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Rough Beer Clarification by Crossflow Microfiltration in Combination with Enzymatic and/or Centrifugal Pretreatments

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In this work, crossflow microfiltration performance of beers, characterised by different turbidity levels (at 20 °C) in the ranges of 2.4-52.4 EBC unit, was assessed in a bench-top plant, appropriately designed and equipped with a 0.8-μm ceramic tubular membrane module, under constant feed superficial velocity (v_S = 6 m s^{-1}), transmembrane pressure difference (TMP = 3.74 bar), temperature (~10 °C), and periodic CO_2 backflushing. The quasi steady-state flux ($J_{v,ss}$) was reached after about 1 h, independently of the turbidity (H) of rough beer. Its decline was drastic as H increased from 0 to 2-3 EBC unit and tended to an asymptotical value of 91 ± 8 dm^3 m^{-2} h^{-1} for H > 7 EBC unit. By removing yeast cells and larger aggregates via centrifugation, or hydrolyzing firstly the gel forming polysaccharides and, secondly, get rid of the suspended solids by centrifugation, it was possible to increase $J_{v,ss}$ to 137 ± 13 or 294 ± 30 dm^3 m^{-2} h^{-1}. Periodic CO_2 backflushing of rough beer resulting from combined enzymatic and centrifugal pre-treatments was able to lower the overall hydraulic resistance to (5.2 - 8.1) x 10^{11} m^{-1}, values near to the resistance of clean membrane.

1. Introduction

The environmental and safety issues associated with handling of filter-aids (*i.e.*, diatomaceous earth DE or Kieselguhr) and disposal of spent filter sludge are pushing the beer industry towards novel DE-free crossflow microfiltration (CFMF) systems. Unfortunately, in the latter the average beer permeation flux (J_v) of 50 - 100 dm^3 m^{-2} h^{-1} are by far smaller than that (250 - 500 dm^3 m^{-2} h^{-1}) achievable with powder filters (Buttrick, 2007).
The three commercial CFMF systems currently used for beer clarification resort to hydrophilic, polyethersulphone (PES) membranes, with porosity of 0.45-0.65 μm, and hollow-fibre (Norit and Pall) or flat-sheet (Alfa Laval) modules. While the PROFI or Alfabright process developed by Pall or Alfa Laval relies upon a preliminary centrifugation step to remove yeast cells and large aggregates from rough beer so as to minimise membrane fouling, the Norit system makes use of just a retentate tank to separate most of the suspended matter from the retentate before its re-circulation through the CFMF modules.
At present, it is unclear whether the centrifuge stage is effectively convenient to minimise membrane fouling by yeast cells and larger aggregates, especially because of its quite high additional investment and operation costs (Buttrick, 2007). Similar to some DE-filtered beers, microfiltered ones are still affected by chill and permanent haze. In fact, cooling the rough beer (during CFMC) down to 0 °C is often insufficient to get rid of the chill haze problem, because this separation process is unable to retain the soluble haze precursors responsible for virtually all post-filtration hazes, and thus a further finishing process may be needed. Use of polyvinylpolypyrrolidone (PVPP) alone or combined with selected carrageenan (i.e., Polyclar Brewbrite ®) or silica xerogel (i.e., Polyclar® Plus) stabilizes the permeate beer once bottled (Rehmanji et al, 2005).
The aggregates in fermented beer are complexes consisting of about 10% polyphenols and 90% proteins, that are generally regarded as responsible for chill and permanent haze (van der Sman et al., 2012).

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Whereas chill haze only forms at temperatures of 0 - 4 °C and consists of small (0.5 - 2.0 μm) aggregates that disappear at higher temperature than 10 °C, the permanent haze is due to the formation of larger (2 - 10 μm) aggregates persistent at room temperature. Removal of such aggregates from rough beer is a primary goal to assure beer stability during storage, as well as its serving at 4 °C.

The main foulants present in rough beer are yeast cells, proteins, polysaccharides and polyphenols. Generally speaking, yeast cells tend to form a cake layer over the membrane surface, the thickness of which being controlled by the beer superficial velocity. The internal fouling of membranes was attributed to arabinoxylans, β-glucans, hydrophilic (haze-forming) proteins and polyphenols (Taylor et al., 2001; Gan et al., 2001), as they penetrated inside the membrane itself and were adsorbed onto the membrane pore walls, leading to pore constriction or blocking. The polysaccharides may also form complexes with proteins or gels, these entrapping other macromolecules or aggregates either in the cake layer or inside the membrane itself.

Whereas the cake layer and some of the aggregates accumulated within the porous membrane structure can be removed by short-term back-flushes, most of the proteins and aggregates adsorbed onto or inside the membrane are only removable by acid and/or alkaline cleaning enriched with oxidative agents (Gan et al., 1999).

Previously (Cimini, 2012; Cimini and Moresi, 2013), the CFMF performance of laboratory-made green beers, obtained from a commercial hopped-malt extract, was studied at ~10 °C in a bench-top plant, appropriately designed and equipped with a 0.8-μm ceramic tubular membrane module by varying the initial green beer turbidity, feed superficial velocity (v_S) and trans-membrane pressure difference (TMP) in the ranges of 1.1 - 12.0 EBC unit, 2 - 6 m/s, and 0.96 - 4.73 bar, respectively. By operating at TMP = 3.73 bar and v_S = 6 m/s with green beers having turbidity of 1 to 4 EBC unit, an average permeation flux of 258 or 199 $dm^3\ m^{-2}\ h^{-1}$ was respectively achieved, thanks to a series of periodic CO_2 back-flushing.

The aim of this work was to test the effectiveness of the aforementioned operating conditions when using an experimental beer obtained in the pilot-scale brewery by the Italian Brewing Research Centre (CERB, Casalina di Deruta, Perugia, Italy), as such or after a series of pre-treatments to minimise the fouling contribution of yeast cells, aggregates, and polysaccharides. In particular, the rough beer was centrifuged to remove yeast cells and larger aggregates and thus to lessen cake layer formation. Alternatively, it was pre-treated with a commercial mixture of hemicellulases and β-glucanases to degrade the arabinoxylans and β-glucans and then centrifuged to limit cake and gel layer formation, as well as aggregate entrapping over and inside the membrane.

2. Materials and Methods

Twenty-five litres of wort (density 1.045 kg dm^{-3}) were obtained by mashing 100% pils malt (Durst-Malz, Bruchsal-Heidelsheim, Bruchsal, D) and hopping with traditional bitter Hallertau Magnum hop pellets. Then, they were fermented by adding 11.5 g of dry yeast (Saflager W-34/70, Fermentis, Marcq-en-Barœul, F). The fermentation temperature was kept constant at ~12 °C for about 10 days, then gradually lowered to 2 - 4 °C over the following 4 days. The phase maturation was prolonged for about 30 days. After racking, the rough beer was stored in a stainless-steel maturation vessel and kept at 4 °C. The rough beer was used as such or clarified using a laboratory centrifuge (Beckman model J2-21) at 6000 x g at < 4 °C for 10 min once collected in 0.3-L plastic bottles.

Commercial Beerzym PENTA preparation (Erbslöh Geisenheim AG, Geisenheim, Germany), consisting of a mixture of β-glucanases and pentosanases excreted by a selected strain of *Trichoderma sp*., was added (0.15 cm^3 /L) to the above rough beer, and the enzymatic treatment at 4 °C was prolonged for 24 h so as to degrade almost all the pentosans and β-glucans present. The resulting liquor was then centrifuged as reported above.

A typical temperature- and pressure-controlled bench-top CFMF plant was assembled and used. It was equipped with a 0.8-μm ceramic tubular membrane module, made of Zr and Ti oxides bound to an Al-Ti oxide support (US Filter, Warrendale, PA, USA), with 6 mm inside diameter, 500-mm length, and 94.2 cm^2 effective membrane surface area. As suggested by Gan et al. (1999), membrane cleaning included a combined synergic caustic and oxidation cleaning, followed by acidic cleaning. During its use, the membrane module exhibited a water permeability of 773 ± 17 $dm^3\ m^{-2}\ h^{-1}\ bar^{-1}$ (r^2 = 0.99) at 20.0 ± 0.1 °C.

A stainless steel Lowara centrifugal pump (Montecchio Maggiore, I) was piloted using a 0.75 kW electric motor via a frequency inverter Commander SK (Control Techniques, Powys, UK) so as to vary TMP under constant v_S. The process temperature was monitored and automatically controlled by an on-off temperature-controller. Feed and retentate pressure and flow rates were monitored using digital and analogical pressure and flow rate sensors, respectively. By using a technical-grade scale (B), type Europe

4000 AR (Gibertini, Elettronica Srl, Novate, Milan, I), interfaced to a personal computer (PC) via a RS-232 serial port, it was possible to estimate the permeation flux (J_v). Several total recycle runs were carried out at ~10 °C by setting TMP and v_S at 3.74 bar, and 6 m/s, respectively. Membrane cleaning with CO_2 backflushing was carried out at a backflush pressure difference of +3 bar for 2 min when the permeation flux dropped below a prefixed value.

The beer and permeate samples were assayed for pH, density, viscosity, colour, β-glucans, real original and extract, and ethanol contents in accordance with Analityca EBC (2010). In particular, turbidity or haze (at 20 °C) was determined using a bench-top turbidity meter HD25.2 (Delta OHM SrL. Caselle di Selvazzano, PD, Italy) at a wavelength of 470 μm, and expressed in Nephelometric Turbidity Units (NTU). Four formazine-based calibration standards having turbidity of 0, 8, 80 and 800 NTU were used. The turbidity, as calculated from the light detected by a photodiode positioned at 90° compared to the emitted light direction, was then multiplied by 0.25 to be converted into EBC units.

3. Results and discussion

Table 1 summarizes the average characteristics of the rough beers used here (*i.e.*, RB1 - 4), their turbidity values varying from 2.4 in RB1 to 52.4 EBC unit in RB2, as well as their corresponding permeates (*i.e.*, P1 - 4). Owing to the natural sedimentation of suspended matter in the storage tank at 4 °C, some variability from batch to batch and within the same beer batch was noticeable. All beer permeates, except those obtained by enzymatic treatment, exhibited a turbidity slightly higher than the limiting turbidity level (< 0.6 EBC unit) suggested by the European Brewery Convention standards, even if all permeate samples appeared to be brilliantly clear. This might be explained by accounting for the fact that the aforementioned EBC threshold value results from turbidity measurements at 560 nm, while the data listed in Table 1 were taken at 470 nm. In fact, it is well known that long wavelengths are always less intensely scattered than short ones (Chapon, 1993). Finally, the density, colour, and alcohol degree of samples RB2 and P2 were typical of the *Lite* or *Standard American Lager* (BJCP Style Guidelines, 2008).

Table 1: Mean and standard deviations of the main characteristics (pH; density, ρ; viscosity, η; turbidity, H; colour, C; β-glucans, β-G; real extract, RE; original extract, OE; alcohol, A) of the rough beer samples, as such (RB), precentrifuged (RBC) or enzymatically treated and centrifuged (RBEC), together with the corresponding micro-filtered (P, PC, PEC) samples.

Sample	pH	ρ	η	H	C	β-G	RE	OE	A
		[kg dm^{-3}]	[mPa s]	[EBC]	[EBC]	[g m^{-3}]	[°Plato]	[°Plato]	[% v/v]
RB1	4.39±0.05	1.0048±0.0004	1.41±0.01	2.4±0.1	6.9±0.1	140±4	3.5±0.2	14.4±0.1	5.1±0.1
P1	4.39±0.05	1.0048±0.0004	1.40±0.01	0.8±0.05	6.3±0.1	135±12	2.9±0.1	12.6±0.1	5.1±0.2
RB2	4.39±0.05	1.0047±0.0004	1.41±0.01	52.4±0.1	6.9±0.1	140±4	3.5±0.2	14.4±0.1	5.1±0.1
P2	4.39±0.05	1.0044±0.0005	1.39±0.01	1.1±0.05	6.3±0.1	135±12	2.9±0.1	12.6±0.1	5.1±0.2
RB3	4.39±0.05	1.0048±0.0004	1.39±0.01	18.0±0.1	6.9±0.1	140±4	3.5±0.2	14.4±0.1	5.1±0.1
P3	4.39±0.05	1.0043±0.0005	1.37±0.01	1.2±0.01	5.5±0.1	135±12	2.9±0.1	12.6±0.1	5.1±0.2
RB4	4.21±0.05	1.0050±0.0004	1.36±0.01	7.1±0.1	6.9±0.1	140±4	3.5±0.2	14.4±0.1	5.1±0.1
P4	4.21±0.05	1.0045±0.0005	1.33±0.01	0.9±0.01	6.3±0.1	135±12	2.9±0.1	12.6±0.1	5.1±0.2
RBC5	4.39±0.05	1.0043±0.0004	1.36±0.01	1.5±0.1	5.8±0.1	140±4	3.5±0.2	10.3±0.1	3.9±0.1
PC5	4.39±0.05	1.0040±0.0005	1.36±0.01	0.8±0.01	5.1±0.1	135±12	1.7±0.1	9.3±0.1	3.9±0.2
RBEC6	4.39±0.05	1.0052±0.0004	1.21±0.01	0.9±0.1	5.1±0.1	1.3±0.6	3.5±0.2	10.3±0.1	3.9±0.1
PEC6	4.39±0.05	1.0041±0.0003	1.21±0.01	0.4±0.01	4.8±0.1	0.8±0.5	1.7±0.1	9.3±0.1	3.9±0.2

In CFMF under constant TMP (3.74 bar), v_S (6 m/s) and T (10 °C) for up to 4 h, the permeation flux tended to a quasi steady-state value ($J_{v,ss}$) after about 1 h, independently of the turbidity (H) of the rough beer tested in this work (Figure 1A). From Fig. 1 B it can be noted a drastic reduction in $J_{v,ss}$ or in the average permeation flux ($J_{v,av}$), as turbidity increased from 0 to 2 - 3 EBC unit with an asymptotical value of 91 ± 8 (or 117 ± 14) dm^3 m^{-2} h^{-1} for H > 7 EBC unit. Both trends were empirically described with an average standard error of 4.2 or 6.2% by the following regressions:

$$J_{v,ss} = J_{v,w} [1 + 0.97 (e^{-1.90\ H} - 1)] \quad (1)$$

$$J_{v,av} = J_{v,w} [1 + 0.96 (e^{-1.59\ H} - 1)] \quad (2)$$

where $J_{v,w}$ is the permeation flux (3061 ± 67 dm^3 m^{-2} h^{-1}) with deionised water (H=0).

Figure 2 compares the time course of the permeation flux (J_v) at 10 °C under constant TMP (3.74 bar), v_S (6 m/s), and periodic CO_2 backflashing when using a rough beer sample as such or after preliminary treatments aimed at removing yeast cells and larger aggregates by centrifuging or at hydrolyzing firstly the gel forming polysaccharides and secondly get rid of the suspended solids by centrifugation.

It can be noted that the centrifugation step resulted in about 50 or 75% increase in $J_{v,ss}$ (137 ± 13 dm^3 m^{-2} h^{-1}) or $J_{v,av}$ (205 dm^3 m^{-2} h-1), thanks to the recovery of suspended matter with size larger than 0.5 μm. In all probability, such a pretreatment was unable to counteract the gel layer formation, as well as the entrapping of the smaller aggregates within the membrane porous structure. The preliminary use of the commercial enzyme preparation appeared to be capable of degrading almost all the β-glucans (Table 1), thus lowering their tendency to aggregate and making the smaller molecular mass fractions easier to permeate across the membrane undisturbed (Stewart et al., 1998). Once the enzymatically-treated beer had been centrifuged, the resulting $J_{v,ss}$ or $J_{v,av}$ values (294 ± 30 or 336 dm^3 m^{-2} h^{-1}) was more than two or one and a half fold higher than that achieved when the rough beer was centrifuged and then microfiltered.

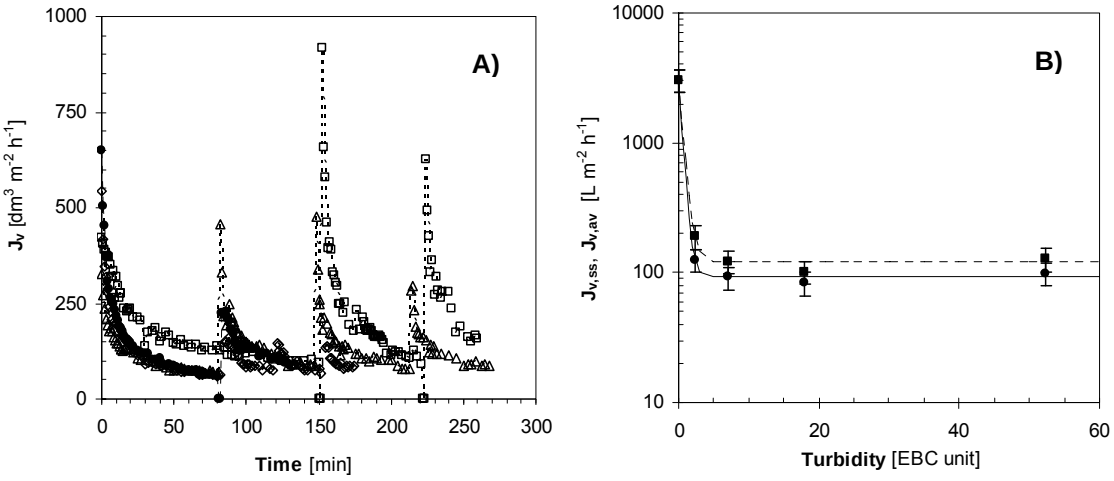

Figure 1: Effect of the turbidity (H) of a few rough beer samples (□, 2.4; △, 7.1; ◇, 18; ●, 52.4 EBC unit) on A) the time course of the permeation flux (J_v) at 10 °C under constant TMP (3.74 bar), v_S (6 m/s), and periodic CO_2 back-flushing and B) quasi steady-state ($J_{v,ss}$).and average($J_{v,av}$) permeation fluxes. The continuous and broken lines in Fig. 1B were calculated using Eq.s (1) and (2), respectively.

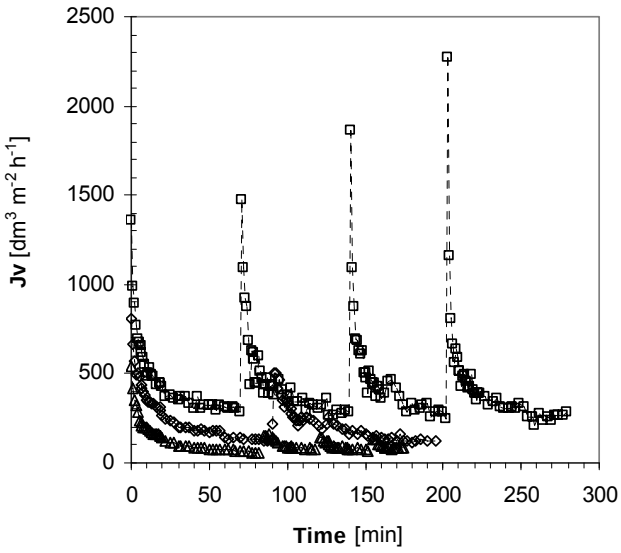

Figure 2: Time course of the permeation flux (J_v) of rough beer samples as such (△, 18 EBC unit), precentrifuged (◇, 1.5 EBC unit) or after enzymatical and centrifugal pretreatments (□, 0.9 EBC unit) under constant TMP (3.74 bar), v_S (6 m/s), temperature (10 °C), and periodic CO_2 back-flushing.

To discriminate the fouling mechanisms, the flux decline behaviour during rough beer microfiltration can be examined by estimating the time course of the overall resistance (R_T) to filtrate flow as follows:

$$R_T = \frac{TMP}{\eta_P J_v} \quad (3)$$

where η_P is the permeate viscosity, and TMP the transmembrana pressure difference. According to Tracey and Davies (1994), the upward or downward concavity of the R_T-vs-t curve is related to internal fouling due to pore constriction or intermediate blocking or external fouling due to cake filtration.

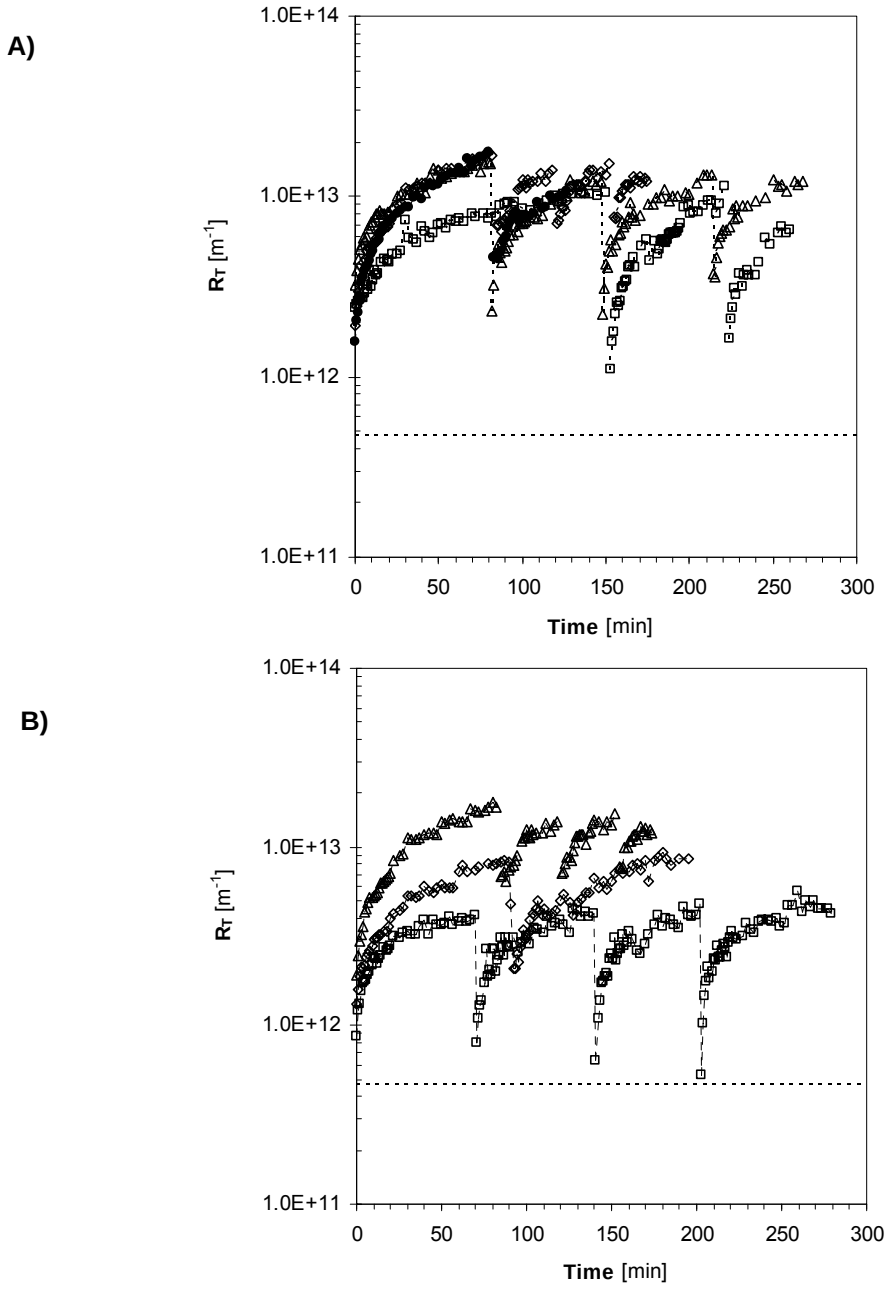

*Figure 3: Time course of the overall hydraulic resistance (R_T) when operating at 10 °C under constant TMP (3.74 bar), v_S (6 m/s), and periodic CO_2 back-flushing, as reported in the Materials and Method section, and using: **A**) rough beer samples of different turbidity (□, 2.4; △, 7.1; ◇, 18.0; ●, 52.4 EBC unit); **B**) rough beer samples as such (◇, 18.0 EBC unit), pre-centrifuged (◇, 1.5 EBC unit) or enzymatically pre-treated and centrifuged (□, 0.9 EBC unit). Both broken lines refer to the clean membrane hydraulic resistance (R_m).*

As shown in Figure 3A, once the rough beer had entered the membrane module, R_T [=(2.3 ± 0.7)x10^{12} m^{-1}] was found to be definitively greater than the resistance of the clean membrane [R_m= (4.7 ± 0.1) x 10^{11} m^{-1}]. To counteract membrane fouling, the electro-valve connecting the permeate tank to the 10-bar CO_2 cylinder was automatically opened to boost suddenly the pressure in the permeate side of the tubular module to +3 bar, with respect to that in the retentate side for as long as 2 min. In this way, the swift flush of CO_2 was capable of lowering R_T to (1.1-2.3) x 10^{12} m^{-1}, especially when a rough beer of low turbidity (H = 2.4 EBC unit) was undergoing filtration.

Regardless of the initial turbidity of rough beer, the time course of R_T exhibited a downward concavity typical of external fouling (i.e., cake filtration and complete pore blocking) and this explains the efficacy of CO_2 backwashing to restore the original permeation flux

Figure 3B shows the effect of some rough beer pre-treatments on R_T.

The pre-centrifugation step not only reduced the growth rate of R_T, but also approximately halved its value from 1.67 x 10^{13} m^{-1}, typical of a sample of rough beer with H = 18 EBC unit, to 0.88 x 10^{13} m^{-1}. The enzymatic pre-treatment followed by centrifugation further reduced R_T to ~0.41 x 10^{13} m^{-1}. Moreover, CO_2 backflushing was able to lower R_T such as low as (5.2-8.1) x 10^{11} m^{-1}, these values being near to the resistance of clean membrane [R_m = (4.7 ± 0.1) x 10^{11} m^{-1}].

4. Conclusions

By using a laboratory-scale plant, equipped with a 0.8-µm ceramic tubular membrane module and a centrifugal pump driven via an asynchronous motor piloted by a frequency inverter to control simultaneously the feed flow rate and input pressure, it was possible to establish the effectiveness of the microfiltration operating conditions (i.e., TPM = 3.74 bar; v_S = 6 m/s; ~10 °C) when using rough beers as such or differently pre-treated. The preliminary centrifugation step yielded an average permeation flux ($J_{v,av}$) of 205 dm^3 m^{-2} h^{-1}, whereas the enzymatic pre-treatment enhanced $J_{v,av}$ to 336 dm^3 m^{-2} h^{-1}, a value falling within the average beer permeation flux (250 - 500 dm^3 m^{-2} h^{-1}) achievable with DE-filters (Buttrick, 2007). Further work is still needed to establish the efficacy of the CO_2 backflushing technique when using tubular ceramic membranes of different porosity.

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