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Implementation of an automated process for *Limnospira indica* harvesting and culture medium recycling for space applications

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Future long-term space exploration missions require the implementation of circular life support systems for the supply of water, oxygen and food from mission wastes. Therefore, separation systems dealing with multi-phasic streams need to be addressed. The BioHarvest (BHV) study focused on solid/liquid separation in space with the aim to demonstrate the continuous separation and harvesting of the cyanobacterium *Limnospira indica* from its culture broth under axenic conditions. The cyanobacterium biomass is intended to be used for further food processing while the broth free of organic matter and resupplied with nutrients should be directly recycled into the photobioreactor (PBR). In this study, an automated breadboard model based on a two-step process was built. First, a Biomass Harvesting Unit (BHU) separates the biomass produced in the PBR from the culture medium with dead-end filtration. Second, the Medium Filtration Unit (MFU) further treats the culture medium to retain the dissolved organic compounds using crossflow filtration. The performances of BHU and MHU met the requirements in batch mode and in short continuous mode: the BHU was able to retain all the biomass and the MFU could retain more than 90% of organic matter while being permeable to nutrients. The productivity of the MFU was also very good, with a high permeation flux allowing treating the targeted 80 L of culture per day. However, continuous operation of the BHV technology could not be achieved in the long term due to biomass accumulation as a sticky cake with a high specific resistance on the BHU filter, despite backwashing cycles and intense vibrations. Future work shall therefore focus on this critical step, to improve process performance by preventing fouling of the filter sheets.

KEYWORDS

regenerative life support, solid-liquid separation, filtration technology, harvesting of microalgae, biomass for food

1 Introduction

Future long-term space exploration missions require the implementation of circular life support systems for the supply of water, oxygen and food from mission wastes. MELiSSA

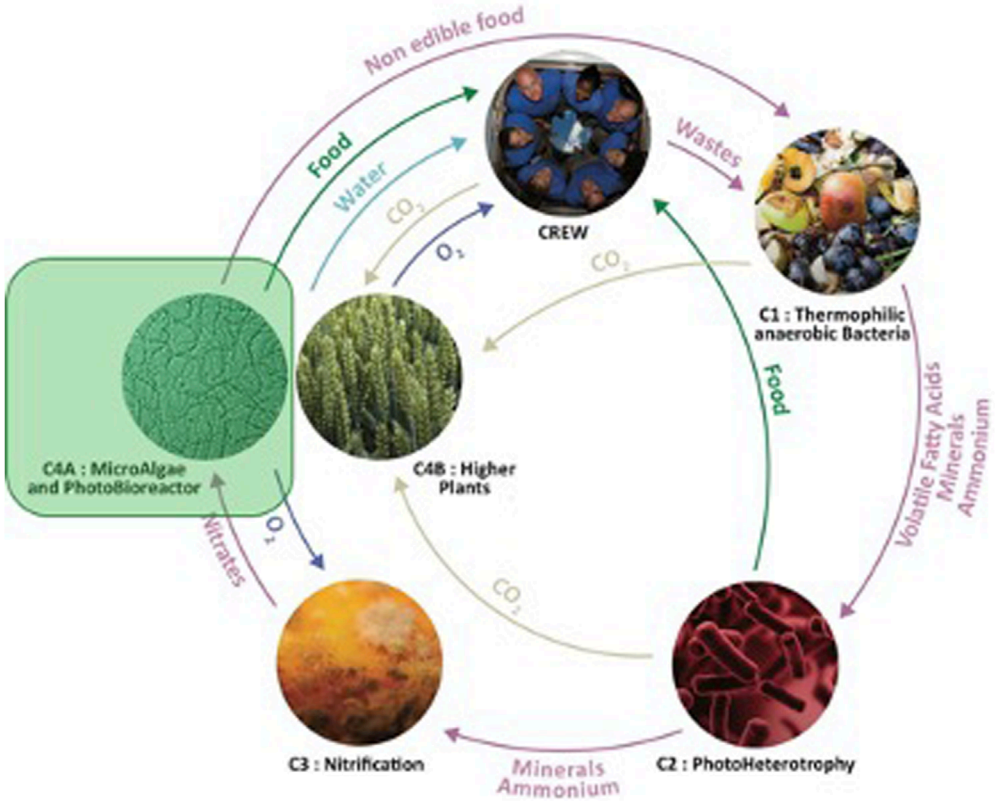

FIGURE 1
Diagram of the MELiSSA Loop with the compartment C4A highlighted in green.

(Micro-Ecological Life Support System Alternative) is the European project of circular life support systems led by the European Space Agency (ESA) since 1987. The MELiSSA loop as depicted in Figure 1 is composed of five interlinked compartments. The compartment C4A is responsible for air revitalization and edible biomass production. It relies on a photobioreactor growing the cyanobacterium *Limnospira indica*. Besides its high radiation resistance, this microorganism shows a high nutritional quality and therapeutic value which are beneficial in space but also on Earth. Microalgae and cyanobacteria have lately gained interest worldwide due to their extensive application potential on Earth in various fields such as renewable energy, pharmaceutical, construction, food and feed industries.

The BioHarvest (BHV) project aimed to study solid/liquid separation in space dedicated to the Solid Loop of the compartment C4A. The objective was to demonstrate the separation and harvesting of the cyanobacterium *Limnospira indica* from axenic cultures in photobioreactors under space compatible conditions. After separation using the BioHarvest technology, the biomass (i.e., the solid outlet stream) is intended to be used for further food processing while the culture broth (i.e., the liquid outlet stream) resupplied with nutrients shall be suitable for reinjection into the photobioreactor (PBR) where *the strain* is cultivated.

Starting with a literature review of the potential separation principles and associated technologies, a trade-off based on the requirements of the harvested product and the space constraints were performed. In the last 6–7 years, the following dewatering/harvesting techniques have been mainly studied to enhance the harvesting of microalgae.

Considering the processes based on density difference, centrifugation remains the reference (faster, more effective, and useable on most microalgae strains). However, less energy consuming methods were also investigated. Settling or flotation are exploited after the addition of classical inorganic salts, or more original coagulating agents such as cellulose nanocrystals for the recovery of *Nannochloropsis oculata* (Verfaillie et al., 2020) or cooking oil-surfactant emulsion for harvesting *Chlorella vulgaris* with high pH (Potocar et al., 2020). The addition of microspheres has been investigated for the enhancement of Ballasted Dissolved Air Flotation (DAF) on *Scenedesmus obliquus*, *Chlorella vulgaris* and *Arthrospira maxima* with up to 95% coagulant reduction and 99% cells separation (Ometto et al., 2014). Depraetere et al. (2015) could also achieve separation by spontaneous settling of *Arthrospira platensis* by enrichment in carbohydrates in nitrogen starved culture conditions. Spirulina settling through flocculation has been implemented in the form of bioflocculation using fungi biomass (with efficiencies ranging from 90% to 100% after pH adjustment) (Nazari et al., 2021), autoflocculation (pH-induced flocculation) in salt-rich culture medium (Formosa-Dague et al., 2018), and using a combination of salts and extra-polymeric substances such

as polysaccharides in the medium (Rashid et al., 2019). For these techniques, even if efficiency and low energy are widely recognized, the presence of additives contaminating the biomass and hindering culture medium recycling are a brake on the development for certain applications (Singh and Patidar, 2018).

Membrane processes based on steric separation by crossflow filtration (Rossignol et al., 1999) or by shear-enhanced (dynamic) filtration (Frappart et al., 2011) have been studied for many years and show very good performances, sometimes in combination with a preliminary coagulation (Zhao et al., 2020a). Here, ensuring sufficient water recovery and a final high biomass concentration (up to 180 g/L for combined sedimentation and dynamic filtration (Hapońska et al., 2018)) are the key points. The main limitations lie in the possible membrane fouling reducing the process performances (productivity and selectivity) and the energy consumption (due to pumping in this pressure-driven process). The most innovative studies of the recent years rely on membrane design: a membrane with a wave pattern structure allowed to accumulate biomass in the hollows of the structure and maintain membrane zones devoid of fouling layers to promote permeation (Zhao et al., 2020b), and another membrane was highly negatively charged to prevent fouling by microalgae cells (Huang et al., 2020). Human urine has been employed as a forward osmosis solution exploited first as a nutrient source for cultivation of *Chlorella vulgaris* and then for biomass dewatering and concentration, but the concentration factor remains low (Volpin et al., 2019). For the dewatering/harvesting of *Arthrospira* species at industrial scale, sieving is the reference as the cells are large (Belay, 1997) and it was shown that centrifugation was less efficient (GEPEA and Algosolis expertise). In a MELiSSA context, microfiltration and ultrafiltration with organic and mineral membranes were studied by Rossi and others (Rossi et al., 2004; Rossi et al., 2005; Rossi et al., 2008). Among the selection of membranes, the ones related to ultrafiltration (40–50 kDa) showed the best performances in terms of permeation fluxes (~40–50 L/h/m^2 for organic membranes; ~30–40 L/h/m^2 for inorganic membranes). The authors also highlighted the major role of exopolysaccharides in the fouling phenomenon.

Most of microalgae harvesting studies were conducted for terrestrial applications, where specific aspects of the space environment such as the operability under microgravity were not approached. Furthermore, these studies lack information regarding the quality and composition of the solid and liquid fractions recovered from the separation process. The BioHarvest study aimed to partly fill this gap by studying solid/liquid separation in space compatible conditions. Focusing on the C4A compartment of the MELiSSA loop, the objective of BioHarvest was more specifically to demonstrate the separation and harvesting of the cyanobacterium *Limnospira indica* from axenic cultures in photobioreactors under space conditions. To this end, a BioHarvest automated breadboard model (BBM) attached to a photobioreactor was developed to separate the cyanobacterium biomass from its culture broth. The BBM was designed to operate under microgravity and axenic conditions, in order to deliver edible biomass of 4%–8% suspended matter and recycle more than 90% of the culture medium back to the photobioreactor. The maximal limit of biomass concentration in the liquid outflow was 0.01 g/L. The target was to achieve a continuous life test period of at least 40 days.

The final selection of the technologies composing the BBM was conducted according to the trade-off methodology ALiSSE (Advanced Life Support System Evaluator). ALiSSE is a decision tool dedicated to life support system comparison and evaluation. This methodology is based on multiple criteria: mass, energy and power, efficiency, risk to humans, reliability, crew time, sustainability, and life cycle cost.

Based on the literature review, the requirements and the ALiSSE criteria, two steps were selected and introduced in the BHV as distinct units. Firstly, the Biomass Harvesting Unit (BHU) consists of the dead-end filtration of the culture on a vibrating stainless steel medium, inspired by the usual sieving, able to recover the biomass in a continuous way and release a maximum of water. Secondly, the Medium Filtration Unit (MFU) based on ultrafiltration removes organic matter (polysaccharides) from broth before culture medium recycling and ensures sterility of medium in case of punctual contamination. The design of the harvesting process is detailed in the following paragraphs and the performances are demonstrated.

2 Materials and methods

2.1 Breadboard configuration

The BioHarvest demonstration unit was made of three subsystems (see Figure 2). 1- the photobioreactor (PBR) to cultivate *Limnospira indica* in a dedicated culture medium. It possessed its own control system and electronics. 2- the biomass harvesting unit (BHU) to collect *Limnospira indica* and evacuate it into a drain, while the clarified culture medium is sent to filtrate tank 3- the medium filtration unit (MFU) dedicated to the filtrate purification by ultrafiltration, i.e., the concentration of extracellular organic matter and potential cell fragments in the retentate and the recovery of the culture medium in the permeate to be recycled into the PBR. The nutrient content in the permeate was verified for culture medium adjustment by addition of nutrients if needed. The steps 2 and 3 were automated using a control system and electronics (CSE) unit giving access to the measured and calculated values (e.g., temperature, pressure, liquid level, weight, operation time, mass balance, flow rates). A loop allowed an automated cleaning in place while the PBR was sterilized using chemicals and steam.

2.1.1 Photobioreactor

The cultivation of *Limnospira indica* was ensured in HECTOR (see Figures 3A, B), an airlift photobioreactor with a useful capacity of 170 L and a culture thickness of 6 cm. It was equipped with a LED lighting system, pH, temperature and dissolved oxygen probes connected to its own control system. The control software (C-BIO2) recorded each minute the monitored parameters (pH, temperature, dissolved oxygen, light intensity) and controlled the fixed parameters (pH, temperature, light intensity). In this way, automatic regulation of the culture conditions was ensured according to the setpoints fixed by the operator (i.e., for temperature, pH, and light intensity). Temperature regulation was performed by circulation of industrial water in the internal heat exchanger. Regulation of pH was performed by injections of CO_2 from a gas bottle into the gas inlet line. The target was to produce 80 ± 10 L/day

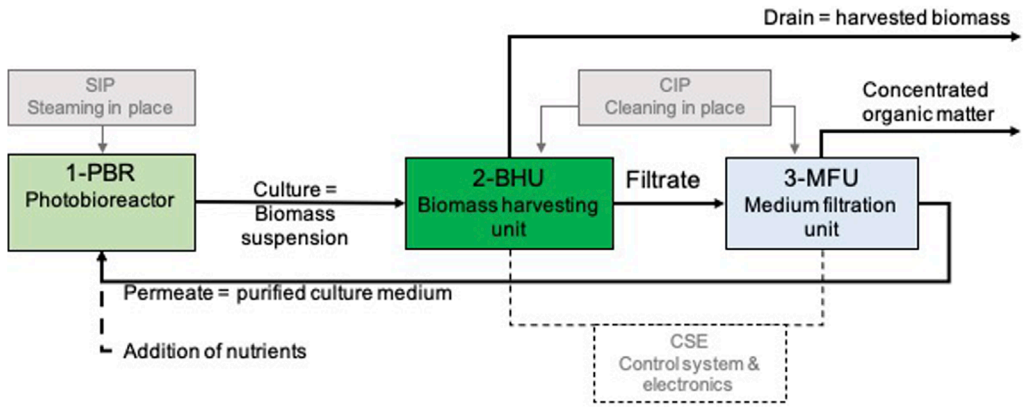

FIGURE 2
Overview of the BioHarvest sub-systems.

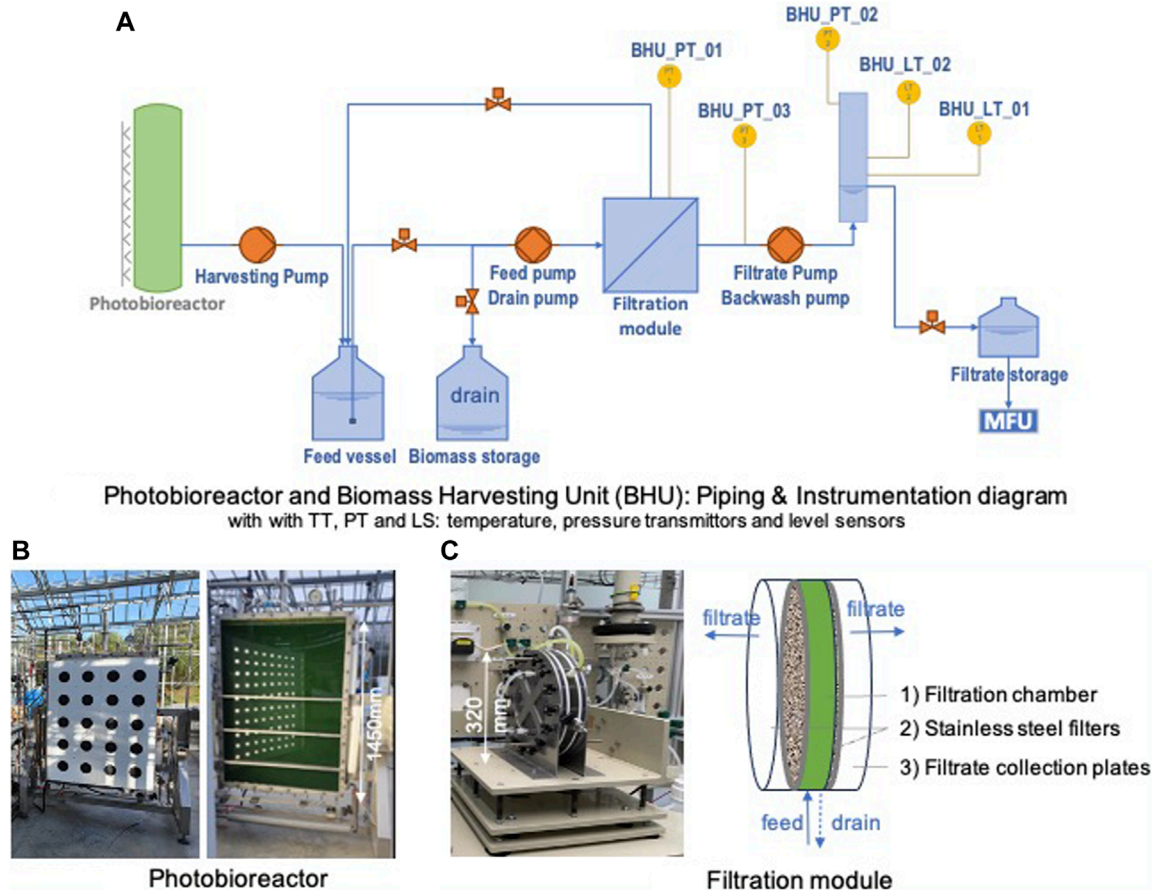

FIGURE 3
HECTOR photobioreactor and Biomass Harvest Unit system (BHU) with detailed filtration module.

of culture with 2 g/L of suspended matter (SM) corresponding to biomass.

2.1.2 BHU: biomass harvest unit

The dead-end filtration module [see Figures 3A, C)] was made of 1) a central filtration chamber (0.79 L) to collect the harvested biomass before draining, with 2) on both sides two stainless steel non woven filters (effective filtration area of 0.025 m^2 each) and 3) at the external sides two polycarbonate filtrate collection plates from which the filtrate was evacuated. Two nonwoven filters were tested, made of a stainless-steel mesh with pore distributions around 5 and 10 µm (Bekaert).

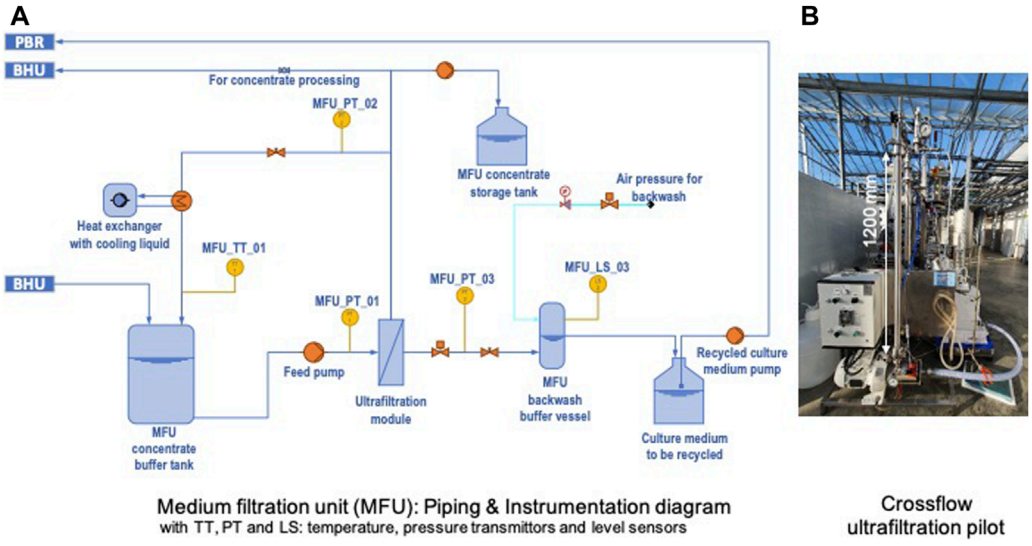

FIGURE 4
Medium filtration unit system (MFU).

The biomass suspension was pumped from the PBR into the central filtration chamber. The filtrate was directed to a buffer volume, from which it could be injected to the MFU or used for backwash. Based on a timer or on observed pressure acting on the CSE, the BHU was drained of the harvested biomass before being submitted to a backwash using the filtrate coming from the buffer volume. The target was to recover at least 90% of the culture medium and to harvest the biomass with a SM (suspended matter) of 4%–8%.

2.1.3 MFU: medium filtration unit

The crossflow pilot XLAB 5 (PALL) was equipped with an INSIDE CéRAM™ membrane from Tami industries (ref. MTB200511U015) containing an active layer in zirconium dioxide with a 15 kDa cut-off to limit the fouling by polysaccharides (see Figure 4). This ultrafiltration ceramic membrane measured 1,178 mm long and 20 mm in diameter, with 5 channels of 6 mm hydraulic diameter, for a total membrane area of 0.13 m^2. The temperature was regulated using a heat exchanger. The pure water flux with the clean membrane was 120 L/h/m^2 at 0.8 bar, 30°C. The filtration unit was designed to allow a crossflow velocity higher than 2 m/s. At the outlet of this unit, concentrated organic "waste" was collected, while a buffer vessel was foreseen collecting the permeate. The target volume reduction rate (VRR) for the crossflow filtration step was 15, with a continuous extraction of the permeate. Like the BHU, drainage and backwash were applied based on a timer or on observed pressure.

The pure continuous mode experiments were limited by the productivity of the BHU. Therefore, a fed-batch mode was deployed: 1- filtration of 20 L of filtrate for VRR between 1 and 2.4; 2- a new volume of filtrate (15 L) was added in the feed tank to reach experimentally a VRR = 10. The limited volume of filtrate produced by BHU, the size of the feed tank (20 L) and the dead volume of MFU also limited the VRR.

2.2 Online measurements and control

The control logic was divided into control loops (master control, BHU control, MFU control, cleaning). The master control loop was the general start and stop of the whole BBM. The automated operation of BHU control relied on the succession of sequences of filtration, draining (i.e., collecting the harvested algae), and backwash. The transition from filtration to draining and backwash was time-based or triggered by the pressure buildup in the filtration module. The same principle was applied to the MFU. In addition, the cleaning loop allowed to initiate a chemical cleaning to restore the BHU/MFU performance in case the backwash was not sufficient to counteract pressure buildup. Daily nominal operation (i.e., filtration) was foreseen during about 16 h, the remaining hours being dedicated to draining, backwashing and cleaning.

2.3 Offline measurements

To monitor the culture in the photobioreactor, the suspended matter concentration corresponding to the biomass (SM), the pigments concentration and the inorganic composition of the medium was measured during culture. To monitor the performance of the BHU, the suspended matter but also the dry matter concentration was measured in the filtrate and the drain. To monitor the performance of the MFU, the organic carbon concentration was measured in the permeate and the retentate. The biomass concentration (in grams of suspended matter per liter of culture) was obtained by filtering a known volume of culture on 0.7 µm cellulose filter, drying and weighing, while the dry matter concentration was obtained by direct drying and weighing of the samples. Chlorophyll a (Chl-a) and carotenoids were determined by an extraction with methanol then using a UV-Vis spectrophotometer according to Ritchie (2006) and Strickland and Parsons (1968). Dissolved inorganic and organic carbon concentrations were measured

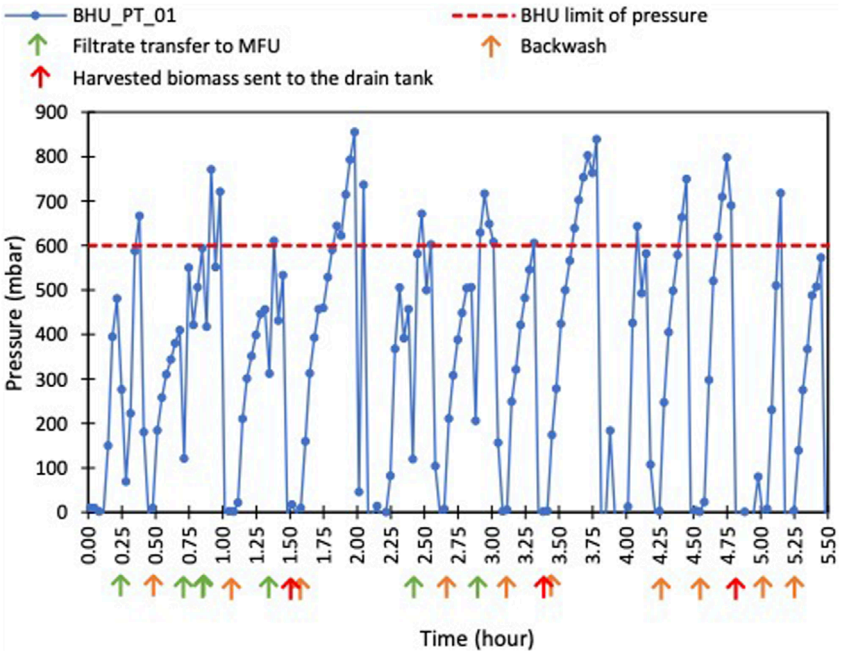

FIGURE 5
Continuous filtration of suspended matter (no vibration during filtration, only during backwash; drain cycle after 3 backwashes).

with a TOC-meter (Shimadzu TOC 5000) on filtered culture at 0.7 µm (Minisart, Sartorius tedium, Germany). Anions and cations concentrations were measured using an anionic chromatograph (Dionex) and cationic chromatograph (Dionex) respectively (DX-120 ion chromatograph).

3 Results and discussion

The performances of the biomass harvesting in BHU and the culture medium purification in MFU were first evaluated separately in batch mode. Then the continuous mode including several cycles of filtration, drainage and backwash was deepened.

3.1 Validation of the BHV performances in batch mode

3.1.1 Biomass harvest unit (BHU)

The aim of the BHU step is to separate the biomass from the culture medium. Two mesh filters (5 and 10 µm) were used to filtrate a microalgae suspension of 0.71 ± 0.04 g_{SM}/L. The pilot unit was used here at a controlled filtration flux to match with the flow target corresponding to the filtration of half the photobioreactor per day (3.33 L/h = 80 L/day). The filtration was very efficient because, with both meshes, no microalgae cells were found into the filtrate and the dry matter retention rate was 94 ± 1%. The suspended matter into the filtrate (50 mg/L) corresponded to the released organic matter, probably the polysaccharides described in the literature.

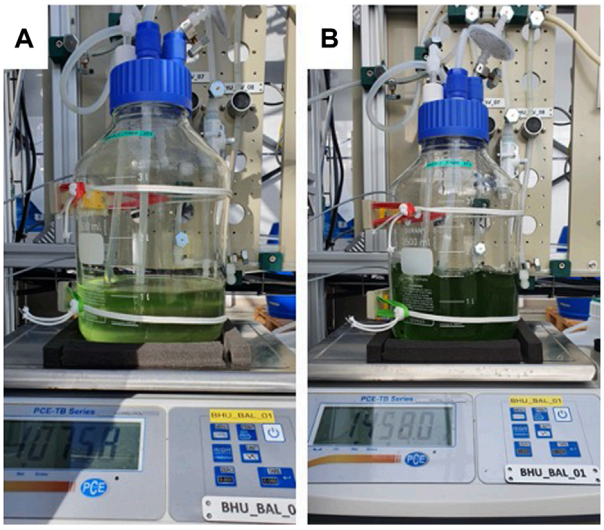

FIGURE 6
Impact of the vibrations during backwash/drain on the recovery of biomass in the BHU hydraulic circuit **(A)**: no vibration: clear solution; **(B)** presence of vibration: more suspended matter recovered.

3.1.2 Medium filtration unit (MFU)

The aim of the MFU unit is to purify the BHU filtrate from the organic compounds to avoid culture drifts when the medium is recycled. Based on literature (Hadj-Romdhane et al., 2013), a cut-off of 15 kDa, a transmembrane pressure (PTM) of 0.8 bar and a tangential speed of 2.3 m/s were used. The filtrate used to test MFU

TABLE 1 Repartition of the biomass (suspended matter) and the culture medium (liquid phase) after 6 h of filtration in BHU.

	V (L)	%v. liquid	Suspended matter concentration (g/L)	Mass of suspended matter (g)	%w. Suspended matter
Drain	5.8	32.8	2.8	16.5	71.2
Filtrate	10.5	59.5	0.06	0.6	2.7
Cake	0.04	0.2	75.0	3.1	13.4
BHU	1.3	7.4	2.3	2.9	12.6

came from a culture maintained in batch mode for a longer period and contained 182 mg/L of organic carbon. In terms of productivity, the initial permeate flux (at VRR = 1) was 62.8 L/h/m^2, 52% of the water flux. This flux was sufficient to ensure the treatment of more than 190 L/day of filtrate. In terms of selectivity, at VRR = 1, the membrane was able to decrease the organic carbon concentration from 182 mg C/L in the feed to 53 mg C/L in the permeate with a retention rate of 71%.

The batch tests demonstrated that the selected conditions allowed to fulfill the requirements: a full retention of the biomass by BHU and a strong reduction of organic matter by MFU. Consequently, the continuous mode was carried out to evaluate the performances of several cycle sequences.

3.2 Continuous mode: separation performance on biomass harvesting unit (BHU)

During this sequence the main objectives were to separate the biomass from the culture medium, to achieve a biomass concentration between 40 and 80 g_{SM}/L in the drain tank and to maintain the productivity and selectivity performances with backwash and draining to allow a continuous production. The culture concentration varied during the tests between 1.5 and 2 g_{SM}/L, so that the target concentration of biomass into the drain corresponded to a concentration factor ranging from 25 to 55.

3.2.1 Filtration cycle

An example of continuous filtration of biomass in BHU is depicted in Figure 5, where the pressure in the filtration chamber versus the filtration time is presented. The BHU worked as expected during the first 3.5 h. The pressure in the filter chamber increased during filtration because of the accumulation of the biomass on the filter. This is normal for dead-end filtration. At the same time, a quantity of filtrate was generated and transferred to the MFU (green arrow when 500 mL of filtrate was transferred). When the limit of pressure was reached (600 mbar in this test), a backwash cycle was automatically initiated to remove the cake from the mesh (orange arrow). To facilitate the unclogging effect of the backwash, the filter was equipped with a vibration system which was activated during backwash cycle allowing more suspended matter recovery (Figure 6). The activation of the drain cycle was fixed after 3 backwashes cycles (red arrow). The filter kept during several hours good performances concerning the separation of the suspended matter (cells) from the medium. After 6 h, only 3% of the suspended matter from all the culture treated passed through the filter.

The concentrated biomass was distributed between different compartments: 1) inside BHU hydraulic circuit, 2) as a filtration cake deposited on the filter and 3) inside the drain tank. This distribution changed during the filtration and depended on the number of drain cycles that occurred. After 6 h of filtration, as expected, most of the suspended matter (71%) was recovered in the drain tank (Table 1). However, a concentration between 40 and 80 g/L was expected in the drain (concentrated harvested biomass) but it reached only 2.8 g/L. This can be explained by the fact that the biomass was not maintained in suspension, despite vibrations. Indeed, a sticky cake of biomass was formed on the filter within a concentration of 75 g_{SM}/L. The adhesion of the cake to the filter limited the collection of the biomass in the drain tank. The accumulation of matter on the filter induced an increasing pressure in the filtration chamber. This rise initiated the backwash cycle and the drain cycle. After 3.5 h, the mesh was partially clogged and a new increase of pressure in the filter house appeared directly and started new backwashes and drain cycles (Figure 5, orange and red dots) that promoted the dilution of the drain tank. When the clogging appeared, it became difficult to generate filtrate for MFU because the produced filtrate was used for the repeated backwashing (no green dots anymore).

3.2.2 Improvement of BHU operation

In order to improve this harvesting phase, three enhancements were combined during the filtration cycle and compared to the initial conditions (case A): introduction of vibrations during the filtration cycle and not only during the backwash cycle, as well as the reduction of the maximum pressure in the filter chamber before a backwash cycle (case B); case B with the reduction of the mesh cut-off from 10 to 5 µm (case C). In Figure 7, the required time to produce 500 mL of filtrate is followed in the different conditions, versus the volume of filtrate sent to MFU. In case B, the filtrate recovery was twice higher than in initial conditions (case A) before a drastic increase in the time required to send the 500 mL of to MFU, because of the necessary backwash. Reducing the membrane cut-off from 10 to 5 µm (case C) allowed producing 17 L of filtrate before clogging. The optimization of the procedure allowed to multiply the volume of recovered filtrate by 5.7 times. However, clogging was still present, limiting the operation.

A second approach was to add an internal recirculation diaphragm pump to the BHU filter chamber. The aim was to create a low shear at the filter surface and to prevent biomass accumulation. This solution led to cellular deterioration, allowing a portion of the

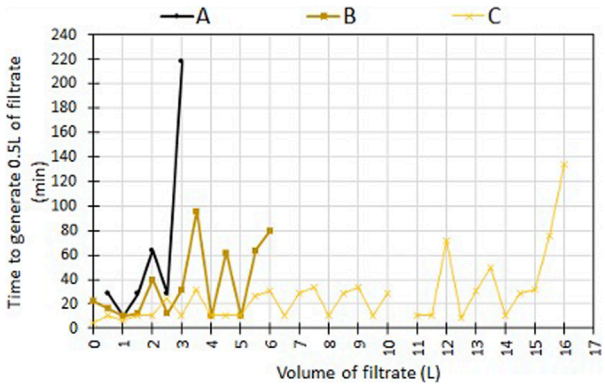

FIGURE 7
Time required to recover Vf = 500 mL of filtrate versus the total volume of filtrate sent to MFU. Comparison of solution for BHU improvement: **(A)** initial conditions (10 µm; no vibration; maximum pressure before backwash at 600 mbar): only 6 mL × 500 mL of filtrate recovered; **(B)** vibration during filtration phase + reduction of maximum pressure before backwash to 500 mbar: 12 mL × 500 mL of filtrate recovered; **(C)**: vibration during filtration phase + reduction of maximum pressure before backwash to 500 mbar + membrane cut-off at 5 µm: 34 mL × 500 mL of filtrate recovered.

biomass passing through the filter, which impacted the retention quality. Thus this solution was not retained.

3.2.3 Analysis of the characteristics of the biomass accumulated on the filter (cake resistance)

During dead-end filtration, there is an accumulation of the biomass on the filter, generating a cake. Using classical equations of the dead-end filtration, it is possible to calculate the cake specific resistance which is a useful parameter to estimate the filterability of the product.

This calculation was performed:

- using the results of the batch tests,
- using the results obtained at constant flow in specific periods on the BHU
- using the results of the complementary tests.

At constant flow, Eq. (1) can be used to calculate the cake specific resistance:

$$\Delta p = \frac{\eta . R_s}{S} . Q + \frac{\eta . r_c . C . Q^2}{S^2} . t \quad (1)$$

Where t is the time (s), V the filtrated volume (m^3), Q the flow ($m^3.s^{-1}$), η the dynamic viscosity of filtrate (Pa.s), S the filter area (m^2), Δp the pressure (Pa), C the dry matter concentration ($kg.m^{-3}$), R_s the clean filter resistance (m^{-1}) and r_c the cake resistance ($m.kg^{-1}$).

During the batch tests, the mean cake resistance estimated with the last 0.5 L filtrated using Eq. 1 was: 10^{14} m kg^{-1}. It is a bit high for dead-end filtration, that is why the addition of vibration was decided to facilitate the cake disruption and removal even during the filtration phase.

The results obtained with the C configuration (vibration during filtration phase + reduction of maximum pressure before backwash at 500 mbar + membrane cut-off at 5 µm) between 17 h and 29 h were analyzed. In Figure 8, the evolution of the pressure versus time for the different filtration cycles (1–11) are drawn. Three main phases can be observed. The first phase during the first 10 min (0.20 h) and below 30 mbar, filtrations were similar, the pressure was low, a slow fouling occurred. The second phase occurred between 0.2 and 0.3–0.4 h, until 450 mbar with a strong rise of the pressure meaning a strong clogging of the filter. During the third phase, above 0.3–0.4 h and 450 mbar, the cake was built with biomass accumulation above the filter leading to a bundle of lines for the different cycles.

The resistance of the clean filter was low (2.9×10^{10} m^{-1}). In the first phase, the accumulation of material led to an apparent specific resistance of initial cake equal to 5×10^{13} m kg^{-1}. In the second phase, the total resistance of the filter reached 7.5×10^{12} m^{-1} at 450 mbar by deposit of biomass and/or organic material in the filter. In the third phase, the biomass accumulated on the filter formed a more compact cake with a specific resistance reaching 4.1×10^{14} m kg^{-1}. That means that the cake is denser than in the batch tests. No strong modification of the slope is observed when the vibrations start at 200 mbar.

According to literature (SUEZ, 2023), a r_c between 10^{14} and 10^{15} m kg^{-1} is too high for a classical press filter and a specific resistance lower than 10^{12} m kg^{-1} should be reached. Some adjuvants like diatomite can be used to reduce and obtain a very low specific resistance near 10^{10} m kg^{-1} to facilitate juice clarification, for example, but this kind of additive does not fit to the initial space requirements.

These results confirm that the reduction of the pressure to 500 mbar for the realization of the backwash seems beneficial because from 450 mbar, one can observe the compaction of the cake which will undeniably reduce the productivity of this harvesting phase. In addition, finding a strategy to limit or even mechanically eliminate the cake is most likely the next step. Indeed, preliminary tests at lab scale with mechanical agitation inside the filtration chamber reduced the specific resistance of the cake to the interval 1.7×10^{13}–2.7×10^{13} $m.kg^{-1}$.

First results demonstrated that BHU was efficient for the separation but needed adjustments to fully meet the expected performances, notably the increase of harvested biomass concentration. The accumulation of suspended matter on the filter surface and its limitation and/or removing is a key point. The interaction between specific *Limnospira* properties and morphology and filter characteristics needs to be further investigated. The adhesion of the biomass to the filter could be mitigated by the integration of spacers, or by the modification of the design of the filtration cell or filter material. The cake could also be removed by the addition of a mechanical action. Some of these solutions are currently studied at laboratory scale and seem promising but will require further investigations.

3.3 Culture medium recycling by MFU

3.3.1 Selectivity of MFU

The main objective of the MFU step is to treat the filtrate from BHU to recover a purified culture medium in the permeate that

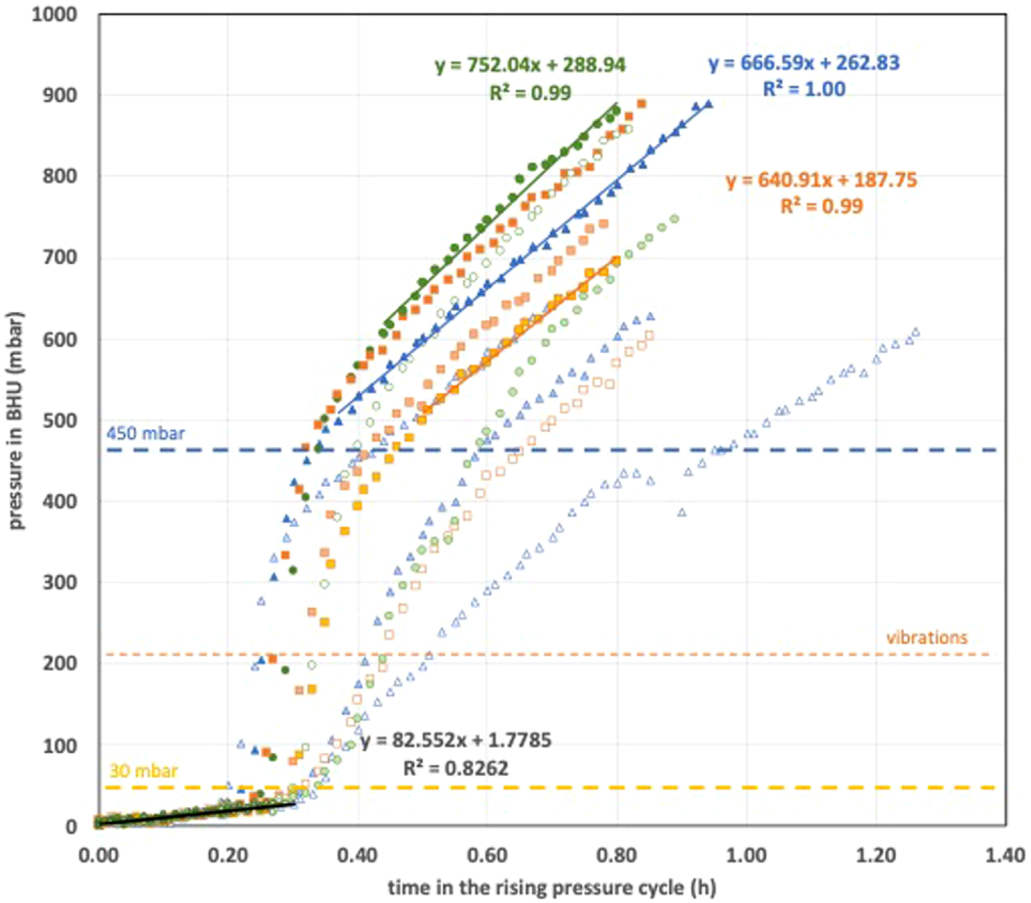

FIGURE 8
Filtration of algal suspension in pilot scale BHU at constant flow (3.3 L/h); triangles, squares and circles corresponding respectively to cycles 1–4, 5–8, and 9–11.

can be recycled to the culture, and concentrate organic components using a 15 kDa ceramic membrane (Hadj-Romdhane et al., 2013). Depraetere et al. (2015) have already shown the negative effect of the accumulation of organic materials on further dewatering if the culture medium is not treated. Figure 9 shows the mass balance of the organic carbon and the concentration of the organic carbon in the permeate and the retentate respectively, during the ultrafiltration of 20 L + 15 L of filtrate to a volume reduction ratio (VRR) of 10. The total quantity of carbon that has been introduced in the MFU pilot is equal to the sum of carbon in the permeate and retentate, within the analysis variability. A small difference can be noted at VRR = 3.5 because the corresponding addition of filtrate in the MFU feed led to experimental variations on concentration and volume measurements. The mass balance shows that the organic matter accumulation on the membrane is negligible. The results show that a small quantity of organic carbon went through the membrane. The concentration in the permeate stayed relatively constant, confirming the results obtained during batch tests. The majority of the organic carbon was retained in the retentate (Figure 9) with a rejection rate between 93% and 96%. At the same time, the rejection rate of inorganic carbon remained low, less than 4%. At a VRR of 10, 17%–20% of the initial organic carbon was in the permeate.

The concentration of organic carbon in the permeate was maintained between 45 and 50 mg C/L. With the increase of VRR, the concentration reached nearly 1,600 mg C/L at VRR 10, and compared with the initial concentration after BHU system which is between 160 and 182 mg C/L, it confirms an almost total retention of organic carbon with the 15 kDa membrane, as expected (Hadj-Romdhane et al., 2013).

3.3.2 Productivity of MFU

In Figure 10, the productivity of MFU is analyzed through the permeate flux (A) and the volume distribution (B). The initial permeation flux during the ultrafiltration of the BHU filtrate through the 15 kDa mineral membrane was 60 ± 1 L/h.m^2 (Figure 10A). During the concentration, the flux of permeate decreased slightly. At VRR = 4 and 10, the permeate flux was respectively 59 ± 3 L/h.m^2 and 48 L/h.m^2. The variation of the flux due to the culture conditions (initial concentration of organic matter, age of the microalgae culture) was limited. 5 h were required to treat the 35 L of initial filtrate.

At the end of operation (Figure 10B), at VRR 10, 90% of the filtrate was recovered into the permeate and 83% of initial organic carbon (even the eventual residue of biomass issued from BHU) was

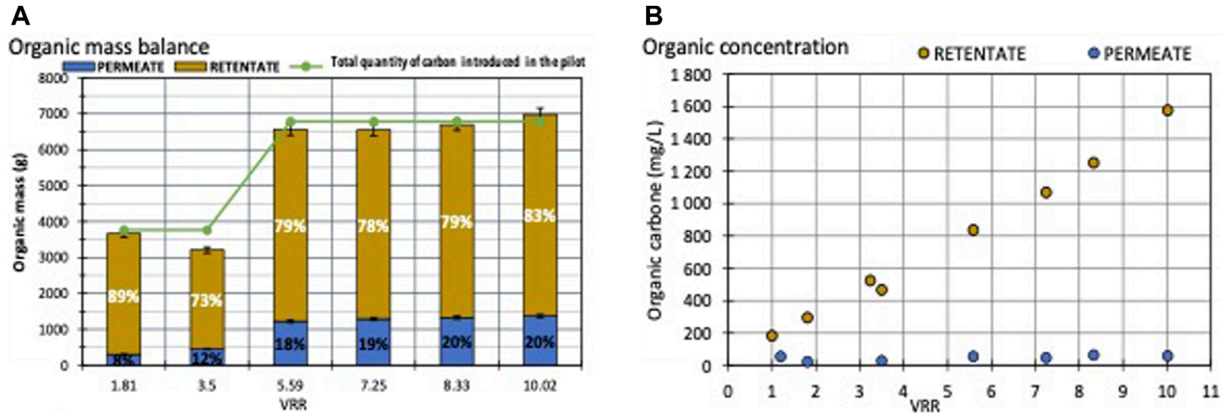

FIGURE 9
MFU selectivity: organic carbon mass balance **(A)** and evolution of the organic carbon concentration in the retentate and permeate **(B)** during the ultrafiltration of filtrate in MFU (15 kDa–T = 30°C–PTM = 0.8 bar–v = 2.3 m/s).

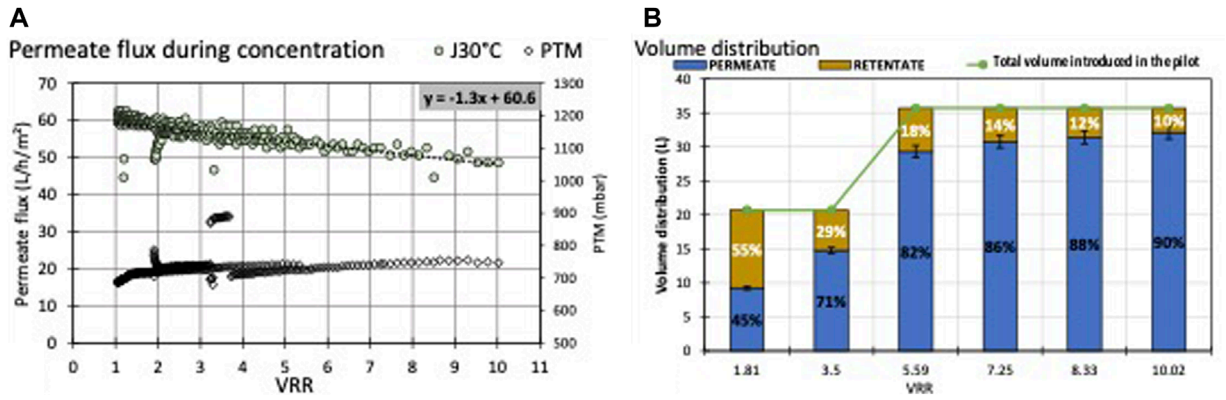

FIGURE 10
MFU productivity: permeate flux **(A)** and Volume distribution **(B)** during MFU (15 kDa–T = 30°C–PTM = 0.8 bar–v = 2.3 m/s).

concentrated in 10% of the initial volume. The high performances of the MFU were in accordance with the expectations relying of the former studies (Rossi et al., 2008; Hadj-Romdhane et al., 2013).

A scale up for the treatment of 80 L/day of filtrate from BHU (half of the photobioreactor to respect the dilution rate of the culture) can be done. If the flux remains stable, 12 h will theoretically be necessary to reach a VRR = 15 corresponding to the production of 75 L of recycled culture medium. This range of time shows that the chosen membrane surface of 0.13 m^2 is appropriate to achieve this objective during the day, including the cleaning operations.

Finally, the selected BHU dead-end filtration process with adaptations such as vibrations and mechanical cake removal will be well adapted to the harvesting of *Limnospira indica*, and the coupling with MFU ultrafiltration will allow a high quality water recovery, which is very interesting for space applications. However, BHU would not be adapted to strains like *Nannochloropsis oculate, Chlamydomonas reinhardtii or Chlorella vulgaris* also studied for space applications, because those microalgae would not be retained by the filter due to their smaller size. Another process satisfying ALiSSE criteria should be developed, for example, micro or ultrafiltration, with related questions among which the limited suspended matter concentration that could be reached, the potential fouling or a potential higher retention of nutrients.

4 Conclusion

The BioHarvest (BHV) project aimed to study solid/liquid separation in space dedicated to the Solid Loop of the compartment C4A of the MELiSSA program. The objective was to perform the harvesting of the cyanobacterium *Limnospira indica* from axenic cultures in photobioreactors under space conditions. The BioHarvest demonstration unit was made of three subsystems: a PBR to produce the biomass, the BHU based on dead-end filtration to harvest the biomass and clarify the culture medium and MFU based on ultrafiltration to purify the culture medium before recycling. The capacity of BHU to separate the biomass from the culture medium was demonstrated. Only 3% of the suspended matter went through the filter. However, the biomass accumulation on the mesh filter directly impacted the volume distribution and

the suspended matter concentration in the harvesting tank. Several enhancements were obtained based on the selection of the mesh cut-off, the introduction of vibrations and pressure regulation. However, complementary methods to mitigate the cake accumulation are still needed.

With regards to the performance of MFU in fed-batch mode, the demonstration test showed that the selectivity of the filter met the expectations: the system allowed to decrease the concentration of organic components by a factor 4. Likewise, the productivity of the filter membrane was within the expected range. By extrapolating the test results, the MFU as designed in the breadboard can indeed theoretically support a daily harvesting target of 80 L/day. However, as the MFU is dependent from BHU, its performance could not be fully evaluated.

From this study, it appears that the most critical step is the accumulation of the biomass as a cake on the filter of the BHU, limiting the whole process productivity. In future works, the physicochemical properties of the concentrated biomass and their evolution during the process (rheology, adhesion properties) shall be deepened to better understand the paste behavior and select the best BHU modifications. The selection of a new mesh to limit the adhesion and a mechanical cake removal may be the most promising enhancements among several possibilities.

Data availability statement

The datasets presented in this article are not readily available because the analyzed datasets for this study are available upon request to interested researchers, on condition of a prior agreement from ESA. Requests to access the datasets should be directed to EC, estelle.couallier@univ-nantes.fr.

Author contributions

JT was involved in the conception of the work, the building of the units, the acquisition, analysis and interpretation of the data and the drafting of the paper. MV participated to the conception of the work, the building of the units, the acquisition, analysis and interpretation of the data. CC participated in the drafting and reviewing of the paper. BL-L participated to the conception of the work, bringing her knowledge of the MELiSSA pilot plant, former works and space constraints. DD was involved in the conception of the work, the building of the units, the acquisition, analysis and interpretation of the data and the drafting of the paper. MF participated to the conception of the work, the building of the units and the data analysis and the drafting of the paper. EC was involved in the conception of the work, the building of the units, the acquisition, analysis and interpretation of the data, the drafting, reviewing and submission of the paper. All authors contributed to the article and approved the submitted version.

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Conflict of interest

Author MV was employed by GlaxoSmithKline Biologicals. Authors CC and DD were employed by Redwire Space NV.

The remaining authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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