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# Submerged membrane photobioreactor for the cultivation of *Haslea ostrearia* and the continuous extraction of extracellular marennine

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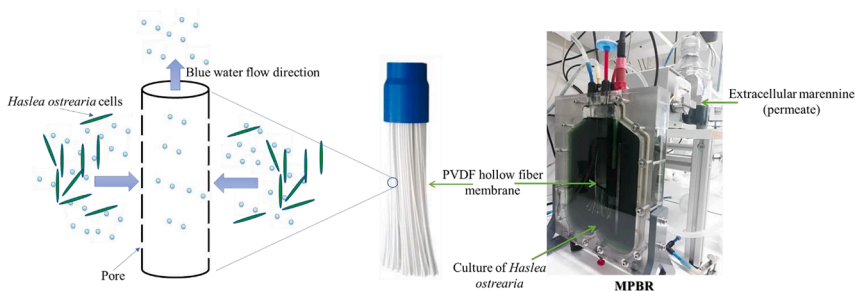
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## HIGHLIGHTS

- Successful implementation of membrane photobioreactor (MPBR) applied to *H. Ostrearia*.
- Simultaneous *H. Ostrearia* cultivation and marennine continuous extraction.
- No cell leaching even at high dilution rates.
- High cell and marennine concentrations at low dilution rate.
- Marennine productivity enhanced at high dilution rates.

## GRAPHICAL ABSTRACT



## ABSTRACT

### Keywords:

*Haslea ostrearia*  
Extracellular marennine  
Photobioreactor  
Submerged membrane

*Haslea ostrearia* is a marine diatom known to produce and excrete the marenine blue pigment. Its controlled, continuous and intensified cultivation remains a challenge. Thus, a submerged membrane photobioreactor (MPBR) was implemented in order to simultaneously and continuously cultivate *H. ostrearia* and extract marennine. The MPBR was compared with a similar air-lift photobioreactor (without membrane), both working at a dilution rate equal to 0.1, 0.3 and 0.5 d<sup>-1</sup>. Contrary to the air-lift photobioreactor, the MPBR successfully operated at high dilution rate without biomass washout. The MPBR allowed continuously recovering marennine and reaching high cell density ( $555 \pm 25 \times 10^6$  cells L<sup>-1</sup> at D = 0.1 d<sup>-1</sup>), marennine concentration ( $36.00 \pm 0.02$  mg L<sup>-1</sup> at D = 0.1 d<sup>-1</sup>) and marenine productivity ( $7.20 \pm 0.01$  mg L<sup>-1</sup> d<sup>-1</sup> at D = 0.5 d<sup>-1</sup>).

## 1. Introduction

The cultivation and exploitation of marine microalgae have long been studied. Nevertheless, only few species whose physiology is well known are cultivated and exploited (Morais Junior et al., 2020). Among

diatoms, *Haslea ostrearia*, an eukaryotic microalga of the class *Bacillariophyceae* remains difficult to cultivate at high productivities. Thus, the industrial exploitation of this microalga remains limited except for aquaculture. For the latter, the blue-green pigment ‘‘marennine’’ produced and excreted by the microalga is used for the oyster greening,

giving them a better organoleptic quality. This biological activity has led to an increase in the market value of oysters by 20 to 30% for the French oyster industry (Falaise et al., 2019). This pigment could also be used for its antioxidant, antiproliferative, antibacterial, antiviral and antitumor properties (Falaise et al., 2016; Gastineau et al., 2018, 2014a, 2012b; Hussein and Abdullah, 2020; Prasetya et al., 2020b).

So far *H. ostrearia* has been mainly cultivated in open culture systems of 200 L and 10 m<sup>3</sup> or in photobioreactors (PBRs, less than 7 L) (Gastineau et al., 2014b; Rossignol et al., 2000a; Turpin et al., 2001). Whatever the system, high volumes of culture often have to be set-up in order to produce a consistent amount of biomass or marennine, as their concentrations are usually low (Bélangier et al., 2020; Francezon et al., 2021). Thus, cultivation process intensification remains a challenge, notably to increase marennine or biomass concentration but also the productivities. Currently, the photobioreactors (PBR) used for *Haslea ostrearia* cultivation are mostly developed for growing microalgae in suspension (Nghiem Xuan et al., 2021; Rossignol et al., 2000a, Rossignol et al., 2000b) whereas this diatom settles and forms biofilms in natural environments. Moreover, the nutrient requirements of this species have long been questioned, as well as the culture parameters (temperatures, pH and light). In general, enrichment solutions such as those proposed by Provasoli et al. (1957) and containing nitrates, phosphorus, sulfur, vitamins and trace metals, are added to artificial or natural filtered seawater to feed microalgae cultures. Culture mediums have also been developed specifically for *H. ostrearia* such as the diatom artificial medium (DAM) (Gagneux-Moreaux et al., 2007), a modified version of the Harrison et al. (1980) artificial seawater (ASW) (Mouget et al., 2009) and the NX medium (Nghiem Xuan et al., 2020). Techniques of immobilized cultures (active and/or passive) have also been developed such as culture in tanks for the greening of oysters (Turpin et al., 2001) and culture on alginate beads (Lebeau et al., 1999). An immersed membrane PBR (MPBR) was also implemented for the continuous extraction of extracellular marennine (EMn) while maintaining algal cells within the photobioreactor (Rossignol et al., 2000a). This kind of photobioreactor is promising because the biomass and hydraulic residence times can be decoupled (Castaing et al., 2011, 2010; Massé et al., 2006). Furthermore, the cells are not submitted to any shear stress due to pumping or circulation. With this cultivation system, Rossignol et al. (2000a) improved the production of biomass and marennine with marennine concentration, productivity and specific productivity equal to 25 mg L<sup>-1</sup>, 3 mg L<sup>-1</sup> d<sup>-1</sup> and 33 × 10<sup>-9</sup> mg cells<sup>-1</sup> d<sup>-1</sup> respectively. Moreover, this system induces low energetic costs compared to tangential filtration (Humeau et al., 2011; Massé et al., 2011; Mo et al., 2015; Zhang et al., 2019).

Recently, Nghiem Xuan et al., 2020 and Nghiem Xuan et al., 2021) showed that growing *Haslea* in a stirred flat panel PBR allows better access of the cells to nutrients and light. Till now, they reached a better productivity of extracellular marennine of 4.5 ± 0.2 mg L<sup>-1</sup> d<sup>-1</sup> (Nghiem Xuan et al., 2020) against 0.7 ± 0.1 mg L<sup>-1</sup> d<sup>-1</sup> for immobilized cell culture (Nghiem Xuan et al., 2021).

On the basis of a literature review, it appears that air-lift as well as submerged membrane photobioreactor could be of interest for *Haslea ostrearia* cultivation. Therefore, in this study, an integrated air-lift membrane photobioreactor was implemented and compared to a conventional air-lift photobioreactor. This work aims at studying the versatility of the membrane process facing different conditions of dilution rates (0.1, 0.3, 0.5 d<sup>-1</sup>) as well as its performances (productivities) compared to the same air-lift photobioreactor without membrane.

## 2. Material and methods

### 2.1. Microalgae and culture maintenance

A diatom strain *Haslea ostrearia* (NCC 529), isolated from oyster-ponds in the Bay of Bourgneuf, France and provided by the Nantes culture collection of the Mer-Molécules-Santé (MMS) laboratory, was

used in this study.

Provalosi medium (ES 1/3) was used as culture media. It was prepared in 1 L of natural seawater (Le Croisic Océarium, Loire-Atlantique, France) filtered using 0.7 µm then 0.45 µm filters (Whatman GF/F). pH and salinity were adjusted at 7.8 and 28 g L<sup>-1</sup> respectively. The medium was sterilized by filtration at 0.2 µm.

The microalgae were maintained in 500 mL flasks filled with 250 mL of ES 1/3 medium, at 20 °C. Light was supplied by white led light for the day/night cycle 14/10 at 150 µmol photons m<sup>-2</sup> s<sup>-1</sup>. 500 mL of these starter cultures were used to inoculate a 1 L PBR.

### 2.2. Photobioreactor system

All experiments, with or without immersed membrane, were sequentially carried out in a lab-scale airlift PBR with a volume of 1 L, as described in Nghiem-Xuan et al. (2021). Mixing resulted from gas injection at the bottom of the PBR and the pH, fixed at 7.8, was controlled by CO<sub>2</sub> injection, supplied by an automated regulating valve. The PBR or MPBR was placed in an air-conditioned room with temperature adjusted to 20 °C. Cultures were conducted with modified Provasoli ES 1/3 medium, enriched in macronutrients with a NaNO<sub>3</sub> concentration of 0.15 g L<sup>-1</sup>, NaHCO<sub>3</sub> equal to 0.42 g L<sup>-1</sup> and Na<sub>2</sub>SiO<sub>3</sub> · 5H<sub>2</sub>O of 0.03 g L<sup>-1</sup>, needed for cell metabolism. In order to prevent any precipitation phenomenon and to have a chemically stable growth medium, carbon (NaHCO<sub>3</sub>) and silica (Na<sub>2</sub>SiO<sub>3</sub> · 5H<sub>2</sub>O) were directly and separately added in “fed-batch” to the photobioreactor. Light was supplied by a led panel across the day/night cycle 14 h/10 h at 200 µmol photons m<sup>-2</sup> s<sup>-1</sup>.

Two kinds of cultivation experiments were carried out using one airlift PBR without membrane (thereafter named PBR, as control) and one with membrane of filtration (MPBR) (Fig. 1). The latter was equipped with an immersed ultrafiltration membrane. For the present work, PolyVinylidene Fluoride (PVDF) hollow fiber membranes were chosen with an average pore diameter equal to 0.05 µm and a total membrane surface area equal to 0.0373 m<sup>2</sup>. Culture medium containing extracellular marennine was sucked through the membrane, from the outside to the inside of the fibers, while biomass was retained. An equivalent volume of nutrient medium was supplied from the top of MPBR through a peristaltic pump. No backwashes were implemented.

For both photobioreactors, air-lift alone or with a membrane, three dilution rates (D) equal to 0.1, 0.3 and 0.5 d<sup>-1</sup>, were fixed (renewal rate of the culture medium equal to 10%, 30% and 50%). Experiments at different dilution rates were performed separately.

The dilution rate was calculated as follows:

$$D(\text{d}^{-1}) = Q/V_r \quad (1)$$

With Q the flow rate (mL d<sup>-1</sup>) and V<sub>r</sub> the reaction volume (mL).

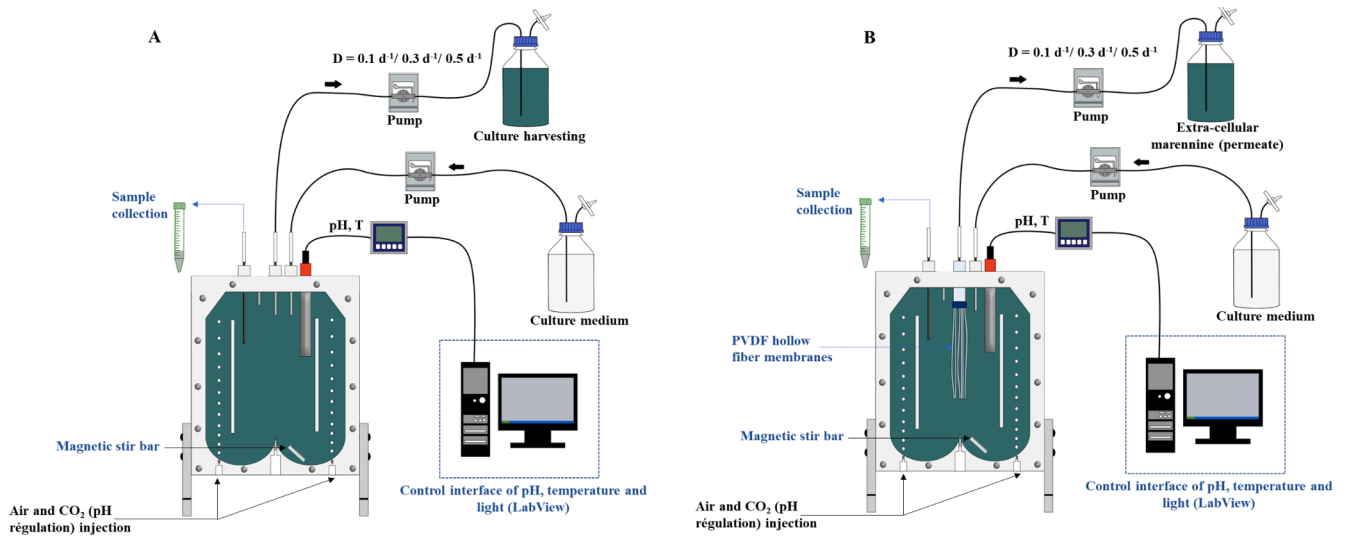
### 2.3. Analytical methods

Before each sample, stirring with a magnetic bar was carried out to push over the cells remaining stuck to the walls of the photobioreactor and around the fiber of the filtration membrane. The cell number in suspension was determined using a Nageotte counting chamber (Marienfeld, AQRA, India). The cell concentration (N) was calculated using the following formula:

$$N(\text{cells L}^{-1}) = n/V \quad (2)$$

With n: the number of cells, V: represents 5 µL for 4 bands of 1.25 µL. All measurements were carried out in triplicates for a minimum of 300 cells.

Extracellular marennine (EMn) concentration was determined by measuring the optical density (A<sub>669</sub>) at 669 nm, as described by Pouvreau et al. (2006). The pigment concentration (mg L<sup>-1</sup>) is then calculated by the following equation, using the Beer-Lambert law:



**Fig. 1.** The schematic illustration of the microalga cultivation process using MPBR and PBR. A) PBR without membrane filtration, B) PBR with membrane filtration. Both of them are operated at different dilution rate (D) equal to 0.1, 0.3 and 0.5 d<sup>-1</sup>. Before sampling stirring with a magnetic bar on the photobioreactor walls as well as manual stirring of fiber bundle was carried out.

$$[EMn](\text{mg L}^{-1}) = (A_{669}/\epsilon) \times M \quad (3)$$

With A: the absorbance of the sample at 669 nm,  $\epsilon$ : the molar extinction coefficient ( $\epsilon = 12 \times 10^4 \text{ L mol}^{-1} \text{ cm}^{-1}$  for the extracellular marennine), l: the optical path of the cell in cm and M: the molar mass of the EMn which is equal to  $9893 \times 10^3 \text{ mg mol}^{-1}$  according to [Pouvreau et al. \(2006\)](#).

The marennine volumetric ( $P_v$ ) and specific ( $P_s$ ) productivities were calculated from the dilution rate, according to the following equations Eq. (4) and Eq. (5) respectively:

$$P_v(\text{mg L}^{-1} \text{d}^{-1}) = C_m \times D \quad (4)$$

$$P_s(\text{mg cells}^{-1} \text{d}^{-1}) = P_v/C \quad (5)$$

With  $C_m$ : the average concentration of EMn at the steady state ( $\text{mg L}^{-1}$ ), D: dilution rate ( $\text{d}^{-1}$ ), C: the average cell number in suspension at the pseudo-steady state ( $\text{Cells L}^{-1}$ )

#### 2.4. Retention rate

The retention rate (RR) of marennine by the membranes was calculated as follows:

$$RR(\%) = \left(1 - \frac{[EMn]_p}{[EMn]_r}\right) \times 100 \quad (6)$$

With  $[EMn]_p$ : the concentration of EMn in the permeate,  $[EMn]_r$ : the concentration of EMn in the PBR (retentate).

### 3. Results and discussion

#### 3.1. PBR and MPBR performances

##### 3.1.1. Biomass cultivation follow-up

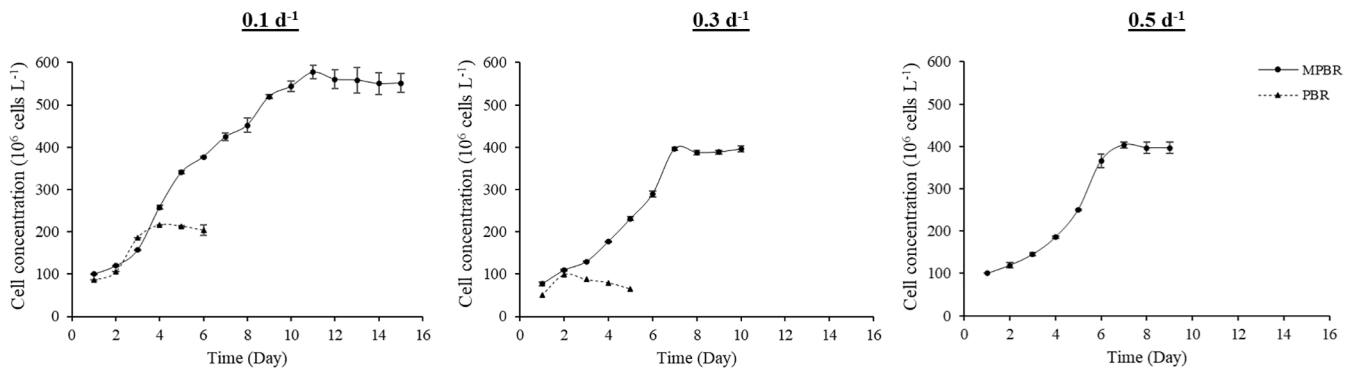
[Fig. 2](#) shows the evolution of the suspension cell concentration of *Haslea ostrearia* in the PBR and MPBR operating at different dilution rates. For both photobioreactors, cell concentrations first increased then stabilized. The stabilization of cell concentration occurred sooner for the PBR and reached lower values. As shown in [Table 1](#), whatever the

**Table 1**

Growth parameter of *Haslea ostrearia* in MPBR at different dilution rate.

Dilution rate (d <sup>-1</sup> )	MPBR		
	0.1	0.3	0.5
$\mu_{\max} = \frac{\ln(N_t) - \ln(N_{t-1})}{t - t_{(t-1)}}$	0.31	0.32	0.28
$t_g = 2/\mu_{\max}$	2.25	2.19	2.49

$\mu_{\max}$ : growth rate,  $N_t$  cell number at time (t),  $N_{t-1}$  cell number at time (t-1).  $t_g$ : generation time (day).



**Fig. 2.** Growth kinetics of *Haslea ostrearia* in PBR (◆) and MPBR (●) operating at different dilution rates of 0.1, 0.3 and 0.5 d<sup>-1</sup>. Washout occurred in the PBR, indicated by the continuous decrease of the cell concentration. Results were expressed as mean  $\pm$  standard deviations of triplicate determinations.

dilution rate adopted for the MPBR, the specific growth rates were almost similar (from 0.28 to 0.32 d<sup>-1</sup>). These values depend on the hydraulic and biomass residence times as well as the assimilation level of photons and nutrients. In the case of PBR, the biomass and hydraulic residence times are similar whereas they are equal to infinite and 10, 3.3 or 2 days respectively for the MPBR. As a consequence, the PBR did not allow maintaining a high cell density.

Cell washout was observed with the PBR from a dilution rate equal to 0.3 d<sup>-1</sup>. From this value, no cell concentration stabilization was observed (Figs. 2 and 3). Therefore, for the PBR, the maximum stabilized cell concentration reached 211 ± 5 × 10<sup>6</sup> cells L<sup>-1</sup> after 4 days of operation (Fig. 2). This value of cell density at low dilution rates (0.1 d<sup>-1</sup>) seems to be congruent with previously published results. Indeed, a maximum cell density of 150 ± 0.1 × 10<sup>6</sup> cells L<sup>-1</sup> was reached at day 5 when *Haslea ostrearia* was cultured under mixed conditions in a crystal PBR (i.e. airlift PBR) with a dilution rate equal to 0.1 d<sup>-1</sup> and under a light intensity of 300 μmole photons m<sup>-2</sup> s<sup>-1</sup> with a periodicity of 14 h light/10 h dark (Nghiem Xuan et al., 2021).

Nowadays, different types of microalgae cultivation systems exist such as open raceway ponds and closed PBR like vertical column, flat panel and tubular PBR. When classically operated in continuous mode, biomass concentration and then productivity depend on the dilution rate (Bilad et al., 2014). In 2012, Tang et al. (2012) demonstrated that by increasing the dilution rate of a continuous PBR up to 8-folds (from 0.076 to 0.64 d<sup>-1</sup>), the cell concentration of the microalga *Chlorella minutissima* decreased from 14.7 × 10<sup>7</sup> to 2.1 × 10<sup>7</sup> cells mL<sup>-1</sup>. In addition to these classic PBRs mentioned above, closed or semi-closed PBR with an integrated membrane have also been studied for the cultivation and harvesting of microalgae and mainly for the waste water treatment (Bilad et al., 2014; Zhang et al., 2019). Whatever the type of membrane bioreactors, for microalgae or bacteria, the membrane often prevents cell washout and allows increasing the concentration of biomass while keeping a renewal of the growth medium (Bilad et al., 2014; Marbelia et al., 2014; Mo et al., 2015; Zhang et al., 2019). Moreover, MPBR allow to reduce energy costs (Akkoyunlu et al., 2021). Nevertheless, few works deal with the interest of MPBR for the simultaneous extraction of exometabolites, which is of interest if those exometabolites can be recovered from the membrane while cells are kept in the culture vessel. Thus, for the present study, a self-designed airlift PBR was used in order to maintain a better access of cells to nutrients and light through aeration and thus better metabolite production (Nghiem Xuan et al., 2021; Pruvost et al., 2016). In addition, this PBR was equipped with a submerged membrane in order to increase *Haslea ostrearia* cell density and simultaneously to extract the marennine

exometabolite. As a consequence, the specific MPBR made it possible to cultivate the *Haslea* strain at high concentrations never reported in the literature with a maximum suspension cell concentration of 555 ± 25 × 10<sup>6</sup> cells L<sup>-1</sup> obtained at a dilution rate equal to 0.1 d<sup>-1</sup> (Fig. 2), a value 3 times higher than the PBR device (Fig. 3). It was also able to maintain relatively higher cell concentration over a wider range of dilution rates (at least up to 0.5 d<sup>-1</sup> with a cell concentration of 399 ± 11 × 10<sup>6</sup> Cells L<sup>-1</sup>) (Figs. 2 and 3). In fact, high cell density in the photobioreactor will facilitate the downstream processing of biomass in the event of refining. Moreover, at the end of a culture period, the biomass could be more concentrated thanks to a last suction of the medium through the membrane. This method could then also be an effective approach to reduce harvest costs as demonstrated by Bilad et al. (2014). Then, the recovered biomass can be exploited for the extraction of intracellular marennine as well as the siliceous exoskeleton called frustule which could change the ways of manufacturing devices for energy storage, optoelectronics, solar cells and batteries (Abdusatorov et al., 2020).

In this study and as shown in Fig. 2, the pseudo-stabilization phase depends on the dilution rate applied. The higher dilution rate is, the faster the stabilization of the culture (10, 6 and 5 days for 0.1 d<sup>-1</sup>, 0.3 d<sup>-1</sup> and 0.5 d<sup>-1</sup> respectively, Table 2). Despite the total retention of cells within the MPBR, without the presence of a purge and/or a biomass outlet, a pseudo-steady state was reached for all the experiments performed with the MPBR. However, our results are consistent with the study of Rossignol et al. (2000a) where they show that during a three-week experiment at a dilution rate equal to 0.1 d<sup>-1</sup>, the viable biomass of *H. ostrearia* and the marennine productivity reached a plateau phase at the end of the first week. This behavior may be due to the nutritional composition of the culture medium used in this study which may no longer be sufficient for their multiplication but only to keep them alive. This state can be explained by a cell renewal in which the number of living cells makes up for the number of dead cells. Moreover, it's noted that the values of the pseudo-stabilized cell concentrations are different between the dilution rates 0.1 d<sup>-1</sup> (555 ± 25 × 10<sup>6</sup> Cells L<sup>-1</sup>) and the couple 0.3 d<sup>-1</sup> and 0.5 d<sup>-1</sup> (390.4 ± 6 × 10<sup>6</sup> Cells L<sup>-1</sup> and 399 ± 11 × 10<sup>6</sup> Cells L<sup>-1</sup> respectively). In fact, researchers suggest that EMn is synthesized as a light shielding molecule (Gastineau et al., 2014a; Pouvreau et al., 2008; Rossignol et al., 2000b). Consequently, the more the medium is renewed, the more EMn concentration in the PBR decreases and therefore the cells will be more exposed to light which stresses them and affects their growth.

### 3.1.2. Extracellular marennine production

For the MPBR device, marennine concentrations increased then stabilized at higher values for the lowest dilution rates (Fig. 4.A, 4.B, 4.C). Thus, at 0.1 d<sup>-1</sup>, a maximum concentration of EMn equal to 36 ± 0.02 mg L<sup>-1</sup> was obtained for the MPBR versus 14 ± 0.01 mg L<sup>-1</sup> for the PBR (Fig. 4.A). As presented in Fig. 4.A and 4.B, the stabilized EMn concentrations were always lower for the PBR compared to the MPBR. By plotting the stabilized concentration of EMn as a function of pseudo-stabilized cell concentration (Fig. 4.D), it appears that EMn concentration increased with cell density for both photobioreactors. The stabilization was always faster for the highest dilution rate.

In addition to the continuous agitation provided by gas injection, manual agitation with a magnetic stirred, already present in the bioreactor, was carried out every day and before each sample. This manipulation allowed to push over the cells which remain stuck on the photobioreactor walls and around the fibers of the filtration membrane. Thus, the two devices (PBR and MPBR) were compared on the basis of the cells in suspension which are the easiest to recover for an industrial application. It should be noted that very few cells remain attached to the filtration membranes (barely visible to the naked eye), comparing to those in suspension. The MPBR achieved the highest volume productivity of EMn (Fig. 5.A). Indeed, its maximum productivity was equal to 7.2 ± 0.01 mg L<sup>-1</sup> d<sup>-1</sup> (D = 0.5 d<sup>-1</sup>), that means 2 to 5 times higher than the productivity of the PBR (Fig. 5.A and 5.B), but also higher than cell

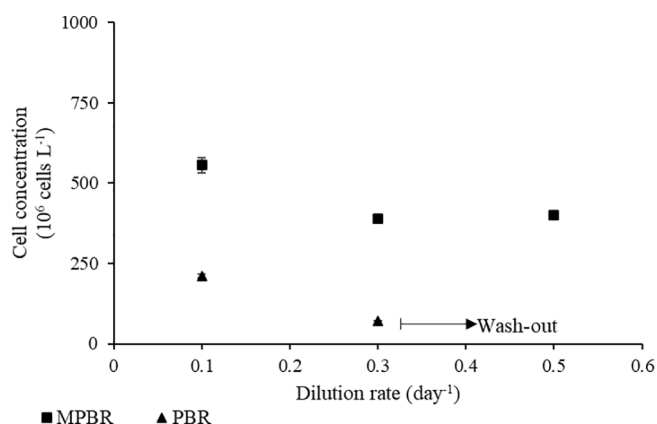
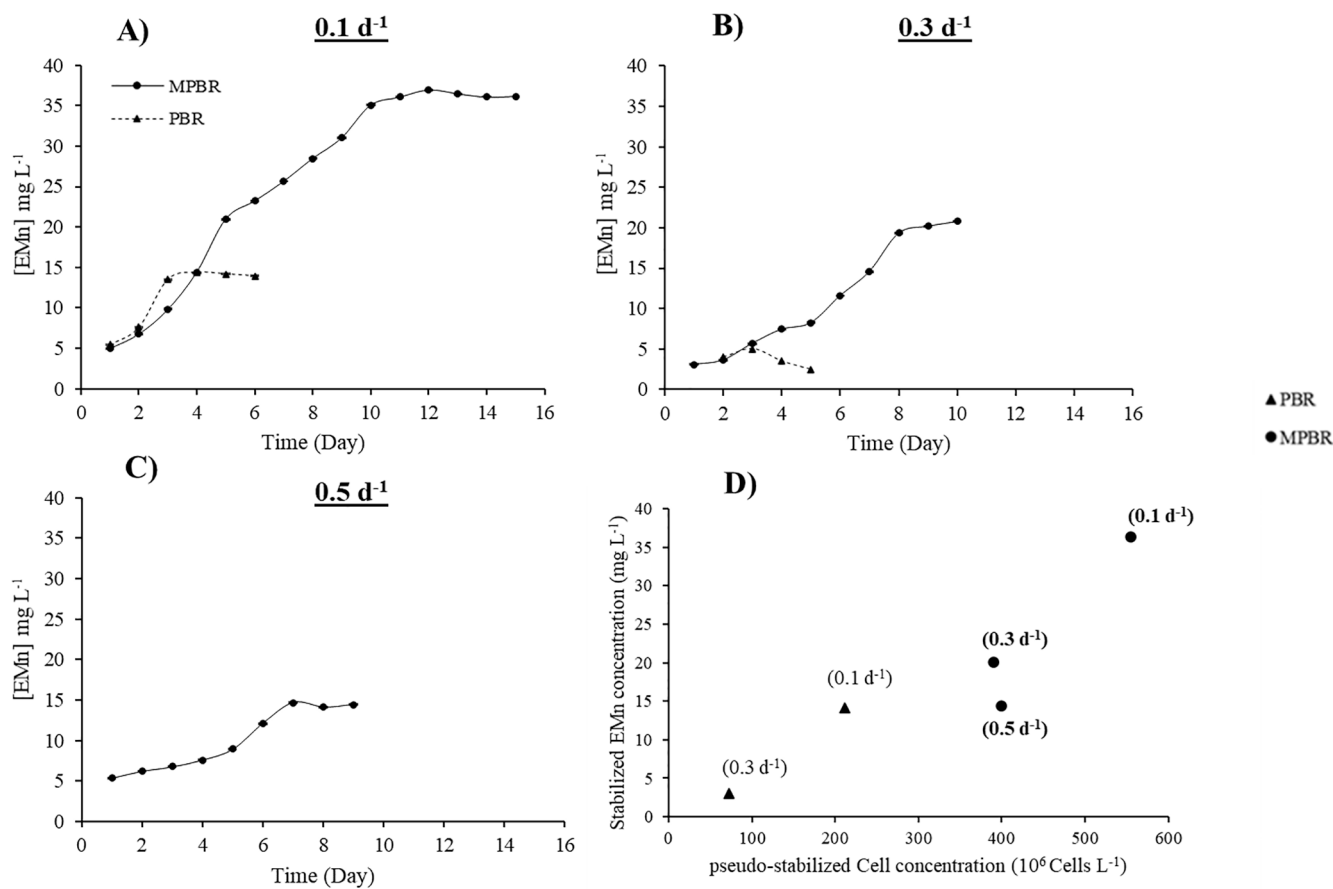


Fig. 3. Stabilized cell density of *Haslea ostrearia* in both PBRs operating at different dilution rates. Washout in PBR (◆) at dilution rate D equal to 0.3 d<sup>-1</sup>. MPBR (■) was able to prevent washout up to D = 0.5 d<sup>-1</sup> allowing higher cell concentration. Results were expressed as mean ± standard deviations of triplicate determinations.

**Table 2**

Cell or marennine concentrations as well as marennine productivity following operating conditions of the MPBR of this study.

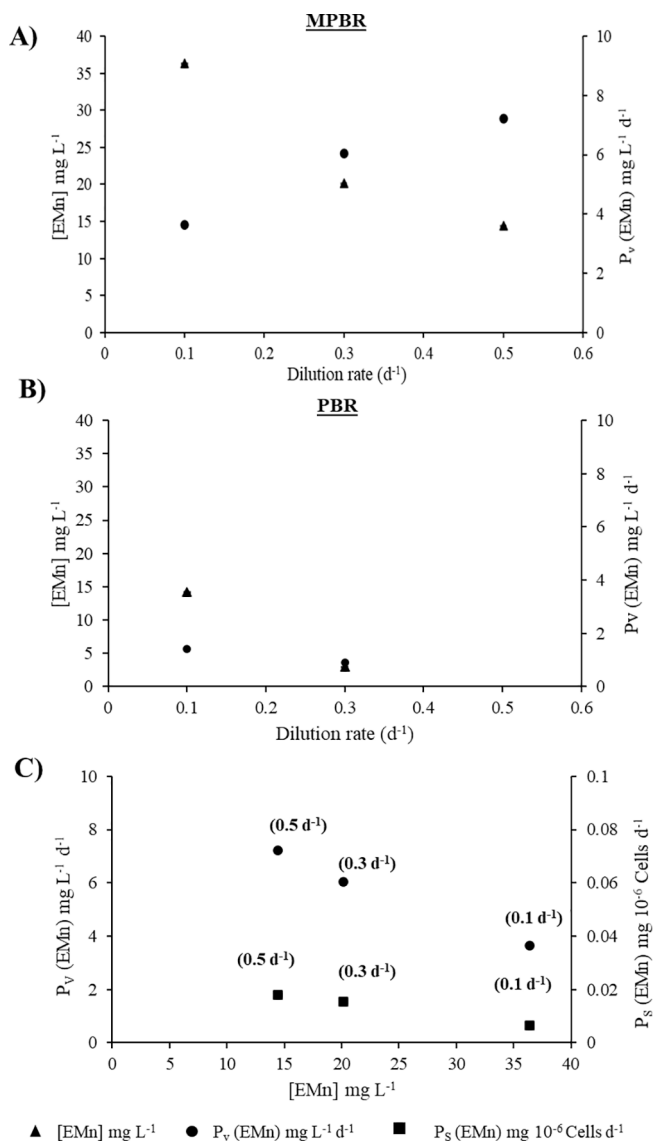
Operating system	Photobioreactors	Free cells/immobilized cells	With/without Membrane filtration	Volume (L)	Renewal rate (%)	Cell concentration ( $\times 10^6$ cells $L^{-1}$ )	[EMn] ( $mg L^{-1}$ )	$P_v$ (EMn) ( $mg L^{-1} d^{-1}$ )	Cultivation time (Day)	Time of stabilisation (Day)
Continuous	Flat panel PBR	Free	With	1	10	561	36	3.6	15	10
					30	390	20	6.0	10	6
					50	399	14	7.2	9	5



**Fig. 4.** Extracellular marennine production curve in the PBR (◆) and the MPBR (●). A, B and C: production kinetics of EMn in the PBR and MPBR at different dilution rates 0.1, 0.3 and 0.5 d<sup>-1</sup>. D: correlation between the stabilized concentration of EMn and the pseudo-stabilized cellular concentration at 0.1, 0.3 and 0.5 d<sup>-1</sup> in the two types of PBR, EMn concentration increased with cell density for both photobioreactors. Results were expressed as mean  $\pm$  standard deviations of triplicate determinations.

culture systems used so far. For example, for agar/alginate immobilized cells under a light intensity of  $3 \times 10^{16}$  quanta  $cm^{-2} s^{-1}$  with a 14/10 h light/dark cycle, Lebeau et al. (2000); Lebeau et al. (2002) found a maximum marennine productivity of 0.5–0.6  $mg L^{-1} d^{-1}$  at a dilution rate equal to 0.25 d<sup>-1</sup>. Passive cell immobilization at the bottom of open ponds of 10 m<sup>3</sup> resulted in the same range of EMn concentration (3.4  $mg L^{-1}$ ) and productivity (0.4  $mg L^{-1} d^{-1}$ ) (Turpin et al., 2001). Nghiem Xuan et al. (2021) also found a lower productivity equal to  $4.5 \pm 0.16$   $mg EMn L^{-1} d^{-1}$  during the cultivation within a conventional mixed PBR fed with optimized culture medium and with an irradiance equal to 300  $\mu mol photons m^{-2} s^{-1}$  with a photoperiod 14/10 h light/dark. Finally, the volume productivities of marennine from the study presented here, were 0.7 to 2 times higher than the productivity obtained by Rossignol et al. (2000a) who used a cylindrical photobioreactor ( $L = 40$  cm and  $d = 15$  cm) equipped with a submerged membrane. The cylindrical PBR is known to be one of the most practical PBR due to its low energy consumption. Nevertheless, it presents a small specific illumination ( $a_s$ ) area

of  $26.66 m^{-1}$  ( $a_s$  cylindrical PBR =  $2/R$ , with  $R$  is the internal radius of the cylinder) which may result in a high dark fraction inside the reactors. This value is below the specific illumination area of our air-lift PBR which is equal to  $33.33 m^{-1}$  ( $a_s$  flat plate PBR =  $1/L$ , with  $L$  is the thickness of the PBR). In addition, Airlift PBR (also called gas/liquid reactor) allows a good constant mixing, by gas injection, which improves light access to cells. This was not the case with the study of Rossignol et al., (2000a) who only mixed with magnetic stirrer for 5 min when they injected the culture medium. With the MPBR configuration, the stabilized concentration and volume productivity of extracellular marennine are inversely proportional (Fig. 5.A and 5.C). A dilution rate of 0.1 d<sup>-1</sup>, resulted in the highest EMn concentration ( $36.00 \pm 0.02$   $mg L^{-1}$ ) and the lowest productivity ( $3.60 \pm 0.01$   $mg L^{-1} d^{-1}$ ) whereas it is the opposite for 0.5 d<sup>-1</sup> ([EMn] =  $14.00 \pm 0.03$   $mg L^{-1}$ ,  $P_v = 7.20 \pm 0.01$   $mg L^{-1} d^{-1}$ ). This is in agreement with the study of Rossignol et al. (2000b) who increased the dilution rate by 10 times (0.025 and 0.25 d<sup>-1</sup>) to improve the volume productivity of marennine from 0.17 to 2.7  $mg L^{-1}$



**Fig. 5.** Concentration (◆) and volume productivity (●) of extracellular marennine at different dilution rates 0.1 d<sup>-1</sup>, 0.3 d<sup>-1</sup> and 0.5 d<sup>-1</sup>. A, B) EMn concentration and productivity in the MPBR and the PBR. The stabilized concentration and volume productivity of extracellular marennine are inversely proportional. C) Evolution of volume (●) and specific (■) productivity of EMn in the MPBR depending on the marennine concentration. Results were expressed as mean ± standard deviations of triplicate determinations.

d<sup>-1</sup> in free-cells bioreactor associated with an external flat membrane module and from 0.12 to 0.7 mg L<sup>-1</sup> d<sup>-1</sup> in immobilized-cells bioreactor, under light intensity equal to 80 μmole photons m<sup>2</sup> s<sup>-1</sup> with the following cycles: 14 h light/ 10 h dark. Moreover, Fig. 5.C shows that the specific productivity of marennine, calculated from the number of cells in suspension, increases by increasing the dilution rate. The highest EMn specific productivity was equal to 0.018 × 10<sup>-6</sup> mg cells<sup>-1</sup> d<sup>-1</sup> at D = 0.5 d<sup>-1</sup>, 3 times higher than the specific productivity obtained at D = 0.1 d<sup>-1</sup>. In fact, an increase of medium renewal rate would allow to stimulate the synthesis of EMn by increasing the accessibility of light and nutrient to microalgal cells. Besides, marennine, which presents a significant bioactivity, could have inhibited the growth of *H. ostrearia* cells at high concentrations (Nghiem Xuan et al., 2020). This phenomenon was not observed during this study. In fact, at high concentration of 36.00 ± 0.02 mg L<sup>-1</sup>, a high suspension cell concentration of 555 ± 25 × 10<sup>6</sup> cells L<sup>-1</sup> was still reached, higher than the literature values (Gastineau et al., 2012a; Gaudin et al., 2006; Lebeau and Robert, 2003; Nghiem Xuan

et al., 2020, 2021; Prasetya et al., 2020b; Rossignol et al., 2000a).

All these results show that membrane technology can play a crucial role in microalgae biorefinery processes and depending on the biotechnological application envisaged. As shown above, low dilution rate operations (0.1 d<sup>-1</sup>) induced a high concentration of marennine in the photobioreactor. In this case, the entire culture (cells plus the culture medium) could be harvested just after the stabilization of the concentrations. The cells would then be separated from marennine by centrifugation. This operating mode, which resembles a batch operation, would allow to recover a small volume of marennine-rich solution as well as pellet of cells.

On the other hand, high dilution rate operations (0.5 d<sup>-1</sup>) may also have an interest. Indeed, even if the concentration of marennine into the MPBR is lower, but already high compared to classical photobioreactors, this makes it possible to continuously produce a solution of marennine. In that case, the marennine solutions will already be pre-purified since the membrane will have retained all the biomass. This type of operation could be exploited in oyster farming in order to continuously supply ponds with marennine solutions at concentrations below the toxicity level. The process of greening oysters during the final farming stage before marketing could be improved (Prasetya et al., 2019). In addition, this process can be integrated into aquaculture industries where marennine could serve as a source of antimicrobial substances to control pathogens affecting certain larval, juvenile or adult stages of farmed animals (bivalves, fish) (Prasetya et al., 2020a).

Finally, marennine is known for its multiple biological activities (Gastineau et al., 2014b). One of the scientific and technological issues related to its extraction concerns the preservation of its properties and activities. For the present study, EMn is recovered in its native configuration, in a medium with the same composition of soluble compounds, pH and ionic strength. The use of the filtration membrane enables to pre-purify EMn without adding chemicals and under relatively mild operating conditions. In contrast to cross-flow filtration, the molecules do not pass at high speeds through pumps, which makes them less susceptible to be damaged.

However, the performance of the submerged membrane photobioreactor strongly depends on several parameters including light, carbonization of CO<sub>2</sub> and nutrient concentrations which can be affected by the presence of a membrane in MPBR systems. It would therefore be interesting to study the influence of these parameters. In addition, membrane fouling could constitute a major challenge for extended application in the culture of microalgae notably for long term filtrations where a biofilm could settle. Backwashes with permeates could be used to alleviate severe fouling (Massé et al., 2015). Moreover, the molecular weight cut-off of the membrane as well as the conditions of its use (shear stress at the surface of the membrane) could be changed in order to enhance the hydraulic performances and the transmission rates (Balti et al., 2021; Gifuni et al., 2020). Nevertheless, for industrial applications, larger-scale operations will be needed even if the process of EMn recovery using membranes is optimized. This scale-up remains a challenge, because the behavior of the culture of microalgae will change.

### 3.2. Membrane performance

The membrane retention rate was estimated in this study at each dilution rates after stabilization. As shown in Fig. 6, the membrane allowed marennine to pass relatively easily since retention rates were less than 40%. Furthermore, an increase of the dilution rate induced a decrease of the retention rate (Fig. 6). Put another way, the higher the renewal rate of the culture medium, the more marennine passes freely through the membranes. In fact, at a dilution rate of 0.1 d<sup>-1</sup>, 35% of EMn were retained by the filtration membranes against 10% at 0.5 d<sup>-1</sup>. The highest suspension cell concentration at 0.1 d<sup>-1</sup> (555 ± 25 × 10<sup>6</sup> cells L<sup>-1</sup> against 399 ± 11 × 10<sup>6</sup> cells L<sup>-1</sup> at 0.5 d<sup>-1</sup>) as well as EMn concentration (36 ± 0.02 mg L<sup>-1</sup> against 14 ± 0.03 mg L<sup>-1</sup> at 0.5 d<sup>-1</sup>) could induce a higher membrane fouling or polarization concentration at the

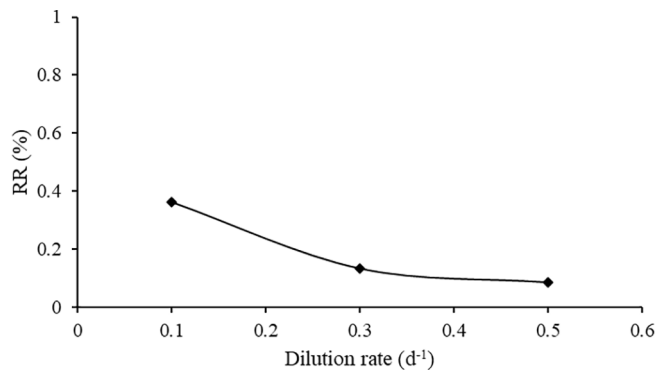


Fig. 6. Membrane retention rate with the MPBR operating at different dilution rates.

membrane surface. In addition, since the retention rates were calculated at the end of the stabilization period, it should be noted that the membrane was submerged for the longest time in the case of the 0.1 d<sup>-1</sup> before evaluation of the retention rate. Thus, a different membrane fouling could occur due to different residence time of membrane. In order to mitigate this mass transfer decrease, a backwash could be implemented with a fresh growth medium.

#### 4. Conclusion

This work demonstrates the high potential of air-lift membrane photobioreactors for simultaneous cultivation of the diatom *H. ostrearia* and extraction of its valuable exometabolite marennine. High cell concentrations in the photobioreactor were achieved, depending on the dilution rate. Productivities and concentrations of extracellular marennine are significantly increased compared to previous works using classical photobioreactor. Thus, the operation of MPBRs at high dilution rates is a promising field for continuous extraction of pre-purified algal exometabolites.

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#### CRedit authorship contribution statement

**Nesrine Gargouch:** Conceptualization, Methodology, Validation, Investigation, Writing – original draft. **Raphaëlle Touchard:** Resources, Investigation. **Hélène Marec:** Resources, Investigation. **Jean Luc Mouget:** Funding acquisition, Writing – review & editing. **Jérémy Pruvost:** Conceptualization, Supervision, Writing – review & editing. **Anthony Masse:** Conceptualization, Methodology, Validation, Supervision, Writing – original draft.

#### Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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