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A vibrating membrane bioreactor operated at supra- and sub-critical flux: Influence of extracellular polymeric substances from yeast cells

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Abstract

A vibrating membrane bioreactor, in which the fouling problems are reduced by vibrating a hollow fiber membrane module, has been tested in constant flux microfiltration above (supra-critical) and below (sub-critical) an experimentally determined critical flux. Suspensions of bakers yeast cells were chosen as filtration medium (dry weight 4 g/l). The influence of extracellular polymeric substances (EPS) from the yeast cells is evaluated by UV absorbance measurements of the bulk supernatant during filtration. The critical flux seems to be an interval or a relative value rather than an absolute value. Filtration just below the critical flux (sub-critical) seems to be a good compromise between acceptable flux level and acceptable increase of fouling resistance and trans-membrane pressure (TMP) in a given time period. EPS from the yeast cells causes the membrane module to foul and part of the fouling is irreversible. A fraction of the EPS content is loosely bounded in the yeast cells and is easily and fast washed out when suspended in water. Another fraction of the EPS content is more tightly bound in the yeast cells and is therefore not washed out as easily. A part of this tightly bounded EPS content is continually washed out during supra-critical flux operation whereas the washing out at sub-critical flux operation is not observed. This might be due to locally different hydrodynamic conditions at the membrane surface and pore entrances at supra- and sub-critical flux respectively.

Keywords: Vibrating microfiltration; Membrane bioreactor; Critical flux; Yeast cell suspensions; Extracellular polymeric substances.

1. Introduction

Submerged suction pressure driven membrane units are of expanding interest and are often reported in relation to waste water treatment. However, applications in other

areas are also seen. When connected to, or submerged into, a fermentation tank, waste water tank or another tank or reactor, from which water or eventually a solute has to be continually removed, such a set-up is called a submerged membrane bioreactor (SMBR) or simply just a membrane bioreactor (MBR). In contrast to more conventional membrane filtration systems, which are often operated at constant pressure, MBR's are often operated at constant flux, controlled by a suction pump. The pump creates a lowered pressure on the permeate side, thereby inducing a pressure driving force, which often is relatively low. MBR's in different configurations have been described widely in the literature in filtration of waste water [Derance & Jaffrin, 1999; Clech et al., 2003; Ognier et al., 2004; Kimura et al., 2005; Yamato et al., 2006], yeast cell suspensions and biomass suspensions in general [Chang & Fane, 2001; Cho & Fane, 2002; Fane et al., 2002] and different artificial particles (latex, bentonite) [Kim & DiGiano, 2006]. Many of these studies are based on experimental apparatus, which uses hollow fiber membranes. The critical flux concept formulated by Field et al. [Field et al., 1995] back in 1995 is widely used as a guideline flux, below which, long term operation without cleaning is possible. Much have later been said and stated about the strong and weak form of this critical flux hypothesis and in the later years there seem to be a general agreement that the term "normally sub-critical flux" or just "sub-critical flux" is a term that can be used as a guideline level for the flux, at which only an acceptable TMP increase in a given period of time is observed when the flux is kept constant [Cho & Fane, 2002; Ognier et al, 2004; Hughes & Field, 2006].

A widely used approach to avoid fouling in MBR's is the use of air bubbles [Fane et al., 2002; Kim & DiGiano, 2006], but vibrations of a hollow fiber membrane module, which induces shear at the membrane surface, can also be used in order to avoid or reduce fouling problems. Such a system have been described and tested by Genkin and co-workers in the filtration of yeast cell suspensions [Genkin et al., 2006] and we have also earlier tested such a vibrating submerged membrane bioreactor, that consists of a vibrating hollow fiber membrane module, in the filtration of yeast cell suspensions [Beier et al., 2006] and in the separation of an α-amylase enzyme from yeast cells [Beier & Jonsson, 2007]. Our vibrating MBR system is also referred to as a *dynamic microfiltration system*. This system has the advantage of being able to operate at a very low feed flow velocity (< 1 cm/s in the module cylinder) and very low trans-membrane pressures (TMP < 100 mbar) with critical fluxes (depending on the surface shear rate) from 10 – 50 L/(m^2·h) for a membrane module consisting of 54 PES hollow fibers placed vertically in a bundle. Till now, almost only experiments concerning a stepwise increasing flux has been conducted in order to determine critical fluxes and the dependency on the surface shear rate (degree of module vibration) [Beier et al., 2006; Beier & Jonsson, 2007]. The aim of this paper is therefore to present data from constant flux experiments conducted with yeast cell suspensions on the vibrating MBR. The critical flux concept is evaluated and discussed by comparison of constant flux filtrations above (supra-critical) and below (sub-critical) an experimentally estimated critical flux. The influence of extracellular polymeric substances (EPS), which is diffusing or being washed out from the yeast cells, is also evaluated by comparing the permeability drop and fouling resistance

obtained by flux increasing experiments done on a clean and a pre-fouled membrane module respectively. The relative amount of EPS from the yeast cells is measured as the UV absorbance of the bulk supernatant during filtration. The impact of EPS is also investigated by running identical constant flux filtrations on pre-washed and unwashed yeast cell suspensions. Both supra- and sub-critical flux behaviors are investigated in order to get an idea of the extent and nature of EPS fouling from the yeast cells at these two different operational conditions.

2. Theory

The water permeability of the clean and fouled membrane module is used to evaluate the different operational conditions. The permeability of the membrane module is calculated according to Darcy's law [Beier, 2006].

$$J_v = l_p \cdot \Delta P \qquad (1)$$

Thus, the permeability (l_p) is the proportionality factor between the volumetric (J_v) flux and the pressure difference across the membrane (ΔP). In order to evaluate the extent of membrane fouling and fouling resistance, Darcy's law can be rewritten into a resistance-in-series model [Mulder, 1996].

$$J_v = \frac{1}{R_{tot}} \cdot \Delta P = \left(\frac{1}{R_m + R_f} \right) \cdot \Delta P \qquad (2)$$

The total resistance towards transport through the membrane (R_{tot}) can be divided into different sub-resistances. In this work we are only dealing with the membrane resistance (R_m), which is a membrane constant, and the fouling resistance (R_f), which is considered as the additional resistance to the membrane resistance caused by membrane fouling. The membrane resistance is determined from pure water experiments in which no fouling of the membrane occurs.

The permeability drop of the membrane is also used to evaluate the filtration performance. The permeability drop is defined as follows [Beier et al., 2007]

$$Permeability\ \ drop = \frac{l_p(initial) - l_p(final)}{l_p(initial)} \cdot 100\% \qquad (3)$$

The initial and final permeabilities are determined from water flux experiments and Darcy's law. The final permeability is measured after the experiment has been stopped and the system and membrane module has been rinsed only with water.

3. Materials and methods

The vibrating MBR used in this work consists of a module with 54 hollow fibers placed vertically in a bundle. The system is described in details by Beier et al. [Beier et al., 2006]. Relevant membrane parameters are listed in Table 1.

Table 1: Characteristics of the membrane module

Hollow fiber Manufacturer	Membrane material	Number of fibers	Length of fibers (cm)	Total membrane area (cm^2)	Pore size (μm)	Permeability of module, ($L/(m^2 \cdot h \cdot bar)$)
X-flow, Netherlands	PES/PVP (98%/2%)	54	12.5	487	0.36-0.50	1518 ± 124

The fibers are made of a polyethersulphone (PES) and polyvinylpyrrolidon (PVP) blend in a 98%/2% ratio. The 2% PVP is added in order to make the fibers more hydrophilic, since hydrophilic membranes tend to foul less than more hydrophobic ones. The average water permeability of the clean membrane module has been measured 1518 $L/(m^2 \cdot h \cdot bar)$ according to equation (1) with a standard deviation of 124 $L/(m^2 \cdot h \cdot bar)$ based on five measurements. A sketch of the system is shown in Figure 1.

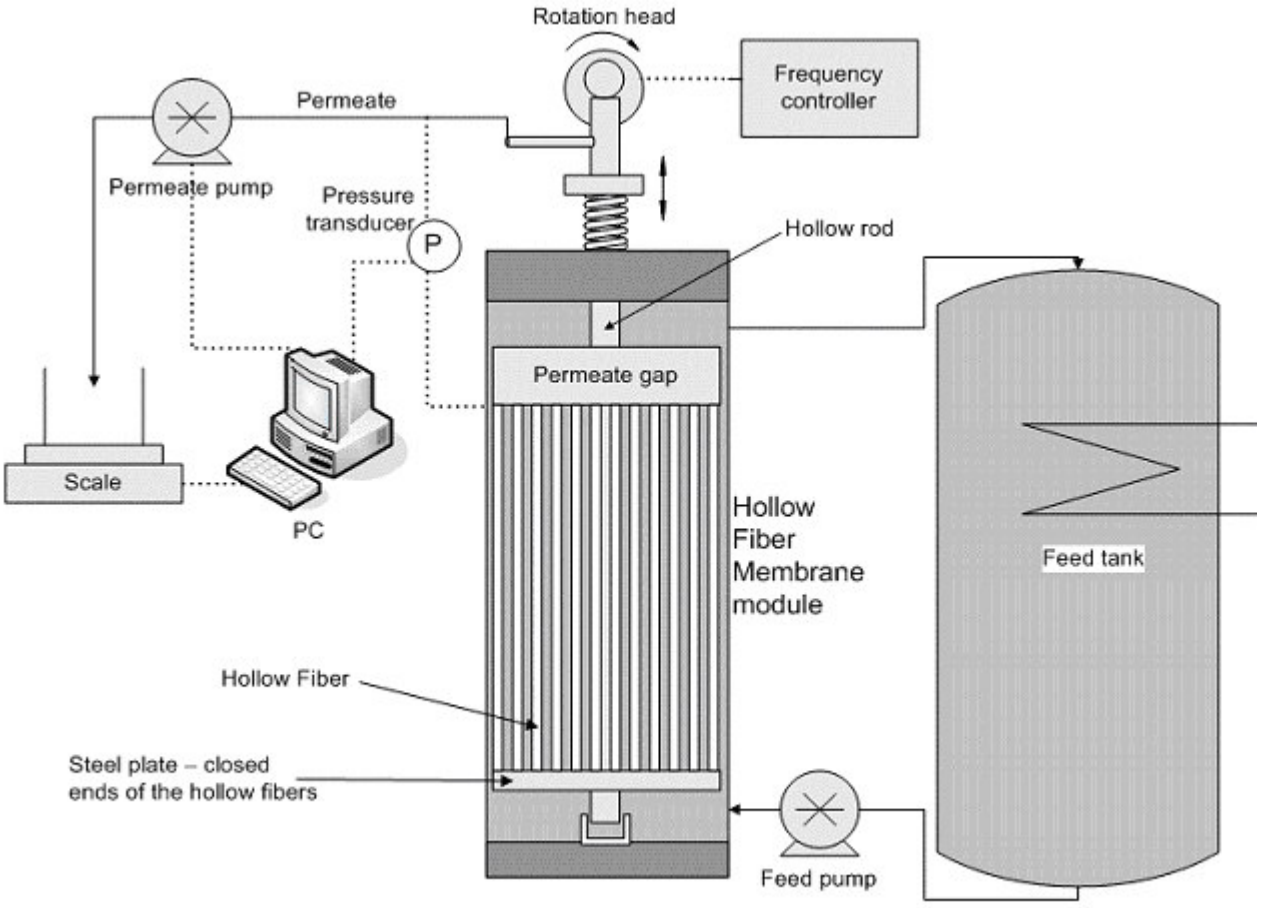

Figure 1: Sketch of the experimental apparatus [Beier et al., 2006].

The skin layer is located on the outside of the fibers, which are all closed in the bottom ends through the steel plate. The top ends of the fibers are via a permeate gap and the hollow rod connected to a suction pump that sucks permeate through the fibers at constant rate. Permeate is collected in a beaker on an electronic scale connected to the PC. The permeate pump is controlled by a PC and the corresponding TMP is monitored and logged by the PC by use of a pressure transducer. The module is placed in a plastic cylinder connected to a feed tank. The feed fluid (3 L in total) is circulated between the feed tank and module cylinder by a feed pump at very low pumping rate corresponding to a velocity in the module cylinder below 1 cm/s. The membrane module can be vibrated in the module cylinder at variable frequency and amplitude by a “rotation head”. Suspensions of bakers yeast are filtrated. Experimental parameters are given in Table 2.

Table 2: Experimental parameters.

Feed flow velocity (cm/s)	Vibration frequency (Hz)	Vibration amplitude (mm)	Dry yeast content of feed (g/L)	Critical flux ($L/(m^2 \cdot h)$)
0.91	25	0.7	4.0	15

At the experimental parameters given in Table 2, a critical flux has earlier been determined to 15 $L/(m^2 \cdot h)$ by Beier et al. [Beier et al., 2006] by a stepwise flux increasing method. Constant flux experiments are conducted above the critical flux (supra-critical) and just below the critical flux (sub-critical). The effect of EPS from yeast cells, that fouls the membrane, is investigated by measuring the UV absorbance of the bulk supernatant during the filtration and by running identical filtrations on pre-washed and unwashed yeast suspensions.

- *Unwashed*: The dry yeast is suspended in water.
- *Washed*: The dry yeast is suspended in 1 liter of water and centrifuged. After centrifugation the supernatant is removed (and UV absorbance is measured) and the remaining bottom yeast slurry is resuspended in 1 liter of water. The centrifugation procedure was repeated 6 times. The suspension was left in the refrigerator for 24h between the 5^{th} and 6^{th} centrifugation in order to investigate the time effect of the EPS washing-out.

The bulk supernatant has an absorption maximum at 260 nm. This is in agreement with the absorption maximum at 260-264 nm for yeast suspension supernatant reported by Hughes and Field [Hughes & Field, 2006]. Before and after each filtration, the permeability of the membrane module is measured after rinsing with water. The chemical cleaning of the membrane module was done with a 0.05% caustic solution (NaOH) at $50^{o}C$ for 1 hour.

4. Results and discussion

4.1. Supra- and sub-critical flux filtrations

The critical flux concept [Field et al., 1995] is evaluated by running two constant flux filtrations for approximately 5 hours with yeast suspensions of 4 g/L (unwashed). One filtration is conducted at supra-critical flux (30 L/(m^2·h)), which corresponds to a level twice as large as the critical flux. The second filtration was done at sub-critical flux (14 L/(m^2·h)), which is just below the critical flux. TMP data for the two filtrations is shown in Figure 2.

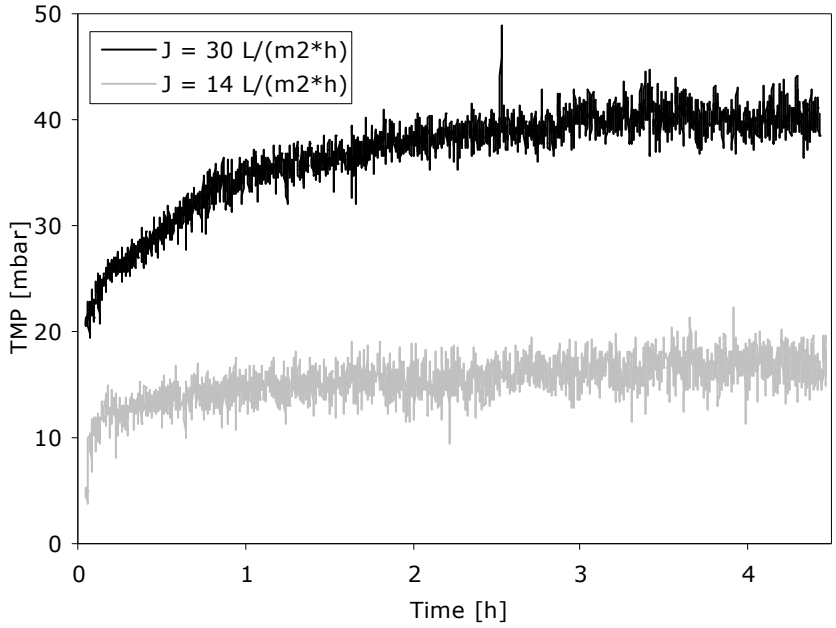

Figure 2: TMP for constant flux filtrations at supra-critical flux (30 L/(m^2·h)) and at sub critical flux (14 L/(m^2·h)). Feed dry yeast content = 4 g/l.

In Figure 2 it is seen that in both cases the pressure initially needs some time to stabilize. It is also seen that in both cases the TMP continually increases but at sub-critical flux the increase is only marginally compared to at supra-critical flux. The initial sequence of the TMP curve at supra-critical flux seems to be slightly different from the TMP curve at sub-critical flux since the initial slope of the curve is larger for a longer period (0-2h). This might indicate that the nature of the fouling and the fouling mechanism during the filtration (will discussed later) at supra- and sub-critical flux differs. Such behavior is also reported by Defrance and Jaffrin during filtration of waste water with a MBR [Defrance & Jaffrin, 1999]. Overall, the TMP is larger and increases more at supra-critical flux than at sub-critical flux.

The initial permeability of the membrane module after chemical cleaning is around 1500 L/(m^2·h·bar). For the two 5h filtrations shown in Figure 2, the final permeability is measured after rinsing with water. The membrane resistance is determined from the clean module water permeability according to equation (2), in which the fouling resistance is zero. The total resistance is determined from the final permeability according to equation (2). From the total resistance and the membrane resistance, the fouling resistance is determined. The permeability data and the resistances are shown in Figure 3 for the two 5 hours constant flux filtrations.

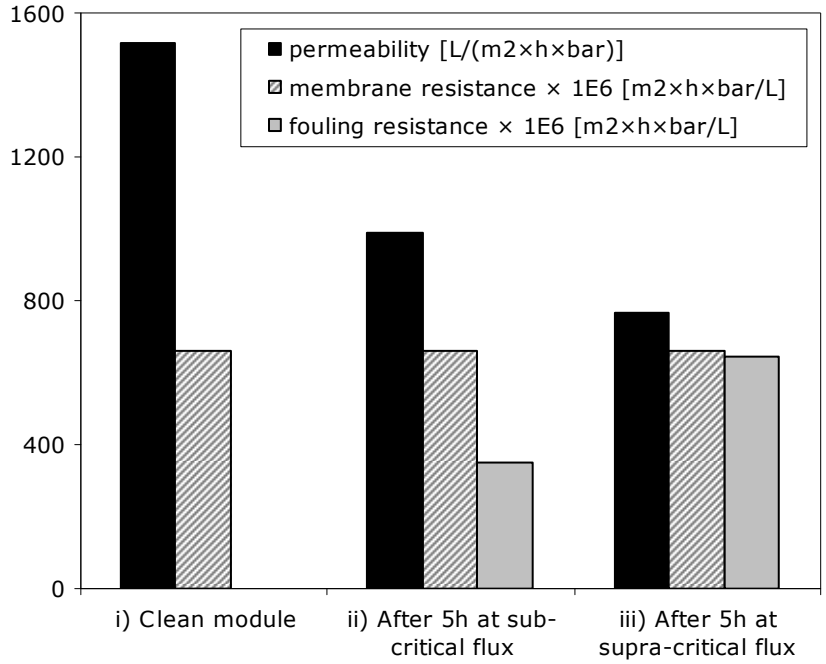

Figure 3: Permeabilities and resistances for i) clean module, ii) for constant flux filtration at sub-critical flux (14 L/(m^2·h)) for 5 hours and iii) for constant flux filtration at supra-critical flux (30 L/(m^2·h)) for 5 hours. Feed dry yeast content = 4 g/l.

In Figure 3 it is seen that when filtrating at sub-critical flux the final permeability is higher (990 L/(m^2·h·bar)) than when the filtration was done at supra-critical flux (766 L/(m^2·h·bar)). The permeability drop caused by exceeding the critical flux by 100 % for 5 hours, however, does not seem severe, but by looking at the fouling resistance it is seen that at supra-critical flux the fouling resistance is almost twice as large as the fouling resistance at sub-critical flux. At supra-critical flux the fouling resistance is at the same level as the membrane resistance. The results from these two experiments can be summarized:

- At supra-critical flux a rather large increase in TMP in the first 2 hours is observed. After 2 hours the TMP increase is not that severe. The final permeability is 766 L/(m^2·h·bar) after 5 hours (the initial permeability is 1518

L/(m^2·h·bar)). The fouling resistance after the experiment has been stopped is almost at the same level after the experiment as the membrane resistance.

- At sub-critical flux the rapid TMP increase in the first 2 hours is not observed. Only the rather slow TMP increase is observed. The final permeability is 990 L/(m^2·h·bar) after 5 hours. The fouling resistance after the experiment is only around half the value compared to at supra-critical flux.

The results and TMP-curves indicate that the critical flux is not sharply defined, but a clear difference in fouling resistance is observed. Field and co-workers, who in 1995 introduced the critical flux concept (hypothesis) [Field et al., 1995], have in 2006 together with Hughes [Hughes & Field, 2006] introduced a more weak or loose definition of the critical flux, which they refer to as a "normally sub-critical flux". This is a flux, below which only a minor and acceptable TMP increase is observed. Such a definition of the critical flux is a compromise between acceptable TMP increase and acceptable flux level. This is also mentioned by Defrance and Jaffrin [Defrance & Jaffrin, 1999] and Le Clech and co-workers [Le Clech et al., 2003]. Our results support this newer (and weaker) definition of the critical flux as a flux below which the TMP (at constant flux) only increases marginally in a given time period. The critical flux is then more likely a flux interval than an absolute flux value. This could be due to the fact that the local flux can vary along the fiber length because of a pressure loss inside the hollow fiber. Thus, the local flux in one fiber end is larger than in the other end and therefore the largest local flux can exceed the critical flux, since the critical flux is often determined as an average flux over the whole fiber length. Longer fibers lead to larger flux distributions along the fiber length because of larger pressure losses. This phenomenon has been described by Kim and DiGiano [Kim & DiGiano, 2006] who have investigated microfiltration of latex particles using single hollow fibers. Their conclusions support the idea of referring to the critical flux as being length averaged. However, the problem with length distribution of the flux in our case is not considered to be large, since we only use fibers with a length of 12.5 cm, whereas the fibers used by Kim and DiGiano [Kim & DiGiano, 2006] are 30 and 100 cm respectively.

4.2. Influence of EPS

EPS have been reported extensively in the literature to have a rather large impact on membrane fouling [Ye et al. (i), 2005; Ye et al. (ii), 2005; Hernandez Rojas et al., 2005; Chen et al., 2006; Hughes & Field, 2006]. This is also the case with the vibrating MBR in filtration of yeast cell suspensions where the fouling is referred to as EPS from the cells. Two similar experiments have been conducted at which the flux was stepwise increased from 0 to 40 L/(m^2·h) over a period of 2.5 hours according to a procedure described by Beier and Jonsson [Beier & Jonsson, 2007]. The initial and final permeability of the membrane module was measured after water rinsing according to equation (1). The first experiment was conducted with an initial chemically cleaned membrane and the second experiment was conducted after the first experiments when the membrane module was only rinsed with water. We refer to

the latter state as "fouled" or "pre-fouled", since the membrane module had not been chemically cleaned at this state. The initial permeability of the membrane module in the second experiment therefore corresponds to the final permeability of the first experiment. The permeability drop is calculated according to equation (3). The fouling that has been "added" to the module during the two experiments can be calculated as a fouling resistance according to equation (2). The permeability drop during the two experiments are shown in Figure 4, as well as the fouling resistances and the membrane resistance.

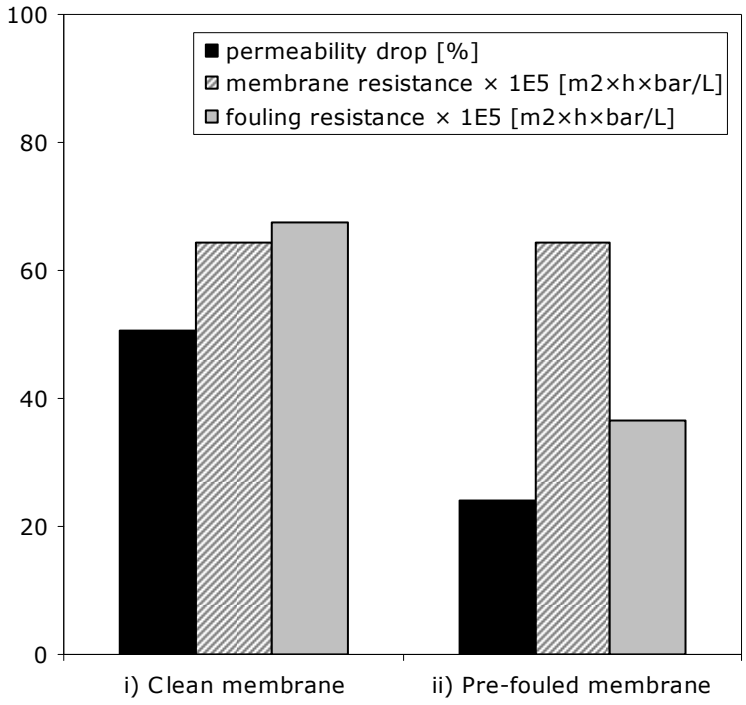

Figure 4: Permeability drop, membrane resistance and fouling resistance for two identically conducted experiments with i) a clean and ii) a pre-fouled membrane module. Flux stepwise increased from 0 to 40 L/(m^2·h) over a period of 2.5h. Feed dry yeast content = 4 g/l.

It is seen that the permeability drop for the clean membrane module is around 50 %. This permeability drop might be caused by EPS from the yeast cells that is "washed" out of the yeast cells during filtration and therefore is able to foul the membrane. Since a permeability drop is observed, part of the EPS fouling must be irreversible attached to the membrane, since it is not removed by water rinsing. This is in agreement with Hughes and Field [Hughes and Field, 2006] who have reported the irreversible fouling of EPS from yeast cells to be rather independent of the hydrodynamic conditions, whereas the reversible fouling is affected by hydrodynamic conditions such as the shear rate or shear stress at the membrane surface. The fouling resistance is at the same level as the membrane resistance for the clean module, which was also seen for the experiment depicted in Figure 3 at supra-critical flux. When the

experiment is conducted with a pre-fouled membrane, the permeability drop is only around 25 % and the fouling resistance is only around half the value of the membrane resistance. An explanation could be that initially the membrane is already fouled to some extent by irreversible bounded EPS from the first experiment with the clean module. This probably creates a dense layer directly on the membrane surface. That way the "new" EPS from the present yeast cells is not able to foul the membrane as much as in the first experiment, since much of the membrane surface is already "occupied" by EPS fouling. Since the fouling resistance on the pre-fouled membrane is only around half the value of the clean membrane fouling resistance, the "new" fouling layer on the pre-fouled membrane must be less dense. The permeability of the fouled membrane module could in all cases be recovered to the initial water permeability of around 1500 L/(m^2·h·bar) by chemically cleaning.

The effect of EPS from the yeast cell is also investigated by measuring the UV absorbance of the bulk supernatant during the previously mentioned 5 hours constant flux filtrations at supra- and sub-critical flux respectively. The bulk supernatant UV absorbance is depicted in Figure 5 together with the fouling resistance, calculated according to equation (2).

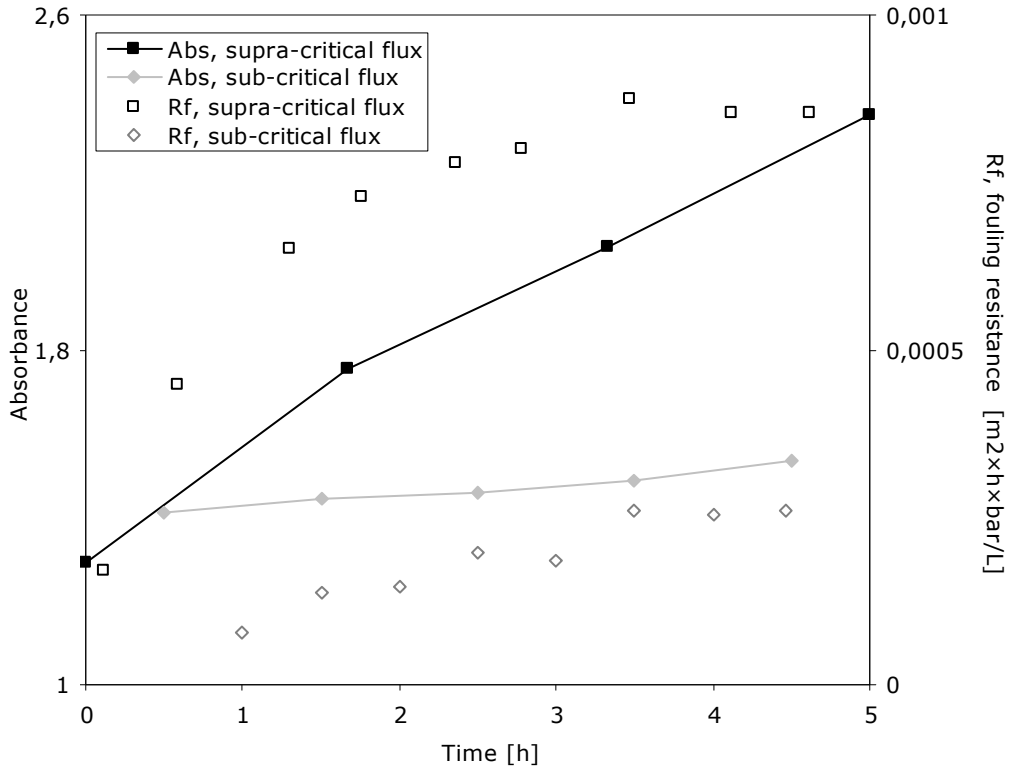

Figure 5: Bulk supernatant UV absorbance (260 nm) and fouling resistance for constant flux filtration at supra-critical flux (30 L/(m^2·h)) and sub-critical flux (14 L/(m^2·h)) for 5 hours. Feed dry yeast content = 4 g/l.

The absorbance increase at sub-critical flux seems to be small, whereas the absorbance continues to increase at supra-critical flux. Also, the increase in fouling resistance at sub-critical flux is much less than at supra-critical flux. Thus, the level of EPS (measured as absorbance) and fouling resistance seem to be related). At supra-critical flux, EPS is continually washed out during filtration, whereas the level of EPS seems to be rather constant at sub-critical flux. Since the bulk hydrodynamic conditions in both experiments are almost identical, much of the EPS "washing out" during filtration might occur at the membrane surface (pore entrances). The local hydrodynamic conditions at the pore entrances might change around the critical flux causing a larger washing-out of EPS at supra-critical flux. This might explain why the absorbance keeps increasing at supra-critical flux, whereas the washing-out of EPS from the yeast cells at sub-critical flux is small, resulting in a rather constant level of EPS content in the bulk. Such a change in local hydrodynamic conditions at a certain flux level, influencing the filtration performance, has earlier been reported by Jonsson and co-workers related to microfiltration and ultrafiltration of BSA solutions [Jonsson et al., 1992].

The behaviour at supra-critical flux is further investigated by filtration of both unwashed and pre-washed yeast suspensions. During the washing process the absorbance of the washing water supernatant after each suspension and centrifugation is measured and depicted in Figure 6.

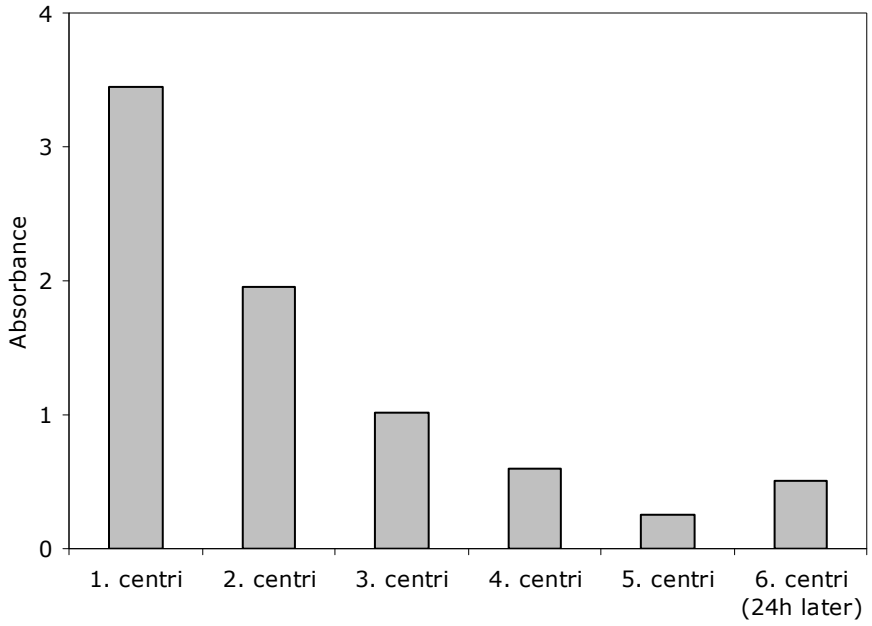

Figure 6: UV absorbance (260 nm) of washing water supernatant during the washing process after each suspension and centrifugation of the yeast cell suspension (12 g/L). "Centri" = Centrifugation.

In Figure 6 it is seen that part of the EPS content from the yeast cells is washed out and removed by the suspensions and centrifugations. It is also seen that not all EPS is washed out immediately since the absorbance is increased after the yeast cell

suspension has been left in the refrigerator for 24h between the 5th and 6th centrifugation. Therefore, part of the EPS content is probably more easily washed out compared to other parts of the EPS content. In Figure 7, the absorbance of bulk supernatant data is shown for the constant supra-critical flux experiments (30 L/(m^2·h)) with the washed and unwashed yeast cell suspensions respectively. Also, the fouling resistances, calculated according to equation (2), are depicted.

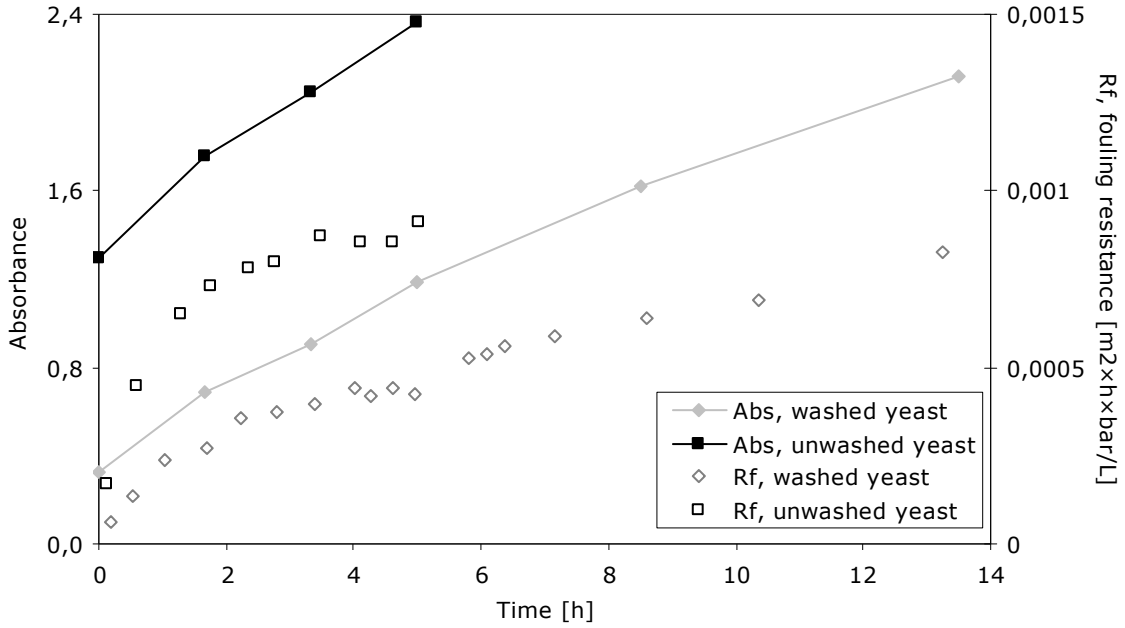

Figure 7: UV absorbance (260 nm) of bulk supernatant and fouling resistance for constant supra-critical flux experiments (30 L/(m^2·h)) of washed and unwashed yeast cell suspensions (4 g/l).

The washing out of EPS (from the washed yeast cells) continues during constant supra-critical flux filtration which is seen in Figure 7 where the bulk supernatant absorbance continues to increase during the 13.5 h filtration period. This, again, shows that the EPS content of the yeast cells is not completely washed out during the initial washing process and that the continually washed-out EPS fouls the membrane and gives a continually increasing fouling resistance. This continually washing out of EPS at supra-critical flux might take place, as mentioned earlier, around the pore entrances where the local hydrodynamic conditions may favor this EPS washing out. The difference in UV absorption is almost constant for the filtration of washed and unwashed yeast suspensions, which might indicate that the loosely bounded EPS probably is washed out initially, rather fast (also seen in Figure 6). The more tightly bounded EPS seems to be continually washed out during filtration in a similar manner for both the washed and unwashed yeast suspensions, since the fouling resistances increase at almost the same rate after the first two hours. The tightly bounded EPS, thus, does not seem to be removed by the initial pre-washing/centrifugation process

but is probably only washed out at the membrane surface (or pore entrances perhaps) during supra-critical flux filtration.

5. Conclusions

Constant supra- and sub-critical flux experiments have been conducted with the vibrating microfiltration membrane bioreactor and the critical flux concept has been evaluated. Bakers yeast cell suspensions have been tested.

- The critical flux seems to be an interval or a relative value rather than an absolute value. Filtration just below the critical flux seems to be a good compromise between acceptable flux level and TMP increase. At this level only a minor increase in fouling resistance is observed compared to at supra-critical flux.

EPS fouling influence and mechanism have been investigated for filtrations at sub- and supra-critical flux.

- EPS causes some irreversible fouling of the membrane module. Therefore a permeability drop is observed during filtration of yeast suspensions at both sub- and supra-critical flux. The permeability drop is largest at the supra-critical flux condition.
- Some EPS is fast washed out of the yeast cells and some is continually washed out during filtration (the latter being the part that is not removed by pre-washing and therefore more tightly bounded in the yeast cells). The continually EPS washing-out during filtration only seems to take place at supra-critical flux. We propose to explain this by saying that the washing out of more tightly bound EPS happens around the pore entrances where the local hydrodynamic conditions (at supra-critical flux only) facilitate the washing-out process. The local hydrodynamic conditions at sub-critical flux at the membrane surface do not seem to facilitate such a washing-out process of EPS from the yeast cells. This is based on UV absorbance measurements of bulk supernatant during the filtration experiments.
- EPS fouling has a larger effect on the TMP and fouling resistance increase at supra-critical flux than at sub-critical flux (the cells are expected not to be able to stick to the fast vibrating module – leaving the EPS as the only matter being able to foul the membrane).
- The loosely bounded EPS fouls the membrane independently weather the flux is supra- or sub-critical. It is the more tightly cell-bounded EPS that probably only fouls the membrane at supra-critical flux because only at this stage the EPS washing-out from the cells is facilitated.

The critical flux seems to be a sensible compromise between acceptable flux level and acceptable TMP and fouling resistance increase. The critical flux seems to be a level below which the continually washing out of tightly cell-bounded EPS from the yeast cells is relatively low leading to a relatively controlled and only slow increasing TMP and fouling resistance.

6. List of symbols

Abs	UV absorbance (260 nm)	[-]
J_v	Volumetric flux	[L/(m^2·h)]
l_p	Water permeability	[L/(m^2·h·bar)]
P	Pressure	[bar]
R_{tot}	Total resistance	[m^2·h·bar/L]
R_m	Membrane resistance	[m^2·h·bar/L]
R_f	Fouling resistance	[m^2·h·bar/L]
TMP	Trans-membrane pressure	[mbar]

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