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ORIGINAL ARTICLE

Separation of phycocyanin from *Arthrospira platensis* (spirulina) by application of ceramic microfiltration membranes

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Abstract

Phycocyanin is a phycobiliprotein that has various pharmacological properties. The nature of phycocyanin is blue, non-toxic, odorless, and slightly sweet when dissolved in water. Considering the importance and uses of phycocyanin, including oral, medicinal, and cosmetic, the aim of this research is finding a new way to extract optimal phycocyanin. In this regard, four new and economical microfiltration membranes: kaolin–zeolite (K–Z), kaolin–zeolite–fly ash (K–Z–F), kaolin–alumina (K–A), and kaolin–alumina–fly ash (K–A–F), were made by extrusion method. Some physical characteristics of the fabricated membranes were investigated. The highest porosity related to K–A–F, and the average size of the pores in the membranes was between 0.8 and 1.537 µm. SEM analysis was also performed to prove the uniformity of the membrane structure. After the cell breaking of *Arthrospira platensis* (spirulina) in water through freeze-thawing and centrifugation, the solution is purified by microfiltration. Finally, the performance of the membranes was compared with each other. K–A–F membrane had the best performance in phycocyanin purification (purity 0.91).

Keywords Ceramic membranes · Microfiltration · Spirulina platensis · Microalgae · Phycocyanin

Instruction

Microalgae are one of the most effective renewable raw materials for healthy edibles and functional food products (Matos, Cardoso et al. 2017). They can be used to increase the nutritional and pharmaceutical value, because they have a favorable effect on human health by improving the quality and also reducing the risks of illness and disease. These biological materials are rich in high value bioactive compounds (Balti, Zayoud et al. 2021). *A. platensis* (*spirulina*) is a blue–green microalgae with fast cell growth, easy harvest, and promising market, so it is often considered as a human food supplement and animal feed (Singh, Tiwari et al. 2011; Jaeschke, Teixeira et al. 2021; Lim, Khoo et al. 2023). Spirulina is an obligate phototroph of cyanobacterium that can be easily monoculture and harvested (PETTERSON 1996). Currently, spirulina is produced in China, California, Hawaii, India, Mexico, Taiwan, and Thailand (Li and Qi 1997). According to the future market insights website, the global phycocyanin market acquired a valuation of 754.40 million US$ in 2022. With consumers gravitating toward natural food-grade substances, the market is expected to reach 1487.7 million US$, garnering a CAGR of 7% from 2023 to 2033. In addition to the proven nutritional properties, spirulina has attracted the attention of the international scientific community due to its possible use as a source of medicinal and nutrient compounds (Fabre, Niangoran et al. 2022). The importance of this microalgae is due to its phycobiliproteins, known as phycocyanins, which act as components of light-collecting complexes in cyanobacteria, and red algae lack β-chlorophyll and absorb light mainly in the blue and red regions of the visible spectrum due to

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α-chlorophyll (Fernández-Rojas et al. 2014a; Cuellar-Bermudez, Aguilar-Hernandez et al. 2015; Kannaujiya, Kumar et al. 2019; Avci and Haznedaroglu 2022). Phycobiliproteins, such as phycocyanin and phycoerythrin, are considered auxiliary pigments of chlorophyll (Fernández-Rojas et al. 2014b). Recent cosmetic applications of spirulina have been reviewed in a review article (Ragusa, Nardone et al. 2021). The effect of different nutrient concentrations of a sample of spirulina planted in distilled water or seawater on the growth rate, protein, lipid, carbohydrate, pigment contents, and photosynthetic parameters of *S. platensis,* was investigated (Hong, Hien et al. 2023). The purity and yield of phycocyanin depends on the extraction, separation, purification, and quality of the raw material. Many procedures of isolating phycocyanin from microalgae have been used previously (Soni, Kalavadia et al. 2006; Silveira, Burkert et al. 2007; Kumar, Singh et al. 2009; Chittapun, Jonjaroen et al. 2020; Prabakaran, Sampathkumar et al. 2020; García, Longo et al. 2021).

Phycocyanin, with a molecular weight of 20 kDa, is known as an auxiliary photosynthetic pigment of the phycobiliprotein society, which has many commercial applications, and its market value is about 10–50 million US$ per year (Abalde, Betancourt et al. 1998; Bhaskar, Gopalaswamy et al. 2005; Chaiklahan, Chirasuwan et al. 2011). Food coloring for chewing gum, cold sweets, beverages, candies, and cosmetics such as lipstick and eyeliner are some of the uses of phycocyanin as a blue color (Herrera, Boussiba et al. 1989). Among the different components of phycocyanin, C-phycocyanin is a type of phycobiliprotein that should find a new commercial outlet when its light sensitivity can be reduced (Vonshak 1990; Kageyama 1994). Many researchers have investigated the potential therapeutic effects of spirulina and phycocyanin (Ink 1983; Iwata, Inayama et al. 1990; Li and Qi 1997; Romay, Armesto et al. 1998). Considering the importance and applications of phycocyanin, we are looking for a better and more economical way to purify this substance. In the classical methods, sonication, extraction, centrifugation, chromatography, ion exchange, and dialysis processes are generally used (Kageyama 1994; Abalde, Betancourt et al. 1998). Centrifuge extraction is usually performed with high efficiency (90%) under a low flux rate and high-energy consumption (Dassey and Theegala 2013). Sedimentation by polyelectrolytes is a time-consuming process in which relatively high biomass recovery has been observed (Granados, Acién et al. 2012). In another study, a green and sustainable technology was assessed: Freeze-dried Spirulina platensis was submitted to ultrasound-assisted extraction (UAE), where a glucose/glycerol-based natural deep eutectic solvent (NADES) was used (Martins, Mouro et al. 2023). Some other papers investigated the efficiency of extraction for nonpolar pigments, chlorophyll, and carotenoids, using SFE as a green method, before water-soluble phycobiliprotein extraction from Arthrospira biomass. The improvement in the sequential phycobiliprotein extraction of cell biomass residue after SFE evaluated by comparing phosphate and water extraction with ultrasound-assisted extraction (Pan-Utai, Iamtham et al. 2022).

The microfiltration system is one of the important membrane processes, the driving force of which is the pressure difference. Different mineral materials are used for making ceramic membranes. Kaolin or China clay is composed of kaolinite or aluminum silicate. This material has features such as high hardness, glossiness and flexibility, catalytic properties, low ability to conduct electricity and heat, whitening ability, softening ability, filling and covering, reasonable price, hardening ability, and high tolerance and invariability in acidic and basic environment (Rashad, Logesh et al. 2021; Idrissi, Elidrissi et al. 2023). Zeolites are crystalline and hydrated aluminosilicates of alkali and alkaline earth metals. Important physical and chemical properties of zeolites include: high degree of hydration, low density, and large void volume when hydrated. The applications of natural zeolite include: removal of ammonium ions from industrial wastes, removal of heavy metal ions from nuclear, mineral, and industrial wastes, gas separation, and catalysis (Fan, Zou et al. 2021). Alpha alumina appears as a white and swollen powder. The specific surface area of alpha alumina is very low, so this material shows good resistance to high temperatures. Alumina is widely used as a basic material for catalytic protection (Fan, Zou et al. 2021; Aouadja, Bouzerara et al. 2022). Fly ash is produced by rapid cooling and solidification of molten ash. Therefore, a large part of fly ash particles are in amorphous and non-crystalline state. Fly ash particles are usually spherical with a diameter of less than 1–150 μm, while cement particles are smaller than 45 μm (Sonwani, Gupta et al. 2023). The advantages of fly ash include: Fly ash is a cost-effective alternative to Portland cement, it is inherently hydrophilic, fly ash is a by-product or a waste product; hence, the use of fly ash concrete reduces CO_2, and it is environmentally friendly (Li, Chang et al. 2022). The development of microfiltration ceramic tubular membranes was studied by using activated carbon in the membrane structure to treat oily wastewater. In this research, kaolin, kaolin–zeolite, and kaolin–zeolite-activated carbon membranes were made as ceramic membranes. The results showed that the presence of activated carbon and zeolite in the membrane structure increases the permeate flux and reduces the amount of total organic carbon (Jafari, Abbasi et al. 2020). Zhu et al. studied the synthesis of kaolin-coated fly ash ceramic membrane as a low-cost raw material for oil–water emulsion separation. This group of researchers succeeded in reducing the price of raw materials and the energy consumption of cooking and construction time (Zou, Fan et al. 2021). In 2021, another group of researchers studied the design and development of a disk ceramic membrane

with fly ash waste material. The characteristics of this material include: its sphericity, cheap price, low alkalinity, pozzolanic properties, good shear strength, good quality, low carbon content, easy to use, and insensitivity to moisture (Suresh and Katara 2021). Rashad et al. researched the synthesis of kaolin microfiltration ceramic membrane to separate oil–water emulsion. In this research, they used cheap materials including: China clay, aluminum fluoride trihydrate, and alumina to make the membrane (Rashad, Logesh et al. 2021). Hong et al. studied the effect of fly ash particle size on the microstructure and mechanical strength of fly ash-based ceramic membrane. In this research, the effect of particle size on porosity, pore size, mechanical strength, and gas permeability of the membrane was investigated. The results showed that the wider particle size distribution of fly ash makes the constructed membrane have a denser structure with less porosity (Huang, Chen et al. 2023).

Despite their interest, membrane processes are rarely used for the relative purification of phycobiliproteins (Gudin 1991). The microfiltration process by cellulose membrane was tested for microfiltration of *Chlorella sp.* suspension. The result showed that the permeate flux increases with transmembrane pressure and feed rate (Ahmad, Yasin et al. 2012). The study of Babul and Takizawa (Babel and Takizawa 2010) showed that the hydraulic resistance that increases due to algae cells causes a significant increment in fouling. The price of phycocyanin products differs according to their purity ratio; it is defined as the ratio of absorbance at 620 nm to absorbance at 280 nm. The value of phycocyanin with a purity higher than 0.7 (food grade) is about 0.13 US$/mg, while analytical grade (purity above 4.0) costs up to 15 US$/mg. Herrera et al. produced phycocyanin powder with a purity ratio of 0.74 (food grade) using ultrafiltration followed by activated carbon absorption and then spray drying (Herrera, Boussiba et al. 1989). Jaouen et al. evaluated the possibility of using microfiltration and ultrafiltration to clarify the extract obtained from sonication (Jaouen, Lépine et al. 1999). For clarification and concentration steps, five membranes were used in the range of microfiltration to reverse osmosis. The content of phycocyanin in product can be reached by using drying methods such as sunlight, oven, spray, and drying with cross flow (Sarada, Pillai et al. 1999; Doke Jr 2005). The yield of phycocyanin from a combined aqueous extraction–ultrafiltration process of a food-grade strain of Arthrospira maxima was investigated (Nisticò, Piro et al. 2022).

Accordingly, after cell breaking down of the spirulina and releasing of blue–green color in water, an economical method should be found to separate the solid blue cells from the solution, so that the blue phycocyanin can be extracted from the solution with a high degree of purity. In this research, four new and economical extruded ceramic membranes: kaolin–zeolite (K–Z), kaolin–zeolite–fly ash (K–Z–F), kaolin–alumina (K–A), kaolin–alumina–fly ash (K–A–F), were used for the purification of phycocyanin, and their performance was compared with each other. In order to achieve a membrane with a good structure and without cracks, the membrane fabrication test was repeated until the best composition percentage was obtained.

Material and methods

Preparation of ceramic membranes

Kaolin was obtained from Zenus mine in Marand city of the East Azarbaijan Province, from Iran's China Soil Industries Company. The analysis of the kaolin is reported in the natural zeolite which was obtained from the Semnan mine. Alpha alumina was obtained from the Iranian Nano Materials Company in Mashhad, Khorasan Razavi Province. The fly ash was obtained from Shahr Beton Company in Tehran. The analysis of these material is reported in Table 1. In order to extract and purify phycocyanin, four types of ceramic microfiltration membranes: kaolin–natural zeolite, kaolin–zeolite–fly ash, kaolin–alpha alumina, and kaolin–alpha alumina–fly ash. These materials were chosen due to their availability, applications, and reasonable price. Repetition of the test determined the best combination ratio of these materials to make a strong membrane without cracks and breakage. The best composition ratios for each membrane are:

K–Z: Kaolin 90%–zeolite 10%

K–Z–F: Kaolin 80%–zeolite 10%–fly ash 10%

K–A: Kaolin 90%–alumina 10%

K–A–F: Kaolin 70%–alumina 10%–fly ash 20%

The materials were mixed in the proportion mentioned in Table 2 and thoroughly mixed until homogenous. Edible starch was added as an adhesive and cavity maker for all membranes in 8%, then distilled water added (40% of the weight of the soil). Extrusion is one of the shaping methods used to reduce the thickness or cross-sectional area of materials. The prepared dough is sent into the machine by manual pressure, and at the end of the machine, it comes out in the form of tubes with an inner diameter of 10 mm and an outer diameter of 14 mm. After the extrusion process, the membranes were placed at the room temperature for 48 h to dry. Then, it was cut into 23–25 cm. The membranes were baked in an electric furnace with a specific temperature gradient (final temperature: 1250 °C). The RS 80/300/13 Nabertherm model electric furnace with a programmable range made in Germany, which can adjust temperature and time, was used for the membrane calcination process. After baking and increasing the physical strength of the membrane, the cooling process begins in a way that prevents thermal shock to the membrane. In this step, the membrane reaches the

Table 1 The chemical analysis of the material

Analysis	Kaolin	Zeolite	Alpha alumina	Fly ash
Specific surface area [m^2g^{-1}]	–	26.458	5–10	0.5078
Mean pore diameter	–	15.675 nm	75 µm	17.574 A^0
Moist%	–	–	0.3	–
AL_2O_3%	24	11.412	99	28.239
SiO_2%	63	70.937	0.012	62.86
Fe_2O_3%	0.55	1.479	0.012	3.774
TiO_2%	0.04	–	–	1.886
CaO%	1.2	3.251	0.012	0.787
MgO%	0.4	1	–	0.82
Na_2O%	0.3	2.177	0.43	–
K_2O%	0.4	0.907	–	1.128
P_2O_5	9	–	–	0.508
L.O.I	9	8.2	0.5	1.2

Table 2 The chemical composition of Zaruk cultivation medium in terms of g L^{-1}

Nutrients	Amount per liter of water
$NAHCO_3$ (sodium bicarbonate)	16.8 g
K_2HPO_4 (dipotassium hydrogen phosphate)	0.5 g
$NaNO_3$ (sodium nitrate)	2.5 g
K_2SO_4 (potassium Sulfate)	1.0 g
$MgSO_4$. 7 H_2O (Magnesium sulfate)	0.20 g
$CaCl_2$ (calcium chloride)	0.04 g
$FeSO_4$. 7H_2O (ferrous sulfate)	0.01 g
EDTA (ethylenediaminetetraacetic acid)	0.08 g
Solution 1	1 ml
Solution 2	1 ml

Table 3 The chemical composition of solution 1

Nutrients (solution 1)	Amount per liter of water
H_3BO_3 (boric acid)	2.86 g
$MnCl_2$. 4 H_2O	1.81 g
$ZnSO_4$ (zinc sulfate)	0.22 g
$CuSO_4$ (copper sulfate)	0.08 g
MoO_3 (molybdenum oxide)	0.01 g

Table 4 The chemical composition of solution 2

Nutrients (solution 2)	Amount per liter of water
NH_4VO_3 (ammonium vanadate)	229. 10^{-4} g
K_2Cr $(SO_4)_4$. 24 H_2O (chrome alun)	960. 10^{-4} g
$NiSO_4$. 7 H2O (nickel sulfate)	478. 10^{-4} g
Na_2WO_4 (sodium wolframite)	179. 10^{-4} g
Co $(NO_3)_2$. 7 H_2O (cobalt nitrate)	44. 10^{-4} g

ambient temperature for 3 h. Figure 1 shows the final fabricated membranes.

Preparation of Spirulina platensis

The cyanobacteria microalgae Spirulina *platensis* was a gift from Persian Gulf Algae Development and Technology. Spirulina contains significant bioactive molecules, proteins with essential amino acids, unsaturated acids such as linoleic, vitamin (E, B12), polysaccharides, mineral salts (Na, K, Ca, Fe, Mn, and Se), and pigments (phycocyanin, chlorophyll, lutein, beta-phycocyanin, aloe zasanthin, and carotene), which in the past decades have been the focus of researchers and industrialists in addition to food items for industrial use (Sanchez, Bernal-Castillo et al. 2003; Chen 2011; Ravelonandro, Ratianarivo et al. 2011; Patel and Goyal 2013; Andrade, Cardoso et al. 2019; Ragaza, Hossain et al. 2020). Spirulina strain was prepared as 100 ml of liquid medium from Fars Gulf Algae Technology Development Company located in Bushehr, Iran. The culture medium was prepared and 10 ml of it was used for cultivation in a volume of 100 ml. Five ml of the original stock with an absorption coefficient of 0.9 was added to a 250 ml Erlenmeyer flask; double distilled water at a temperature of 121.8 °C and a pressure of 2 bar was used to increase the volume to 100 ml. This strain was first grown and kept in a 250 ml Erlenmeyer flask with Zaruk medium at room temperature. The chemical composition of Zaruk cultivation medium in terms of g L^{-1} is shown in Tables 3, 4, and 5. The chemical solution of culture medium was prepared with distilled water. During the cultivation period, the temperature was kept constant at 26 °C and fluctuating ± 2 °C. Illumination was done with an LED lamp with a light intensity of 40µE

Table 5 The average pore size of the ceramic membranes

Membrane	The average pore size r_m (μm)
K–Z (90–10)	1.537
K–Z–F (80–10–10)	0.881
K–Al (90–10)	0.912
K–Al–F (70–10–20)	1.016

Fig. 1 The final fabricated ceramic membranes

m-2 s-. The exposure time was considered based on 16 h of light and 8 h of darkness. The cultures were regularly aerated with an air pump uniformly. The culture medium was sterilized by autoclaving at 121 °C. Inoculation was done from the original culture medium in the amount of 10% to each Erlenmeyer. Spirulina was cultured for 14 days. The pH of the culture medium was set to 10 and measured daily with a pH meter. Sampling of the culture medium was done regularly to measure the growth rate by measuring the optical absorption (OD) using a spectrophotometer at a wavelength of 560 nm with a visible and ultraviolet spectroscopy spectrophotometer (UNICO MODEL: UV2100). After sampling, distilled water was added to the culture medium as much as the harvested volume. After a period of 14 days, the samples were poured into centrifuge tubes with a volume of 50 ml and then centrifuged at 1500 rpm. The samples were washed with distilled water and filtered with Whatman filter paper and then dried at 45 °C for 24 h. Various culture mediums have been introduced for Spiro Lina, but the best one is Zaruk culture medium. The basic needs of spirulina are:

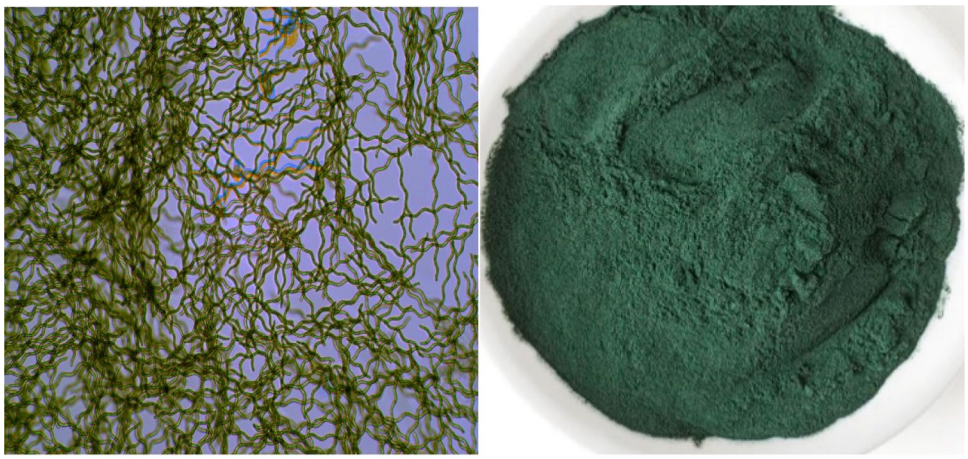
- Alkaline water
- Fixed nitrogen source
- Source of iron, potassium, phosphorus, and sulfur
- Carbon source (to provide carbon dioxide)
- Source of calcium, chlorine, and magnesium

Photo of Spirulina platensis under light microscopy and the green spirulina powder used in this research can be seen in Fig. 2.

Twenty-five gr of biomass poured into 500 ml of 0.1 M phosphate buffer solution, which was placed in a rotary stirrer for 6 h. Then, it was placed in the freezer for 12 h (Fig. 3). After twice freezing and thawing, the sample centrifuged at a 4800 rpm for 10 min. Finally, after centrifugation, the sample entered the membrane microfiltration system.

Membrane microfiltration and experimental system

The membrane microfiltration process was carried out using a feed tank, pump, pressure gauge, membrane module, product collection container, and digital scale. The Italian Pentax model pump directs the effluent from the tank to the module.



(a) (b)

Fig. 2 **a** The photo of Spirulina platensis under light microscopy + 60- day 10 of culture and **b** the green color Spirulina platensis powder

Fig. 3 The crude extract of spirulina

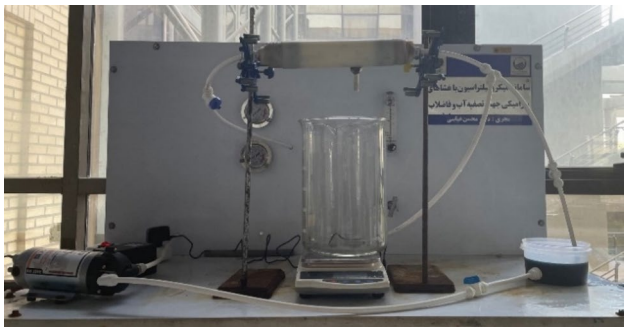


Fig. 5 Microfiltration membrane system

A part of the fluid is removed as a product, and the rest is returned to the feed tank as the residue of the module. A valve is installed at the output of the module to adjust the pressure. The schematic of the experimental setup is shown in Fig. 4. A tubular module of Teflon material is designed for 25 cm tube membrane, with 10 mm inside diameter and 14 mm outside diameter. This module includes input, residual output, and product output (filtrate) (Fig. 5). The process was carried out in the pressure range of 1–4 bar. By measuring the amount of permeate by a digital scale every 5 min (for 1 hour), and having the membrane area, the amount of flux was calculated.

Characterization

To calculate the porosity percentage of each membrane, the length, inside and outside diameter of the membrane were first measured. The weight of the membranes was measured after drying for 5 h at 150 °C. After that, the membranes are placed in distilled water for 3 h until all the holes are filled. Then, the water of all membranes was dried by filter paper, and its weight was measured again to obtain the weight of the wet membrane. The following relationship was used to determine the porosity of the ceramic membranes (Bhattacharya, Ghosh et al. 2011):

$$\in = \frac{v_s}{v_t} \tag{1}$$

$$v_s = \frac{M_w - M_d}{\rho_w} \tag{2}$$

In Eq. (1), $\in$ is the porosity in percent, v_s is the volume of the empty spaces of the membrane in cm^3, and v_t is the total

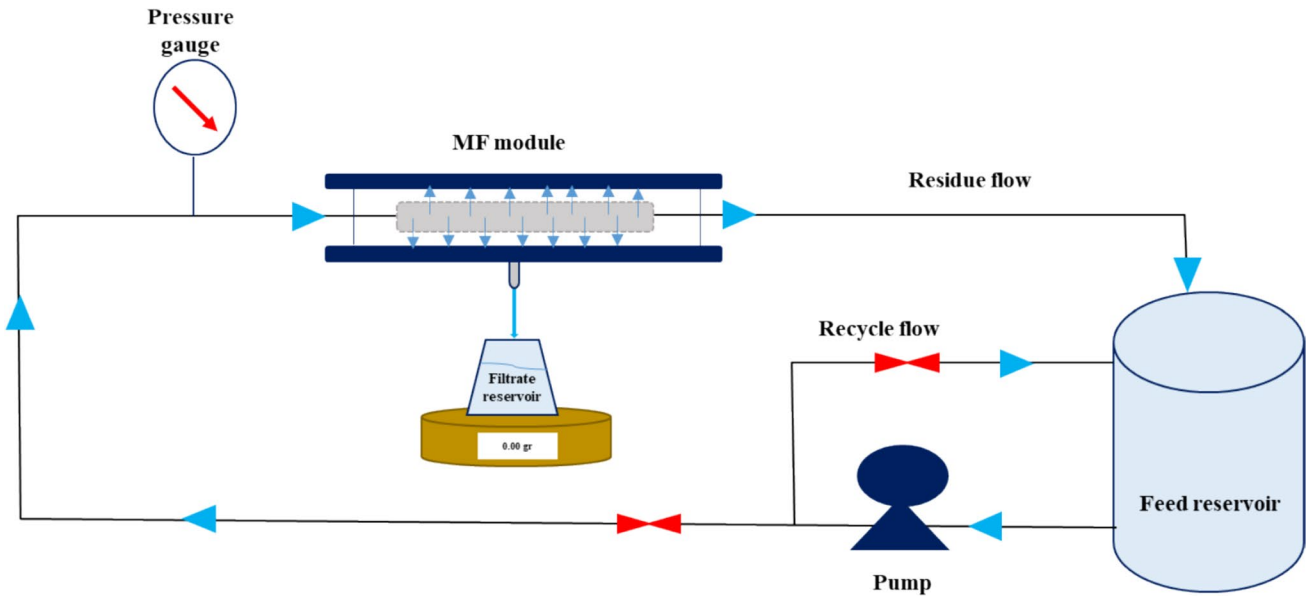


Fig. 4 The schematic of the experimental system

volume of the membrane in cm^3. In Eq. (2), M_w is the weight of the wet membrane in g, M_d is the weight of the dry membrane in g, and ρ_w is the volumetric mass of water in g/cm^3.

In order to calculate the average pore size, the permeability flux of distilled water at pressures between 1 and 4 bar and the percentage of membrane porosity were first calculated. Then, the average pore size was calculated based on the Guerout–Elford–Ferry equation (Li, Cao et al. 2020):

$$r_m = \sqrt{\frac{(2.9 - 1.75 \in)8\mu Lj}{\in TMP}} \quad (3)$$

Which r_m in Eq. 3 is the average pore size in μm, ϵ is the percentage of porosity, μ is the viscosity of water at the operating temperature (8.9×10^{-4} Pa.s), L is the membrane thickness in meters, j is water permeability flux in L/m^2 h, and TMP is the transfer pressure of the membrane (transmembrane pressure) in kpa.

A Perkin Elmer Lambda 25 UV/VIS spectrophotometer used to analyze the content of phycocyanin in the filtrate and residue (1979) (Boussiba and Richmond 1979). The analysis was carried out at wavelengths of 280 and 620. The purity ratio was determined by spectrometry as A620/A280.

Results and discussion

Ceramic membrane porosity

Figure 6 presents the porosity test results of K–Z, K–Z–F, K–A, and K–A–F membranes.

It can be seen, that by adding fly ash, the percentage of membrane porosity has increased slightly. The membrane with alpha alumina, instead of natural zeolite, has more porosity since alpha alumina particles are more significant than natural zeolite. This is probably due to the process of calcination and reaction for natural zeolite at temperatures below 1250 °C, while it happens at a higher temperature for alpha alumina. The more porosity of the ceramic membrane resulted in better mechanical resistance, durability and selectivity, and easier cleaning of the membrane (Surya, Sembiring et al. 2024).

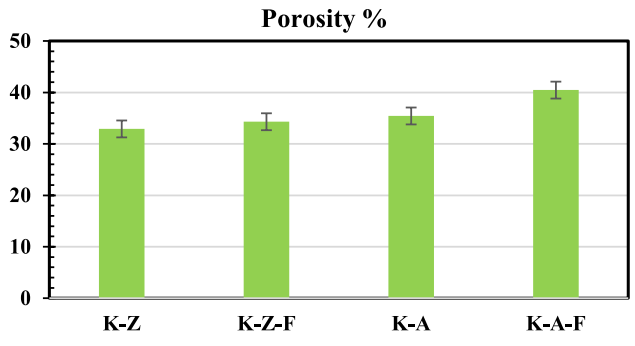


Fig. 6 The porosity percentage of the four ceramic membranes

Average pore size of membranes

The average pore size of the four ceramic membranes is presented in Table 2.

Scanning electron microscope analysis (SEM)

Figure 7 shows the scanning electron microscope (SEM) images for the inner surface of K–Z–F and K–A–F, before the microfiltration operation. This figure shows that the porous surface has a uniform structure without cracking. According to the SEM results, the average diameter of the holes is about 2 μm, which is consistent with the calculations. The K–A–F membrane has a higher percentage of porosity due to the larger size of the alpha alumina particles than natural zeolite. Obviously, for both of the membranes, fly ash is well dispersed in the membrane structure.

The scanning electron microscope image for the inner surface of the membranes after the microfiltration operation is shown in Fig. 8. According to this figure, most of the cavities of the both membranes are precipitated after microfiltration. In K–Z–F, due to the higher roughness, more sedimentation occurred.

Permeate flux

Color extraction from green algae is somewhat tricky, due to the resistant of the cell wall and the small size of the cell, so in order to extract phycocyanin from spirulina algae, the microalgae cell wall must be firstly broken. There are different methods to break the cell wall and release phycocyanin. Among the existing methods, using buffer in extraction was better due to maintaining the protein nature of phycocyanin and its less degradation under these conditions. Furthermore, among buffers, phosphate buffer has shown better results regarding the amount and purity of phycocyanin, and it is chosen as the best extracting solution (Kamble, Gaikar et al. 2013; Munawaroh, Anwar et al. 2023; Zhuang, Chew et al. 2023). After the spirulina cell breakdown in water, the microfiltration process was carried out by all four types of membranes at an operating pressure of 4 bar and ambient temperature, the permeate flux passing through the membrane was calculated at different times. Figure 9 shows the changes in the permeate flux in terms of time for the K–Z, K–Z–F, K–A, and K–A–F membranes.

The difficulty of fractionation through tangential microfiltration cell fragments and high molecular weight soluble proteins including c-phycocyanin was explained. The larger particles cause clogging and inside fouling by the

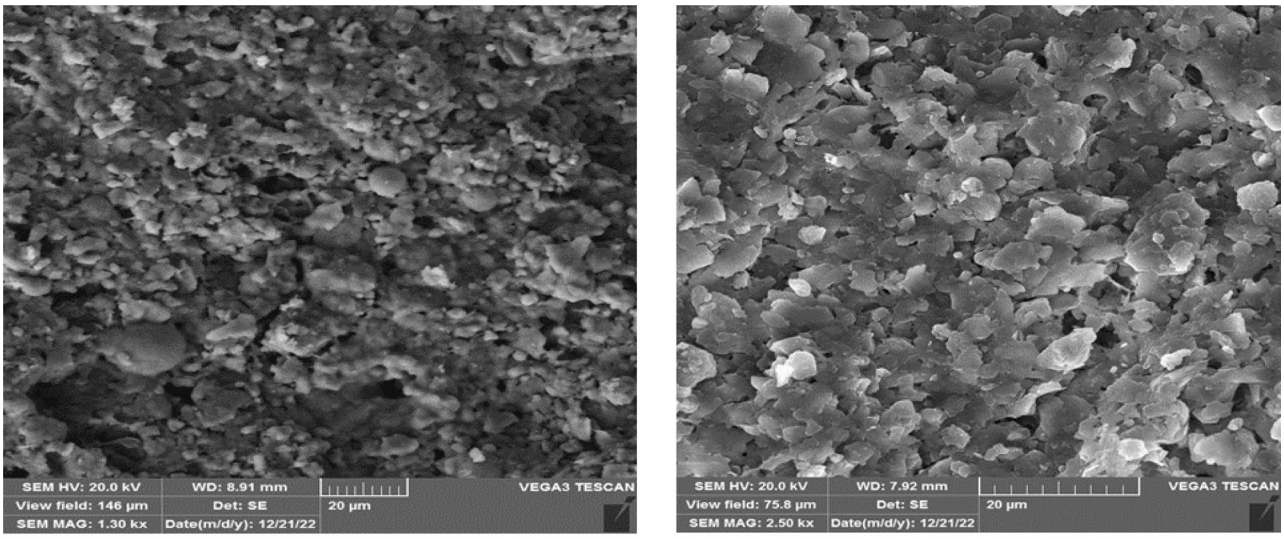
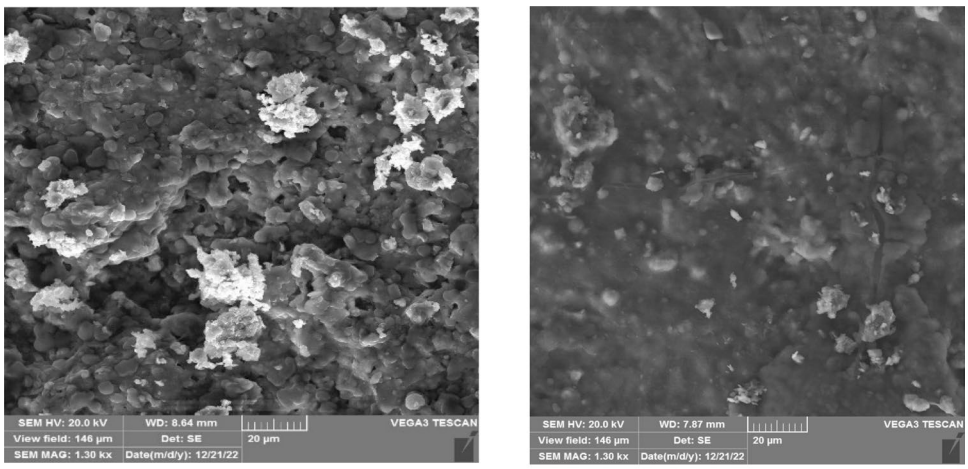


Fig. 7 The SEM images of inner surface of the **a** K–Z–F and **b** K–A–F before microfiltration

Fig. 8 The SEM images of inner surface of the **a** K–Z–F and **b** K–A–F after microfiltration



time (Jaouen, Lépine et al. 1999). The permeability flux decreased with time, due to the spirulina particles blocking the inner surface of the membrane. Moreover, after the addition of fly ash particles, the permeability flux increases due to the increase in the average size of the holes and the inherent hydrophilicity of fly ash. According to Fig. 10, the highest permeation flux is related to the K–Z–F and K–A–F membranes.

Purity of phycocyanin

Spectrophotometric analysis was performed to determine the purity ratio of phycocyanin. The purity was 0.33 before microfiltration. After the process, the purity of phycocyanin in the permeate solution was measured, and the results are shown in Table 6.

As can be seen, the purity ratio in the solution coming out from the alumina membrane was higher than that of the zeolite membrane. This issue can be due to the presence of more porosity in the membranes that were made of alumina. For the membranes with fly ash, the purity ratio of phycocyanin is increased. Therefore, the highest purity ratio was obtained from the K–A–F membrane (0.91), which can be used in food industries. The extracted blue solution from the membrane microfiltration process is shown in Fig. 10.

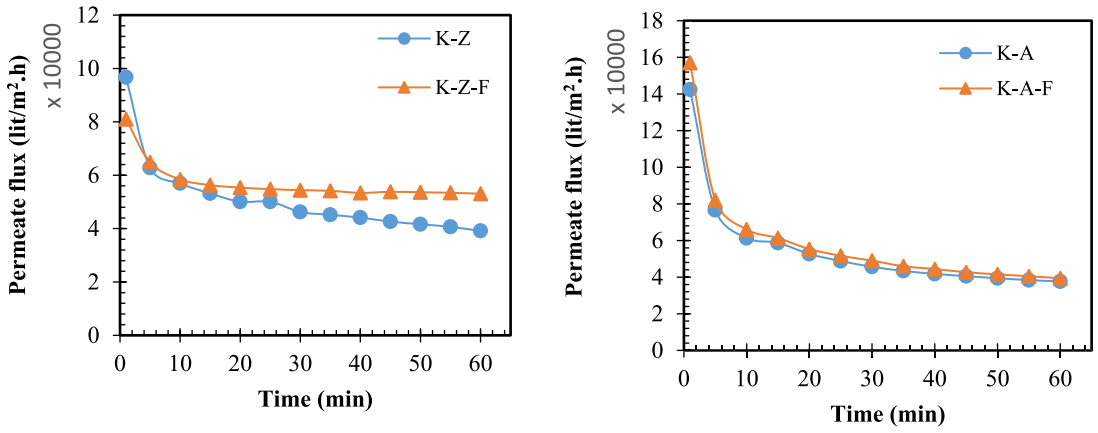


Fig. 9 Permeate flux through K–Z, K–Z–F, K–A, and K–A–F membranes in terms of time

Fig. 10 The extracted blue color phycocyanin

Table 6 Results of the spectrophotometric analysis

Membrane	A620	A280	Purity ratio (A620/A280)
K–Z	2.1141	3.2525	0.65
K–Z–F	3.2917	3.7909	0.87
K–A	2.8899	3.8026	0.76
K–A–F	3.3709	3.6876	0.91

Conclusion

Four new and economical ceramic membranes were made and utilized to extract and purify phycocyanin from Spirulina platensis by microfiltration. The physical characteristics of the fabricated membranes were investigated. SEM analysis before and after the microfiltration showed the uniform structure of the membranes without any cracks. The permeate flux of membranes decreased with time, because the spirulina particles clogging the inner surface of the membrane. The highest permeation flux was related to K–Z–F and K–A–F membranes.

It is concluded that the presence of fly ash in the membrane structure, in addition to being economical, can increase the purity of the extracted phycocyanin. The highest purity of phycocyanin was related to the output from K–A–Z and K–Z–F membranes, and spectrophotometric analysis showed that this value reached 0.91 (the highest value) in K–A–F membrane. Considering the importance and application of phycocyanin, microfiltration by K–A–F ceramic membrane can be used for better and more economic extraction of this substance on larger scales.

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Declarations

Conflict of 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.

Ethical standard For this type of study formal consent is not required.

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