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Assessing Reverse Osmosis For Water Recycling In Alcoholic Fermentation Processes

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Marjorie Gavach, Camille Sagne, Claire Fargues, Marielle Bouix, Martine Decloux, and Marie-Laure Lameloise

Abstract

Recycling the stillage condensates to dilute worts in the fermentation step would represent an effective way to decrease wastewater production and ground water consumption. However, condensates contain fermentation inhibiting solutes, such as volatile acids, alcohols and aromatic compounds that should be removed. Reverse osmosis was investigated as a clean process for such a purpose. Experiments were carried out at pilot scale with industrial condensates and using Hydranautics ESPA2 membrane. Influence of transmembrane pressure (TMP), volume reduction factor (VRF) and pH on permeate flow rate and rejection rates of inhibitory compounds were investigated. The optimal operating conditions were TMP=10 bar to get the maximal admissible permeate flow, a low VRF to produce the less concentrated permeate and a $\text{pH} \geq 6$ to obtain the highest rejection rates of the acids. Results were confirmed by trials at pre-industrial scale in a distillery. However, the permeate produced at pH 6 proved to be less fermentable than the permeate produced at natural pH because of an increase in the osmotic pressure. Natural pH permeate displayed a fermentation activity almost equivalent to tap water chosen as the blank. The remaining inhibitory acids did not seem to hinder significantly yeast growth nor yeast physiology.

KEYWORDS: Condensates, Fermentation, Inhibition, Recycling, Reverse Osmosis

1. INTRODUCTION

Industries including fermentation processes use large volumes of ground water to dilute their worts. In beet distilleries, water is recovered as condensates during stillage concentration. Due to their organic content (COD from 5 to 10 g O₂.L⁻¹), these condensates cannot be discarded without a treatment. They are generally sent to wastewater treatment plant or stabilisation ponds before being spread on land. With the increase in ethanol production, better management of water resource is needed. Recycling the stillage condensates to dilute worts in the fermentation step would represent an effective way to decrease wastewater production and spare ground water. However, as assessed by previous work¹, condensates contain many fermentation inhibiting solutes, such as volatile acids, alcohols and aromatic compounds, released from the raw material or formed during alcohol production process. Eight molecules were proved to be especially problematic because of their concentration and/or their inhibitory effect and chosen as targets for further investigations: formic (fa), acetic (aa), propanoic (pa), butanoic (ba), valeric (va) and hexanoic (ha) acids, furfural (f) and 2-phenylethanol (phol). These molecules led to fermentation inhibition even at low concentration, e.g. 4 mmol.L⁻¹ of butanoic acid¹. Moreover, when mixed they showed synergistic effects¹ that are expected to be emphasized in the presence of ethanol. The treatment process should match the industrial scale, be robust, environmental friendly and cost-effective. As restrictive rules in EU limit the re-use of biologically treated water for food contact, only physico-chemical treatments could be considered here.

Many studies underline the interest of reverse osmosis (RO) for re-use applications in agro-industries. Previous studies showed the interest of RO for condensates detoxification² and the Hydranautics ESPA2 membrane appeared as one of the most appropriate³. In the following, the influence of transmembrane pressure (*TMP*) and volume reduction factor (*VRF*) is studied at pilot scale in order to find the best compromise between permeate flow density and inhibitory compounds rejection rate. As most of the inhibitory compounds are acids, the influence of pH condensate on rejection rate is also investigated. Feasibility of the process will be assessed by fermentation tests and experiments at a pre-industrial scale in a French distillery.

2. MATERIAL AND METHODS

2.1. Condensate characterization

The condensate was sampled in a French beet distillation plant; its pH was between 3 and 4. Average concentrations of the inhibitory solutes are summarized in Table 1. Furfural was always absent.

	fa	aa	pa	ba	va	phol
C (mmol.L ⁻¹)	1 – 1.5	10-20	1-2	1-2	0-0.5	0.1-0.2

Table 1. Inhibitory compounds concentration of the industrial condensate studied

2.2. RO experiments

Optimal operating conditions determination. *TMP*, *VRF* and pH studies were carried out with a 2540 spiral-wound RO pilot (2.6 m² of membrane surface area) from Polymem (France) equipped with ESPA2 membrane from Hydranautics (cross-linked polyamide active layer membrane, low energy-brackish water type) and operating batch-wise. Each experiment was preceded by a cleaning step with KOH, followed by a rinsing step with filtered demineralized water and the industrial condensate to treat was filtered through 10 μm and 3 μm cartridges before feeding the RO pilot. Retentate flow rate at the outlet was set at 400 L.h⁻¹ and temperature was maintained at 20°C with a thermostated water bath.

TMP impact was studied in the range 5-30 bar. Experiments were run in the recycling mode in which retentate and permeate streams were recirculated to the feed tank (*VRF* = 1) (Figure 1).

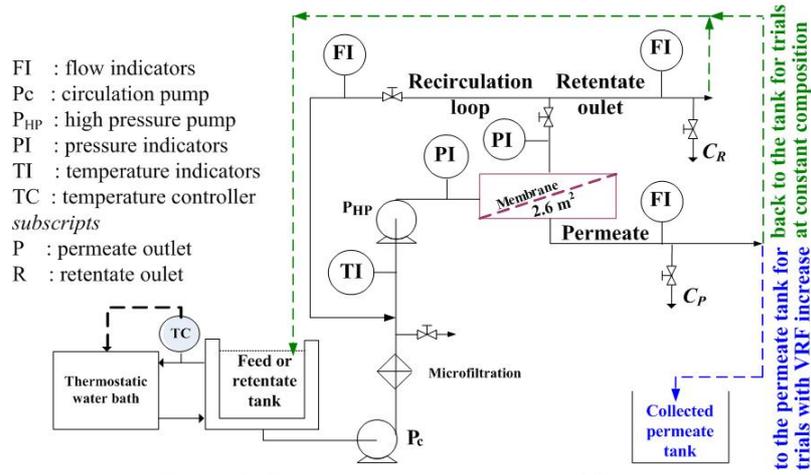


Figure 1. Scheme of the spiral-wound RO pilot

VRF influence (VRF up to 14) was evaluated at TMP 10 bar for all membranes. Experiments were run in the concentration mode in which permeate was continuously drained out and collected into a tank where the total permeate volume extracted was regularly measured (Figure 1). In this batch mode, VRF can be expressed as:

$$(VRF)_t = \frac{V_F}{(V_R)_t} = \frac{V_F}{V_F - (V_P)_t} \quad (1)$$

with: V_F : Initial condensate volume in the feed tank (L)
 $(V_R)_t$: Retentate volume remaining in the system at t time (L)
 $(V_P)_t$: Extracted permeate volume at t time (L)

The influence of pH was evaluated in the best TMP and VRF conditions, i. e. 10 bar and 2. Two pH values were tested: condensates natural pH = 3.4 and neutral pH adjusted to 6 with NaOH 1N (16.7 mmol of NaOH per litre of condensates). Experiments were run in the continuous “feed and bleed” mode, where a fraction of the retentate is recycled in the module thanks to a recirculation loop; the remainder is extracted continuously. In feed-and-bleed mode, VRF is calculated as follows:

$$VRF(\text{continuous}) = 1 + \frac{Q_P}{Q_R}$$

(2)
with: Q_P : Extracted permeate flow ($\text{m}^3 \cdot \text{h}^{-1}$)
 Q_R : Extracted retentate flow ($\text{m}^3 \cdot \text{h}^{-1}$)

Efficiency of RO was evaluated from the permeate flux density J_P ($\text{L} \cdot \text{h}^{-1} \cdot \text{m}^2$) normalized at 20°C using Darcy’s Law, and the inhibitory compounds rejection rate calculated from the concentrations at each side of the membrane as follows:

$$R_s = \left(1 - \frac{C_P}{C_R}\right) \cdot 100 \quad (3)$$

with: C_P : solute concentration in permeate ($\text{meq} \cdot \text{L}^{-1}$)
 C_R : solute concentration in retentate at the membrane outlet ($\text{meq} \cdot \text{L}^{-1}$)

Pre-industrial trials RO experiments were conducted at pre-industrial scale in a French beet distillery in order to confirm the results obtained. A triple 4040 spiral-wound pilot (LTH Dresden, 23.7 m^2 of membrane surface area) equipped with ESPA 2 membranes was implemented. A retentate recirculation loop allowed the VRF to increase. Experiments were run in continuous mode, the pilot tank being continuously fed by condensates coming from the evaporator. Operating conditions were fixed according to the results of the above pilot study.

2.3. Analytical methods

Inhibitory compounds fa, aa, pa, ba, va, ha, f and phol were quantified by an HPLC method developed at AgroParisTech (Massy, France). The chromatographic separation was performed on a high density C18 column Thermo-Electron Corporation BetaMax Neutral heated at 50°C . The mobile phase flowrate was $1 \text{ mL} \cdot \text{min}^{-1}$. The mobile phase was a gradient of (A) H_2SO_4 $5 \cdot 10^{-4} \text{ mol} \cdot \text{L}^{-1}$ aqueous solution and (B) acetonitrile. The gradient program went from 5 to 40% of acetonitrile in 10 min and returned to

5% after a 5 min plateau. After each run, the column was equilibrated under the starting conditions for 10 min.

2.4. Fermentation tests

The yeast strain was 46 EDV *Saccharomyces cerevisiae* (Martin Vialatte Oenologie). The growth medium was prepared so as to get a saccharose concentration of 180 g.L⁻¹. It was composed of syrup 236 g.L⁻¹, molasses 59 g.L⁻¹, yeast extract 0.5 g.L⁻¹, peptone 2 g.L⁻¹, (NH₄)₂SO₄ 2 g.L⁻¹, H₃PO₄ 0.3 g.L⁻¹, MgSO₄ 0.1 g.L⁻¹, and qsp 1L with a) tap water, b) raw condensate, c) RO permeate at natural pH, d) RO permeate at pH 6. The pH was adjusted to 3.6 with H₂SO₄.

Yeast growth was studied by monitoring the cell multiplication over time with a Bioscreen C device (Labsystems). Wells of a microplate were filled with 200 μL of each of the growth media (20 replicates) and inoculated with *Saccharomyces cerevisiae* at 10⁵ cells per mL. For each well, the optical density (OD) reflected the yeast development.

Yeast physiology was assessed during continuous fermentation in a 1.75 L chemostat where ethanol concentration was stabilized at 50±3 g.L⁻¹. Three chemostats were carried out with a), b), c) media. The specific growth rate of yeast μ (h⁻¹) was calculated as the ratio between feed flowrate and chemostat volume. Samples from the chemostat were collected twice a day for viability staining. A double staining was applied: about 10⁶ cells were suspended in 1 mL Mc Ilvaine buffer (citric acid 100 mM and disodium hydrogen phosphate 200 mM) set to pH 4; 1μL of 1mg.mL⁻¹ propidium iodide was added and the suspension was incubated 20 min at 40°C; then 2μL of carboxyfluorescein diacetate (Chemchrome, Chemunex) were added and the suspension was incubated another 10 min before flow cytometer analysis was performed with a Partec flow cytometer.

3. RESULTS AND DISCUSSION

3.1. Influence of TMP

Figure 2 shows high permeate fluxes for the ESPA2 membrane (Hydranautics), increasing linearly with TMP as predicted by the theory:

$$J_p = A(TMP - \Delta\Pi) \quad (4)$$

Where A is the permeability to the solution (L.h⁻¹.m⁻².bar⁻¹), and $\Delta\Pi$ the osmotic pressure difference between retentate and permeate (bar). Nevertheless, in order to limit the membrane's fouling manufacturers advise not to exceed a permeate flux $J_p = 30$ L.h⁻¹.m⁻². This enforces to run the treatment at a maximum of 10 bar with the ESPA2 membrane for this application.

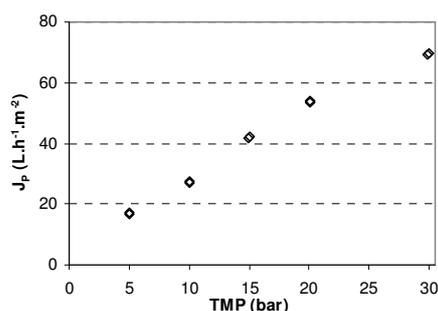


Figure 2. Influence of TMP on permeate flux density at 20°C and VRF = 1

For all solutes, solutes rejection R_s increases with TMP, as shown in Figure 3. This result is in good accordance with literature data⁴. Actually, J_p increase with TMP corresponds to enhanced transfer of water through the membranes compared to the solutes, leading to the dilution of the latter in the permeate and to their concentration in the retentate. The smallest and more polar acetic and propanoic acid molecules are the less retained (between 69 and 92% for pa).

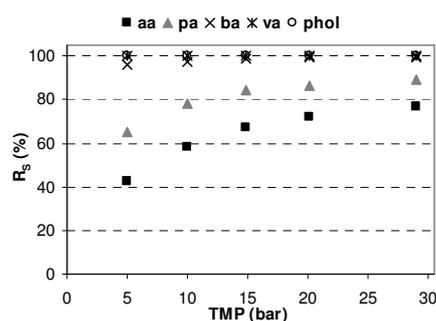
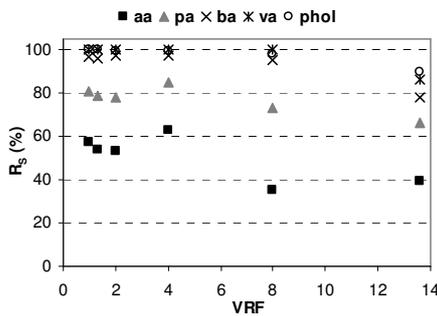


Figure 3. Influence of TMP on solutes rejection for ESPA2

3.2. Influence of VRF

As shown in Table 2, the permeate flux decreases steadily as VRF increases. Actually, the increase of the concentrations on the retentate side leads to an increase of the osmotic pressure and a decrease in effective transmembrane pressure $TMP-\Delta\Pi$.

VRF	1,33	2	4	8
J_p (L.h ⁻¹ .m ⁻²)	23.8	22.2	19.0	13.5

Table 2. Influence of VRF on permeate flux density J_p at 20°C and $TMP = 10$ bar for ESPA2Figure 4. Influence of VRF on rejections for ESPA2 membrane

This solutes concentration increase on the retentate side also impacts on the solutes rejection: constant until $VRF = 4$ (Figure 4), ESPA2 performances decrease after. Moreover, above $VRF = 4$, aa and pa concentrations are higher in permeate than in initial raw condensate, making the filtration operation useless.

Hence, in order to obtain reasonable permeate concentrations and flux with this membrane, further experiments were run at $VRF = 2$ and $TMP = 10$ bar.

3.3. Influence of pH

Results presented in Table 3 show that the acids rejection rates increase with the pH, especially for the low molecular weight formic and acetic acids^{5,6}: for $pH < 5$, a classical polyamide surface exhibits a positive charge due to free amine groups, attracting the ionized form of aliphatic acids: small acids then cross the membrane in a certain extent.

	fa	aa	pa	ba	phol
Natural pH RO permeate (pilot-scale)	0,0%	48,5%	64,0%	91,2%	93,1%
Natural pH RO permeate (pre-industrial pilot)	0%	45,0%	66,5%	92,6%	95,7%
pH 6 RO permeate (pilot-scale)	92,0%	93,9%	>92,4%	>95,9%	93,6%

Table 3. Influence of pH on rejection rates R_s (%) at $VRF = 2$ and $TMP = 10$ bar for ESPA2 membrane

At pH 6, all acids show a rejection rate higher than 90% explained by electrostatic repulsions between the ionized form of the acids, predominant for pH above 5, and the negative net charge of the membrane surface at that pH value^{6,7}. This repulsion is as more important as the acid is small, due to its higher charge density. For acids and regardless of pH conditions, the higher the molecular weight, the better the rejection rate, in agreement with literature observations⁵. As expected, no pH effect can be observed on the rejection rate of the neutral compound (phol). Rejections were confirmed with the condensate at natural pH on the pre-industrial pilot.

According to these results, treatment appears more efficient at pH 6 than at natural pH.

3.4 Influence on yeast fermentation

Table 4 sums up the composition in inhibitory target compounds of the condensate before and after pilot scale RO treatment. As permeate obtained at pH 6 contains less inhibitory target compounds than the natural pH permeate, it is expected to be less inhibitory.

	fa	aa	pa	ba	phol
Raw condensate	1.19	15.6	1.43	1.99	0.14
Natural pH RO permeate	2.9	8.0	0.5	0.3	0.02
pH 6 RO permeate	0.2	1.4	<0.10	<0.08	0.02

Table 4 : Concentration (mmol.L^{-1}) in inhibitory compounds in raw and treated condensates used for fermentation tests

Influence on yeast growth. Figure 5 confirms that the raw condensate inhibits strongly the yeast growth. The lag phase lasts quite 24h instead of a few hours for the other media. Compared to other media, the specific growth rate (represented by the slope of the OD during the growth phase) and the maximal OD are lower for the raw condensate.

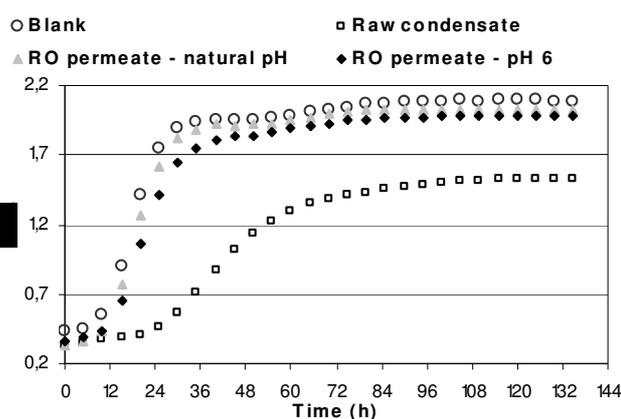


Figure 5. Optical density OD obtained with RO treated and raw condensates versus fermentation time

Natural pH RO permeate presents results equivalent to the blank during the first 60 hours. pH 6 RO permeate shows a lower specific growth rate, which was unexpected considering its lower concentration in inhibitory compounds. Such a result can be explained considering that this permeate showed a higher pH and overall a higher mineral level due to the soda addition. A comparatively higher quantity of H_2SO_4 had hence to be added when preparing the growth medium (d) (cf. 2.4). The increased osmotic pressure resulting impacts significantly on the fermentability, by stressing the yeast⁸ and disrupting its growth⁹.

Maximal OD reached for both permeates are similar each other and almost equivalent to the blank.

Influence on yeast physiology. The % of viable, dead and stressed yeast cells as given by the flow cytometry analyses are presented Table 5. Unstained cells (i.e. ghosts) correspond to lysed cells that can no more be stained. The harmful influence of raw condensate on yeast activity is confirmed. Natural pH RO permeate presents a specific growth rate similar to the blank. However, a slight difference on physiological state is noticeable. If it were due to the remaining inhibitory acidic compounds, fermentation carried out at higher ethanol concentrations should emphasize the effect on physiology.

	% Viable	% Dead	% Stressed	% Unstained cells	μ (h^{-1})
Blank	56.6	18.3	17.3	7.8	0.04
Natural pH RO permeate	51.5	23.0	16.0	9.5	0.04
Raw condensate	19.2	17.4	44.3	19.1	0.02

Table 5. Viable, stressed and dead cells and specific growth rate during fermentation with RO permeate and raw condensate

4. CONCLUSION

Influence of *TMP*, *VRF* and pH on permeate flow rate and rejection rates of inhibitory compounds were investigated for the Hydranautics ESPA2 membrane. The optimal operating conditions were *TMP* = 10 bar to get the maximal admissible permeate flow, a low *VRF* to produce the less concentrated permeate and a $\text{pH} \geq 6$ to obtain the highest rejection rates of the acids. However, the permeate produced at $\text{pH} = 6$ proved to be less fermentable than the permeate produced at natural pH because of an increase in the osmotic pressure due to the addition of soda for pH modification. Natural pH

permeate displayed a fermentation activity almost equivalent to tap water chosen as the blank. The remaining inhibitory acids did not seem to hinder significantly yeast growth nor yeast physiology.

Fermentability experiments were achieved at ethanol concentrations twice lower than in industrial conditions. Further experiments at concentrations $>100 \text{ g.L}^{-1}$ are therefore needed to conclude definitely on the feasibility of the RO process.

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