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Membrane based separation and purification of fusarubins from *Fusarium solani*

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ABSTRACT

The use of fugal secondary metabolites is extensive throughout several industries, and the compounds are often extracted using loads of harmful organic solvents. The issues with several different and also similar products produced by the same biosyntethic pathways are challenging the downstream separation and purification, especially when scaling up production for the industry. The main objective of this study was to investigate the separation, concentration and purification possibilities of four different valuable fungal pigments produced by *Fusarium solani*. We present a full membrane based filtration train to elucidate which membrane type can be useful in the separation and concentration of the compounds. This visualized the possibility if using microfiltration to concentrate bostrycoidin and also to separate fusarubin from the rest of the pigments. Also, a comparison study between three types of microfiltration membranes is presented, showing little to none difference in the investigated membranes. Lastly, a high concentration- high recovery filtration study is presented, concentrating bostrycoidin in an even higher concentration. It is shown in this paper, that it is possible to use membrane filtration to separate, concentrate and purify the fusarubins investigated.

1. Introduction

Natural products, such as secondary metabolites, represent a large variated collection of chemical compounds, that can be attributed to a wide display of biological activities [1,2]. A subdivision of natural products is called polyketides, which accommodate large structural diversity and have been investigated extensively for many years, due to their bioactivity and industrial applicability in the pharmaceutical industry, agriculture and food industry [3–5]. In recent years, other fields have shown interest in polyketides such as electrolyte production for energy storage [6–8]. Filamentous fungi have been attributed to the production of many different polyketides with several specific uses such as antibacterial properties like penicillium [9], carcinogenic toxicity like aflatoxin [10], fusarielins [11] and lovastatin which is used for lowering cholesterol [12,13]. Some of these polyketides are also produced as pigments, which has shown great potential for additional value in production in biorefineries [14–16], but also as a natural alternative for syntactically produced pigments which show limitations [17]. *Fusarium* species have long been studied for the vast amount of secondary metabolite production [18–20], which also includes several quinone-like pigments depending on subspecies [21–23]. The PKS3; *fsr1* gene cluster is present in all genome sequenced *Fusaria* and has been assigned to the production of the pigments 8-methylfusarubin in *F.fujikoroi* [24], and bostrycoidin in *F.graminearum* [25]. Both compounds, however, share the same precursor in their biosynthetic pathway, and the common intermediate is believed to be a C14 heptaketide-aldehyde [23,26]. A common pigment also found is fusarubin, which can be produced from dihydrofusarubin by a spontaneous nonenzymatic oxidization step occuring under alkaline conditions [18,27]. The compound anhydrofusarubin has been shown to be produced by a nonenzymatic dehydroxylation under acidic conditions [28].

Fungal secondary metabolites, polyketides and pigments are produced by the fungi as supplementary products to a variety of different compounds, which from a production perspective makes the purification difficult. Conventionally, large amounts of organic solvents as chloroform, acetone, methanol and hexane are being used for the extraction of secondary metabolites and pigments [29–32]. For industrial-scale applications, the use of large amounts of low selectivity organics extraction

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solvents is often used which both present economic, safety and environmental challenges. Membrane filtration offers competitive, economical [33], less time and energy consuming down steam processing steps for upscale production in biotechnological industries [34]. Pressure-driven membranes have been used for separation and extraction of natural products such as vitamin C [35,36], and pigments (betaxanthines [37] and betacyanines [38]). Benzylpenicillin or penicillin G is also produced using membrane filtration of the fermentation broth a part of the downstream process [39,40] as is the production of lactic acid [41–43]. Pressure-driven membrane filtration has also been used for concentration of compounds at large-scale productions, which is presenting a mature technology possessing multiple uses [44]. Membranes are often characterized by the molecular weight cut-off, MWCO, which is determined by the molecule weight where the compound is rejected by 90% [45].

Conventionally, microfiltration (MF) is used to remove organic matter and large impurities, recover larger macromolecules such as carbohydrates and proteins, and is often used as a pretreatment step [45]. Ultrafiltration (UF) has been used to recover specific compounds, such as insulin and other valuable compounds [34,39], whereas nanofiltration (NF), has been used for recovery of specific low molecular weight molecules e.g. polyphenols [34,46], NF is also widely used in water softening, desalination and wastewater treatment, but also for separation of amino acids from fermentation broths, e.g. L-glutamine [47]. NF has shown high separation of small charged molecules due to selectivity in the membranes, which has made NF an important advance in the industry [48]. The reverse osmosis membranes (RO), are used to remove ionic compounds. The two standard operation modes for membrane filtration are used widely, dead-end filtration and cross-flow filtration. At cross-flow mode, the fluid flows in parallel with the membrane surface, and permeates through due to pressure difference, this way reducing the filter cake formation significantly when compared with the dead-end mode where the fluid flows directly through the membrane, hence the flux can be increased for a longer period of time [45,49]. Membranes are often used as a downstream process in connection with other types of extraction, such as solvent extraction and the use of polymeric resins [50,51], thereby making it possible to combine membrane filtration with earlier published production methods for fungal pigments [32].

The aim of the study was to evaluate the potential of integrating cross-flow membrane filtration systems as a part of the extraction and purification of pigments produced by *Fusarium solani*. Stepwise filtration in the sequence MF-UF-NF-RO were used to study the fractionation of the compounds and different polymeric membrane materials were used in order to study the influence of the membrane material properties on the performance of the process with respect to specific compound rejection and permeation flux.

2. Materials and methods

2.1. Secondary metabolite production and properties

For the production of the compounds in this paper, we used the *Fusarium solani* mutant 77–13-4 OE::*fsr6* G418^R (Nielsen et al 2019 [52]).

The production of secondary metabolites or pigments was based on earlier published studies [32], which elucidated the optimized media composition to selectively produce chosen quinone pigments. In this study, we applied the media composition that favored the production of bostrycoidin. Three other pigments were produced in parallel and still present in the media, however in low concentrations levels when compared to bostrycoidin. The amount of media was scaled up to produce 4.0 L and 4.5 L fermentation broth, for the first and second experimental run, respectively, the pH was adjusted to 5.5 ± 2 and the fungi incubated for 7 days. For the third experiment, we used the combined retentates of experiments 1 and 2, to lower the waste of valuable products and media components, and to achieve as a high concentration of the desired products as possible. Data for the four different pigments are listed in Table 1.

The water solubility of the molecules is not listed in the literature, however, earlier published data [6], give an indication of the relative aqueous solubility by the solubility descriptor, ΔG_{solv}, where the more negative the value, the higher the solubility. Estimated pK_a values have been computed by the program Marvin sketch [53], and the numbers refer to the specific functional group on the molecule as indicated in Fig. 2B. The hydrophobicity/hydrophilicity of the pigments is also presented in Table 1 based on the octanol/water partition coefficient, Log K_{ow}.

2.2. Membrane properties

In the first part of this study, we investigated four commercially available flat sheet membranes, Table 2; the MF membrane, Synder V0.1 (Sterlitech), the UF membrane, Synder MQ (Sterlitech), the NF membrane, Alfa Laval NF99HF (Alfa Laval) and the RO membrane, Dow Filmtech XLE (Sterlitech). The pure water flux was measured prior to every experiment, however, it was not possible to measure the pure water flux for the MF membranes. The rest of the data was reported by the manufacturers.

The pure water flux was determined using the cross-flow filtration set up, measured over a time of 5 mins where Milli-Q water was filtered through the membrane and monitoring the mass of permeate. For the second experiment, two additional MF membranes, as presented in Table 3, were compared together with the MF membrane used in experiment one. The second MF membrane was a Trisep MF01 (Sterlitech) and the third was Microdyn Nadir MV020 (Sterlitech). The main difference between V0.1 and MF01 is the polymer used in the membrane, where V0.1 is polyvinylidene fluoride (PVDF) and MF01 is polyethersulfone (PES). MV020 is also made from PVDF, but a larger declared pore size, and a slightly different pH stability window.

For the third part of the study, The V0.1 membrane was chosen to investigate long term filtration.

2.3. Membrane filtration

The cross-flow filtration set-up, FT17-50 unit, Armfield (UK), illustrated in Fig. 1, was used in all experiments. The system is controlled by a computer and all data is logged. For each experiment, a membrane disc of 90 mm in diameter (effective surface area 63.6 cm^2) was used. Prior to each experiment, all membranes were flushed for two minutes in Milli-Q water to ensure the protection film was removed. At each experiment, Milli-Q water was used to establish pure water flux. The system temperature was controlled by a cooler element and was stabilized at 22 °C. The system was thoroughly flushed with Milli-Q water and 70% ethanol after each experiment.

The first experiment was conducted as a filtration train (MF-UF-NF-

Table 1
. Data for the pigments investigated in this study. The pk_a values I-III refers to Fig. 2B, where the related functional group is indicated. Pka values are estimated using Marvin sketch [53], at 25C°.

	MW [g/mol]	ΔG_{solv} [kJ/mol] [6]	pK_a I	pK_a II	pK_a III	Log K_{ow}
Fusarubin	306.27	−51,82	8.14	9.94	12.20	1.4 [54]
Javanicin	290.27	−35,60	8.42	10.35	–	2 [55]
Bostrycoidin	285.26	−29,26	8.61	10.56	2.63	2.6 [56]
Anhydrofusarubin	288.26	−45,48	7.94	9.69	–	2.4 [57]

Table 2
. Data for the membranes used in the first part of the study as obtained from the material data sheets.

Flat sheet membrane	MF	UF	NF	RO
	Synder V0.1	Synder MQ	Alfa Laval NF99HF	Dow Filmtech, XLE
Typical feed	Industrial/Dairy	Industrial	Dairy, pharma/ biotech, beverage	Brackish Water
Type	"Intermediate", Fat/microbial removal, protein fractionation	Protein, Beverage clarification	Concentration and purification	Extra-low energy
pH range	1–11	1–10	3–10	2–11
Pure water Flux [$Lm^{-2}h^{-1}$]/ bar	–	21 ± 2	17 ± 1	8 ± 1
MWCO/ Pore size	0.1 µm	50.000 Da	300 Da	≈ 100 Da
Polymer	PVDF*	PES**	PET***	PA-TFC****

* PVDF: Polyvinylidene fluoride ; ** PES: Polyethersulfone ; *** PET: Polyethylene terephthalate ; **** PA-TFC: Polyamide thin film composite

Table 3
. Data for the additional two MF membranes, for the second part of the study as obtained from the material datasheets.

Flat sheet membrane	Trisep, MF01	Microdyn Nadir, MV020
Typical feed	Dairy/Process	Environment, paint, paper, metal, chemical, pharma/ biotech
Type	Food & Dairy, Concentration of macromolecules and large organic solutes	High stability against oxidizing agent
pH range	1–12	2–11
Pore size	0.1 µm	0.2 µm
Polymer	PES	PVDF

RO), where all individual cross-flow batch filtrations was conducted with 50% recovery, which indicates that 50% feed volume goes to both the permeate and the retentate respectively. The minimum volume of feed in the vessel for the pumps to operate was approximately 250 mL. The initial feed volume was 4.0 L, where 2.0 L passed through the MF at 2 bar transmembrane pressure (TMP), leaving 2.0 L as the retentate. The 2.0 L permeate was used for the next batch filtration with the UF membrane at 4 bar TMP, yielding 1.0 L retentate and 1.0 L permeate. The 1.0 L permeate was used for the NF batch filtration, which was run at 10 bar TMP resulting in 500 mL retentate and 500 mL permeate. This permeate was used for the RO membrane filtration, which was conducted at 10 bar TMP. 10 mL samples was taken from the feed vessel for further extraction and analysis at each step, starting from the initial feed sample. The experiment was performed three times (triplicate). A flow chart of the experiment is seen in Fig. 2D.

The second part of experiments with the three different MF membranes was conducted in a similar fashion to the first experiment, and all batch filtrations were performed at 2 bar TMP. However, the initial feed volume was adjusted to the minimum needed 500 mL to save fermentation broth. 50% recovery was used in this experiment as well. Samples of 10 mL were taken at each step. The experiment was performed in triplicate, using a fresh membrane coupon every time. A scheme of the experiment is found in supplementary information Figure S1.

The purpose of the third experiment was to investigate how the process behaved during a high concentration high recovery experiment. All the retentates from the completed MF batch filtrations were collected and mixed into a highly concentrated feed solution. Triplicate samples of 10 mL were taken from the feed, the retentate and the permeate. The experiment was conducted with a permeate recovery of 90%. The feed volume was 8.1 L, 7.3 L was filtered through the MF1 membrane as permeate, and 800 mL was maintained as retentate. The experiment was performed at 2 bar TMP. A scheme of the experiment is found in supplementary information Figure S2.

The permeate flux was logged by a computer and was calculated using Eq. (1).

$$J = \frac{V_P}{A \times t} \tag{1}$$

where, V_P is the volume of permeated water, A is active membrane surface area and t is the time of filtration.

The apparent rejection of the fusarubins was caluculated using Eq. (2).

$$\text{Rejection}\% = \left(1 - \frac{C_P}{\frac{1}{2}(C_F + C_R)}\right) x100 \tag{2}$$

Where, C_P is the concentration of permeated compound, C_F is the feed concentration and C_R is the concentration in the retentate.

The relative mass percentage is described using Eq.3

$$\text{Relativemass}\% = \frac{m_S}{m_F} x100 \tag{3}$$

where m_s is the mass in the sample, either permeate or retentate, and m_F is the mass in the feed solution.

2.4. Extraction and analysis

All samples taken out during experiments were extracted using liquid–liquid extraction procedure and analyzed, for concentration levels of fusaruibin, javanicin, anhydrofusarubin and Bostrycoidin, using HPLC-DAD system as described in our previously published paper [32].

3. Results and discussion

In Fig. 2A the initial MF scheme of filtration is illustrated. In order to investigate whether it was possible to concentrate and separate the four quinone pigments produced by *F.solani,* Fig. 2B**,** we used the optimized media found in our previously published work, [32]. We used the media optimized to favor the production of bostrycoidin. Fig. 2C shows the chromatograms of the MF feed, retentate and permeate samples illustrating the change in composition of the four different quinones indicated by colour. It is seen that there is a clear visual separation of the compounds, where fusarubin passed through the MF membrane to a much greater extent than the other compounds. Bostrycoidin remained primarily in the retentate and got concentrated. A number of unidentified peaks is also seen in the chromatograms, these peaks can be attributed to different other compounds, which is produced by the same biosynthetic pathway. The peaks have not been included in the calibrations and are therefore not quantified. In Fig. 2D the full separation train is illustrated as a scheme. The significant colour change throughout the separation is visualized, from the initial sample to the dark concentrated retentate and light orange permeate from the MF membrane. The NF membrane almost removes all of the pigments from the solution, leaving only little to be retained in the RO retentate, permeating no pigments at all.

Analysis of the initial fermentation broth sample show indeed that as intended, bostrycoidin was present in the highest concentration (144.1 mg/L), fusarubin and anhydrofusarubin followed with 5.2 and 5.5 mg/L respectively, and javanicin was present in the lowest concentration (2.4 mg/L on average). All specific concentrations for all experiments can be seen in the supplementary information Table S1-3 and visualized in

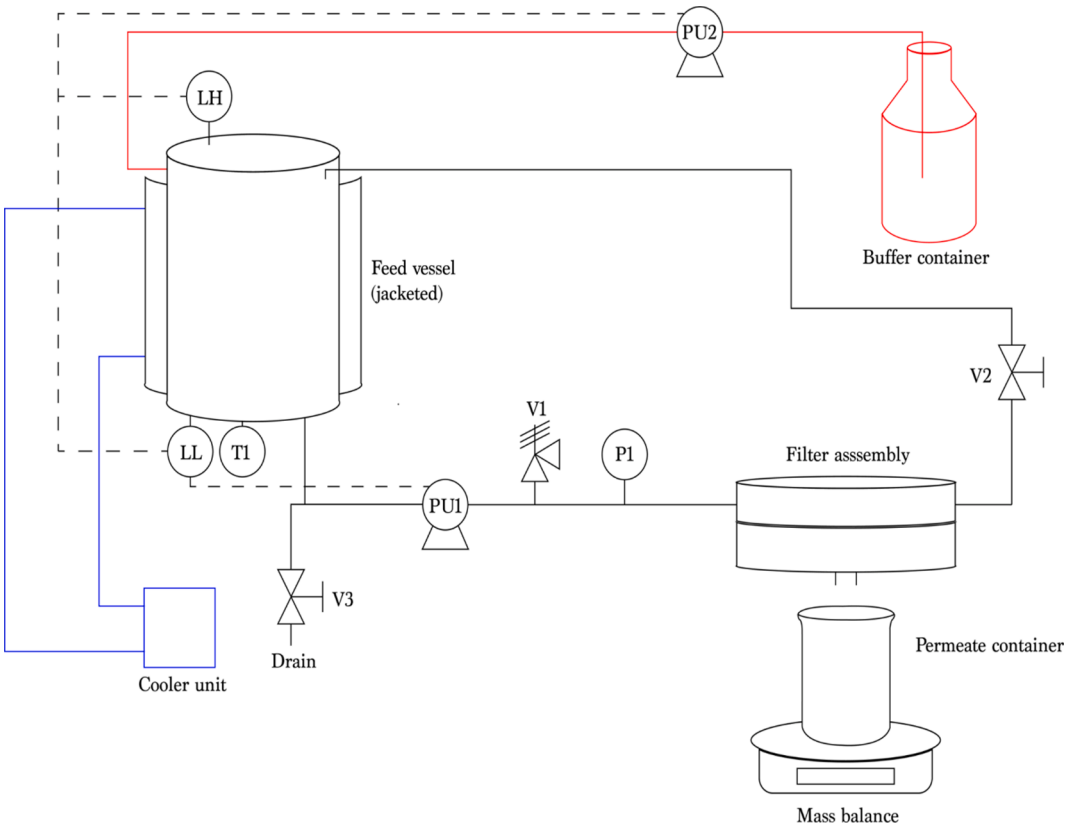

Fig. 1. Overview of the cross-flow membrane set up used in this study. Fluid runs from the buffer container, via the pump, PU2, to the feed vessel. This is adjusted by the LH and LL floater indicators, when the feed level is low PU1 will stop and PU2 will go until the LH indicates that the level is high, this ensure the feed vessel to be filled up continuously. Feed runs through the filter assembly via the pump, PU1, and down to the permeate container, which is placed on a mass balance. The feed vessel is jacked and connected to a cooler for ensuring a constant temperature, which is measured at T1.

supplementary information Figure S3-S4. The absolute masses in the 4 L of feed, Fig. 3A, corresponds to 576.3 mg of bostrycoidin, 31,3 mg of fusarubin, 21.9 mg of anhydrofusarubin and 9.6 mg og javanicin. After MF, operated at 50% recovery, bostrycoidin gets concentrated to 537.1 mg in the retentate, and only 10.8 mg in the permeate, which corresponds to an average rejection of 97.5%. Fusarubin is not present in the retentate and shows an absolute mass of 24.2 mg in the permeate. Anhydrofusarubin and javanicin were both concentrated in the retentate to 27.6 and 10.1 mg respectively, showing average rejection percentages of 91.4% and 52.4%.

The MF membrane with a pore size of 0.1 μm was capable of removal of the targeted pigment, bostrycoidin, with a molecular weight of 285.26 Da significantly higher than expected based on size exclusion mechanism where the species are rejected by a membrane with respect to molecular size and membrane pore size. This is because the solution contains other suspended cells as well as proteins and organic cell matter which might cause fouling on the membrane surface and resulting in the pore blocking effect [58–60]. Using a visual inspection, the formation of a foulant layer could also be observed after the filtration on the surface of V0.1 membrane as presented in Figure S10. Moreover, the permeate flux data of the V0.1 membrane in Fig. 3B demonstrates a flux decline within 200 min of the filtration test. The average permeate flux declined 20% from the initial value of 25 L/(m2h) to 20 L/(m2h). The initial permeate flux value of the MF test per se also suggests that fouling has occurred since the permeate flux for MF membranes is known to be notably higher than 25 L/(m2h). On the other hand, Adsorption also plays a minor role in the high rejection value of bostrycoidin. As presented in Table 1 by Log Kow, bostrycoidin is a hydrophobic molecule (Log Kow $>$ 2) and it is well known in the literature that hydrophobic species tend to adsorb more on the membrane surface resulting in an enhanced rejection performance [61–63]. Using a mass balance for bostrycoidin over the MF step, nearly 3% of bostrycoidin was adsorbed onto the V0.1 membrane (See supplementary information Table S4).

The UF step seemed to have little separation effect on the remaining compounds, as all compounds were found in both retentate and permeate. The data from the NF filtration clearly showed that the NF membrane retained the majority of the remaining compounds, concentrating fusarubin to 13.6 mg, javanicin to 1.1 mg, bostrycoidin to 2.9 mg and anhydrofusarubin to 0.5 mg, where little to none, were to be found in the permeate. The XLE RO membrane filtered out the last small amounts and left no pigment in the permeate.

These tendencies become clearer when investigating the relative mass in percentage. The initial feed mass is set as being 100%. For fusarubin 100.2% is to be found in the permeate from MF, and at the UF step, 56.5% is found in the permeate and 63.1% is to be found in the retentate. The NF membrane retains afterwards 45.9% and permeates 4.3%. Similar tendencies are seen for javanicin. For bostrycoidin it is seen that 95.1% is retained by the MF membrane, leaving 1.9% to permeate, which corresponds to the rejection of 97.5%. This tendency is also seen for anhydrofusarubin. For some of the molecules, it is seen that over 100% can be achieved in the relative mass %, this can be explained by the change in pH throughout the experiment, starting out acidic, making bostrycoidin ionic at the -N, making the permeate more alkaline, oxidizing additional compounds to fusarubin when exposed to air, as explained by Kurobane and Vining [18,27], which also corresponds to anhydrofusarubin is to be found in the acidic retentate. These compounds are however in very low concentration and the variation can also be explained by this. Statistical anlysis verified the explained tendincies, however no significance in means was found in the filtration steps for javanicin due to very low concentrations. For fusarubin the statistical analysis showed a significant difference in the NF step where fusarubin was retained. The anaylsis yielded a significance in difference of

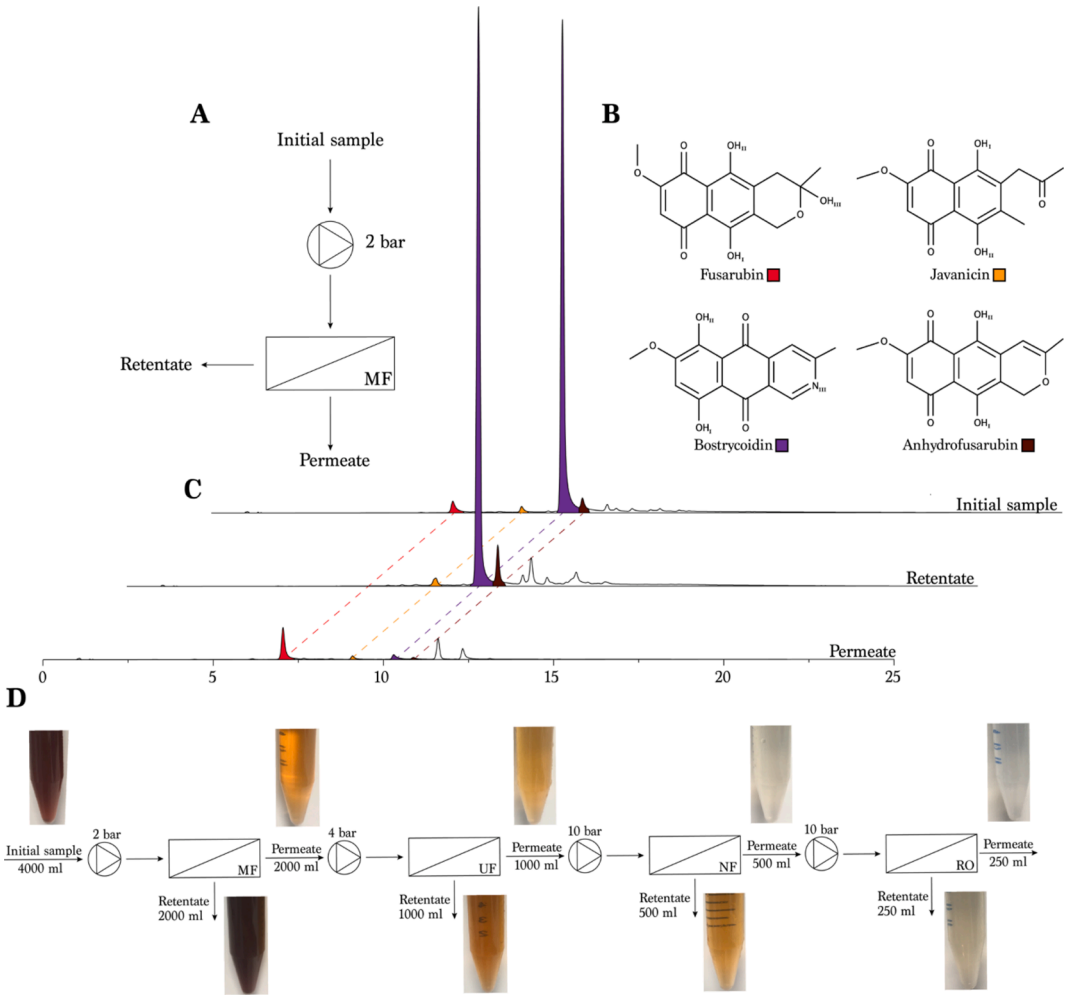

Fig. 2. A) Scheme for the initial MF experiment. B) structural formulas for the four quinones of interest, colours represent the peaks in the chromatogram, numbers on the molecules, refer to the Pka values listed in Table 1. C) chromatogram of the filtration steps, showing clear separation and the magnitude of the different molecules in the media. D) scheme for the full separation train, which also shows the colour difference between the filtration steps.

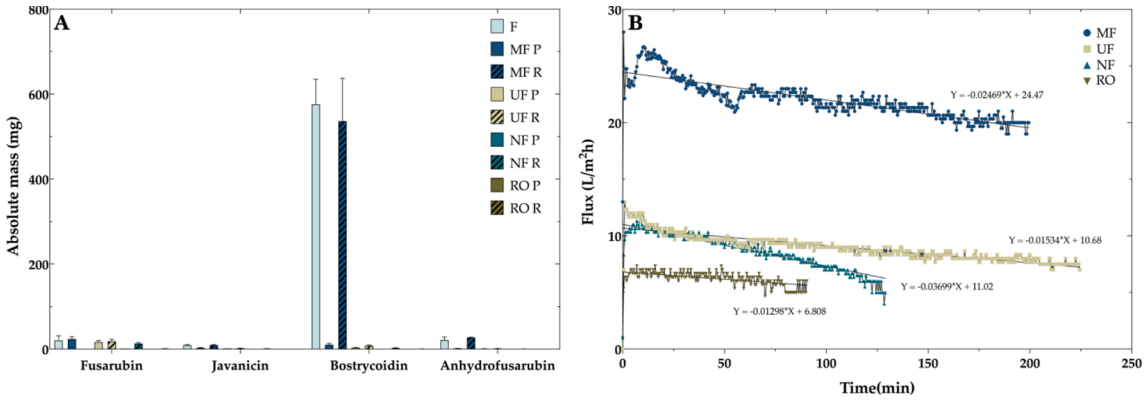

Fig. 3. Experimental data for the separation train, A) Absolute mass, indicate the amount of each pigment is filtrated at which filtration step. B) permeate flux data.

bostrycoidin at the MF step, correspoinding with previously explained tendencies. Due to large variation of the data, some of the tendencies is not found as significant when analyzed, as for anhydrofusarubin, however the statictical analysis can be challenging when conversion products is present and concentrations a low in the sample matrix. Statistical results can be seen in Figure S5. Fig. 3B illustrates the average permeate flux for the individual filtration steps. The MF step starts at 25 L/(m^2h) and increases to 26.5, whereafter it decreases over 200 min to 20 L/(m^2h) as a result of fouling as described earlier. The experiment using UF membrane have an initial value of 13, which fast decreases to 10 and after this it slowly decays to 7.5 L/(m^2h). The NF membrane shows starting values of 11.5, decreasing to 5.0 L/(m^2h) over the experiment. The RO membrane also shows a decrease from 7.0 to 6.0 L/(m^2h) over the experiment. These tendencies were expected, as there might be fouling occurring on the membrane surface. The decrease was not something we consider crucial as the feed volumes filtered were

relatively large, going from starting volume at 4.0 L, to 250 mL. Specific data for the relative mass% can be found in the supplementary information for all experiments in Table S4-S6 and Figure S6-S8.

Because it was possible to retain bostrycoidin in the MF retentate, it was interesting to investigate different types of MF Membranes. We saw similar patterns with the three investigated MF membranes, where fusarubin was permeated through the membrane completely yielding nothing in the retentate and small concentrations in the permeate. Javanicin was found in both permeate and retentate of all membranes, yielding rejection percentages of 55.9%, 52.9% and 57.7% for the three membranes respectively. Bostrycoidin was concentrated from 70.4, 63.1 and 63.0 mg in the three feed solutions, Fig. 4A, V0.1 membrane, Fig. 4B, MF01 and Fig. 4C, MV020, respectively to 67.0, 65.4 and 65.8 mg in the retentate, concluding in 98.0% , 97.8% and 98.2% rejection for the three membrane types respectively. The same tendencies were observed for anhydrofusarubin, which initially had an absolute mass of 2.6, 2.5 and 3.1 mg, whereas in the retentate 2.5, 2.4 and 2.9 mg was found, which adds up to 91.6%, 90.6% and 92.5% of rejection for the three membrane types respectively. When investigating the relative masses it is seen that fusarubin is being oxidized in all cases, exceeding 100% in the permeate, leaving nothing in the retentate, this again indicate that aeration throughtout the filtration experiments leads to formation of fusarubin from oxidation and conversion of "not identified compounds" from the solution. Javanicin, showing 26.5%, 28.7%, and 26.4% in the permeates for the three membranes respectively, and 69.9%, 71.6% and 74.8% were found in the retentates for each membrane. Bostrycoidin was found as 1.4%, 1.7% and 1.4% in the permeate and 95.1%, 105.0%, and 104.1% for V0.1, MF01 and MV020 respectively for the retentate. The reason for the slightly higher amount of bostrycoidin in the MF01 and MV020 experiments, can be explained by the higher deviation in the datasets due to irregularities in samples. The tendencies was varified by the statistical analysis, where no significance besides what has been stated earlier was found in the multible comparisons carried out in the study. The results of the statistical anlysis can be seen in Figure S9. Visually, the permeate was significant more transparent than the retentate, which can be seen in Figure S10. In Fig. 4D the average permeate flux for the three membranes is visualized, all showing a significantly decreasing curve. The initial flux of the V0.1 membrane was 25.3 which decreased to 18 corresponding to the MF flux in Fig. 3B. The initial flux of the MF01 membrane was slightly higher at 28, and decreased to 19.6 over the 21 mins, being the fastest of the three membranes. The MV020 membrane showed the highest initial flux measured at 29.3 L/(m^2h) which corresponded to the higher pore size but also showed the fastest decline to 19 L/(m^2h) over the time of 22 min. This rapid flux decline for all three MF membranes over nearly 20 min of filtration is attributed to the fouling as a result of the presence of other species in the solution e.g. proteins and cellular parts, as discussed previously. It was not possible to determine any significant difference in the filtration behavior of the three analyzed membranes when comparing them to each other.

To investigate long term, high concentration, high permeate recovery, the retentates from previous experiments were combined to a starting feed volume of 8.1L and used for MF filtration with the V0.1 membrane and operated with a permeate recovery of 90%. In the initial samples, there was no trace of fusarubin, as this was filtered off by previous experiments. Therefore, data on fusarubin is not present in Fig. 5A. We found javanicin in the initial sample to be 90.4 mg and after filtration 17.9 mg was in the permeate or 19.8% of the relative mass. In the retentate, the absolute mass was 34.3 mg or 38.0% of the relative mass. Bostrycoidin was found in the initial sample with an average absolute mass of 2744.7 mg and only 55.4 mg was found in the permeate, leaving 744.6 mg in the retentate. This however only corresponded to 28.9% of the relative mass. A possible explanation is related to the subsequent liquid–liquid extraction used for the chemical analysis. Due to the high amount of cell tissue and fatty acids present in the highly concentrated retentate as well as the pigments it was harder to perform the extraction as utilized before, leaving some of the pigments still in the media after extraction. Furthermore, the pattern was the same for all the

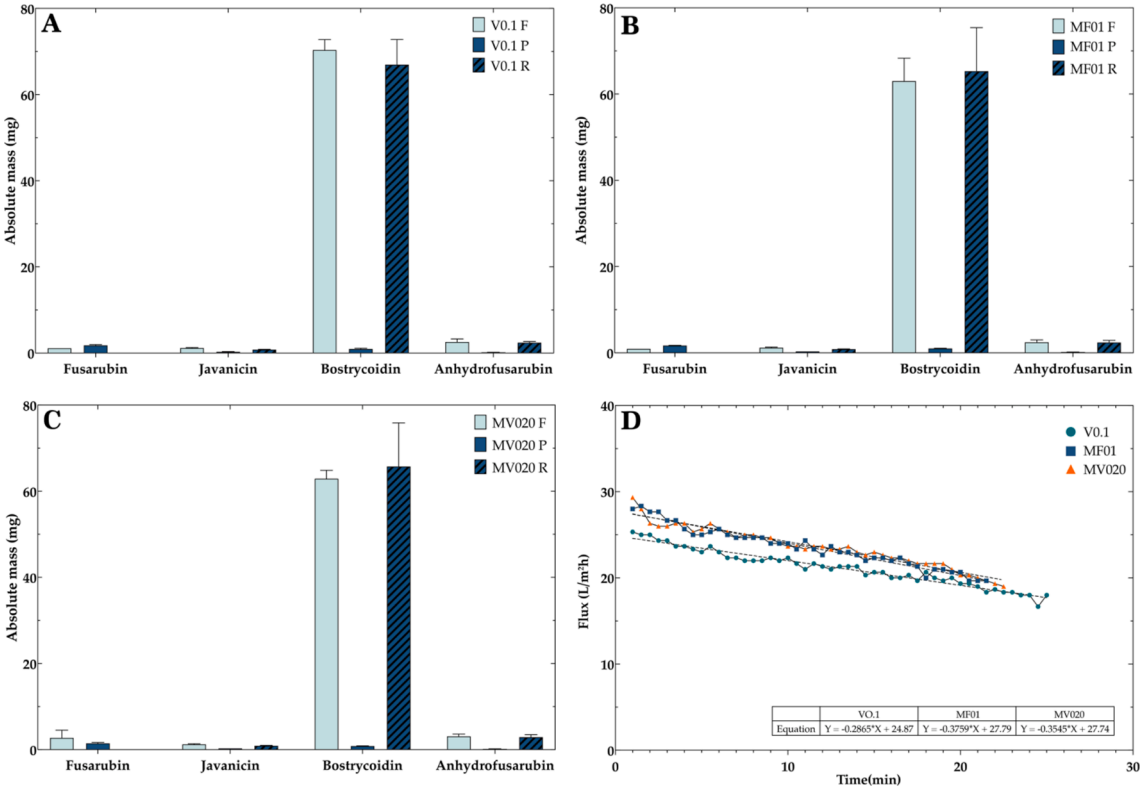

Fig. 4. experimental data for the three investigated MF membranes. A) V0.1 membrane, B) MF01 membrane, C) MV020 membrane. D) Permeate flux for all three membranes.

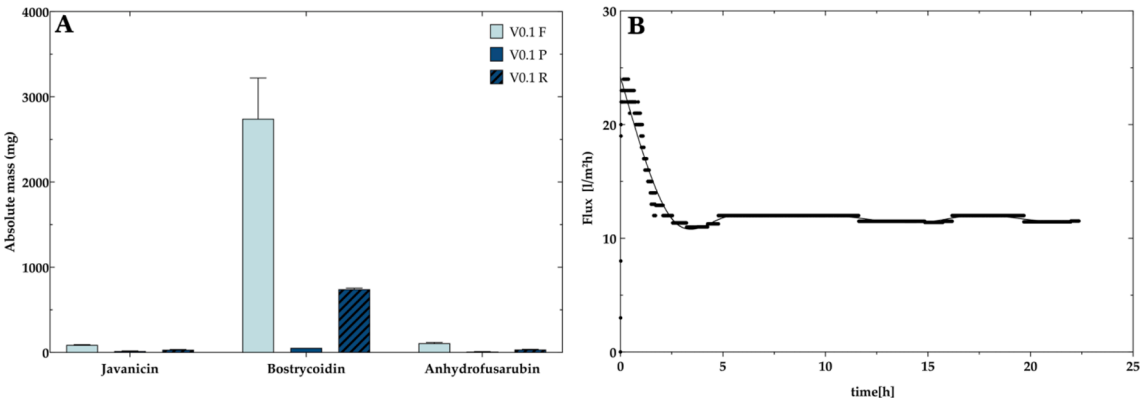

Fig. 5. Large volume separation experiment, A) absolute mass, B) permeate flux data, which has been smoothened.

quinones, in the study, and thereby it is thought to be plausible. Another hypothesis was tested, as the samples could have overloaded the column of the HPLC system, but even when samples were diluted a thousand times, similar results were obtained. So even though the relative mass percentage did not add up to 100% it is still plausible that near to 100% of the desired pigments, was concentrated in the retentate. With the exception of javanicin, as this compound was present in both permeate and retentate.

When comparing with results from experiments 1 and 2, the amount of bostrycoidin in relative percentage matched, it can therefore be assumed that almost all the bostrycoidin should be present in the retentate. For this to be concluded, a new extraction method should be investigated or additional filtration steps could be relevant. The average permeate flux of the experiment, Fig. 5**B**, is illustrating an initial value of 24.8 L/(m2h) similar to values obtained before. After four hours of steady decrease, the average permeate flux stabilize around 12 L/(m2h), where the flux is maintained until the end of the experiment after 22.5 h. This behavior was expected, as dealing with heavily concentrated feed the membrane will over time be fouled with particles, whereas the pattern of the flux will stabilize and a steady-state of the filtration will occur.

It is reasonable to assume that it is possible to concentrate bostrycoidin at a recovery of 90%, as the results shows an increase of average concentrations from 338.8 mg/L to 930.8 mg/L in the retentate and only 2.1% of the relative mass was found in the permeate. The same could be assumed for the compounds javanicin and anhydrofusarubin. The 800 mL retentate was extracted and analysis yielded sample purity of 94.1% when analyzed at 495 nm. The amount of bostrycoidin extracted was concluded to be 10.4 g, which is higher than the 930.8 mg/L which also concludes the assumption of the challenges with the extraction of highly concentrated liquids. Chromatogram measured at 495 nm can be found in Figure S11.

4. Conclusion

In this study, we highly focused on the compound bostrycoidin and performed experiments based on a media optimized to favor the production of bostrycoidin. However, we also found three other quinone compounds, in significantly lower concentrations though. It has been shown that it is possible to use membrane filtration to separate the compound fusarubin completely from bostrycoidin, by microfiltration, yielding rejection of bostrycoidin of 98% and 0% for fusarubin. In the study we investigated three different MF membranes, which showed rejection of bostrycoidin of 98%, 97.8% and 98.2% respectively, fusarubin was only found in the permeate. Leaving bostrycoidin in the retentate, and being concentrated with a purity of 94.1%, and permeating fusarubin. We, therefore, conclude that it is plausible to concentrate and purify the compounds investigated in this study. This can be achieved in a less harmful, more environmentally friendly way for larger-scale applications when compared to previously used methods.

Declaration of Competing Interest

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

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.seppur.2021.119576.

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