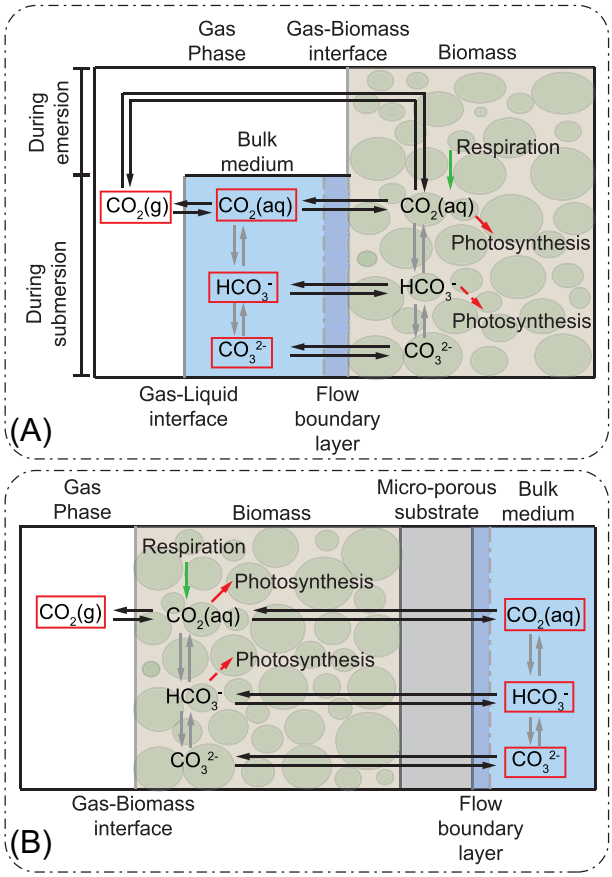


Figure 2. Schematic representations of dissolved inorganic carbon (DIC) dynamics in different biofilm-based photobioreactors (PBRs). (**A**) Continually or periodically submerged biofilm-PBRs. (**B**) Porous substrate photobioreactors (PSBRs). In both panels: solid black arrows show the mass transfer processes; solid gray arrows represent conversion processes; green arrows show production of DIC, red arrows and dashed red arrows represent consumption of DIC due to CO_2 and HCO_3^- uptake, respectively. The different compartments and boundaries of the PBR systems are noted at the top and bottom of each panel. Possible strategies to supply the systems with extra DIC are marked in red boxes.

overlying bulk medium, or, only having direct contact with the biofilm intermittently. Also, due to the direct DIC influx as CO_2 at the biofilm-gas interface, if not (excessively) buffered, the elevated pH due to CO_2 consumption at the surface of the biofilm promotes DIC influx by dissolving more CO_2 from the gas phase. If inorganic carbon could be made available in a dissolved form and supplied to the system via the culture medium, a non-submerged system, or systems that are only periodically submerged in the medium, may actually be at a disadvantage: since in the first case, the biomass in the dark and/or weakly illuminated region acts as an additional diffusion barrier, and in the second case, the emersion phase removes the biofilm from its external inorganic carbon source.

Enhancement of DIC availability in biofilm-based PBRs

Most biofilm-based PBRs designs aim to maximize the transport of CO_2 into the biofilm. During the design, detailed knowledge on the dynamics of the pH and DIC gradients inside the biofilm is usually lacking. These dynamics are usually only investigated in detail after a system demonstrated its potential in application. Thus, the practical value of investigating dynamic interactions in biofilm PBR is currently to achieve a more efficient operation within the constraints of the original design. For example, the knowledge of the dynamics of light and DIC inside the biofilms may be used to tailor the DIC supply strategy now to a specific light condition. The goal is to optimize the utilization efficiency of both light and DIC (i.e. minimizing both photorespiration and DIC over-supply, thus achieving the 'optimal ratio', as discussed in the previous section, Fig. 1). Several previous studies have applied external inorganic carbon sources to boost the productivity of biofilm-based PBRs (e.g. Schultze *et al.* 2015; Schnurr *et al.* 2016). However, systematic studies focusing on finding the 'optimal ratio' for a specific system are rare. Nevertheless, possible strategies for supplying DIC to different types of biofilm PBRs are summarized in the following paragraphs.

Continually submerged biofilm-based PBR systems normally possess only a relatively shallow liquid layer above the biofilm. By reducing the thickness of the liquid above the biofilm, the path from gas phase to the biofilm is shortened and mass transfer can be enhanced (Guzzon *et al.* 2008; Cao *et al.* 2009; Posadas *et al.* 2013; Zamalloa, Boon and Verstraete 2013; Boelee *et al.* 2014; Zhang *et al.* 2017). In such systems, extra inorganic carbon can also be supplied as CO_2 gas or DIC in the bulk medium. This is especially true for closed biofilm-BPRs (Schnurr *et al.* 2016), as in such systems, CO_2 cannot escape into the gas phase and could not be lost to the atmosphere. Schnurr *et al.* (2016) attempted to optimize the productivity of a flow-cell biofilm by sparging the bulk medium with CO_2 gas and by changing the photon flux density. Their data showed that by increasing the CO_2 concentration from 0.04% to 2%, (v/v) at 100 μmol photons $m^{-2} \cdot s^{-1}$, the productivity increased from 0.5 to 2 $g \cdot m^{-2} \cdot d^{-1}$. Also, in their study, a mathematical model based on the experimental data suggested, the 'optimum point' for their system to be 7.1% CO_2 in the medium in combination with an illumination light intensity of 400 μmol photons $m^{-2} \cdot s^{-1}$. Another approach to enhance CO_2 transfer from gas phase to the biofilm is the 'algal turf scrubber' system (e.g. Setlik, Sust and Malek 1970; Adey, Luckett and Jensen 1993; Doucha and Livanshky 1995; Mulbry and Wilkie 2001; Valeta and Verdegem 2015). This system utilizes the better mixing inside the bulk medium created by a more turbulent flow, increasing both the transport of CO_2 from the gas phase to the bulk medium, and from the bulk medium to the biofilm. Such systems maximize inorganic carbon (i.e. CO_2 from air) supply with minimum costs (e.g. no extra inorganic carbon source) and are especially suitable for applications demanding low costs, e.g. waste water treatment. Recently, based on the fact that alkaliphilic phototrophs can utilize bicarbonate and tolerate highly alkaline environments, a novel biofilm-based PBR has been developed by Sharp *et al.* (2017). This system was designed to enable separation of CO_2 transfer from gas to medium from biofilm growth altogether. By using a highly alkaline medium (pH > 9, DIC > 0.5 $mol \cdot L^{-1}$), CO_2 can first be captured from the gas phase (e.g. normal air or flue gas, driven by pH equilibrium) with the help of a gas scrubber. Then, the DIC-enriched medium can be fed into the biofilm PBR to support biomass growth.

In periodically submerged systems, instead of permanent submersion, biofilms are submerged only intermittently and are directly exposed to the gas phase during the remaining time. Thus, the mass transfer between the gas phase and the biofilm is improved. Examples of such systems (Table 1) are rotating-disk PBRs (e.g. Torpey *et al.* 1971; Przytocka-Jusiak *et al.* 1984; Blanken *et al.* 2014), revolving belt PBRs (Gross and Wen 2014), rotating drum PBRs (e.g. Christenson and Sims 2012; Bernstein *et al.* 2014), and oscillating PBRs (e.g. Johnson and Wen 2010; Gross *et al.* 2013). For such systems, inorganic carbon can be

supplied directly from the gas phase to the biofilms and/or via DIC in the bulk medium. Blanken *et al.* (2014) showed that when CO_2 was supplied in the gas phase of a rotating-disk biofilm PBR, the productivity could be increased to 20 g · m^{-2} · d^{-1} compared to 2–4 g · m^{-2} · d^{-1} when no extra CO_2 was provided. However, Gross *et al.* (2013) found no increase in productivity when the CO_2 concentration in the gas phase above an oscillating biofilm-PBR was increased from normal air concentration to 3%. Similarly, Kesaano *et al.* (2015a) found that the productivity of their rotating biofilm PBR did not increase after addition of bicarbonate into the bulk medium. Possible explanations for these contradictory results could be, as discussed in the previous section, an elevated pH inside the biofilm, presence of different DIC uptake mechanisms, or, as reviewed by Schnurr and Allen (2015), differences in reactor design and/or light intensities. Another recent study proposed a model for optimizing CO_2 supply to biofilm-PBR (Blanken *et al.* 2017). This study suggested that concentrated CO_2 streams and plug flow behavior of the gaseous phase over the biofilm surface are essential for high productivity and CO_2 utilization efficiency.

PSBR are a special case in biofilm-based PBRs. Because of the permanent exposure of the biofilm to the gas phase, the mass exchange between the biofilm and the gas phase is inherently maximized (Li *et al.* 2016a; Li, Podola and Melkonian 2016b; Podola, Li and Melkonian 2017). Direct transfer from the gas phase avoids mechanical stress due to shear forces from the flowing liquid media. Consequently, increasing CO_2 concentration in the gas phase is a straightforward approach to enhance DIC availability. Schultze *et al.* (2015) investigated the biomass productivity of a PSBR system exposed to high light intensity (~1000 μmol photons m^{-2} · s^{-1}) at different CO_2 concentrations in the gas phase. Their results showed that by increasing the CO_2 concentration from atmospheric to 3% more than doubled the biomass productivity (from 12 to 31 g · m^{-2} · d^{-1}). Interestingly, a further increase to 5% led to a 20% decrease in productivity. Kiperstok *et al.* (2017) performed a similar study on the same PSBR system with a different microalgal strain. In their study, at 1000 μmol photons m^{-2} · s^{-1}, the optimum CO_2 concentration was found to be 5%. Compared to atmospheric levels, the addition of 5% CO_2 in the gas phase almost tripled the biomass productivity. Their result also demonstrated that the addition of CO_2 increased biomass productivity significantly only when a relatively strong illumination was applied. For lower light intensities (<50 μmol photons m^{-2} · s^{-1}), the addition of 5% CO_2 did not lead to a significant increase in biomass productivity. In a recent study, Ji *et al.* (2017) confirmed these observations.

The productivity of a combination of identical PBRs can be optimized by maximizing the productivity of each individual PBR. However, for some applications, this may not be the most efficient solution. A less obvious solution is to decrease surface irradiance instead of increasing DIC availability. This may seem counterproductive, however, in a commercial production setting, instead of a single PBR, an array of PBRs is often used (Liu *et al.* 2013). By increasing the number of PBR units per unit of ground (i.e. footprint) area, the light dilution rate per unit footprint area is increased (i.e. the light intensity on the surface of each PBR is reduced). Now more microalgal cells are exposed to the highest possible DIC concentrations (i.e. at biofilm medium/gas phase interface), and the diluted irradiance does not saturate the available DIC. Although the DIC availability in each individual PBR is not changed, its utilization efficiency per foot print area is increased. This ultimately leads to an increase in productivity per footprint area (Liu *et al.* 2013). With this strategy, no additional inorganic carbon supply is required. Of course, this strategy would not be useful for applications demanding a high surface irradiance, e.g. biofilm-based astaxanthin production (Kiperstok *et al.* 2017).

SUMMARY

The designs of existing biofilm-based PBRs and previous studies on DIC dynamics in various biofilm-based PBRs are summarized in this mini-review. Based on this information, discussions on strategies to enhance DIC availability for the immobilized cells in biofilm-based PBRs were made.

Advances have recently been made in understanding the dynamics of DIC in biofilm-based PBRs through both mathematical modeling and experimental studies. The dynamics of DIC and its interaction with other factors in biofilms can now be better addressed and predictions about the behavior of DIC in specific systems can be made. Nevertheless, more accurate descriptions/predictions of the behavior of biofilm-based PBRs under various environmental conditions are still needed, especially with regard to biofilm inhomogeneity. Advances in our understanding in DIC dynamics can be applied to achieve a more efficient and/or cost-effective operations and/or lead to design improvements of the existing biofilm PBR systems, as well as the future development of new biofilm-based PBRs.

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