Separation Science and Technology
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713708471

Harvesting of Cyanobacterium *Arthrospira platensis* Using Inorganic Filtration Membranes

N. Rossi[a]; I. Petit[a]; P. Jaouen[a]; P. Legentilhomme[a]; M. Derouiniot[a]
[a] Laboratoire de Génie des Procédés Environnement-Agroalimentaire, GEPEA UMR CNRS, Nantes, France

To cite this Article Rossi, N. , Petit, I. , Jaouen, P. , Legentilhomme, P. and Derouiniot, M.(2005) 'Harvesting of Cyanobacterium *Arthrospira platensis* Using Inorganic Filtration Membranes', Separation Science and Technology, 40: 15, 3033 — 3050
To link to this Article: DOI: 10.1080/01496390500385046
URL: http://dx.doi.org/10.1080/01496390500385046

Separation Science and Technology, 40: 3033–3050, 2005

ISSN 0149-6395 print/1520-5754 online
DOI: 10.1080/01496390500385046

Harvesting of Cyanobacterium *Arthrospira platensis* Using Inorganic Filtration Membranes

N. Rossi, I. Petit, P. Jaouen, P. Legentilhomme, and M. Derouiniot
Laboratoire de Génie des Procédés Environnement-Agroalimentaire, GEPEA UMR CNRS, Nantes, France

Abstract: The present work deals with the concentration and the separation of *Arthrospira platensis* from a diluted culture medium. Among the different ways to operate this liquid/solid separation, this paper is focused on the membrane alternative. The general framework of this experimental study is the MELISSA project from the European Space Agency (ESA) for the development of life support systems in Space. The performances of fourteen inorganic membranes (microfiltration and ultrafiltration) were evaluated. According to the results, the operating conditions and the influence of phycocyanin and exopolysaccharides on the fouling phenomenon were investigated on the best membrane. A critical aspect to monitor along the process is the quality of the product in terms of composition of the main cell macro-components, such as proteins and exopolysaccharides. The ultrafiltration membrane ATZ-50 kD exhibited the best permeation flux and cleanability. An increase of fluid velocity and transmembrane pressure is energy-consuming. A good compromise between this consideration and the gain in terms of permeation flux is close to $3\,m \cdot s^{-1}$ and $2 \cdot 10^5$ Pa with the selected membrane and with a cyanobacteria suspension concentration ranging from $50\,mg \cdot L^{-1}$ to $1\,g \cdot L^{-1}$.

Keywords: *Arthrospira platensis*, cyanobacterium, fouling, harvesting, microfiltration, ultrafiltration

Received 25 January 2005, Accepted 2 September 2005
Address correspondence to I. Petit, Laboratoire de Génie des Procédés Environnement-Agroalimentaire, GEPEA UMR CNRS-6144, Pôle mer et littoral, 2 rue de la houssinière, BP 92208, F-44322, Nantes, France. E-mail: isabelle.petit@univ-nantes.fr

INTRODUCTION

If natural populations of photosynthetic microorganisms have been exploited for centuries (*Nostoc* in Asia, *Spirulina* in Africa and North America for sustenance), the potential of microalgae biotechnology has only been recently investigated. Among commercially used species, the cyanobacterium *Arthrospira platensis* (formerly *Spirulina*) is one of the most widely employed and well-studied. Applications have been found in many fields: food (owing to high nutritional quality), cosmetics, environmental uses (pollutants removal), aquaculture and maybe, in the future, therapeutic interest. Because of its nutritional qualities, *A. platensis* has been selected by the European Space Agency (ESA) in another application: the development of auto-regenerative biological life support systems for future manned missions in space, such as Moon or Mars exploration. In fact, the realization of long term space missions requires a number of critical technologies to be developed in order to supply the most important needs. One of them is, obviously, life support for the crew. Basically, the term "life support" comprises four main functions (1): atmosphere regeneration for respiration, water recycling, waste treatment, and generation of food. For these kinds of interplanetary missions, involving several crew members and long distances, resupply of water, food, and oxygen from earth is not feasible, in both technical and economical terms. For this reason, any life support system be as regenerative as possible. Such a system will be based on photosynthetic organisms, such as higher plants and algae, providing edible material. This model system, called MELISSA (Micro Ecological Life Support System alternative), consists of a loop of interconnected photobioreactors and a higher plant chamber (Fig. 1) (2). Compartment I ensures the degradation of the waste material, Compartment II allows the fatty acids treatment before Compartment III, where the transformation of the nitrogen source from ammonium ions to nitrate (more easily assimilable by higher plants and *A. platensis* in the last compartment) is achieved. Finally, Compartment IV is devoted to the production of food and oxygen (3, 4).

The present work is dedicated to the study of Compartment IVa, in charge of the culture of *A. platensis*, and more precisely the harvesting step of this edible biomass. It is relevant to the LSSS (Liquid Solid Separation System), a part of the MELISSA project which aims to concentrate and separate microorganisms from a diluted culture medium. Usually, this liquid-solid separation is processed using sieving, centrifugation, and filtration (5, 6). The present paper is focused on the performance of different inorganic membranes (microfiltration and ultrafiltration) which represent a valuable alternative to the use of the previous conventional techniques. According to the results, the operating conditions and the fouling phenomenon were investigated on the best membrane. A critical aspect to monitor along the process from the photobioreactor to edible material is the quality of the product in terms of composition of the main cell macro-components, such as proteins or exopolysaccharides.

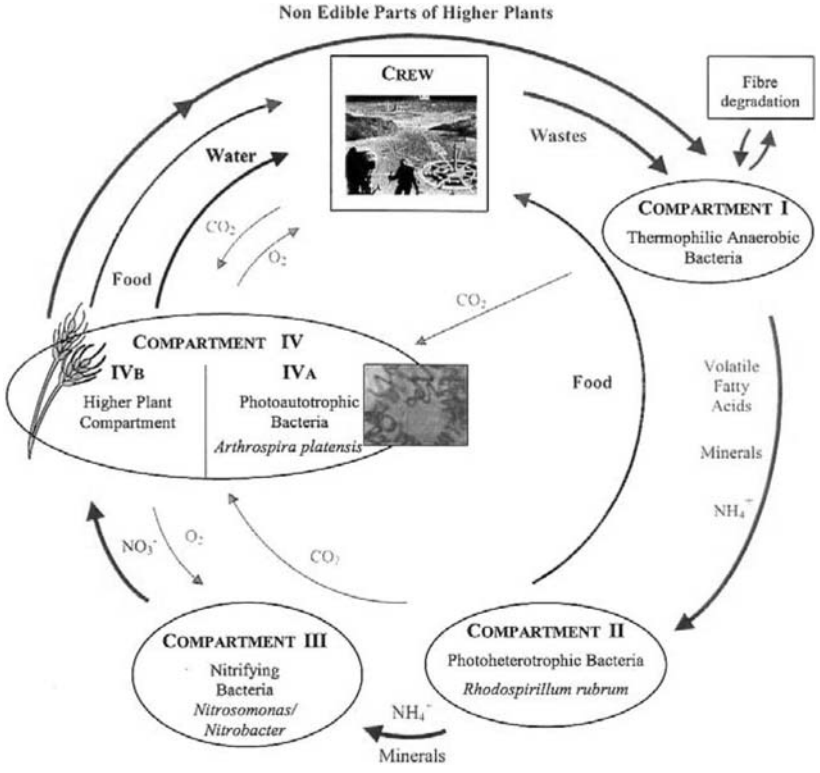


Figure 1. MELiSSA Advanced Loop Concept (2).

MATERIALS AND METHODS

Biological Material

The cultures of *A. platensis*, grown in a modified Zarrouk-medium have been, for a large part, provided by Alpha Biotech company (Assérac, Loire Atlantique, France). The production mode in Alpha Biotech is semi-continuous; the processed culture was taken in the middle of the exponential growth phase. The pH of the culture was equal to 9.5 ± 0.5. In order to compare the performance of the tested membranes, all comparative experiments have been carried out with the same cell concentration level in dry weight. The strain of *A. platensis* is PCC 8005 (Institut Pasteur, Paris, France). Another part of A. *platensis* was cultivated in our laboratory, GEPEA-UMR CNRS-6144 of the University of Nantes, with the same strain PCC 8005, $pH = 9.5 \pm 0.5$, 30°C. The photobioreactor employed in this case was the Luxfors model (from Infors, Massy, France), having a total volume of 3.6 litres (Fig. 2). Instrumentation includes pH, temperature, and dissolved

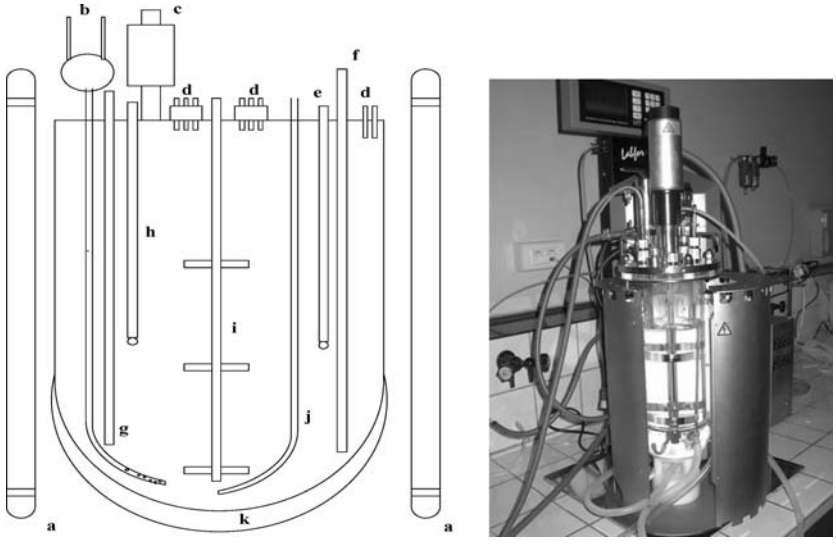


Figure 2. Photovioreactor INFORS used for A. platensis cultivation (GEPEA-CNRS). a–fluorescent tube, b–sparger, c–condenser, d–inlet, e–O_2 sensor, f–harvesting tube, g–temperature sensor, h–CO_2 sensor, i–rotor, j–harvesting tube, k–external cooling jacket.

oxygen measurements. Illumination was provided through 16 external fluorescent tubes (Gro-lux F8W/T5, France) distributed all around the bioreactor. Illumination was measured with a LI-Cor 1400 quantum sensor (Lincoln, NE, USA).

Analytical Methods

Cell concentration was determined by suspended solids measurements carried out at 105°C until a constant weight was obtained (Whatman glass microfiber filters GF/F, Maidstone, UK) and by measuring the absorbance of the medium at 750 nm (Spectrophotometer Lambda 10, Perkin-Elmer, Wellesley, USA) which allows, using a calibration curve, the determination of the concentration of the suspended materials.

Phycocyanin concentration in the culture medium was estimated by the method of Siegelman and Kycia (7). The amount of phycocyanin was calculated from optical densities (OD) of permeate measurements at 615 nm and 652 nm: [phycocyanin] $(g \cdot L^{-1}) = (OD_{615} - 0.474\ OD_{652})/5.34$.

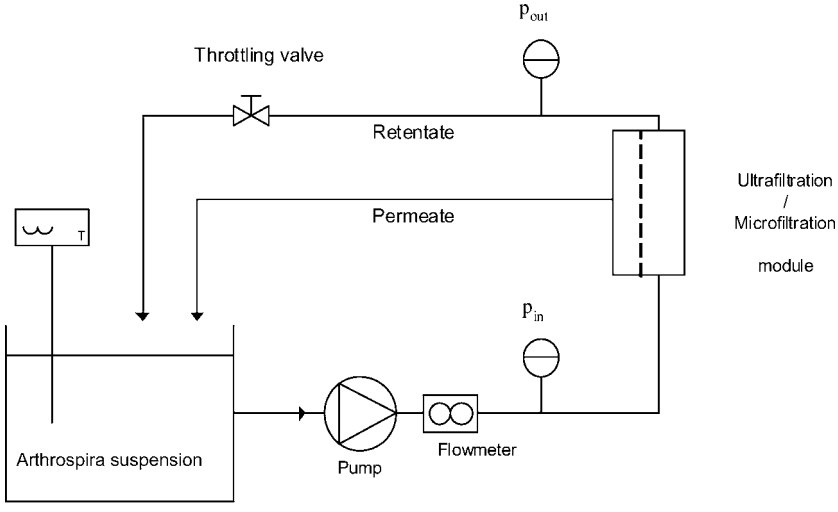
The determination of exopolysaccharide content in the culture medium is carried out using a phenol-sulphuric acid method (8, 9).

Cells viability tests consisted of doing a coloration test, MTS, and a reviviscence test. The MTS [3-(4,5-dimethylthiazol-2-yl)-5-(3-

carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium] allows to detect living cells. The method is based on the reduction of the MTS *in vivo* by the cells having an active metabolism, and the reaction produces formazan released in the culture. This red compound has a maximum absorbance at 490 nm. Then it is possible to estimate the quantity of living cells by means of absorbance measurements and comparisons with a model sample (10). Samples of *Arthrospira platensis*, which are taken before, during and after filtration experiments, are used to re-inoculate a medium culture (Zarrouk modified). Then the cells growth is observed and quantified during a 10-day period at $T = 30^{\circ}C$ (incubator).

Experimental Set-up and Operating Parameters

The experimental set-up (Fig. 3) is identical for all experiments. Operating pressure is adjusted with a throttling valve and controlled by a pump. This pump is a monoscrew one, Moineau type (PCM Pump, Vanves, France), the main feature of which is to generate low shear stress (11, 12). The instantaneous measurements of the permeation flux are carried out using a Mettler PM 4600 balance, linked to a personal computer fitted with a data acquisition software.



p_{in} pressure before MF/UF module

p_{out} pressure after MF/UF module

T temperature

Figure 3. Microfiltration/ultrafiltration experimental set-up.

Membranes were of the inorganic tubular three-channel type from Tami-industries (Nyons, France) with a 93.75 cm^2 effective permeation area. Two materials have been tested:

i. ATZ ($Zr\text{-}O_2\text{-}TiO_2$ filtering layer on a Al_2O_3 support, commercial designation: Céram inside) and
ii. Titanium (TiO_2 filtering layer on a Al_2O_3 support, commercial designation: Pure Titanium) with various cut-off/pore size (Table 1). Titanium membranes exhibit hydrophobic behavior, whereas ATZ membranes are less hydrophobic.

After each experiment, the membrane is rinsed using demineralized water at 20°C, then with warm water at 70°C before the set-up of a chemical cleaning procedure. This chemical sequence includes two steps:

i. first Alkaline solution of soda (NaOH) 70°C, 20 $g \cdot L^{-1}$, 30 min;
ii. second acid solution of nitric acid (HNO_3) 50°C, 4.5 $g \cdot L^{-1}$, 15 min, with a demineralized water rinsing at 20°C between each step (13). The sequence is repeated until the regeneration of the membrane, i.e. the recovery of the initial water flux (±5%). The pure water flux (J_o) of a membrane after cleaning denotes its cleanness.

Characterization of the Membrane State

During the filtration process, permeation flux declines owing to the accumulation of solutes and particles at the membrane surface and/or to pore clogging. Darcy's law reports the solvent passage through the membrane (J_0 expressed in $m^3 \cdot s^{-1} \cdot m^{-2}$) as a function of the applied pressure. This equation involves the hydraulic resistance as follows:

$$J_o = \frac{P_{TM}}{\eta \cdot R_m} \tag{1}$$

where η denotes the solvent dynamic viscosity (Pa·s) and R_m (m^{-1}) the intrinsic membrane resistance, P_{TM} (Pa) is the transmembrane pressure.

Table 1. Cut-off and pore diameters of ATZ and titanium inorganic tubular membranes

	Ultrafiltration	Microfiltration
ATZ	15 kD, 50 kD, 150 kD	0.14 μm
TITANIUM	1 kD, 10 kD, 50 kD, 100 kD, 300 kD	0.14 μm, 0.2 μm, 0.45 μm, 0.8 μm, 1.4 μm

R_m is determined by the resistance-to-flow obtained with ultrapure water and should be a constant value for a given membrane operating at a given temperature. During the filtration of a fluid containing both soluble molecules and suspended solids, additional resistances appear in association with different kinds of fouling phenomena (14, 15):

$$J = \frac{P_{TM}}{\eta \cdot (R_m + R_{irr} + R_{rev})} \quad (2)$$

R_{irr} (m^{-1}) denotes the adsorption resistance due to strong chemical interactions between the membrane, the solutes and the fluid, the removing of this part requires a chemical cleaning.

R_{rev} (m^{-1}) represents the total fouling resistance due to the combination of two phenomena:

i. concentration polarization and
ii. cake formation which induce substantial resistance to permeation; this part is eliminated by means of rinsing steps. Hydrodynamical conditions can also influence R_{rev} (16).

RESULTS AND DISCUSSION

Material and Cut-off/Pore Size Selection

Different commercial membranes from Tami-industries were experimented in isoconcentration mode (with both permeate and retentate recycling) in order to establish a discrimination. Performances can be estimated according to the permeation flux and cells retention. According to our specifications, the membrane must stop all the solid fractions, and this condition is satisfied in all cases: laser granulometric measurements and microscopic observation did not reveal any cell in the permeate. Consequently, the performance of the best membranes can only be assessed from their permeation fluxes.

Whatever the membrane considered in the present study, the flux declines rapidly during the first 30 minutes. After this time, the flux declines slowly to reach an apparent steady state value. Performances of the different membranes are estimated according to the evaluation of this steady state permeation flux at 120 minutes, J_{2h} ($m^3 \cdot s^{-1} \cdot m^{-2}$ or $L \cdot h^{-1} \cdot m^{-2}$).

A strong difference can be observed between ATZ and TITANIUM membranes (Fig. 4). Permeation flux with ATZ is systematically greater, with a difference of 23% between the ATZ 50 kD and the TITANIUM 50 kD and equal to 33% between the ATZ 0.14 μm and the TITANIUM

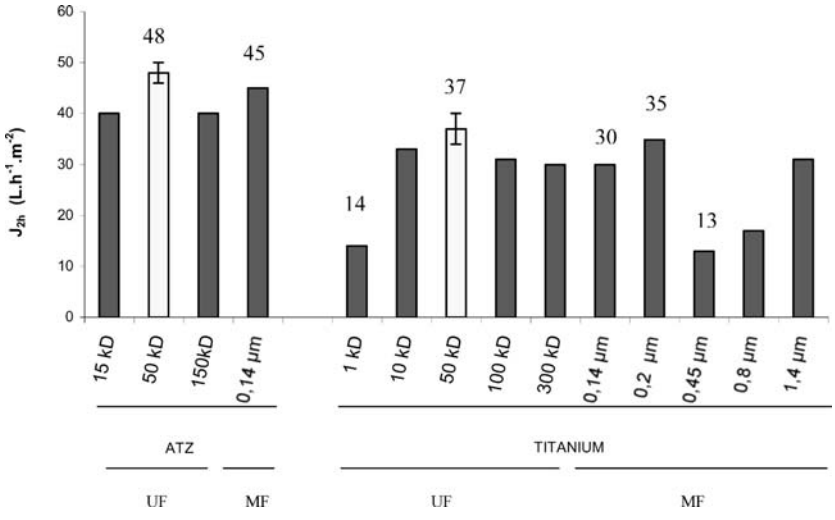


Figure 4. Steady state permeation flux, J_{2h}, with inorganic membranes (isoconcentration: $C_0 = 450\,mg \cdot L^{-1}$; $P_{TM} = 10^5$ Pa; $T = 20°C$; $Re = 1000$). UF = ultrafiltration membranes and MF = microfiltration membranes.

0.14 μm. The most suitable cut-off is around 40–50 kD, whatever the material tested.

Concerning the comparison between ultrafiltration and microfiltration, the best ultrafiltration membranes seem more efficient in terms of permeation flux, even if the difference is not really pronounced, than the best microfiltration membranes after 2 hours of *A. platensis* treatment. Figure 5 illustrates the comparison between ultrafiltration and microfiltration in terms of long time permeation flux.

However, whatever the material constituting the membranes, significant differences of ultrapure water permeation fluxes are observed between ultra and microfiltration membranes. For example, J_0 is respectively $330\,L \cdot h^{-1} \cdot m^{-2}$ for the ATZ 50 kD and of $2240\,L \cdot h^{-1} \cdot m^{-2}$ for the ATZ 0.14 μm. Microfiltration membranes with large pore diameters appear more sensitive to fouling, which is probably increased by the presence of cells fragments and debris.

Consequently, this fouling layer seems to act as a second membrane in series with the real one, and thus quickly masks the intrinsic membrane's characteristics. Consequently, the cleaning of ultrafiltration membranes is easier than for microfiltration ones and therefore requires less chemical products (Table 2).

According to the high fouling potential of microfiltration membranes and the advantage of ATZ comparing to Titanium in terms of permeation fluxes, the most suitable choice is ATZ-50 kD.

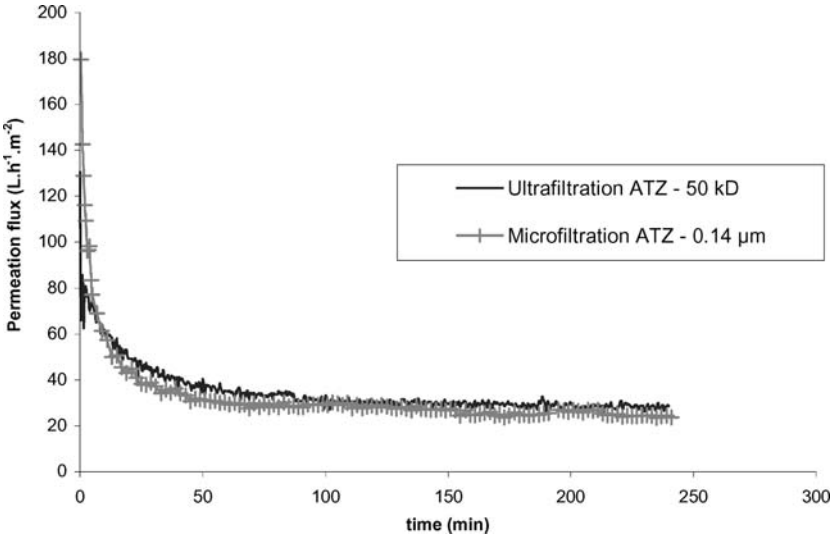


Figure 5. Variations in fluxes with time for ultrafiltration (ATZ–50 kD) and microfiltration membranes (ATZ–0.14 μm). (Isoconcentration: $C_o = 1\,g \cdot L^{-1}$; $P_{TM} = 10^5$ Pa; $T = 20°C$; $v = 1\,m \cdot s^{-1}$; $Re = 1000$).

Operating Parameters

A notable aim of this study is dedicated to the setup of a reliable separation system in order to harvest the edible biomass of *Arthrospira platensis*. After a first step consisting of hardware selection, operating parameters have to be precised and fouling phenomena have to be well-known. The tangential fluid velocity (linked to Reynolds number and shear stress) and the transmembrane pressure are the main parameters which allow the control of the separation efficiency. Biomass concentration in the suspension is also

Table 2. Different regeneration steps after ultrafiltration or microfiltration runs in the same hydrodynamical conditions

	Microfiltration ATZ–0.14 μm	Ultrafiltration ATZ–50 kD
J_0 ($L \cdot h^{-1} \cdot m^{-2}$)	2240	330
$J_{4\,h}$ ($L \cdot h^{-1} \cdot m^{-2}$)	24	29
Number of chemical cycles	12	7
NaOH consumption (g)	480	280
NO_3H consumption (mL)	120	70

(Isoconcentration: $C_o = 1\,g \cdot L^{-1}$; $P_{TM} = 10^5$ Pa; $T = 20°C$; $v = 1\,m \cdot s^{-1}$; $Re = 1000$).

another parameter which has an influence on the ultrafiltration performance. Actually, membrane separation can suit different process configurations: a direct concentration of biomass from the photobioreactor (in this case, the cyanobacteria suspension is concentrated around $1000\,mg \cdot L^{-1}$) or membranes can be used after a first separation step (centrifugation or ultrasonic process (17)) as a finishing technique. In the latter case, the treated cyanobacteria suspension is more diluted (around $50\,mg \cdot L^{-1}$).

In all cases, an increase of fluid velocity leads to an increase of the steady state permeation flux whatever the pressure (Fig. 6). A high fluid velocity reduces membrane fouling thanks to the increase of deposit removal. On the other hand, a higher fluid velocity increases shear stress sustained by cyanobacteria cells. For each fluid velocity, there is an optimal pressure, after which a further increase in transmembrane pressure will not improve performance and even induces a flux decrease (see Fig. 6c at 1 and $2\,m \cdot s^{-1}$). Such a phenomenon has already been observed with other biological suspensions (microalgae, bacteria, apple juice, etc.), which characterize a polysaccharides presence in the medium. Extra-secretion of exopolysaccharides (EPS) can explain such flux drop with pressure. Indeed, these EPS released in the culture medium can induce the formation of a gel layer (by aggregation) which is further compressed and generates an additive resistance to permeation. The EPS excretion by several species is linked to the growing conditions and also to shear stress (18).

The influence of hydrodynamical parameters is shown in Fig. 7 in terms of membrane resistances. The reversible resistance, R_{rev}, and irreversible one, R_{irr}, increase with transmembrane pressure, and decrease with fluid velocity. R_{rev} always represents the major contribution to the overall fouling resistance. The intrinsic resistance, R_m, is constant whatever the experiments. For a given pressure, when the fluid velocity increases, R_{irr} decreases but represents a more important part of the total resistance because R_{rev} becomes less important. We notice that simple water rinsing allows the membrane to recover more than 50% of its initial performance ($R_{rev}$is eliminated by means of water rinsing steps), which appears satisfactory, considering that no chemical product is needed.

Increasing fluid velocity and transmembrane pressure is energy-consuming, a good compromise solution between this consideration and the gain in terms of permeation flux is close to $3\,m \cdot s^{-1}$ concerning the fluid velocity and $2 \cdot 10^5$ Pa for transmembrane pressure with the ATZ membrane 50 kD and with a cyanobacteria suspension concentration ranging from $50\,mg \cdot L^{-1}$ to $1000\,mg \cdot L^{-1}$.

During a 2-hour ultrafiltration run, an average loss of 13% of *Arthrospira platensis* biomass is observed no matter what the hydrodynamical parameters. The deposit of *A. platensis* cells, which are not in circulation at the end of the run, is due to dead zone retention and deposit in low shear stress areas (piping, pumps, and valves). The dead volume of our set-up has been minimized, a very large part (around 90%) of this lost biomass is easily removed and

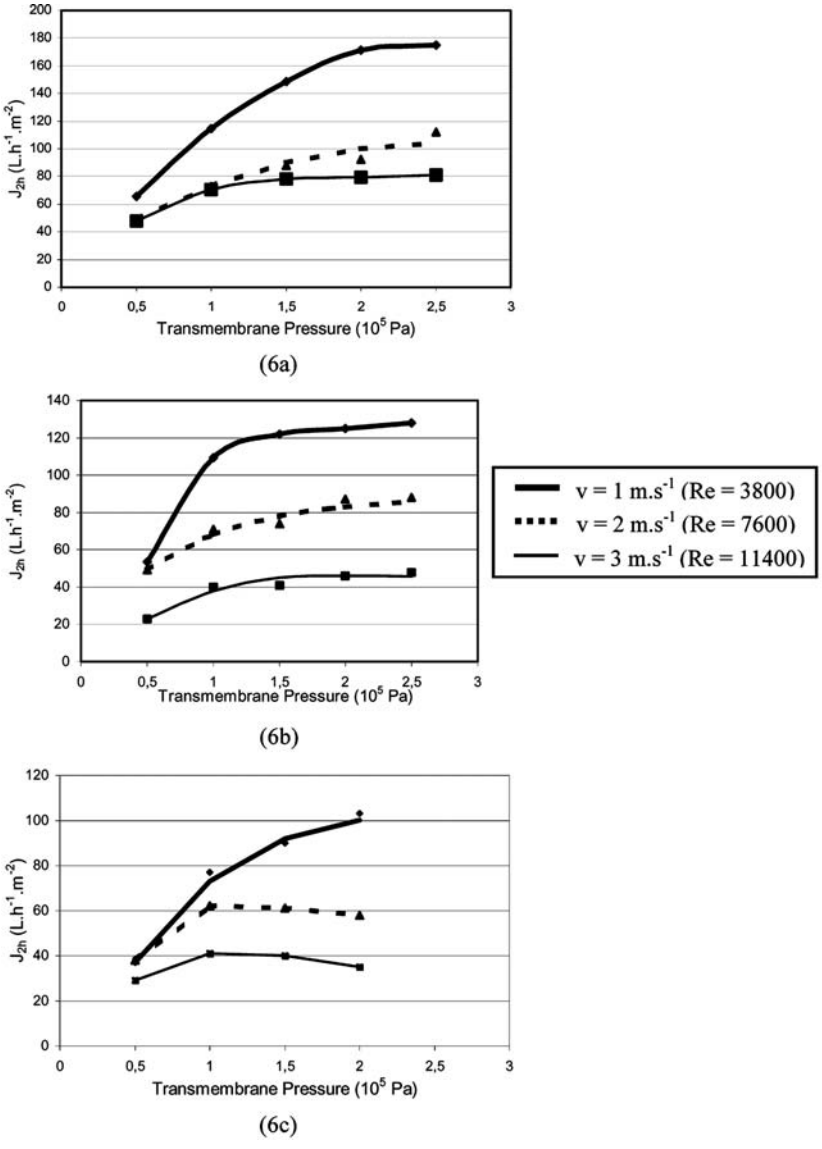


Figure 6. Influence of fluid velocity and transmembrane pressure on the steady state permeation flux for three biomass concentrations, isoconcentration: $C_0 = 50\,mg \cdot L^{-1}$ (6a), $450\,mg \cdot L^{-1}$ (6b) and $1000\,mg \cdot L^{-1}$ (6c), (ATZ–50 kD; T = 20°C).

goes back in circulation after a very short rinsing step (twice the dead volume of the installation).

This temporary retention is not an important drawback, since some reviviscence tests have shown that, after the ultrafiltration run, cells are still alive and can grow again.

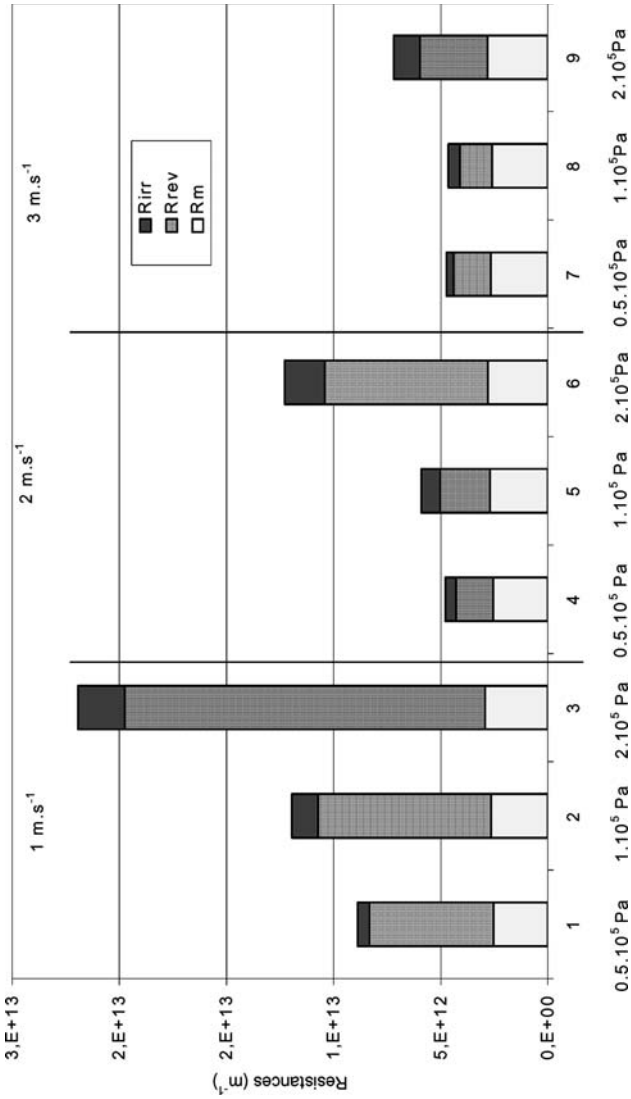


Figure 7. Influence of fluid velocity and transmembrane pressure on the resistances after a 2 h ultrafiltration experiment (ATZ–50 kD, T = 20°C, isoconcentration: $C_0 = 1000\,mg \cdot L^{-1}$)

Throughout ultrafiltration, one can observe the formation of fragments of *Arthrospira platensis* filaments without major cellular degradation. The fragmentation of the filaments is not very traumatizing because very few cells are locally necrosed. According to the coloration tests with MTS (10), whatever the hydrodynamical experimental conditions, the quantity of cells keeping their metabolic activity after a several-hour ultrafiltration run remains around 90–95% compared with that observed before membrane processing.

Membrane Fouling

Membrane fouling is due to the presence, in the suspension to be filtered, of foulant substances released by *Arthrospira platensis*: exopolysaccharides (EPS) and some proteins, particularly the phycocyanin (19). Exopolysaccharides are naturally excreted by the cyanobacteria (20). This excretion can be intensified considering the shear stress sustained by the microalgae in the pilot unit.

Intensive shear stress can also induce a rupture of the cell membrane, thereby releasing its content, especially phycocyanin (21).

Influence of the Culture Conditions on Membrane Fouling

Arthrospira platensis has been cultivated in the Infors photobioreactor (30°C, Zarrouk-medium modified by Alpha Biotech, pH = 9.5) with different culture conditions as follows:

Test 1: light level = $80\,W \cdot m^{-2}$ during 24 hours, maximum irradiance of the PBR during 24h00,

Test 2: light level = $80\,W \cdot m^{-2}$ during 12 hours and darkness during 12 hours, maximum irradiance during 12 hours and darkness during 12 h,

Test 3: light level = $40\,W \cdot m^{-2}$ during 12 hours and darkness during 12 hours, half of the maximum irradiance during 12 hours and darkness during 12 h.

Part of the culture was extracted from the photobioreactor in the middle of the exponential growth phase. Light variations can induce EPS quantity variations because EPS production is closely linked to salinity change and strongly depends on light intensity (22, 23).

Table 3 shows a decrease of the steady permeation flux, $J_{2\,h}$, with an increase of EPS quantity in the medium culture, for the same hydrodynamical ultrafiltration conditions. Between each test, the membrane is cleaned and the initial water flux, $J_0 = 312\,L \cdot h^{-1} \cdot m^{-2}$ ($\pm 5\%$), is recovered. We observe a sharp and systematic effect between a first filtration and a subsequent filtration of the same suspension cultivated in the same conditions (light, mixing, pH,

Table 3. Steady permeation flux and EPS and phycocyanin contents

Conditions	16/09 Test 1 $80\,W \cdot m^{-2}$ during 24 h	04/09 Test 2-a	11/09 Test 2-b	01/09 Test 3-a	08/09 Test 3-b
		$80\,W \cdot m^{-2}$ during 12 hours and darkness 12 h		$40\,W \cdot m^{-2}$ during 12 hours and darkness 12 h	
$J_{2\,h}$ ($L \cdot h^{-1} \cdot m^{-2}$)	38.3	44.7	38.3	42,0	32,0
EPS (mg/g *A. platensis*) before ultrafiltration	450	43	484	40	179
EPS deposit (%)	12.6	–	15.7	–	35.2
Phycocyanin (mg/g *A. platensis*) before ultrafiltration	10	14	63	38	43
Phycocyanin deposit (%)	91	35	46	78	29

(Biomass concentration = $1000\,mg \cdot L^{-1}$; $P_{TM} = 1.5\ 10^5$ Pa; $T = 20°C$; Re = 3800; ATZ membrane 50 kD).

and temperature) but not during the same time. The semi-batch culture mode results in an EPS accumulation which explains the difference between 2a and 2b (id. 3a/3b). One notices a decrease of the J = f(t) profile between the first and the second filtration, of 30% for tests 3 and 17% for tests 2. Operating conditions are yet exactly the same and the initial water fluxes of membranes are identical; the only factors having changed are the quantities of exopolysaccharides and phycocyanin released by the microalgae, which have increased inside the photobioreactor. The results allow to suspect a strong implication of EPS and/or of phycocyanin on membrane fouling.

Chronologically, the quantity of EPS in the culture medium increases, the quantity of phycocyanin also increases at the beginning and then decreases after a given value of secreted EPS is reached. It is well known that a moderate cell density (around $2\,g \cdot L^{-1}$), with high irradiance, results in relatively low cell contents of chlorophyll-a and phycocyanin, whereas high irradiance enhances EPS production (19) (Table 3).

Does Phycocyanin Contribute to Membrane Fouling ?

When *Arthrospira platensis* cells are disrupted, after cycles of freezing, thawing and sonification or crushing with mortar, maximum values of phycocyanin released are around $150\,mg \cdot g^{-1}$. Whatever the adopted hydrodynamic experimental conditions, phycocyanin rates measured during all our ultrafiltration experiments have always been rather low (between 15 and $30\,mg \cdot g^{-1}$). We have never observed a blue coloration, characteristic of the presence of phycocyanin, during our ultrafiltration experiments. In

conclusion, cyanobacteria release a very small amount of phycocyanin. That fits with the previous conclusion: "The fragmentation of the filaments is not very traumatizing, with limited cellular degradation." Therefore, the contribution of phycocyanin in the membrane fouling mechanism is probably minor compared to that of EPS.

Influence of Exopolysaccharides on Membrane Fouling

To measure the impact of exopolysaccharides on membrane fouling we have tried to separate the culture medium from the cells. So, we have settled *Arthrospira platensis*coming from the photobioreactor. Each time, high biomass concentration suspensions have been chosen (more than $2\,g \cdot L^{-1}$) in order to ensure a quick and almost complete settling. The floating liquid obtained through settling was limpid and contained very little biomass.

A floating liquid (containing only $115\,mg \cdot L^{-1}$ of biomass) has been filtered under the same operating conditions as a cyanobacteria suspension with $1000\,mg \cdot L^{-1}$ of biomass. The two suspensions contained $515\,mg \cdot L^{-1}$ of exopolysaccharides and no phycocyanin, and we found very similar $J = f(t)$ curves (Fig. 8).

Figure 9 shows the ultrafiltration of two floating liquids (containing only $45\,mg \cdot L^{-1}$ of biomass) with different EPS concentrations ($65\,mg \cdot L^{-1}$ and $130\,mg \cdot L^{-1}$). These two algal suspensions were cultivated in our photobioreactor in same conditions (light, mixing, pH, and temperature) but not during the same time. These algal suspensions have been settled in order to get the floating liquids. Ultrafiltration runs were realised under the same operating

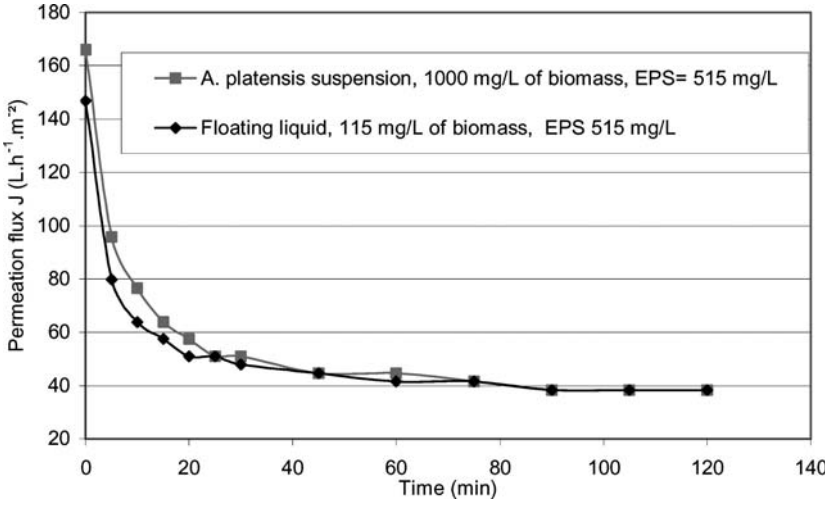


Figure 8. Variation of the permeation flux during ultrafiltration (isoconcentration, $P_{TM} = 1.5\ 10^5$ Pa; $T = 20°C$; $Re = 3800$; ATZ membrane 50 kD).

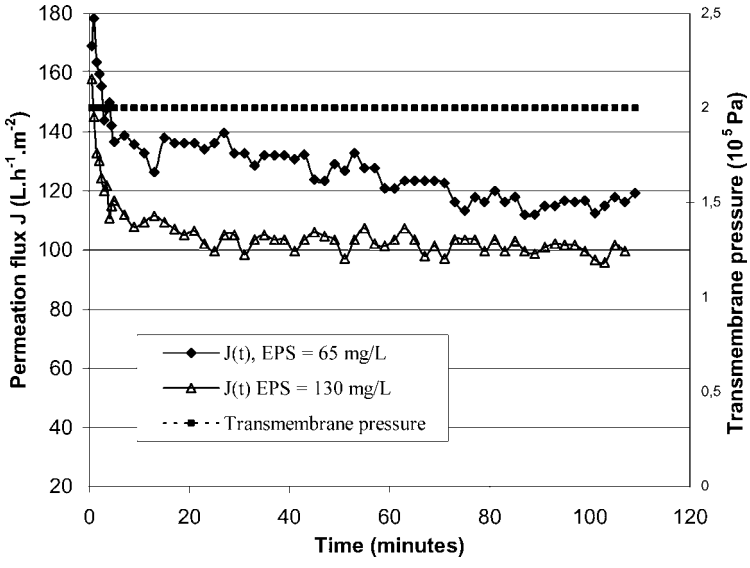


Figure 9. Variation of the permeation flux and transmembrane pressure during ultrafiltration of EPS suspensions (isoconcentration, $P_{TM} = 2 \cdot 10^5$ Pa; $T = 20°C$; Re = 11400; ATZ membrane 50 kD).

conditions and with the same initial water flux. With a transmembrane pressure of $2 \cdot 10^5$ Pa, the permeation flux declines more quickly with a high EPS concentration ($130\,mg \cdot L^{-1}$) and J_{2h} is 15% lower in this case than the one obtained with the $65\,mg \cdot L^{-1}$ EPS concentration. This observation in terms of permeation flux may be explained by the formation of a gel layer of exopolysaccharides which is compressed at the membrane surface and thus generates an additive resistance.

These results clearly show that the quantity of EPS is crucial for the build-up of the fouling layer, much more than the number and the size of the cells and fragments in the suspension.

CONCLUSION

In order to concentrate and to separate *Arthrospira platensis* from a diluted culture medium, the performance of inorganic membranes was evaluated. The ultrafiltration membrane ATZ-50 kD exhibited the best permeation flux and cleanability. It could be underlined that a similar study made on organic membranes has pointed out an ultrafiltration membrane with cut-off of 40 kD among a large panel of ultrafiltration and microfiltration membranes (24).

The operating conditions were investigated. With the selected membrane and a cyanobacteria suspension concentration ranging from $50\,mg \cdot L^{-1}$ to

$1000\,mg \cdot L^{-1}$, a fluid velocity of $3\,m \cdot s^{-1}$ and a transmembrane pressure of 2.10^5 Pa is a good compromise between energy consumption and membrane performance.

Whatever the hydrodynamical ultrafiltration conditions, one can observe the formation of fragments of *Arthrospira platensis* filaments without major cellular degradation. The foulant substances released by the cyanobacteria in the filtered suspension generate the membrane fouling phenomena. The contribution of phycocyanin is minor compared to that of exopolysaccharides. EPS form a gel layer which is compressed at the membrane surface and generates an additive resistance increasing with transmembrane pressure. These results have clearly shown that the quantity of EPS is crucial for the build-up of the fouling layer, much more than the number and size of the cells and fragments in the suspension.

REFERENCES

1. Tamponet, C. and Savage, C. (1994) Closed ecological systems. *J. Biol. Educ.*, 28: 167–173.
2. ESA, MELISSA Project. (2004) http://www.estec.esa.nl/ecls/
3. Godia, F., Albiol, J., Montesinos, J.L., Pérez, J., Pérez, N., Creus, N., Cabello, F., Mengual, X., Montras, A., and Lasseur, Ch. (2002) MELiSSA: A loop of interconnected bioreactors to develop life support in space. *J. Biotech.*, 99: 319–330.
4. Morist, A., Montesinos, J.L., Cusido, J.A., and Godia, F. (2001) Recovery and treatment of *Spirulina platensis* cells cultured in a continuous photobioreactor to be used as food. *Process Biochemistry*, 37 (5): 535–547.
5. Fox, R.D. (1986) *Algoculture: La Spirulina, un Espoir pour le Monde de la Faim*; Aix en Provence. ed: Edisud, 222.
6. Richmond, A. (2004) *Hanbook of Microalgal Culture Biotechnology and Applied Phycology*; Blackwell Publishing. ed, 566.
7. Siegelman, H.W. and Kycia, J.H. (1978) *Algal Bioproteins in Handbook of Phycological Methods*; Hellebust, J.A., ed.; Cambridge University Press: Cambridge. C.J.S, 72–78.
8. Dubois, M., Gilles, K.A., Hamilton, J.K., Rebers, P.A., and Smith, F. (1956) Colometric method for determination of sugars and related substances. *Anal. Chem.*, 28: 350–356.
9. Kopecky, J. (1997) Kinetic model of extracellular polysaccharide production by the unicellular red alga. *Porphyridium purpureum, Algological Studies*, 87: 137–144.
10. Capasso, J.M., Cossio, B.R., Berl, T., Rivard, C.J., and Jimenez, C. (2003) A colorimetric assay for determination of cell viability in algal cultures. *Biomolecular Engineering*, 20: 133–138.
11. Jaouen, P., Vandanjon, L., and Quéméneur, F. (1999) The shear stress of microalgal cell suspension in tangential flow filtration systems: the role of pumps. *Biores. Technol.*, 68 ((2)): 149–154.
12. Vandanjon, L., Rossignol, N., Jaouen, P., and Quéméneur, F. (1999) Effect of shear on two microalgal species (*Skeletonema costatum* and *Haslea ostraria*)

Contribution of pumps and valves in tangential flow filtration systems. *Biotechnol. Bioeng.*, 63 (1): 1–9.
13. Cabero, M.L., Riera, F.A., and Álvarez, R. (1999) Rinsing of ultrafiltration ceramic membranes fouled with whey proteins: effects on cleaning procedures. *J. Memb. Sci.*, 154 (2): 239–250.
14. Fane, A.G., Fell, C.J.D., and Waters, A.G. (1983) Ultrafiltration of proteins solutions through partially permeable membranes—the effect of adsorption and solution environment. *J. Memb. Sci.*, 16: 211–224.
15. Tardieu, E., Grasmick, A., Geaugey, V., and Manem, J. (1999) Influence of hydrodynamics on fouling velocity in a recirculated MBR for wastewater treatment. *J. Memb. Sci.*, 156: 131–140.
16. Morineau-Thomas, O., Legentilhomme, P., Jaouen, P., Lepine, B., and Rince, Y. (2001) Influence of a swirl motion on the interaction between microalgal cells and environmental medium during ultrafiltration of a culture of *Tetraselmis suecica*. *Biotech. Letters*, 23: 1539–1545.
17. Dekker, M. (2002) Ultrasound-based techniques for characterizing concentrated dispersions. *Published in Encyclopedia of Surface and Colloid Science, INC New York Basel,* 5481–5537.
18. Cornet, J.F. (1992) *Etude cinétique et énergétique d'un photobioréacteur. Etablissement d'un modèle structuré. Applications à un écosystème clos artificiel*; Ph.D thesis, Université Paris XI: Paris.
19. Maléríat, J.P., Jaouen, P., Rossignol, N., Schlumpf, J.P., and Quemeneur, F. (2000) Influence of alginates adsorption on properties of ultrafiltration and microfiltration organic membranes. *Rev. Sci. Eau*, 13 (3): 269–287.
20. Borowitzka, M.A. (1999) Commercial production of microalgae: ponds, tanks, tubs and fermenters. *J. Biotech.*, 70: 313–321.
21. Morineau-Thomas, O. (2001) *Mise en œuvre d'un module d'ultrafiltration à écoulement tourbillonaire non-entretenu: application à la séparation d'une suspension modèle de bentonite et de plusieurs cultures de microorganismes marins*; Ph.D thesis, Université de Nantes: France.
22. Cornet, J.F., Dussap, C.J., and Gros, J.B. (1998) Kinetics and energetics of photosynthetic micro-organisms in photobioreactors. In *Advances in Biochemical Engineering/Biotechnology*; Scheper Editor: Heidelberg, 153–224.
23. Cogne, G. (2003) *Conception, mise en oeuvre et modélisation d'un photobioréacteur à membrane pour l'étude du comportement de la cyanobactérie Arthrospira platensis en microgravité*; Ph.D thesis, Université Blaise Pascal: France.
24. Rossi, N., Jaouen, P., Legentilhomme, P., and Petit, I. (2004) Harvesting of cyanobacterium *Arthrospira platensis* using organic filtration membranes. *Food Bioprod. Process.*, 82 (C3): 1–9.