**Direct membrane-carbonation photobioreactor producing
photoautotrophic biomass via carbon dioxide transfer and nutrient
removal**

Hyun-Woo Kim ^{a,*}, Jing Cheng ^b, Bruce E. Rittmann ^c

^aDepartment of Environmental Engineering, Chonbuk National University, 567 Baekje-daero,
deokjin-gu, Jeonju 54896, Korea

^b School of Civil Engineering and Architecture, Wuhan University of Technology, Wuhan,
Hubei, 430070, China

^c Swette Center for Environmental Biotechnology, The Biodesign Institute at Arizona State
University, P.O. Box 875701, Tempe, Arizona 85287-5701, U. S. A.

*Corresponding author phone: +82-63-270-2444; fax: +82-63-270-2449; e-mail:
hyunwoo@jbnu.ac.kr

Abstract

An advanced-material photobioreactor, the direct membrane-carbonation photobioreactor (DMCPBR), was tested to investigate the impact of directly submerging a membrane carbonation (MC) module of hollow-fiber membranes inside the photobioreactor. Results demonstrate that the DMCPBR utilized over 90% of the supplied CO₂ by matching the CO₂ flux to the C demand of photoautotrophic biomass growth. The surface area of the submerged MC module was the key to control CO₂ delivery and biomass productivity. Tracking the fate of supplied CO₂ explained how the DMCPBR reduced loss of gaseous CO₂ while matching the inorganic carbon (IC) demand to its supply. Accurate fate analysis required that the biomass-associated C include soluble microbial products as a sink for captured CO₂. With the CO₂ supply matched to the photosynthetic demand, light attenuation limited the rate microalgal photosynthesis. The DMCPBR presents an opportunity to improve CO₂-deliver efficiency and make microalgae a more effective strategy for C-neutral resource recovery.

Keywords: Membrane-carbonation, permeability, photoautotrophic growth, inorganic carbon flux, resource recovery

1. Introduction

Photobioreactors (PBR) present a promising path to renewable production of biofuel feedstock (Georgianna and Mayfield, 2012). In a PBR, photosynthetic microorganisms absorb photons to create reducing power (NADPH) and energy (ATP), which allows them to reduce inorganic building blocks and synthesize cell components. If implemented on a massive scale, photosynthetic CO₂ fixation by photoautotrophic microorganisms ultimately could make biofuels carbon-neutral alternatives to fossil sources (Rittmann, 2008; Rittmann et al., 2008). A major technological need is for efficient and controllable CO₂ delivery to PBRs.

The usual means to deliver CO₂ to a PBR is gas sparging; however, this conventional method has notable drawbacks, such as contamination from undesired microorganisms, poor pH control, and intensive energy consumption (Contreras et al., 1998; Pegallapati and Nirmalakhandan, 2012). Past literature has demonstrated that it is possible to eliminate CO₂ sparging by using CO₂ delivery via bubbleless gas-transfer membranes (Bilad et al., 2014; Kim et al., 2011a; Kumar et al., 2010).

It was realized with the membrane-carbonation photobioreactor (MCPBR), in which PBR medium was circulated to and from a membrane carbonation (MC) system outside the PBR. This approach allowed for good control over the CO₂-delivery rate, biomass production rate, and pH in the PBR (Kim et al., 2011a), but it introduced additional pumping and energy costs. Cheng et al. (2006), Fan et al. (2007), and Fan et al. (2008) evaluated membrane application for CO₂ supply using microporous membranes, but they produced microbubbles. Cheng et al. (2006) and Kumar et al. (2010) demonstrated the concept of submerged membrane contactor, but the approach consumed substantial energy for circulating liquid and gas.

An appealing alternative is to submerge the hollow-fiber membranes (HFMs) inside the PBR to create a direct membrane-carbonation photobioreactor (DMCPBR), in which water recirculation to an MC unit is unnecessary and diffusion-driven mass transfer delivers CO_2 . Here, we demonstrate the potential of the DMCPBR by evaluating how controlling the CO_2 flux governs the growth pattern of a photoautotrophic cyanobacterium, *Synechocystis* sp. PCC 6803, the efficiency of CO_2 delivery, and the removal of water contaminants, NO_3^- and PO_4^{3-} , from water body. We employ a HFM with distinctively low gas permeability made from economical material. Using a mass-balance analysis, we characterize the relationships among membrane surface area, CO_2 flux, CO_2 delivery efficiency, light availability, nutrient removal and photoautotrophic growth during continuous operation of a DMCPBR.

2. Materials and Methods

2.1. Experimental set-up of the DMCPBR

Fig. 1 is a schematic of the DMCPBR and identifies all the components. DMCPBR consisted of a tubular main reactor (5-L working volume) made of glass (KIMAX, Germany), a HFM module inside the reactor, a magnetic stirrer with a spin bar, a multi-channel peristaltic pump for influent/effluent control, a circular light emission diode (LED) light for irradiation, a sampling port with connection tubing, and a gas (O_2) exchange membrane filter to prevent pressure build-up. The reactor's top was sealed with a rubber stopper having four holes fitted for stainless-steel tubes: medium influent, effluent, pressurized CO_2 supply for the HFMs, and the gas exchange membrane to prevent O_2 -pressure build-up and microbial

contamination of the PBR contents. The DMCPBR contents were stirred at 300 rpm to ensure completely mixed conditions.

The HFM module (Fig. S1B) was fabricated using a single material, non-porous polypropylene (Teijin, Ltd., Japan) HFM material, as characterized previously (Tang et al., 2012). Table 1 presents the operating conditions of the DMCPBR together with physical characteristics of the HFM modules. We connected the HFM module to a 100%-CO₂ tank with Norprene tubing (Masterflex, USA), plastic barbed fittings, and gas-tight rubber seals at both ends, and we controlled the CO₂ pressure to the module with its own regulator (3471-A, Matheson Tri-Gas Inc.; Victor HPT100-80-20-BV, Thermadyne Inc.).

A hollow-shaped LED (LED-144A-YK, AmScope, USA) of 10-cm outer diameter was placed above PBR to supply photosynthetically active radiation (PAR) with a constant illumination level of 79 W/m² (= 363 μE/m²/s) measured at the top of the liquid surface. The illumination included room light, constantly on for safety purposes, of approximately 3 W/m² as PAR.

2.2. Inoculum and culture media

DMCPBR was inoculated with *Synechocystis* sp. PCC 6803 taken from a mother culture grown in a 2-L glass bottle (KIMAX, Kimble Chase) aerated with filtered air at 2 L_{air}/L_{liquid}/min. We illuminated the mother culture continuously using the same kind of LED irradiation as for the DMCPBR. The inoculum was grown in modified BG-11 medium, described elsewhere (Kim et al., 2011a; Kim et al., 2010). To prepare identical growth medium for each experiment and to minimize unresolvable interferences, we utilized ultra-pure deionized water (18.2 MΩ·cm), produced by the Purelab ultra (ELGA lab water, USA), and autoclaved all culture media before use.

For DMCPBR experiments, we modified the medium by removing all inorganic carbon (IC) to ensure that the HFM module was responsible for delivery of any C measured in the reactor. The total alkalinity of this modified BG-11 medium was 1.8 meq/L (90 g/m³ as CaCO₃), and its pH upon preparation was 8.0 ± 0.2.

2.3. Start-up and operating procedures of the DMCPBR

We inoculated a DMCPBR with 2 L of inoculum having an optical density at 730 nm (OD₇₃₀) of 0.4 to 0.6 and then pressurized the HFM module with pure CO₂ gas at a gauge pressure of 15 psig (~1 atm) for the HFM; this pressure was based on preliminary tests. After one day of batch operation, the DMCPBR was operated with continuous flow, similar to a completely stirred tank reactor (CSTR) or a chemostat at a hydraulic retention time (HRT) of 8 d, which corresponds to a dilution rate of 0.125 d⁻¹; the corresponding flow rate of IC-free BG-11 medium was 0.6 L/d. To evaluate biomass stability in the DMCPBR mode, it was ran for over 40 d for one operating condition.

2.4. Sampling and analytical methods

The performance of the MCPBR was monitored by analyzing samples taken from the sampling port according to a prescribed sampling plan. For continuous experiments, one sample was took per day. We either determined all physical, chemical, and biological parameters in duplicate the same day or stored the samples at 4°C before analysis. To represent the steady-state concentrations, the last three days of data were averaged for a run after operation for two HRTs (16 d), when the variability in concentrations was less than 10%.

After filtering samples through a 0.2-μm membrane filter (GD/X, Whatmann, USA),

we analyzed the filtrate for anions (NO_3^- , SO_4^{2-} and PO_4^{3-}) and cations (Na^+ , K^+ , Ca^{2+} , Mg^{2+} and NH_4^+) using an ion chromatograph (ICS-3000, Dionex, USA) equipped with an IonPac AS18 (Dionex, USA) anion exchange column or a CS18 (Dionex, USA) cation exchange column, respectively.

OD_{730} was determined directly using a UV-visible spectrophotometer (Cary 50 Bio, Varian Inc.) at a wavelength of 730 nm, and the OD_{730} value was converted to dry weight (DW) of biomass using calibration curve for PCC 6803 following previous procedures (Kim et al., 2013). We determined pH, total IC, and the concentrations of all carbonate species (i.e., $\text{CO}_{2(\text{aq})}$, HCO_3^- , and CO_3^{2-}) according to the methods of our previous study (Kim et al., 2010). Total alkalinity of the BG-11 medium were calculated using the analytical definition of alkalinity (Snoeyink and Jenkins, 1980), which includes HCO_3^- , CO_3^{2-} , HPO_4^{2-} , H^+ and OH^- , and we converted the equivalents to mg as CaCO_3 using 50 mg as CaCO_3 per alkalinity meq. Soluble chemical oxygen demand (COD) was determined using COD reagents (TNT822, Hach Company) according to manufacturer's digestion protocol. To determine dissolved organic carbon (DOC), we used a TOC analyzer (TOC-VCSH, Shimadzu Scientific Instruments, USA) equipped with combustion catalytic oxidation/non-dispersive Infrared (NDIR) gas analyzer according to the High-Temperature Combustion Method (5310B) in *Standard Methods* (Eaton et al., 2005).

2.5. Mathematical model to determine CO_2 permeability

To estimate the CO_2 permeability through HFM, we modified the method of Tang et al (Tang et al., 2012), who measured H_2 permeability for the same HFM. Appropriate parameter values to CO_2 were applied, and we reported relative permeability. Equation S1-S7 shows the complete set of model equations and the parameter values for CO_2 permeability

estimation. Also, we determined the IC speciation according to the method of our previous study (Kim et al., 2011a).

2.6. Light availability

To evaluate the availability of light inside the DMCPBR, the average light irradiance (LI_{ave}) was computed using the Beer-Lambert light-attenuation model. We assumed that all light entered the PBR at the top, light absorption by the biomass-free medium was negligible, and light attenuation was proportional to the biomass concentration with a Beer-Lambert coefficient (ϵ) of $0.255 \text{ m}^3/\text{g}\cdot\text{m}$ (Cornet et al., 1992; Grima et al., 1997; Suh and Lee, 2003). Following the method of Kim et al. (2011b), we computed the distribution of attenuated LI inside the PBR and integrated the distribution to obtain LI_{ave} .

3. Results and Discussion

3.1 Performances of DMCPBR

Table 2 summarizes IC species concentrations and biomass production rates of PCC 6803 at steady-state for the three surface areas (A_M) of HFM, which were 1.9, 4.7, and 9.4 cm^2 , respectively. Steady-state biomass concentrations were achieved within 10 days, and we confirmed that the DMCPBR could be operated stably for more than 40 days from our preliminary test, as shown in Fig. S3. The HRT and biomass specific growth rate were fixed as 8 d and 0.12 d^{-1} .

When A_M was 1.9 cm^2 , the steady-state IC concentration was as low as 1.5 g C/m^3 , with corresponding $\text{CO}_{2(aq)}$, HCO_3^- , and CO_3^{2-} concentrations were 0.0, 1.4, and 0.1 g C/m^3 ,

respectively (Table 2). Because the average pH of 9.3 made HCO_3^- dominant (94 % of IC), the $\text{CO}_{2(\text{aq})}$ concentration was negligible (Fig. S4). Increasing A_M from 1.9 to 4.7 cm^2 led to higher steady-state biomass concentrations: from 77 to 130 g DW/m^3 , which corresponds to 78% increase in biomass production rate (from 9 to 16 $\text{g DW/m}^3/\text{d}$). This trend points to CO_2 delivery had been rate limiting for the lowest A_M ; providing a higher CO_2 delivery capacity allowed more biomass production.

CO_2 fluxes to the liquid, computed based on the effluent concentrations of IC, soluble microbial products (SMP) as DOC, C in biomass, and CO_2 in the off gas (Kim et al., 2011a; Kim et al., 2011b) improved from 5.0 to 8.2 $\text{g C/m}^3/\text{d}$ (a 60% increase) as A_M was increased (a 2.5-fold increase). The high pH (> 9) at $A_M = 1.9$ and 4.7 cm^2 supports that CO_2 delivery was still rate limiting for photoautotrophic consumption of HCO_3^- and $\text{CO}_{2(\text{aq})}$ by PCC 6803.

High CO_2 permeation allowed rapid consumption of $\text{CO}_{2(\text{aq})}$ and HCO_3^- without pH since the influent alkalinity of BG-11 medium was fixed at 90 g/m^3 as CaCO_3 thus CO_3^{2-} to be 30% of IC. Relatively small proportion of effluent CO_2 (1.9% of total CO_2 captured) at $A_M = 4.7 \text{ cm}^2$ is the evidence of active photosynthetic consumption of transferred CO_2 .

With $\mu (= 1/X_E \cdot dX_E/dt)$ fixed at 0.12 d^{-1} , the biomass concentration (X_E) increased to 130 g DW/m^3 , meaning that most C was fixed into biomass, another sign of active photosynthesis.

The pH values were 9.3 and 9.9 at $A_M = 1.9$ and 4.7 cm^2 , respectively, and the further increase of A_M to 9.4 cm^2 led to an even higher pH (10.1). The steady-state IC was as high as 9.6 g C/m^3 , which is 6-fold higher than that of $A_M = 4.7 \text{ cm}^2$. Our preliminary work found that photobioreactor's alkalinity increased gradually with biomass synthesis when nitrate was used as a sole nitrogen source (Nguyen and Rittmann, 2015). Active photosynthesis might have created additional alkalinity (36, 60, and 76 g/m^3 as CaCO_3) which led to additional increase of pH and CO_3^{2-} concentration. Likewise, the IC

concentration became larger with greater A_M (from 1.5 to 9.6 g C/m³). It indicates that directly submerged MC can made it easy to achieve the active photosynthesis with appropriate CO₂ delivery at least for our experimental ranges of A_M tested. Fig. 2 shows that MCPBR can reduce NO₃-N (6~13 mg/d) and PO₄³⁻-P (0.7~1.5 mg/d), notorious contaminants in water body. **An increase of A_M led to higher uptake of inorganic nitrogen and phosphorus, since biomass production was greater.** These performance results implies that DMCPBR may have a control over the recovery of CO₂ generated from multiple renewable sources because DMCPBR presents clear advantage of reduced energy demand when compared to conventional aerated PBR and even typical side-stream MCPBR (Kim et al., 2011a). Although our approach need pressure energy, it can be relatively low ($\cong$ 1 atm) to promote CO₂ permeation. When the recovery of nutrient or CO₂ from waste stream is considered, DMCPBR may enhance the cost-effectiveness and environmental friendliness simultaneously (Rittmann, 2008).

3.2. Fate of CO₂ captured

Table 3 presents the fate of CO₂ during the experiment. At the A_M of 1.9 and 4.7 cm², 77% (0.024 g C/d) and 82% (0.040 g C/d) of **IC** was transformed into biomass while effluent **IC** was merely 3.0% (0.001 g C/d) and 1.9% (0.006 g C/d), respectively. Ventilation loss was negligible throughout the experiment. In the case of A_M = 9.4 cm², **IC** proportion in biomass gradually increased to 0.051 g C/d possibly due to favorable photosynthetic condition caused by increased CO₂ flux. Throughout the experiment, transformation of **IC** into biomass were kept stable (77~82%) and active photosynthesis did not allow high **IC** in the effluent (< 8.8%) within the tested range of A_M (1.9 ~ 9.4 cm²). Relatively higher level of SMP (20~14%) in the experiment

(Table 3) indicates most CO₂ flux invested to new biomass synthesis, which corresponds to 91~99% of biomass-associated proportion (biomass + SMP) among CO₂ captured (Fig. 3). It explains how MC minimize ventilation loss of CO₂. Average SMP production rate per unit DW were estimated as 0.011~0.016 gC/gDW/d under stable photosynthetic condition in this study.

3.3 CO₂ delivery and fate

The bottom part of Table 3 summarizes fluxes of CO₂-C and estimates of relative permeability. To compute CO₂ fluxes for the HFM, we used theoretical approach (Eq. S1-S7). Results demonstrate that different surface area of A_M (1.4, 4.7, and 9.4 cm²) with constant CO₂ pressure (P₀) 1.02 atm gave CO₂ fluxes of 164, 104, and 70 g CO₂-C/m²/d. Total CO₂ delivery, the product of the flux and the area, showed rather increasing trend from 21 to 46 mg C/d, respectively, in this MCPBR (Fig. 4). From the overall mass balance shown in Eq. S1, a lower flux with increasing A_M is logical, since a given mass/time delivery rate is distributed across a greater more area in the DMCPBR. In one hand, permeability of CO₂ was 3-6 fold higher ($1.2 \times 10^{-6} \sim 5.4 \times 10^{-7} \text{ m}^3 \cdot \text{m}/(\text{m}^2 \cdot \text{atm} \cdot \text{d})$) as compared to earlier report applying the same HFM for H₂ (Tang et al., 2012).

It is evident that a directly submerged MC achieves a high CO₂ capture efficiency and prevents ventilation loss effectively. We also infer that the inherently lower permeability of mono-layer HFM led to sensitive control over the delivery of CO₂, which makes it easier to manage the productivity of PCC 6803, pH, and loss of IC with the HFM. However, excessive A_M might disrupts the IC balance between demand and supply, since the build-up of IC in DMCPBR easily leads to pH drop, which may prevent active photosynthesis (Kallas and Castenholz, 1982; Kurian et al., 2006). This study used a small

surface area of membrane modules normalized to reactor volume (0.4-1.9 cm²/L), compared to other research using membranes: 77 cm²/L (Fan et al., 2008), 143 cm²/L (Fan et al., 2007), 343 cm²/L (Cheng et al., 2006), and 1,400 cm²/L (Carvalho and Malcata, 2001).

The first generation DMCPBR, absorbed at least 90% of the delivered CO₂ at steady-state by minimizing ventilation loss. This high utilization efficiency is better than that obtained in similar PBRs using sparging (50-70%) (Kim et al., 2010). Better performance should be achievable by optimizing the surface area and module construction.

3.4. Effect of LI attenuation

This study also investigated concurrent LI-limitation inside the DMCPBR. Despite balanced IC demand and supply, LI attenuation can slow photosynthesis, preventing rapid reduction of CO_{2(aq)} and HCO₃⁻. Fig. 5 evidences that the increased biomass concentration by the stepwise increase of CO₂ supply via A_M made LI attenuation more significant by decreasing LI_{ave} from 11.9 to 9.4 W/m². Since light is the sole energy source of photosynthesis, it controls the actual rate of biomass synthesis in DMCPBR, especially when IC is not limiting. Our results confirm that the low LI_{ave} (11.9 W/m², 15 % of LI₀) at A_M = 4.7 cm² led to some LI-limitation, as reported half-maximum-rate constants for LI range from about 6 to 14 W/m² for similar cyanobacteria (Kim et al., 2015; Lee and Rhee, 1999; Lee et al., 1987; Wang et al., 1999). The decline of LI_{ave} to 9.4 W/m² accentuated light limitation, as LI_{ave} was in the range of the half-maximum-rate constant.

These results present the direct connection among A_M, LI_{ave}, and biomass productivity. Increasing A_M led to higher X_E, IC, pH, X_E, C in X_E, and IC in the effluent. In contrast, lower membrane surface area led to lower CO_{2(aq)} within the IC, which minimized ventilation of CO₂. Since the overall performance of DMCPBR can be improved not only by optimal

CO₂ transfer, but also by enhanced LI_{ave} (Fan et al., 2007), reactor design must consider reflection, refraction, and scattering of light, together with HFM module configuration. Impaired photosynthesis linking to excessive LI attenuation may slow photosynthesis (Kim et al., 2013), which could result in loss of CO₂ and impair CO₂ recovery. However, LI attenuation, along with turbulence created nearby HFM, should minimize the formation of photosynthetic biofilm or mineral precipitation on the membrane, which are risks in most membrane technology (Bilad et al., 2014).

4. Conclusions

An advanced-material PBR, the DMCPBR, was operated to study the impact of directly submerging an MC unit with varying surface area, which was the key to control biomass productivity. Performance based on biomass productivity validated the effectiveness of the DMCPBR, since CO₂ delivery was the main rate-limiting factor. Moreover, fate of CO₂ explained how the MC module minimized ventilation loss of CO₂ by matching IC demand with supply via the MC module's surface area. Overall, the DMCPBR can minimize the required CO₂ supply and accentuate the ability to harness light energy as carbon-neutral bioresource.

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Figure Captions

Fig. 1 – Schematic of the DMCPBR system using single-material mono-layer HFM (Teijin, TJ).

Fig. 2 –Steady-state experimental measures of nutrient utilization rates and uptake efficiencies.

Fig. 3 – Biomass production rate versus $\text{HCO}_3^- + \text{CO}_{2(\text{aq})}$ in the steady state DMCPBR.

Fig. 4 – CO_2 flux and total CO_2 delivery according to the surface area (A_M) in the steady state DMCPBR.

Fig. 5 – Profiles of available light irradiance (W/m^2 as PAR) according to the height of DMCPBR.

Fig. 1

Fig. 2

Fig. 3

Fig. 4

Fig. 5

Table 1 – Operating conditions of the DMCPBR, including physical characteristics of the HFM modules.

Item	Units	Mono-layer HFM		
<i>Independent variable</i>				
Number of HFM, n	ea	2	5	10
Effective length of each HFM	cm	15	15	15
Membrane volume	cm ³	0.009	0.024	0.047
Membrane surface area, A _M	cm ² (10 ⁻⁴ m ⁻²)	1.9	4.7	9.4
<i>Fixed variables</i>				
Specific surface area, a	m ⁻¹	19,900		
Applied CO ₂ pressure	psig (atm)	15 (1.02)		
Working volume of PBR, V	10 ⁻³ m ³ (L)	5.0		
Height and diameter	cm (10 ⁻² m)	31 and 18		
Hydraulic Retention Time	d	8		
Mixing rate of PBR	rpm	300		
<i>Biomass stoichiometry</i>				
λ _{,C}	g C/g DW	0.51		
λ _{,N}	g C/g DW	0.13		
λ _{,P}	g C/g DW	0.02		
λ _{,O2}	g O ₂ /g DW	2.01		

Table 2 – Steady-state experimental measures of C_i species, biomass, soluble microbial products, and LI_{ave} , based on mass balances during continuous operation of the DMCPBR.

Item	Unit	Mono-layer HFM (TJ)		
<i>Independent variables</i>				
Surface Area of HFMs, A _M	cm ² (10 ⁻⁴ m ⁻²)	1.9	4.7	9.4
<i>Experimental measures</i>				
pH		9.3±0.4	9.9±0.5	10.1±0.2
α ₀		0.0009	0.0002	0.0001
C _i	g C/m ³	1.5	1.6	9.6
CO _{2(aq)}	g C/m ³	0.0	0.0	0.0
HCO ₃ ⁻	g C/m ³	1.41	1.13	6.10
CO ₃ ²⁻	g C/m ³	0.13	0.47	3.50
Average effluent biomass concentration, X _E	g DW/m ³	77±12	130±28	165±29
SMP as DOC	g C/m ³	10	14	15
Average Light Irradiance, LI _{ave}	W/m ²	19.7	11.9	9.4

Table 3 – Fate of CO₂-C captured and membrane mass transport, CO₂-transfer efficiency, and CO₂-utilization efficiency based on mass balances during continuous operation of the DMCPBR.

Item	Unit	Mono-layer HFM (TJ)		
<i>Independent variables</i>				
Surface Area of HFMs, A _M	cm ² (10 ⁻⁴ m ⁻²)	1.9	4.7	9.4
<i>Fate of CO₂-C captured</i>				
Biomass (Q _E ·X _E)	g C/d (%)	0.024 (77)	0.040 (82)	0.051 (77)
Effluent (Q _E ·C _{i,T})	g C/d (%)	0.001 (3.0)	0.001 (1.9)	0.006 (8.8)
Ventilation (Q _G ·C _{i,G})	g C/d (%)	0.000 (0.0)	0.000 (0.0)	0.000 (0.0)
SMP (Q _E ·C _{DOC})	g C/d (%)	0.006 (20)	0.008 (17)	0.009 (14)
<i>C_i transfer rates</i>				
J _m	g C/m ² /d	164	104	70
Relative permeability ^a	%	670	440	300

^a Percentage of permeability in this study over estimated permeability in Tang et al (2012), which used the same membrane.