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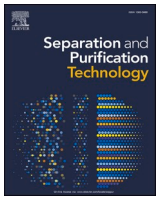

# Effect of backwash as a strategy for biofouling control in the submerged ceramic membrane bioreactor for high-density cultivations: Process optimization and fouling mechanism at pilot scale

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ABSTRACT

Submerged membrane bioreactors are effective tools for synergistic production of valuable compounds, cell retention in the bioreactor, and separation of products from the reaction solution. However, membrane fouling is a problem that impairs efficiency of processes involving submerged membrane bioreactors. One of the mechanical methods for efficient fouling mitigation is backwash. Therefore, this study investigated backwash as a method for mitigating biofouling in a submerged ceramic membrane bioreactor, specifically in terms of separation of *Saccharomyces cerevisiae* from the suspension. To identify the key factors affecting the dynamic flux and their interactions, a Fractional Factorial Design (FFD) was employed based on the key operating variables (2 levels, 5 variables). The average flux model showed a dependency on the flow rate of backwash, time between backwash cycles, backwash duration, cell concentration, and separation process time (adjusted $R^2 = 0.99$). Furthermore, the effect of backwash on flux recovery was assessed in comparison to separation processes without backwash. It was found that after the application of backwash, up to 100 % of the initial flux could be recovered. In contrast, only around 20 % of the initial flux could be maintained after the process without backwash. The optimized conditions of backwash were found to be a flow rate of backwash at 1500 mL $min^{-1}$, time between backwash cycles 5 min, backwash duration 5 sec, cell concentration 20 g dry weight per liter, and separation process time 0.5 h. The mechanism of membrane fouling was determined to be the deposition of yeast cells on the membrane surface and the blocking of pores inside the membrane by sorbitan monostearate as a reagent present in the yeast suspension.

## 1. Introduction

Nowadays, membrane bioreactors (MBRs) are primarily utilized in wastewater treatment due to their efficient combination of biological processes (anaerobic or aerobic processes) and the separation of sludge, which is the result of biological treatment [1–4]. For example, it was demonstrated that hollow-fiber membrane bioreactor with immobilized *Penicillium restrictum* can be used for efficient removal of sulfamethoxazole, erythromycin and tetracycline from pharmaceutical wastewater [5], whereas flat-sheet alumina membranes in anaerobic ceramic membrane bioreactors can successfully treat domestic wastewater [6] and coal chemical wastewater [7]. Therefore, MBRs can be applied to other processes where simultaneous biological reactions and membrane separation are desired. The area where MBRs have also being applied is production of value-added products [8], such as glucose [9], ethanol [10], surfactin [11], polyhydroxyalkanoate [12] or even monoclonal antibodies [13]. Most of the MBRs operate with the membranes positioned outside the bioreactor, which are advantageous because can be isolated without stopping the reactor and also provide extra degrees of freedom for operation. However, this configuration causes cells to be subjected to shear stress and makes it more challenging to control

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**Nomenclature**

| | |
|---|---|
| $A$ | Membrane area ($m^2$) |
| B | Time between backwash cycles (min) |
| C | Backwash duration (sec) |
| D | Cell concentration (dwg/L) |
| $dH_2O$ | Demineralized water |
| E | Separation process time (h) |
| $CE$ | Cleaning efficiency (%) |
| $DY$ | Dry weight cells concentration (dwg/L) |
| $DY_a$ | Dry weight cells concentration after separation process (dwg/L) |
| $DY_i$ | Initial dry weight cells concentration (dwg/L) |
| E491 | Sorbitan monostearate |
| $F$ | Flow rate (L/h) |
| FFD | Fractional Factorial Design |
| $FR$ | Flux recovery (%) |
| GO | Graphene oxide |
| $J$ | Flux (L $m^{-2}$ $h^{-1}$) |
| $J_c$ | Water flux for cleaned membrane (L $m^{-2}$ $h^{-1}$) |
| $J_{c2}$ | Water flux after first cycle of backwash (L $m^{-2}$ $h^{-1}$) |
| $J_f$ | Flux of fouled membrane (L $m^{-2}$ $h^{-1}$) |
| $J_i$ | Initial flux (L $m^{-2}$ $h^{-1}$) |
| $J_{iw}$ | Initial water flux (L $m^{-2}$ $h^{-1}$) |
| $J_0$ | Initial flux of permeate (L $m^{-2}$ $h^{-1}$) |
| $\bar{J}$ | Average flux (L $m^{-2}$ $h^{-1}$) |
| $k$ | Number of factors |
| MBR | Membrane bioreactor |
| $OD600$ | Absorbance at wavelength 600 nm |
| $p$ | Degree of fractionation |
| PLS | Partial Least Squares |
| PVDF | Polyvinylidene fluoride |
| PVP | Polyvinylopyrrolidone |
| $R_c$ | Resistance due to concentration polarization ($m^{-1}$) |
| $R_{cp}$ | Resistance due to cake formation ($m^{-1}$) |
| $R_m$ | Membranes resistance ($m^{-1}$) |
| $R_p$ | Resistance due to pore blockage ($m^{-1}$) |
| $R_t$ | Total resistance of membranes ($m^{-1}$) |
| SCMBR | Submerged ceramic membrane bioreactor |
| SEM | Scanning electron microscopy |
| SMBR | Submerged membrane bioreactor |
| $t$ | Time (h) |
| $t_s$ | Separation process time (h) |
| TMP | Transmembrane pressure (mbar) |
| UV | Ultraviolet |
| $V_s$ | Final volume of permeate (L) |
| $W_p$ | Water permeability (L $m^{-2}$ $h^{-1}$ $bar^{-1}$) |
| $YP$ | Yeast permeation (%) |
| $\Delta P$ | Transmembrane pressure (bar) |
| $\mu$ | Solution viscosity (Pa s) |

reaction conditions [8,14,15]. One possible solution to this problem is to place the membrane inside the bioreactor, known as a submerged membrane bioreactor (SMBR). Moreover, SMBR can bring many other advantages, such as decrease of energy costs [16,17], homogeneity of the liquid phase in the reactor, simplification of the whole bioprocess [8], increased volumetric productivity [18] and volume reduction. Nevertheless, the problem arising is a potentially higher membrane fouling, which can drastically decrease the separation performance of the SMBRs.

One of the methods of fouling mitigation, and therefore prolongation of the membrane's efficient operation, is backwash. This mechanical method consists of periodical reversible flow of permeate through the membrane to remove material deposited on the surface of the membrane and inside the membrane pores [19,20]. The advantages of backwash as a method of fouling control in SMBRs are the no chemicals usage for membrane cleaning, possibility of semi-continuous separation process, and low cost and energy consumption. Lack of chemicals applied for fouling mitigation and possibility of semi-continuous separation process are particularly important in terms of the usability of SMBRs in biomanufacturing, especially in such applications where interrupting the bioprocess to chemically clean the membrane is undesirable. Moreover, periodical backwash of membrane allows to recover *in situ* membrane permeability, compared to the process without backwash, which is desirable to obtain as much bioproduct as possible with lower footprint [21]. The effectiveness of backwashing in mitigating membrane biofouling depends on various factors, such as substances filtered through the membrane and the applied backwash conditions [22]. However, the crucial factor appears to be the type of membrane used. For instance, backwashing can potentially cause irreversible mechanical damage to polymeric membranes placed inside the bioreactor. Consequently, backwashing applied to polymeric membranes might prove ineffective. Therefore, an alternative is to use ceramic membranes instead of polymeric ones. Ceramic membranes are characterized by high permeance, thermal, chemical and mechanical stability, making them long-term stable and potentially superior to polymeric membranes [23,24], however, they are considerably more expensive. An interesting solution is to use ceramic membranes made of single layer, without distinguishing between the support and separation layer (symmetric membrane) [25]. This approach can potentially reduce the cost of ceramic membrane production and facilitate their application in SMBRs. Additionally, the pore size of the membrane plays a pivotal role [26]. Hence, it is crucial to note that employing a different membrane size could result in varied effects during backwashing. This emphasizes the importance of conducting backwashing optimizations tailored to the specific membrane and the targeted substances being filtered.

An overlooked area is the use of submerged ceramic membrane bioreactor (SCMBR) in biomanufacturing, where cultures sensitivity makes the SCMBRs operation more challenging than wastewater treatment processes. Then, in each application there is need to find the best fouling mitigation strategy at the lowest possible cost and reduce the energy consumption while maintaining the continuity of the bioprocess and separation. Although the backwash process is well understood nowadays, a neglected area is the use of this fouling mitigation process in high-density cell retention in SCMBR. A preliminary study by our group has shown very encouraging results when SCMBR was used for polyhydroxyalkanoate production in a high-density cell retention at laboratory scale [27]. However, the values of the operational parameters were selected randomly, and no exhaustive efforts were made to optimize the efficiency of the SCMBR configuration since it was out of the research scope. Therefore, the present research aims tuning backwash as a method of fouling mitigation in a novel custom made submerged ceramic membrane bioreactor for continuous operation of high-density microbial systems (*Saccharomyces cerevisiae* was selected as a representative case study). Herein, multifunctional Fractional Factorial Design (FFD) was used to identify the key factors (i.e., flow rate of backwash, time between backwash cycles, backwash duration, cell concentration, and separation process time) affecting the average flux. To our knowledge, there are no scientific reports on the use of the FFD method which allows finding the best conditions for removing fouling caused by cells in SCMBR. The properties of the custom manufactured ceramic membrane module were presented and discussed. The critical transmembrane pressure was determined to avoid the occurrence of

irreversible fouling on the membrane surface. The experiments helped determine the average flux after the separation process, both before and after the application of backwash, and identify the optimal backwash conditions. Additionally, the cleaning strategy was designed systematically to cope with a particular challenge of this biosystem. Finally, experimental evidence allows proposing the fouling mechanism. It should be emphasized that application of symmetric ceramic membranes placed in one module immersed in bioreactor, applied for retention of low-density and high-density microbial cells (*Saccharomyces cerevisiae*) is an innovative approach in the field of separation processes at pilot scale.

## 2. Materials and methods

### 2.1. Chemicals and materials

Uniform, custom-made ceramic membranes with a pore size of 3 μm, were provided by Paul Rauschert GmbH & Co (Germany) (Table 1) and used for fabrication of ceramic membrane module. *Saccharomyces cerevisiae* yeast were delivered by Saft Instant (France). Bradford reagent, sodium hydroxide, sulfuric acid (96 %) and ethanol (70 %) were delivered by Merck KGaA (Germany).

### 2.2. Characterization of the SCMBR

The morphology of the ceramic membrane was evaluated based on scanning electron microscopy (SEM) photographs (Prisma E-SEM, Thermofisher Scientific, United States) operated at an accelerating voltage of 5–10 kV.

To determine critical transmembrane pressure (TMP), yeast cells separation was conducted using the TMP-step method [28,29]. In this method, three yeast suspensions at the concentrations of 20, 50 and 80 g dry weight per liter (dwg/L) were used at 100 rpm constant mixing speed. The controlled pressure increased with regular steps (10 min step duration for each TMP-step ranged from 20 to 300 mbar). Between each TMP-steps, 10 min of membrane relaxation was applied. The TMP-step method was also conducted for measuring of the water flux (TMP ranged from 20 to 150 mbar). The permeate and water flow rates were measured using a Sonoflow flow sensor (CO.55/080SD V2.0) and recorded using a computer with the Sonoflow C3 software, both delivered by Sonotec GmbH (Germany). The obtained data was used for flux calculations, based on Eq. (1):

$$J(t) = \frac{F(t)}{A} \tag{1}$$

**Table 1**
Main characteristics of the ceramic membrane material and membrane module used in the experiments.

| **Characteristics of the membrane material** | |
|---|---|
| **Material of membrane** | α-$Al_2O_3$ |
| **Density (g/cm$^3$)** | 2.65 |
| **Porosity (%)** | 28 |
| **Average Pore size (μm)** | 3 |
| **Thermal conductivity (W m$^{-1}$ K$^{-1}$)** | 10 |
| **Maximum operating temperature (°C)** | 1400 |
| **Pore volume (mm$^3$ g$^{-1}$)** | 100 |
| **Thermal shock resistance (K)** | 680 |
| **Characteristics of the membrane module** | |
| **Type of filtration** | Microfiltration |
| **Shape of the membrane** | Cylindrical |
| **Number of channels in the membrane** | 1 |
| **Outer diameter of the membrane (mm)** | 10 |
| **Inner diameter of the membrane (mm)** | 7 |
| **Length of the membrane (mm)** | 250 |
| **Number of ceramic membranes in the module** | 25 |
| **Membranes surface area in the module (m$^2$)** | 0.1759 |

where: $J$ denotes flux (L m$^{-2}$ h$^{-1}$), $F$ is the flow rate of water or permeate passed through the ceramic membrane module (L/h), and $A$ is the membrane area (m$^2$).

Water permeability was calculated based on Eq.(2):

$$W_p(t) = \frac{F(t)}{A \bullet \Delta P} \tag{2}$$

where: $F$ is the flow rate (L/h) of water passed through the ceramic membrane, $A$ is the membrane area (m$^2$), and $\Delta P$ is the transmembrane pressure (bar).

### 2.3. Separation process

#### 2.3.1. Operation of the ceramic membrane bioreactor

The submerged ceramic membrane bioreactor system consisted of a BioBench bioreactor (Biostream, Netherlands) equipped with a 10 L (working volume) stainless steel vessel and a ceramic membrane module (Fig. 1). A peristaltic pump (model 530S Watson Marlow, United Kingdom) was used for backwashing. The collected data were used for calculation of flux, based on Eq. (1).

The average flux during separation process was calculated based on Eq. (3):

$$\bar{J} = \frac{V_s}{A \bullet t_s} \tag{3}$$

where: $\bar{J}$ denotes average flux (L m$^{-2}$ h$^{-1}$), $V_s$ is the final volume of permeate passed through the ceramic membrane module (L), $A$ is the membrane area (m$^2$), and $t_s$ denotes is the separation process time (h).

The flux recovery ratio was calculated based on Eq. (4):

$$FR = \frac{J_f}{J_i} \bullet 100\% \tag{4}$$

where: $FR$ denotes flux recovery (%) and $J_f$ (L m$^{-2}$ h$^{-1}$) and $J_i$ (L m$^{-2}$ h$^{-1}$) are flux of fouled membrane and initial flux, respectively.

The separation processes were carried out under constant pressure and mixing speed of 100 ± 10 mbar and 100 rpm, respectively.

#### 2.3.2. Experimental design

An experimental design was selected to identify the key factors affecting the average flux and their interactions. The effect of variables such as cell concentration, time between backwash, backwash duration, flow rate of backwash and the separation process time on average flux during the separation process were evaluated using a Fractional Factorial Design (FFD), denoted as $2^{k\text{-}p}$. In this formula $k$ indicates the number of factors, whereas $p$ denotes the degree of fractionation. In our work, two-level FFD with 5 factors and 1 degree of fractionation was used ($2^{5-1}$). This type of experimental design was selected to reduce the number of experiments performed with 5 or more variables and choose the significant factors and their interactions [30,31]. The Partial Least Squares (PLS) regression analysis was used as a method to model the relationships between variables and responses. Table 2 presents processing input variables and their real values (high level + 1, low level −1) and their center points (level 0) in the presented design. The levels of coded input variables (A, B, C, D, E) were defined by previous experiments and instrumental limitations (data not shown). The necessary experimental runs were computed using MODDE 7 software and are presented in Table S1 (Supplementary information).

### 2.4. Cell size and OD600 measurement

The microbial cell size was measured with a ParticleTech Analyzer (ParticleTech ApS, Denmark). The cell concentration in yeast suspensions and in permeates after separation tests was determined by optical density measurements at a wavelength of 600 nm [32], using a UV-1280

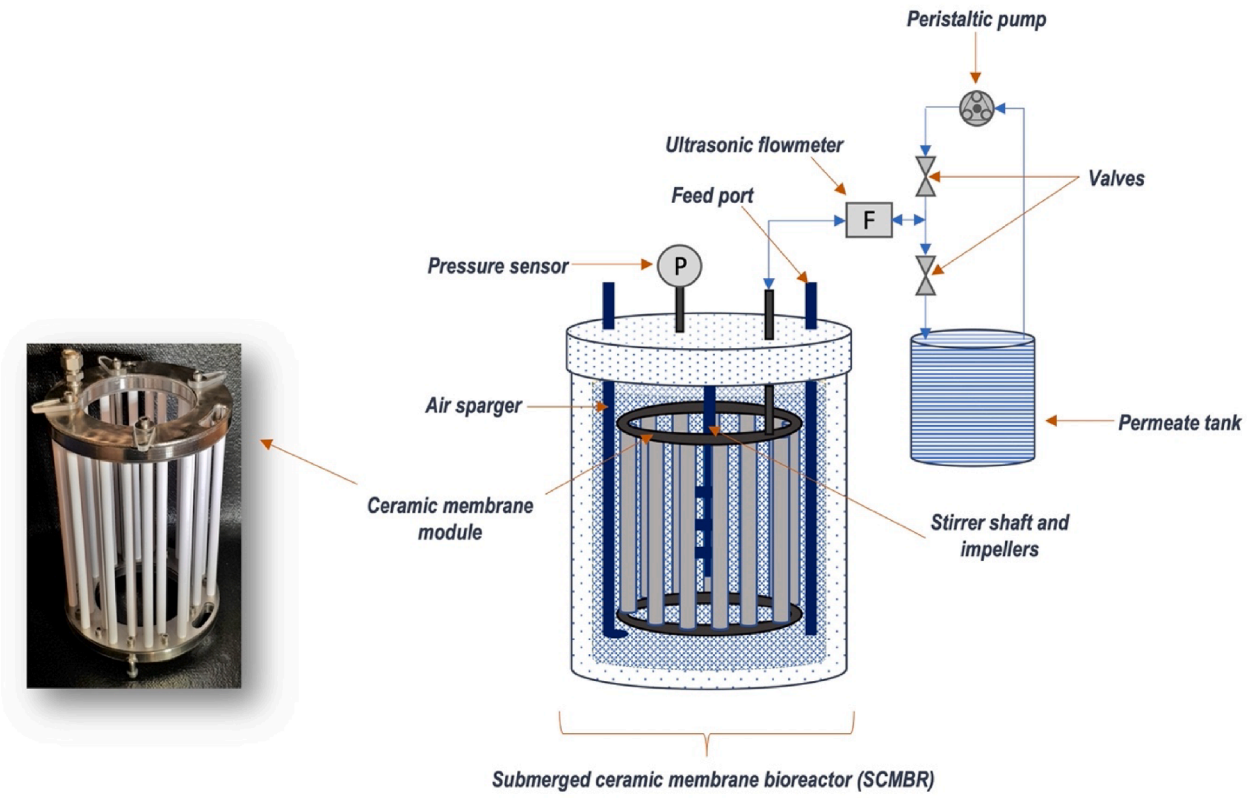


**Fig. 1.** Schematic diagram of operating system with the submerged ceramic membrane bioreactor.

**Table 2**
The values of levels of the input variables in $2^{5-1}$ Fractional Factorial Design.

| Factors | Variables | Levels | | | Variation interval |
|---|---|---|---|---|---|
| | | −1 | 0 | +1 | |
| A | Flow rate of backwash (mL $min^{-1}$) | 375 | 937.5 | 1500 | 1125 |
| B | Time between backwash cycles(min) | 5 | 10 | 15 | 10 |
| C | Backwash duration(sec) | 5 | 10 | 15 | 10 |
| D | Cell concentration (dwg/L) | 20 | 50 | 80 | 50 |
| E | Separation process time (h) | 0.5 | 2.75 | 5 | 4.5 |

spectrophotometer (SHIMADZU Corp., Japan). The concentration of dry weight cells was calculated based on the correlation:

$$DY = 0.3632 \bullet OD600 - 0.0032 \left(R^2 = 0.997\right) \quad (5)$$

where: *DY* denotes dry weight cells concentration (dwg/L) and *OD600* is the absorbance at 600 nm.

Yeast permeation was calculated based on the Eq. (6):

$$YP = \frac{DY_a}{DY_i} \bullet 100\% \quad (6)$$

where: *YP* denotes yeast permeation (%), $DY_a$ and $DY_i$ denote dry weight cells concentration after separation process and initial dry weight cells concentration, respectively.

### 2.5. Bradford method

To determine the quantity of the protein from any damaged *Saccharomyces cerevisiae* cells on the membrane surface, the Bradford method [33] was used. In this case, two steps of investigation were applied–sample preparation and spectrophotometric measurements. In the first step, a specific amount of cake layer was taken from the membrane and left to dry at ambient temperature for 24 h. Next, 100 mg of the dry powder was added to 5 mL of water. The samples prepared in this way were mixed with Bradford reagent in a 1:1 ratio and left for 5 min. After that time, the absorbance was measured at 595 nm using a UV-1280 spectrophotometer. The quantity of proteins was calculated using the bovine serum albumin calibration curve and compared with sample of yeast suspension before separation process.

### 2.6. Cleaning protocol

Before the first use the ceramic membrane module was rinsed with $dH_2O$. To determine the most effective cleaning protocol of the module, separation cycles (cell concentration 50 dwg/L, 30 min of separation process, without application of the backwash) have been conducted. To deal with the challenges of retaining *Saccharomyces cerevisiae cells,* 5 different procedures were tested (Table 3).

Between experiments, the ceramic membranes were cleaned using 2 % NaOH, 0.1 % $H_2SO_4$ and 7 % ethanol. Between application of the mentioned chemicals for membrane cleaning, the ceramic membrane module was rinsed by $dH_2O$. For each of the applied reagents, the cleaning protocol was as follows: soaking, filtering, and backwashing. Each step lasted 30 min. Only for cleaning using NaOH and EtOH, temperature was 50 °C and 60 °C, respectively, compared to the cleaning using $H_2SO_4$ and $dH_2O$ where temperature was ambient. Before each separation experiment, water permeability was measured to evaluate cleaning efficiency (Eq. (7):

$$CE = \frac{J_c}{J_{iw}} \bullet 100\% \quad (7)$$

where: *CE* denotes cleaning efficiency (%) with $J_c$ (L $m^{-2}$ $h^{-1}$) and $J_{iw}$ (L $m^{-2}$ $h^{-1}$) being the water flux for cleaned membrane and the initial

**Table 3**
Systematic cleaning strategy proposed for the SCMBR.

| Cleaning procedure | Chemical agents | Temperature (°C) |
|---|---|---|
| A | $dH_2O$ | 20 |
| B | 1. $dH_2O$ | 20 |
| | 2. NaOH 2 % | 50 |
| | 3. $dH_2O$ | 20 |
| C | 1. $dH_2O$ | 20 |
| | 2. NaOH 2 % | 50 |
| | 3. $dH_2O$ | 20 |
| | 4. $H_2SO_4$ 0.1 % | 20 |
| | 5. $dH_2O$ | 20 |
| D | 1. EtOH 7 % | 60 |
| E | 1. $dH_2O$ | 20 |
| | 2. NaOH 2 % | 50 |
| | 3. $dH_2O$ | 20 |
| | 4. $H_2SO_4$ 0.1 % | 20 |
| | 5. $dH_2O$ | 20 |
| | 6. EtOH 7 % | 60 |
| | 7. $dH_2O$ | 20 |

water flux, respectively.

### 2.7. Characterization of membrane fouling

To characterize membrane fouling, it was decided to perform calculations of total resistance of membranes ($R_t$) as the sum of membranes resistance ($R_m$), resistance due to cake formation ($R_c$), resistance due to pore blockage ($R_p$) and concentration polarization ($R_{cp}$) (Eq. (8)):

$$R_t = R_m + R_c + R_P + R_{cp} \tag{8}$$

The total resistance of membranes was calculated based on flux at the end of the first cycle of separation process (Eq. (9)):

$$J = \frac{\Delta P}{\mu \bullet R_t} \tag{9}$$

where: $\Delta P$ is the transmembrane pressure (bar), $\mu$ denotes solution viscosity (Pa s) and $R_t$ defines total membrane resistance ($m^{-1}$).

The membrane resistance ($R_m$) was determined from Equation (10) by measuring the clean membrane flux with $dH_2O$:

$$J_c = \frac{\Delta P}{\mu \bullet R_m} \tag{10}$$

The combined membrane resistance and resistance due to pore blockage ($R_m + R_p$) at the end of the first separation cycle was estimated based on the $dH_2O$ flux through the membrane after first cycle of backwash (Equation (11):

$$J_{c2} = \frac{\Delta P}{\mu \bullet (R_m + R_p)} \tag{11}$$

Resistance due to pore blockage ($R_p$) was calculated by the difference as $(R_m + R_p) - R_m$, whereas resistance due to cake formation ($R_c$) was determined by difference as $R_t - (R_m + R_p)$.

The concentration polarization effect was neglected, and the solution viscosity was assumed constant at $1{\cdot}10^{-3}$ Pa·s.

## 3. Results and discussion

### 3.1. Characterization of the submerged ceramic membrane bioreactor

The morphology of the single ceramic membrane conduit was evaluated based on a SEM photograph of membrane cross-section (Fig. 2).

It can be observed that the membrane exhibits a homogeneous, sponge-like structure with visible micrometric-sized pores. Moreover, single particles with irregular shapes are visible on the membrane cross-section, which is closely related to the conditions of the sintering process [34]. The homogeneous structure distinguishes the membrane used in this study from commercially available membranes, which typically consist of a selective layer and a support material with different pores sizes [35,36]. The water permeability of the ceramic membrane module equals 3516 ± 140 L $m^{-2}$ $h^{-1}$ $bar^{-1}$, which is significantly higher than the water permeability of ceramic micromembranes with a selective layer [37]. Moreover, as presented by Akhondi et al. [38], backwashing is more effective in removing deposited particles in larger pores compared to smaller ones. This is due to the lower resistance inside larger pores. Therefore, it is likely that the permeate used for backwashing could more efficiently remove fouling from the membrane used in the present study compared to what would be possible with membranes with smaller pore sizes.

The critical transmembrane pressure (TMP) value is crucial in relation to the type of fouling that can occur on the membrane below and above this pressure. Below the critical TMP, no irreversible fouling occurs, while above it, irreversible fouling is present [28,39–41]. Therefore, it was crucial to determine this value in order to select the

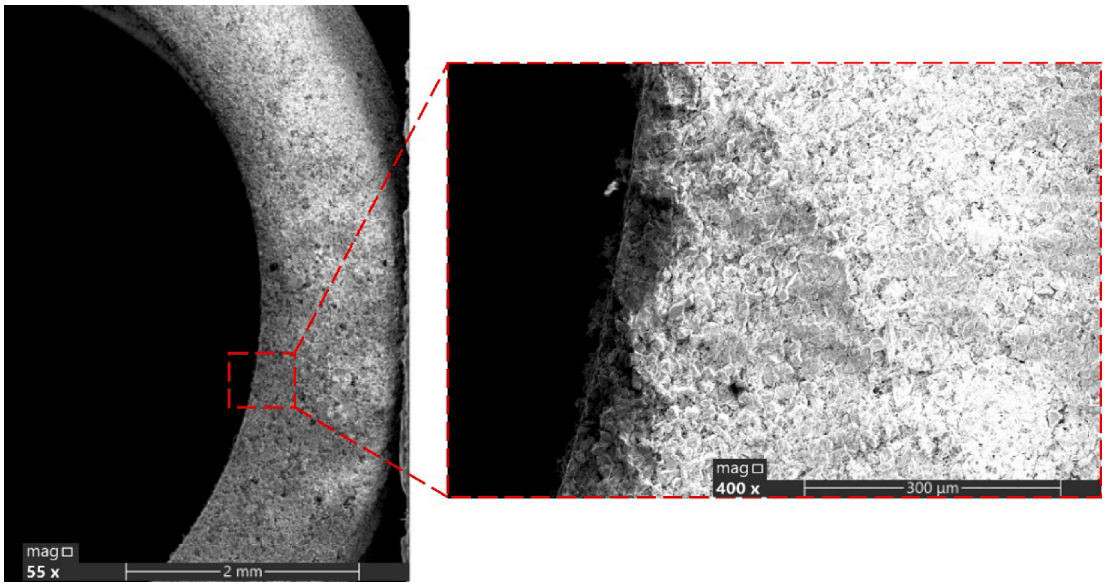


**Fig. 2.** SEM photograph of the single ceramic membrane.

appropriate TMP during the separation process, minimizing irreversible fouling as much as possible and enabling the study of fouling removal. It is also noteworthy that, in contrast to the concept of critical flux (observing changes in TMP under constant flux), critical TMP becomes significant when the separation process in a bioreactor occurs under constant pressure conditions [28]. It was observed that for all concentrations of yeast suspension used, the flux increased linearly with rising TMP (Fig. 3).

However, once the TMP exceeded 100 mbar for yeast suspension at concentration 20 dwg/L, the flux was stabilized or even reduced, suggesting the formation of irreversible fouling. It has also been observed that the values of critical TMP are slightly higher for higher concentrations of yeast (50 dwg/L and 80 dwg/L). The probable reason of this observation may be the fact that during separation of suspensions at high yeast concentrations denser cake layers are formed compared to the cake layers formed after separation of suspension at the lowest applied yeast concentration. Then, small particles which could create irreversible fouling, can attach to these denser cake layers and do not reach the membrane. As was presented by Iritani [42], the amount small particles trapped into the pores of the cake layer of the larger particles during separation process might depend on the density of the cake layer on the membrane. Therefore, in subsequent studies, it was decided to conduct separation experiments at a constant pressure of 100 mbar. This choice was based on the expectation that conducting experiments at this pressure may limit the occurrence of irreversible fouling on the membrane surfaces with higher efficiency, as compared to using higher pressures. Despite the use of this relatively low pressure, the proposed SCMBR could be used for the separation process at low and high concentration of cells (20–80 dwg/L). In contrast to our study, Ogunbiyi et al. [43] observed strong fouling of the ceramic membrane during separation of baker's yeast at concentration of just 0.1 g/L probably due to the very high pressure of 1.5 bar and the very small pore size of 0.5 μm employed in their system.

### 3.2. Separation efficiency of Saccharomyces cerevisiae cells in SCMBR

During the separation processes of *Saccharomyces cerevisiae* cells, two methods of fouling mitigation were initially tested: backwashing using permeate and air backflushing. However, the passage of air through the ceramic membrane module caused intense foam formation, which could not be controlled by antifoaming agents. Similar observations were made previously by Burniol-Figols et al. [27], who stated that air backflushing was not an appropriate cleaning strategy for ceramic membranes used in the separation of cell cultures. Therefore, the study continued only with backwashing using permeate as a method of fouling mitigation. FFD allowed us to examine the effects of variables and their

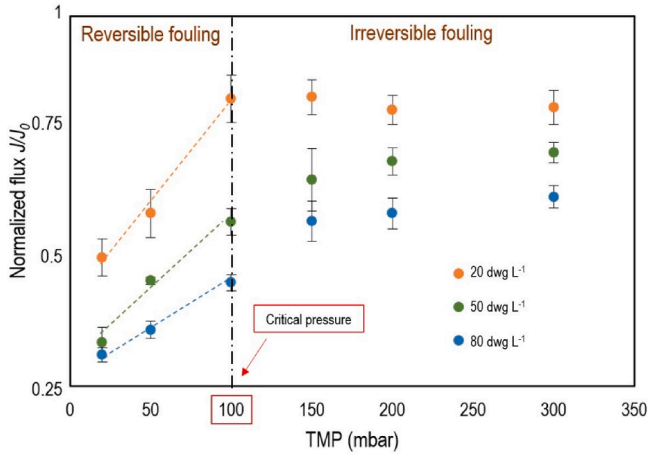


**Fig. 3.** Determination of the critical TMP.

interactions on the average flux during the separation process while reducing the number of experimental runs [44,45].

The average flux in the experiments carried out (Table S1) ranged between 13.5 and 102.34 L $m^{-2}$ $h^{-1}$. The model's parameter count was reduced from 16 to 8 significant parameters (maximum p-value $b_4$ = 0.037) (Table 4). The $R^2$ adjusted for the average flux model equaled 0.999, Q2 was 0.114, which indicates a good model fit with confidence level 0.95. A regression model was constructed for estimation of average flux $\overline{J}$ (L $m^{-2}$ $h^{-1}$) (Equation (12).

$$\overline{J} = b_1 + b_3 \bullet B + b_4 \bullet C + b_5 \bullet D + b_6 \bullet E + b_{11} \bullet A \bullet E + b_{13} \bullet B \bullet D + b_{17} \bullet D \bullet E \quad (12)$$

Plots in Fig. 4 and Fig. 5 show the impact of the significant factors and their interaction, respectively, on the average flux. It can be observed that, individually, factors B (time between backwash cycles), C (backwash duration), D (cell concentration), and E (separation process time) have negative effects on the average flux of permeate (Fig. 4). As these values increase, the average flux decreases. However, the most significant negative slope was noted for separation process time, which is strictly connected to the fact that with increased separation duration, the thickness of the cake layer increases [46].

Consequently, the applied backwash conditions cannot effectively remove fouling, leading to a hindered increase in the average flux. A negative effect has also been observed for time between backwash cycles. An increase in the time between backwash cycles may be related to a longer time of cell separation and, consequently, a greater amount of cells deposited on and above the membrane in the form of cake layer. This increase in cell adsorption is associated with greater difficulty in removing fouling compared to a shorter time between backwash cycles under the same conditions. Regarding backwash duration, a slight negative effect on average flux was observed. This is due to the fact that during this backwash duration, a certain amount of permeate is returned to the fermenter, which affects the final average flux (see Equation (3). These results are consistent with findings from other studies [44,47], where more frequent backwash (shorter time between backwash cycles) and shorter backwash duration positively affect permeate flux compared to longer time between backwash cycles and longer backwash duration. In the case of cell concentration, the negative effect is related to the fact that with a higher concentration of cells, more cells block the pores of the membrane, leading to a decrease in the average flux compared to a lower cell concentration. An interesting finding concerning the factors

**Table 4**
Coefficients of the average flux model. Coefficients with a p-value < 0.05 were considered as significant.

| Factors and interactions | Meaning of factors and interactions | Parameter | Value of parameter | p-value |
|---|---|---|---|---|
| Constant | – | $b_1$ | 48.91 | 7.3E-3 |
| B | Time between backwash cycles | $b_3$ | −8.31 | 1.4E-2 |
| C | Backwash duration | $b_4$ | −3.29 | 3.7E-2 |
| D | Cell concentration | $b_5$ | −9.37 | 1.3E-2 |
| E | Separation process time | $b_6$ | −16.07 | 7.7E-3 |
| A·E | Flow rate of backwash · Separation process time | $b_{11}$ | −3.52 | 3.4E-2 |
| B·D | Time between backwash cycles · Cell concentration | $b_{13}$ | 3.27 | 3.6E-2 |
| D·E | Cell concentration · Separation process time | $b_{17}$ | 5.90 | 2.0E-2 |

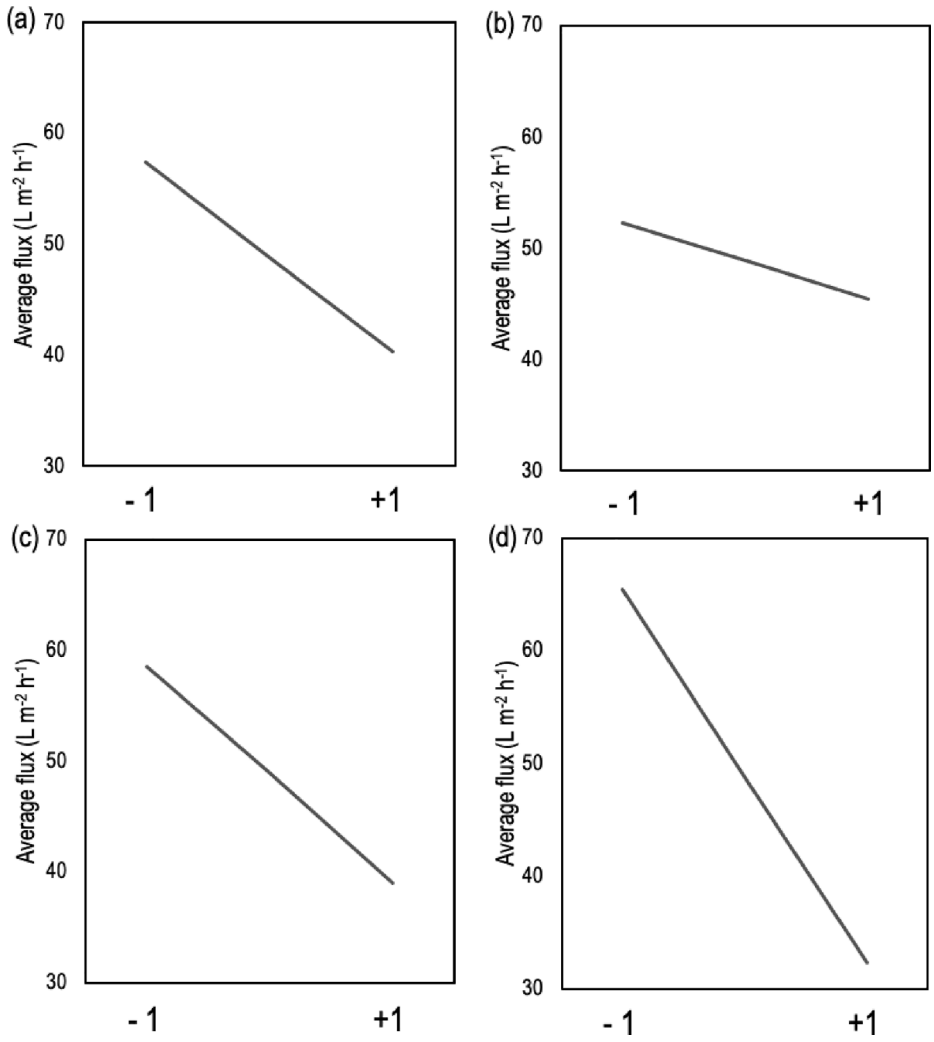


**Fig. 4.** Main effect plots of factors: (a) time between backwash cycles, (b) backwash duration, (c) cell concentration and (d) separation process time and response of average flux. −1 and + 1 mean low level and high level, respectively, of input factors.

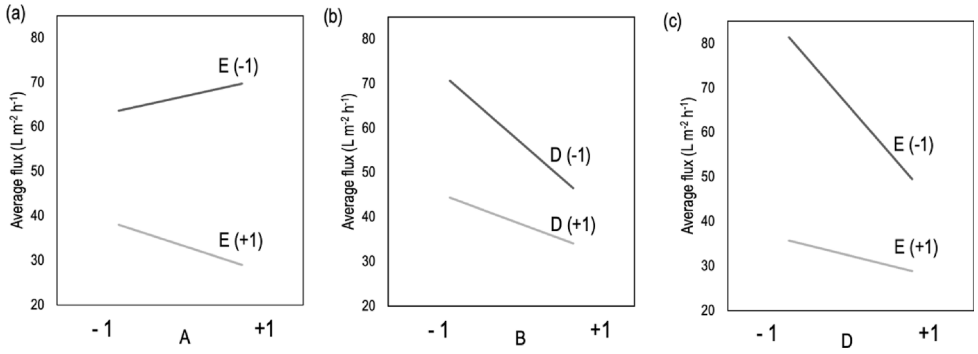


**Fig. 5.** Interaction plots of factors: (a) flow rate (A) and time of the filtration process (E), (b) time between backwash (B) and cell concentration (E) and (c) cell concentration (D) and time of the filtration process (E) and response of average flux. −1 and + 1 mean low level and high level, respectively, of input factors.

affecting the average flux is that the flow rate of backwash (factor A) does not significantly influence the average flux. We assume that other factors (B, C, D, E) may have a dominant effect on the removal of fouling and the average flux of the permeate, outweighing any possible effects of the backwash flow rate on the average flux.

Another explanation could be that the applied range of the backwash flow rate was not sufficient to observe significant changes in the average flux. However, even though the flow rate of backwash does not affect the average flux of permeate significantly, an interaction exists between flow rate of backwash and separation process time (Fig. 5a). When the separation process time is at a low level (-1), an increase in the flow rate of the permeate leads to an increase in the average flux. In contrast, when separation process time is at a high level (+1), and the value of flow rate of backwash increases, the average flux decreases. This is because as the separation process progresses, the cake layer becomes thicker and more difficult to remove, resulting in a decrease in average

flux. However, based on the slope, it can be concluded that these interactions have a relatively small effect. Moreover, interaction effects were observed for time between backwash cycles and separation process time, as shown in Fig. 5b, and for cell concentration and separation process time, as shown in Fig. 5c. When cell concentration and separation process time are at a high level (+1), time between backwash cycles and cell concentration have relatively small effects, respectively. However, if the cell concentration and separation process time are at a low level (-1), the time between backwash cycles and cell concentration have significant effects, respectively. Figure S1 presents surface response contour plots for the above presented interactions.

The obtained model indicates that the average flux decreases as the values of applied factors increase. In the case of values directly related to backwash duration, it may be because some permeate is returned to the fermenter, therefore volume of net permeate does not increase continuously. In case of increasing time between backwash cycles, separation process time and cell concentration, it is due to building up of fouling, and thus, more difficulty in removing it. However, it is worth comparing the results of experiments with and without backwash. It can be seen in Fig. 6a that during separation of cell suspension without backwash rapid decline of flux was observed and after 15 min for each of the used solutions (20, 50 and 80 dwg/L of cell), permeate flux was only around 20 % of the initial permeate flux. However, the accumulated volume results (Fig. 6b) show that highest volume of the permeate was accumulated after separation process of solution at concentration of 20 dwg/L. It may be strongly connected to the higher flux of permeate for first 10 min of the separation process, compared to the solutions at concentrations of 50 and 80 dwg/L.

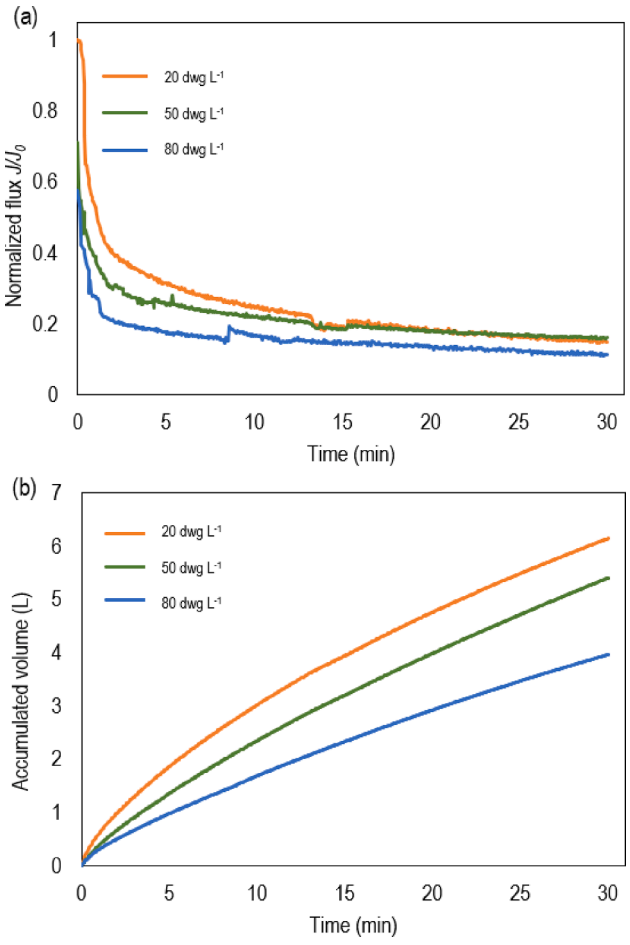


**Fig. 6.** (a) Flux decline for yeast separation process without backwash for 3 different yeast concentrations and (b) amount of collected permeate.

This result can be compared with results when backwash was applied. For example, it can be seen (Fig. 7a) that by applying backwash at 1500 mL $min^{-1}$ flow rate of backwash, 5 min time between backwash cycles and 15 sec backwash duration for the separation process of cell suspension at concentration 20 dwg/L, it was possible to recover 100 % of the initial flux after 1 backwash cycle.

However, during next cycles of backwash at the same conditions the flux gradually decreased reaching around 47 % of the initial flux after 30 min of separation process. In contrary to that, during the separation process of cell suspension at concentration 20 dwg/L without backwash, after 5 min of operation the flux decreased to 30 % on the initial flux value. A similar observation was drawn by Gabrus and Szaniawska [48]. An asymmetric, multilayered ceramic ($TiO_2/Al_2O_3$) membrane with pore diameter equaled 0.8 mm, was used for separation of yeast suspension with yeast concentration at 0.51 dwg/L. It was shown that the values of permeate fluxes when the backwash was applied was around 15 % higher, compared to the fluxes noted for separation process without backwash. Moreover, the interesting fact is that after application of the backwash, over 1 L more permeate was obtained compared to the process without backwash (Fig. 7b).

The definition of input parameters levels was an important step before proper experiments, because, as was mentioned before, they affect amount of the collected permeate. For example, during the yeast cell separation process from a suspension with a concentration of 50

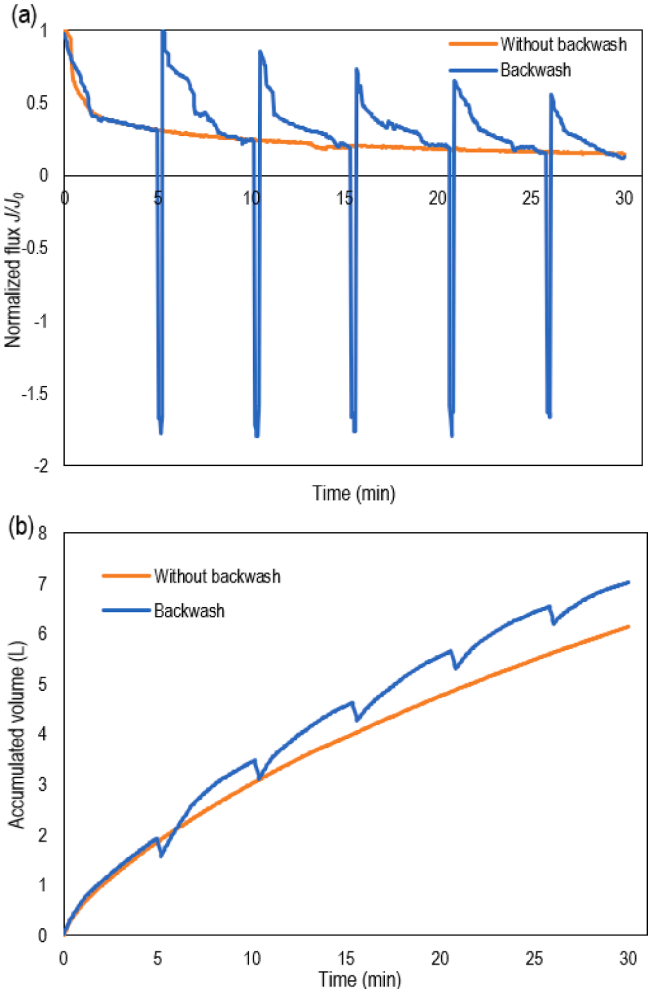


**Fig. 7.** (a) Flux vs separation process time of yeast suspension at concentration 20 dwg/L without and with backwash and (b) amount of collected permeate. Backwash conditions were: flow rate of backwash: 1500 mL $min^{-1}$, time between backwash cycles: 5 min, backwash duration: 15 sec.

dwg/L, backwash was performed using the following conditions: flow rate of backwash: 1500 mL $min^{-1}$, time between backwash cycle: 1 min and backwash duration: 30 sec (Figure S2). It was observed that after the first backwash, it was impossible to obtain any amount of permeate because the entire product obtained in the first separation cycle was returned to the fermenter due to backwashing. In addition, in this work we decided to focus on the average flux in our experiments, because this parameter is dependent on the amount of permeate produced during the time (see Equation (3). Based on the final (net) amount of produced permeate (Table S1) it can be seen that the highest amount of permeate, equaled 18.69 L, was obtained for the following conditions of separation process and backwash: flow rate of backwash: 375 mL $min^{-1}$, time between backwash cycle: 5 min, backwash duration: 5 sec, cell concentration: 20 dwg/L and 5 h of separation process. In contrary to that, the lowest amount of permeate obtained was 2.69 L, when the conditions of separation process and backwash were: flow rate of backwash: 1500 mL $min^{-1}$, time between backwash cycle: 5 min, backwash duration: 15 sec, cell concentration: 80 dwg/L and 0.5 h of separation process. Although the average flux was similar in both cases (56.76 and 52.79 L $m^{-2}$ $h^{-1}$, respectively), the obtained final amounts of the permeate differ from each other. In the first case, when the amount of the obtained permeate was 18.69 L, a high amount of permeate is affected by the long time of the process and a low cell concentration. When the amount of the produced net permeate was 2.69 L, the amount of permeate produced is negatively affected by the short process time (0.5 h) and high cell concentration (80 dwg/L). It is also worth paying attention to time between backwash cycles and backwash duration, which also affects the amount of permeate [49].

Considering the information presented above and in Table S1, it can be concluded that the highest average flux (102.34 L $m^{-2}$ $h^{-1}$) can be obtained for: flow rate of backwash: 1500 mL $min^{-1}$, time between backwash cycle: 5 min, backwash duration: 5 sec, cell concentration: 20 dwg/L and 0.5 h of separation process, whereas the highest amount of the obtained net permeate volume (18.69 L) for: flow rate of backwash: 375 mL $min^{-1}$, time between backwash cycle: 5 min, backwash duration: 5 sec, cell concentration: 20 dwg/L and 5 h of separation process. However, considering the fact that the backwash as a fouling mitigation method in SCMBR can be applied to various cell concentrations, based on the results, we can assume the general conditions of backwash could be flow rate of backwash: 375 mL $min^{-1}$, time between backwash cycle: 5 min and backwash duration: 5 sec. The results obtained may be important when considering the choice of the backwash conditions in SCMBR used in biomanufacturing, especially where specific amount of permeate produced needs to be produced in the shortest possible time.

### 3.3. *Effect of cleaning procedure on efficiency recovery of the ceramic membrane module*

In membrane technology, one of the most important issues is the possibility to regenerate or clean the membrane after a separation process. This allows membranes, particularly ceramic membranes, to be reused multiple times with high efficiency, resulting to a significant cost reduction of the separation process [50]. Although the membrane manufacturers often provide information on how the membranes should be cleaned chemically (type of cleaning reagents and conditions), ways to efficiently clean membranes have been sought for years, which mainly depends on the type of foulant. This is because some membrane foulants can exhibit resistance to standard membrane cleaning methods [43,44,51–53]. Therefore, a set of cleaning procedures and cleaning reagents were investigated to effectively clean the ceramic membrane module after the separation process of the yeast suspension. In the presented study it was decided to investigate 5 cleaning procedures (A–E) (Fig. 8) with the use of cleaning reagents such as $dH_2O$ and solutions of NaOH, $H_2SO_4$ and EtOH at specific concentrations. It was observed that the cleaning efficiency of the ceramic membrane module after using water was 33 % (cleaning procedure A). Consequently, to enhance the membrane cleaning efficiency and restore the ceramic membrane module's initial properties in terms of water flux, it was decided to employ a NaOH solution in the subsequent step after cleaning the membranes with $dH_2O$ (cleaning procedure B). The reason for using NaOH in the cleaning of the ceramic membrane module after the yeast suspension separation process is that alkalis, such as NaOH, hydrolyze organic matter, such as proteins, into smaller molecules. This process can effectively enhance the cleaning of ceramic membranes [54]. The cleaning efficiency significantly increased after applying cleaning procedure B compared to cleaning procedure A, reaching 69 %. Consequently, the logical next step involved using an acidic solution to eliminate inorganic residues from the membrane [55] in order to improve the cleaning efficiency. Therefore, an $H_2SO_4$ solution was chosen for testing, as it is one of the most typical chemical cleaning

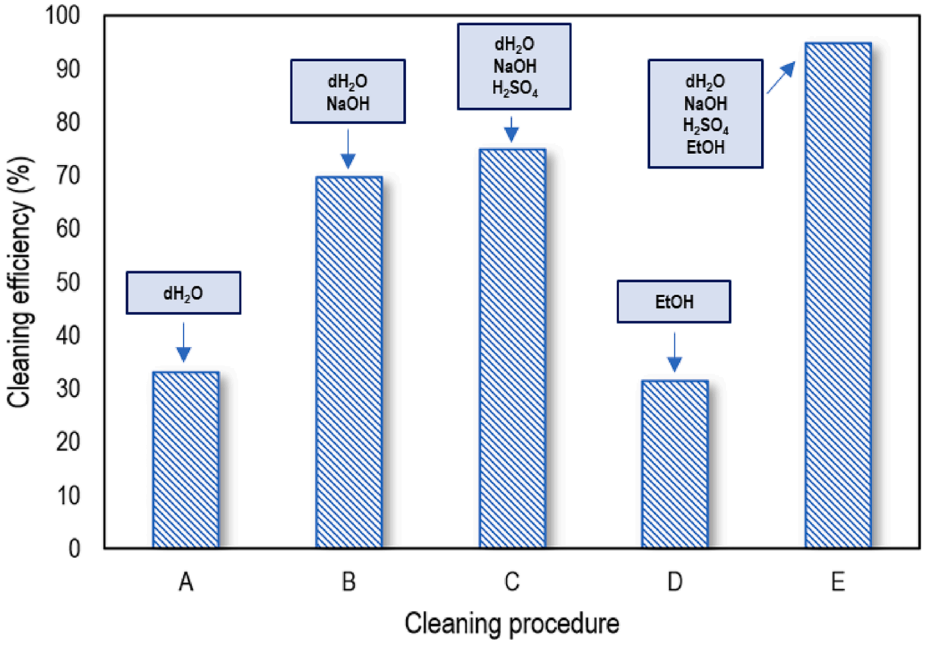


**Fig. 8.** Cleaning efficiency vs applied cleaning procedure and the chemicals applied during specific cleaning procedure.

agents for ceramic membranes [50,56]. Nevertheless, after applying $H_2SO_4$ the cleaning efficiency was only 5 % higher than the utilization of cleaning procedure B. It should be emphasized that the yeast suspension contained an artifact which was sorbitan monostearate (E491), also known as synthetic wax. This food additive plays a role as an emulsifier and when added to baker's yeast, it helps maintain moisture, consequently increasing the shelf life of the baker's yeast [57]. Therefore, it was assumed that this compound caused irreversible fouling of the membrane, rendering it impossible to clean the membrane using cleaning procedure C. Sorbitan monostearate is insoluble in water; however, it can dissolve in organic solvents, such as ethanol, at temperatures above 57 °C [57].

It was observed that the cleaning efficiency was 31 % after using ethanol as the sole cleaning reagent, which yielded a similar result to using only water (cleaning procedure A). Consequently, the final decision was made to combine all methods in cleaning procedure E. The cleaning efficiency after application of cleaning procedure E was 95 %, what allows to conclude that use of the applied cleaning reagents removed yeast cells and sorbitan monostearate from the membrane.

### 3.4. Proposed fouling mechanism and cost analysis

Based on the obtained data, it was decided to characterize membrane fouling during filtration of yeast suspension using a ceramic membrane module. It was shown that yeast permeation ratio was less than 1 %, what allows to state that the separation process of yeast cells with size 9 μm using proposed ceramic membrane module was effective. Based on results presented in Fig. 6, it can be seen that flux decreased with time for each yeast suspension, what is strictly related to gradual reduction of membrane's effective pore size. Table 5 presents the example of estimated membrane resistances calculated based on the flux data at the end of the first separation cycle at specific conditions of backwash. Resistance due to cake formation ($R_c$) seems to be the major fraction of the total resistance ($R_t$) (68.78 %), whereas resistance due to pore blockage ($R_p$) equals 5.82 %.

Conditions of separation cycle: yeast suspension at concentration 20 dwg/L, flow rate of backwash: 1500 mL $min^{-1}$, time between backwash cycles: 5 min, backwash duration: 15 sec. $R_m$, $R_p$, $R_c$, $R_{cp}$, and $R_t$ denote membranes resistance, resistance due to pore blockage, resistance due to cake formation, resistance due to concentration polarization and total resistance of membranes, respectively.

Therefore, based on the obtained flux data and calculations, we can propose the following membrane fouling mechanism. The initial stage of the flux decline (stage I) (Fig. 9a) is caused mainly by pore blocking by yeast cells. However, in addition to pores narrowing cause by yeast cell deposition, it is important to mention the blocking of pores by sorbitan monostearate, which was presented in the yeast suspension at a concentration of 1.5 g per 100 g. In the next stage of flux declining (stage II), the main fouling mechanism was cake layer formation on the ceramic membrane layer. Yeast cells agglomerate on the membrane (Fig. 10) causing flux loss and next, due to consolidation of the fouling material, flux remains quasi-stable (stage III) [58]. However, to confirm whether the yeast cells were damaged due to applied pressure during separation process, a Bradford analysis was conducted. This analysis aimed to investigate whether protein extracted from the destroyed yeast cells appeared in the cake layer during the separation process. It was shown that no proteins were present in the cake layer and in the permeate, which may indicate that the yeast cells were not destroyed and therefore intracellular compounds were not extracted from the yeast. Based on the presented information it can be assumed that during separation process of yeast suspension, fouling model presented by Ho and Zydney [59] and Yuan et al. [60] can be applied, which presents simultaneous pore blockage and cake layer formation for microfiltration process [61]. Taking into account results for periodic backwash of ceramic membrane module during yeast suspension separation process under specific conditions such as flow rate of backwash: 1500 mL $min^{-1}$, TBBW: 5 min, BW: 15 sec (Fig. 7), it can be observed that in the first backwash cycle it was possible to fully recover initial flux. However, according to Le-Clech [62], it was observed that the recovery flux value gradually decreased, indicating the progressive occurrence of irreversible fouling, which cannot be mitigated by the applied backwash method (Fig. 9b).

**Table 5**
Estimated membranes resistances at the end of the first separation cycle.

| | $R_m$ | $R_p$ | $R_c$ | $R_{cp}$ | $R_t$ |
|---|---|---|---|---|---|
| **Membranes resistance ($m^{-1}$) (x $10^{11}$)** | 1.02 | 0.23 | 2.77 | 0 | 4.03 |
| **Relative significance on total resistance (%)** | 25.40 | 5.82 | 68.78 | – | 100 |

The cost analysis plays a significant role in the application of SCMBR in specific industrial contexts and particular fouling mitigation methods. To evaluate the separation process cost, including backwash and cleaning procedures, a cost analysis was conducted for electricity and applied chemical agents (Table 6). The one-time costs, such as those for the ceramic membrane module, bioreactor, and pump, have not been included, as these are one-time expenses that can be utilized for various purposes. Regarding electricity usage and consequent costs, the bioreactor stands out as the most consuming equipment, at $0.5568 per 30 min of the separation process. Additionally, it is evident that the expense of employing the peristaltic pump for backwash is minimal, confirming its lower power consumption and, consequently, affordability as a fouling mitigation method [63]. Therefore, it might be stated that application of backwash in the proposed SCMBR is an almost negligible electrical cost. In terms of applied chemicals, the most expensive component is the use of sodium hydroxide in the cleaning procedure ($16.8), which is directly related to the large quantity of the applied NaOH solution (10 L) at a concentration of 2 %. However, considering the overall process costs and the potential utilization of the proposed SCMBR in the production of value-added products, where the amount of produced product after backwash application may significantly surpass the costs (e.g., in the production of expensive pharmaceutical products like monoclonal antibodies, etc.), this can be a considerable advantage in adopting our proposed fouling mitigation and cleaning procedure.

## 4. Conclusions

In the present work, a submerged ceramic membrane bioreactor was proposed for the separation process of *Saccharomyces cerevisiae* from aqueous suspension and a backwash method for the mitigation of fouling was tested. Homogeneous ceramic membranes with average pore size equaled 3 μm, forming a ceramic membrane module, can be an efficient tool for separation at low pressure (100 mbar) of baker's yeast as a model organism from suspension, at concentrations reaching 80 dwg/L. The Fractional Factorial Design helped to identify and estimate of key factors of backwash process. It was shown that the highest noted average flux was 102.34 L $m^{-2}$ $h^{-1}$, whereas the highest amount of the obtained net permeate volume equaled 18.69 L after 5 h of the process. Furthermore, the study demonstrated a significant increase in flux recovery after application of backwash, compared to separation process without backwash. The tested chemical cleaning procedures and the obtained results demonstrated that the special attention should be paid on the presence of additional substances, as they can significantly impact fouling mitigation and render standard chemical cleaning insufficient. The presented data shows that the proposed SCMBR and a backwash as a fouling mitigation method could be applied top wide range of cell concentration, from low to high-density systems. In this way, the cells care retained in the MBR and there is an improvement in the fermentation process becomes due to the accumulation of active cells inside of the bioreactor. Besides, the downstream processing of the effluent may become easier due to the small concentration of cells in the permeate. Although further studies are required on the larger-scale application of the proposed ceramic membrane module for separation

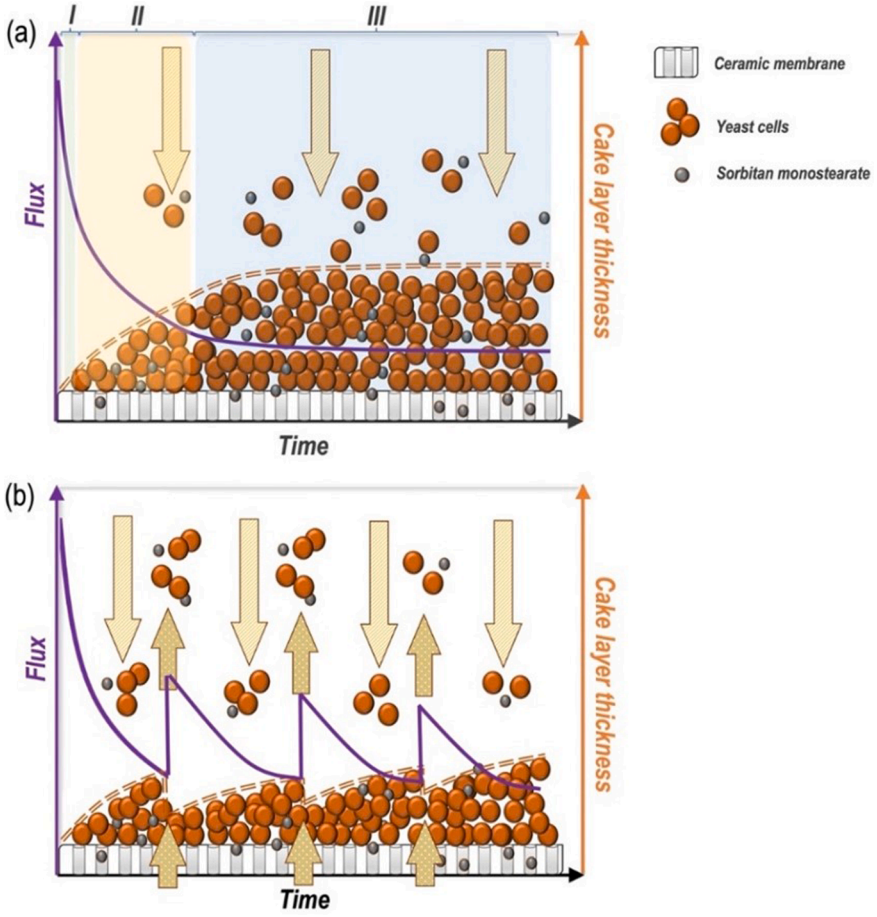


**Fig. 9.** (a) Proposed mechanism of fouling and (b) fouling mitigation using backwash.

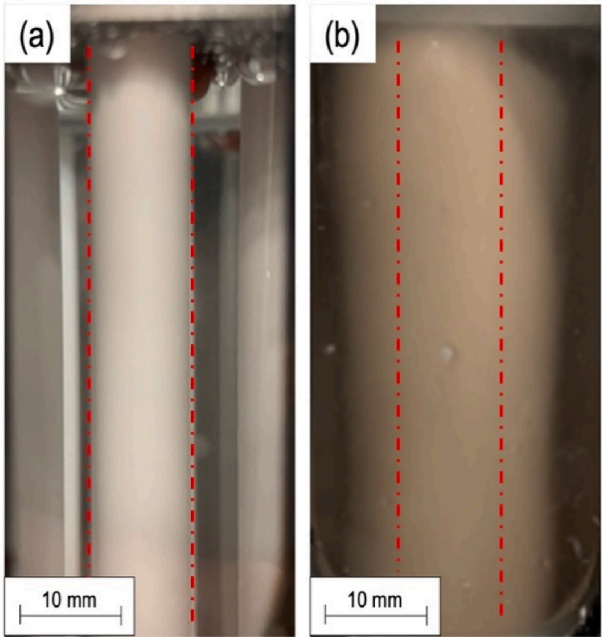


**Fig. 10.** Ceramic membrane (a) before and (b) after yeast suspension (80 dwg/L) separation process (0.5 h) without backwash. View of the interior of the SCMBR enabled by the window located in the vessel. The red mark indicates diameter of single ceramic membrane before and after separation process.

of various bioproducts from the bioreactors, the results showing

**Table 6**
Electricity cost estimation for specific general backwash conditions includes: flow rate of backwash: 375 mL $min^{-1}$, time between backwash cycle: 5 min, backwash duration: 5 sec and separation process time: 0.5 h. The cost estimation for chemical agents is based on the 'E' marked cleaning procedure, involving the application of NaOH, $H_2SO_4$, and EtOH. To calculate electricity costs, information regarding voltage and maximum current consumed by the bioreactor and peristaltic pump was obtained from data sheets provided by equipment suppliers. The electricity rate being considered is from a Danish electricity supplier for a business. The chemical agents' prices were calculated based on reagents' prices available on the market.

| Parameter | | Value ($) |
|---|---|---|
| Electricity cost | Bioreactor | 0.5568 |
| | Peristaltic pump | 0.0003 |
| Chemical agents' cost | NaOH 2 % | 16.8 |
| | $H_2SO_4$ 0.1 % | 1.3 |
| | EtOH 7 % | 1.5 |

effective fouling mitigation using backwash method could be also useful for customization of backwash conditions to conducted bioprocess. Based on the received findings, it can be concluded that the selection of process conditions depends on the concentration of cells and whether we aim to achieve the highest permeate quantity in the shortest possible time. We believe that this study has the potential to enhance existing knowledge on the application of membranes in bioreactors and effective fouling mitigation.

## CRediT authorship contribution statement

**Katarzyna Jankowska:** Writing – review & editing, Writing –

original draft, Validation, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Carlos Domingo-Félez:** Writing – original draft, Supervision, Methodology, Conceptualization. **Oscar Andres Prado-Rubio:** Formal analysis, Methodology, Writing - review & editing. **Ioannis V. Skiadas:** Writing – review & editing, Supervision, Conceptualization. **John M. Woodley:** Writing – review & editing, Supervision, Project administration, Methodology, Funding acquisition, Conceptualization. **Manuel Pinelo:** Supervision, Project administration, Methodology, Funding acquisition, Conceptualization.

## 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.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2024.126428.

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