EXPERIMENTAL STUDY OF THE *Escherichia coli* RETENTION MAKING USE OF CROSSFLOW MICROFILTRATION WITH DIFFERENT MICRO-POROUS STRUCTURES

Renata Natsumi Haneda[1], Carlos Alberto Fortulan[1], Rogério A. Ikegami[1], Benedito M. Purqueiro[1], Elson Longo[2], Sérgio Rodrigues Fontes[1]

[1]São Carlos School of Engineering, University of São Paulo – USP – Department Mechanical Engineering
Av. Trabalhador São-carlense, 400, C.P.359; 13566-590, São Carlos, São Paulo, Brazil – Tel.: 0055-021-16-33739531
e-mail: rnh@sc.usp.br / e-mail: srf@sc.usp.br

[2]University Federal of São Carlos – Department Chemistry São Carlos, São Paulo, Brazil

Abstract*. The crossflow microfiltration process was studied with alumina micro-porous tubes and commercial membrane in the retention and/or elimination of bacteria founded in the buffalo´s milk and in the residual serum of the buffalo´s cheese production. These micro-porous tubes were synthesized with temperature close to 1450ºC to define the pore size. Subsequently, in an of tubes was impregnanted the silvers´s citrate solution in the ceramic tubes, with a new synthesize for the tubes at 600ºC and consequence formation of silver´s nanoparticles. The average pore size and the morphology of the micro-porous structure were analysed respectively through the Technique of Porosimetria for Mercury Intrusion and Scanning Eletronic Microscopy (MEV). The tube´s performance on process was analysed through the trans-membrane flux, at the turbulent regimen and low trans-membrane pressure (150kPa) on microfiltration apparatus. To analyse the decrease of microrganisms on the permeate, counting of total and fecal coliform was made following microbiology parameters standard. The physiology of the bacteria was controlled by parameters of pH and temperature.*

Keywords: *Microfiltration, micro-porous structure, Alumina, Silver, Escherichia coli*

1. Introduction

Microfiltration (MF) is the important membrane technology of filtration. It is a pressure-membrane process for the separation of fine particles, microrganisms and emulsion droplets (Cheryan, 1998). In the crossflow microfiltration, the fluid to be filtered flows parallel to the membrane due to a pressure difference (transmembrane pressure). The crossflow reduces the formation of hydraulic resistance type cake what keeps the process at a low level of filtration. The crossflow microfiltration has become an established process for the separation of microparticles, bacteria and emulsion droplets in a variety of industrial applications (Zeman and Zydney, 1996; Ripperger and Altmann, 2002). The crossflow microfiltration is influenced by a great number of parameters, e.g. crossflow velocity, transmembrane pressure, membrane resistance, layer resistance, size distribution of the suspended particles, particles form, agglomeration behavior and surface effects of the particles etc. Another important feature is the high porosity of membranes which leads to high filtration rates at small pressure differences(Ripperger and Altmann, 2002).

Silver have been used for centuries as bactericide. One of the most fascinating properties of silver is its bactericidal quality (Silver Healthy, 2004). Small concentrations of silver or silver salts kill bacteria by chemically affecting the cell membranes, causing them to break down. Bacteria do not develop resistance to silver, as they do to many antibiotics. The literature (Sondi and Salopek-Sondi, 2004) confirmed that the E. coli cells were damaged, showing formation of "pits" in the cell wall of the bacteria, while the silver nanoparticles were found to accumulate in the bacterial membrane. Even though combined with other metallic composites (zirconium, alumina and titanium), the silver also have attracted a great deal of sanitary attention for restraining harmful microrganisms and viruses from spoiling our living space (Matsunaga *et al.*, 1985 and Kourai *et al.*, 1994). Depending on the silver nanoparticle concentration (50-60µg cm^{-3}), bacterial growth would be inhibited up to 100% (Sondi and Salopek-Sondi, 2004). Moreover, membrane processing techniques have been proposed for the reduction or elimination of microrganisms in fluids.

Other interesting property about microrganism retention on microfiltration membranes is that MF membranes intended for use as a microbial barrier must be characterized by the maximum pore size, bacterial cells can penetrate pores that have a significantly smaller size (Figure 1) and the cell passage through the

smaller pores, given that the cell membrane cannot be stretched (Lightfood, 1974), appears to be possible only due to the cell volume reduction (Suchecka, Biernacka and Piatkiewicz,2003).

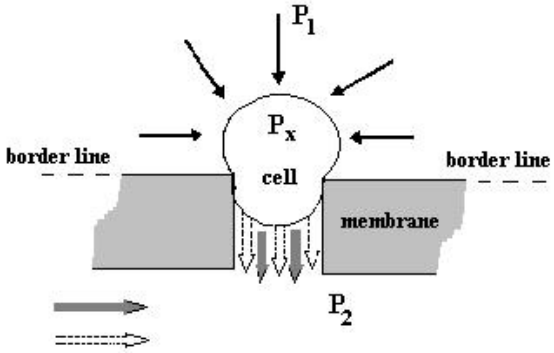


Figure1. Scheme of mechanism (pressure gradient and filtration flux) transporting bacterial cell across the membrane adapted by Suchecka, Biernacka and Piatkiewicz (2003)

Milk, as a complex liquid with high protein content and is exposed to microbiological growth (Guerra *et al*., 1998) as an ideally suited for membrane separation treatment. Application of the crossflow microfiltration in the dairy industry is still at the introductory stage because of pronounced membrane fouling which leads to reduced permeate flux and decreased membrane selectivity. The most significant application of crossflow microfiltration is the "bactocatch" process which uses a 1,4µm membrane to remove 99,6% of the bacteria from skim milk (Krstić *et al*., 2002). During recent years crossflow microfiltration using ceramic membranes has become increasingly important in the dairy industry, both for the purposes of clarification of whey and skim milk (Surel and Famelart, 1995).

In this work was studied the bufffalo´s milk and residual serum in the buffalo´s cheese production, rich in excellent nutrients to bacterial growth. In the buffalo´s milk is found a lot of microrganisms, for example, total and fecal (E. coli) coliform bacteria. The elements of filtration or the micro-porous tubes were manufactured with alumina ($3Al_2O_3$) and burned rigidly in the temperature next of 1450ºC. Later, an of tubes structures were treaty chemistly through the impregnation with silver´s citrate solution by adsorption. Again, the tubes were burned to get silver nanoparticles next to porous ceramic. The attendance of the bacteria decrease in the buffalo´s milk and residual serum of the buffalo´s cheese production was made through the counting of the fecal and total coliform by 20ª Edition of the Standard Methods for Examination of Water.

2. Materials and Methods

The ceramic tubes of alumina with dimensions: 190mm of length and 10mm of internal diameter were molded and studied. One of micro-porous ceramic tube was used to the heat the electric furnace to the final sintering temperature of 1400ºC or 1450ºC (this supplied from Cetebra- Tecnicer Ltda, São Carlos, São Paulo, Brazil). Using the mercury intrusion technique was measurement the average pore size.

The solution of silver´s citrate was prepared following the ratio of 3 mols of citric acid anidro for 1 mol of silver nitrate (Figure 2). The impregnation was made through the capillarity process, with the tube in contact with the solution during 24 hours. After, the tube was to heat in nitrogen atmosphere so that metallic silver was formed, varying it temperature enters 100ºC to 600ºC.

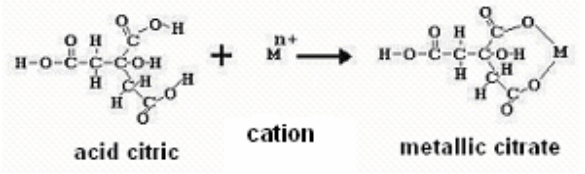


Figure 2. Reaction of the preparation of metallic citrate of silver. M = silver.

Another ceramic tubes of alumina considerate monolayer (homogeneous medium) and bilayer (membrane) were sintering. The tube to sum up with sacarose (50vol.%) in form of membrane (bilayer) was sinterized at same temperature of 1450ºC. Theses tubes were manufactured in Laboratory of Tribology of São Carlos School of Engineering – USP.

The microfiltration apparatus system (Figure 3) was installed in a sterilized atmosphere.All the experimental samples of crossflow filtration (Figure 3) were achieved with properly sterilized environmental. All the parts of apparatus were immersed inside of a container contend a water solution distilled + formaldehyde, during approximately 8 hours for a complete sterilization. To rinse the parts of the group of benches, water distilled in the temperature of boiling (100ºC) was transferred. All the system was displayed in a sterilized atmosphere contends a fluorescent light bulb - GL - Germicida of the ultraviolet type, to prevent contaminations until the beginning of filtration.

The buffalo´s milk emulsion contend bacteria of the fecal coliform group (E. coli) was diluted in water distilled in the ratio of 50%, followed beyond the microbiological parameters and physical-chemical properties as pH, pressure trans-membrane and temperature. The residual serum (wastewater) of the cheese production was not diluted, however all the necessary cares for its microbiological maintenance also were kept. All the material studied was generously supplied by Cooperative of Laticínios of São Carlos, São Paulo, Brazil.

Figure 3. Apparatus of microfiltration process.

The filtration was constant in the transmembrane pressure of ΔP_T = 150 kPa and permeate was collected one to the 10, 20, 30, 40 e 50 minutes to make the microbiological analyses, or counting of fecal coliform based on 20ª Standard Edition Methods for the Examination of Water and Wastewater. The permeates flux had been filtered and passed for a selective membrane for counting of coliformes through of agar Chromo Cult. The membrane was placed in the greenhouse to the temperature of 37ºC during 24horas for incubation and posterior interpretation of the results.

3. Characterization of the micro-porous structure

The technique of Mercury Intrusion was used in this work for characterization of the micron-porous structure. The Figure 4 shows a graph of the distribution of volume in function of the average diameter of the pores of the ceramic tube of alumina sinterized at 1450ºC. The tubular micro-porous structure presented average size of pores of approximately 0,5µm.

The Figure 4b is presented the visualization of the internal surface of tube micro-porous bilayer. This tube presents size porous of 1,5 µm (membrane) and size porous of 30 µm (support). The white box indicated in the figure 4b has dimension of approximately 3,0 µm. The dark gray part where meets the white boxes represents the microporous of membrane (bilayer tube), while the clear part below meets the support of the bilayer tube manufactured with sacarose.

In the Figure 5a is observed the image of electronic microscopy (MEV), where the white part identifies for EDX the presence of silver in the micron-porous structure sinterized at 1450ºC. The Figure 5b represents the

silver mapping (orange) on the surface of the ceramic material objectifying itself to analyze the morphology of the tubular porous microstructure. These analyzes it guaranteed the presence of silver in the tube.

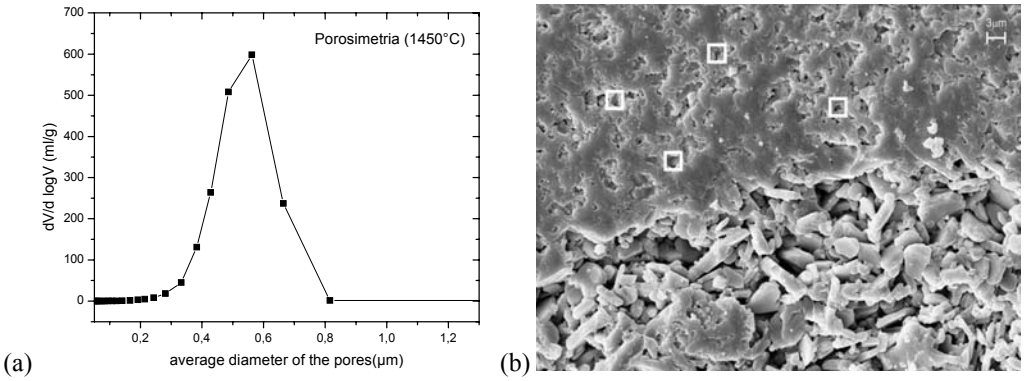


Figure 4. (a) Analysis of porosimetry of the tubular micro-structure sinterized at 1450ºC; (b) Visualization from MEV of the surface of bilayer tubular micro-structure sinterized at 1450ºC.

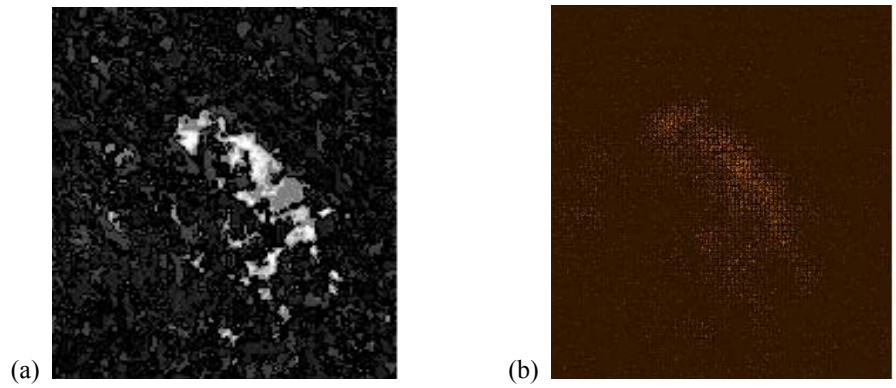
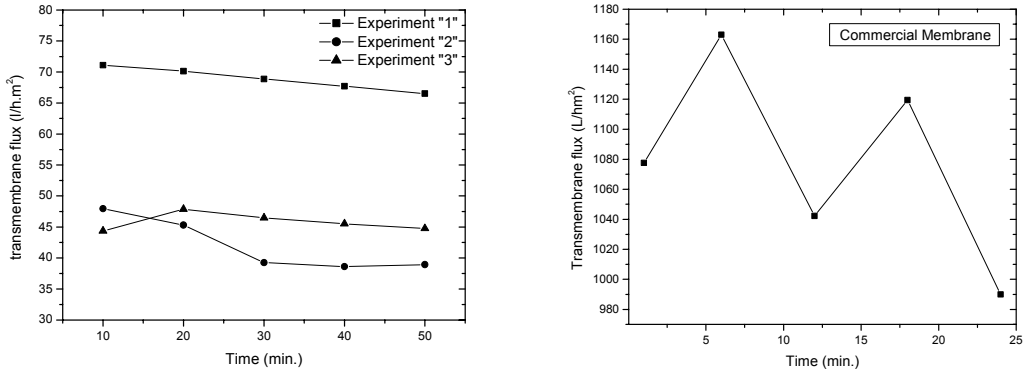


Figure 5. (a) Image of electronic microscopy of the micro-porous alumina structure sinterized at 1450ºC; (b) silver mapping (orange) on the surface of the micro-porous tubular material.

4. Results and Discussion

The Figure 6a shows the graphical of transmembrane flux (l/h.m^2) in fuction of time (minutes) of microfiltration process with the buffalo´s milk. As showed, the behavior of the transmembrane flux vs. time varied between the experiments, but did haven´t occurrences of a reduction of the permeate flux, as occurs typically in the process with formation of cake layer, or polarization layer (Zeman and Zydney, 1996). As also observed in literature (Kristć *et at.,2002),* the curve correspondent to the transmembrane flux(L/hm^2) versus time(minutes) (experiment "1" in figure 6a) with microporous alumina tube (sinterized at 1450ºC and impregnated with silver) shows fouling during the process. The flux decline is usually attributed to concentration polarization and rapid formation of fouling deposit; the first stage appeared to be initial adsorption of bacteria, fats and proteins of the buffalo´s milk in the membrane (Vetier *et al.*, 1988; Savello *et al.*, 1997, Carić *et al.*,2000).

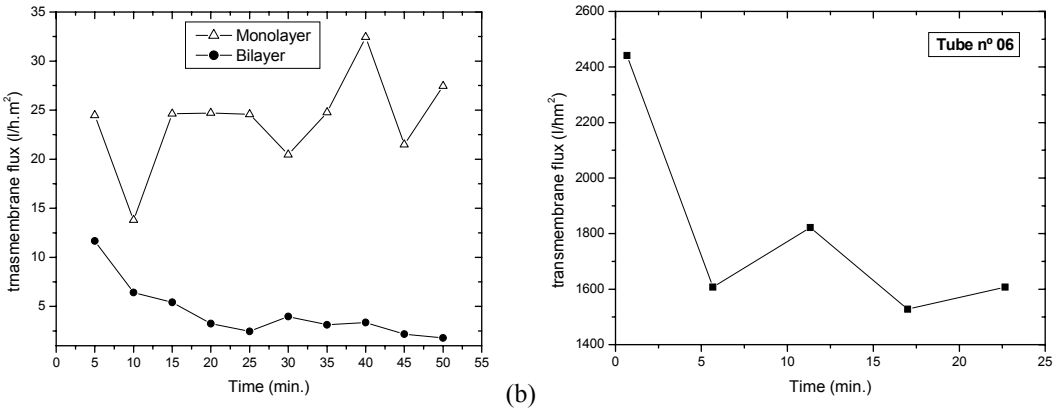
The Figure 6b shows the transmembrane flux v.s. time for the microfiltration process with commercial membrane (cut-of of 0,8µm) in turbulent flow, Re = 31500 and ΔP_I=150 kPa. The reduction of the transmembrane flow, of J= 600 l/h.m^2 in function of the time is the usual polarization phenomena (cake layer) observed in commercial membranes (Zeman & Zydney, 1996).



(a) (b)

Figure 6. (a) Graphical of the membrane flux v.s. time for microfiltration process for the buffalo´s milk in turbulent draining, Re = 31500 and ΔP_T=150 kPa in microporous tube impregnated with silver at 600ºC. (b) Graphical of the transmembrane mass flux v.s. time for the microfiltration with commercial membrane (cut-of 0,8μm) in turbulent flow, Re = 31500 and ΔP_T=150 kPa.

The Figure 7a ("-Δ-") shows the transmembrane flux (l/h.m^2) of the monolayer tube in the beginning of the filtration (J= 24 l/h.m^2) and after 50 minutes (J= 25 l/h.m^2). The intermediate results have the one great variation. The behavior of the permeate flux (figure 7a,"-●-") of bilayer tube despite the oscillations in the results, had presented a certain reduction of permeate in function of time, with typical formation of cake layer.



(a) (b)

Figure 7. (a) Graphical of the transmembrane flux versus time for the microfiltration of residual serum with tube of type monolayer and bilayer (sinterized at 1450ºC); (b) Graph of the transmembrane flux v.s. time for the monolayer tube composted with sacarose.

The Figure 7b shows the graphical of the transmembrane flux (l/h.m^2) v.s. time (min.) of the process with the residual serum of the cheese production with monoporous tube (monolayer) sinterized at 1500ºC. The transmembrane flux (L/h.m^2) in fuction of time shows bigger values than of Figure 7a. This aspect is device to sacarose used in the composition of tube of alumina, as a mechanism to increase the flux of permeate; this result was gotten to Re= 31500 and ΔP_T=150 kPa.

The next figures (8 to 13) presents images of analyzes of Petri´s plate corresponding to the process of the figures 6 and 7. In the plate of Figure 8a is observed dark points indicated for dark arrow; that is the own contamination of fluid of process. In the plates of Figure 8, respectively 8b the surface of the Petri´s plate is observed dark points (for dark arrow), indicating the contamination of permeate. In the case, as the Chromo Cult was used to identify bacteria of coliform group, and can be said that the permeate in the benning of the filtration was contaminated. But in the plate corresponding to the permeate collected at 50 minutes (figure 8c) do not have dark points, evidencing result promising on the microfiltration and the absence of bacteria.

In another process studied the retention of bacteria, the permeate was colleted at 50 minutes (figures 9b and 9c). Also in this process there is the total retention of bacteria.

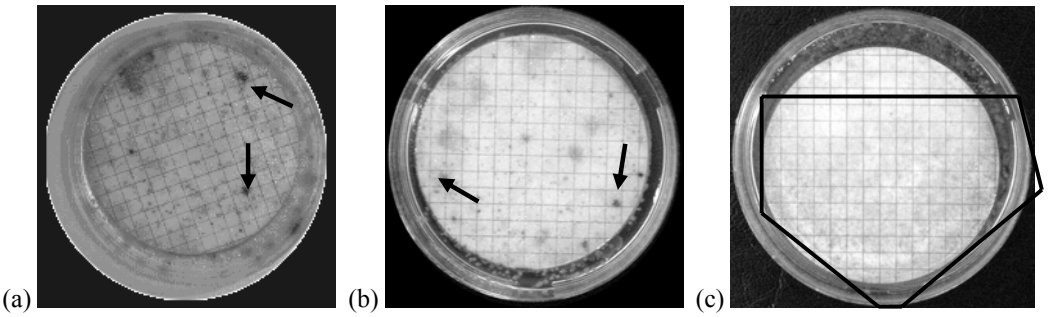


Figure 8. (a), (b) e (c). Bufffalo´s milk diluted in distilled water before the filtration with alumina tube impregnated with silver

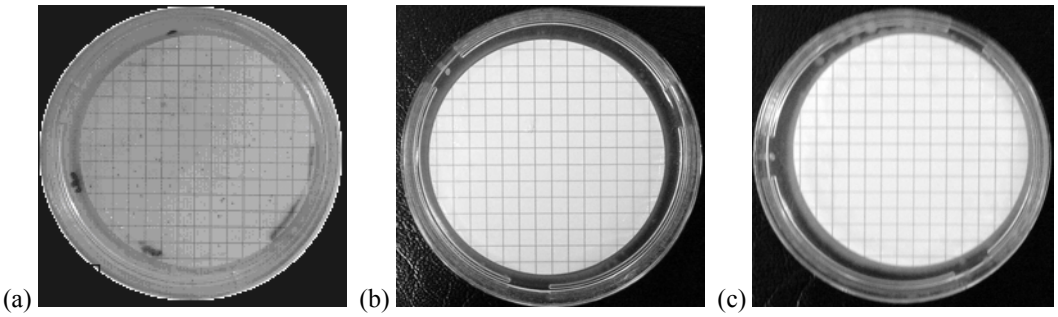


Figura 9. (a), (b), (c). Permeate of the emulsion buffalo´s milk after 50 minutes of filtration with alumina tube impregnated with silver (sinterized at 1450ºC).

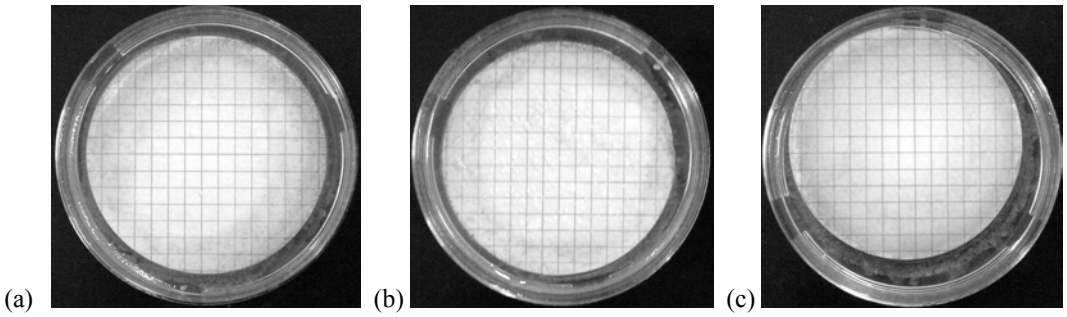


Figure 10 (a) Sample of the residual serum (cheese production) without crossflow filtration, (b) and (c) permeate of residual serum after 20 and 50minutes and of filtration with sacarose (sinterized at 1450ºC) and without silver impregnation.

In all plates of figure 10 gray tones were observed, indicating the presence of bacteria. It indicates that did not have the bacteria retention for the pores of the tube. Can be said that the porous size of 30 μm do not retain bacteria in its surface. With presence of nanoparticles of silver in this structure, one expects is that the future results of this research will be promising.

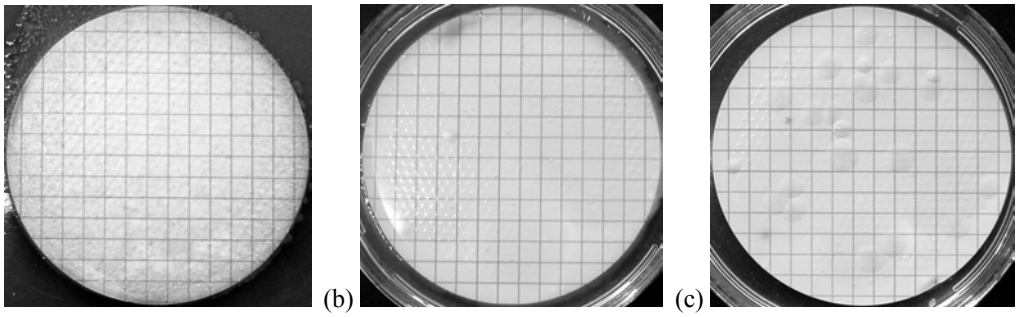


Figure 11. (a) Sample of the residual serum without filtration; (b) and (c) permeate of the emulsion residual serum after 20 and 50minutes. Monolayer tube without silver impregnation (sinterized at 1450º)

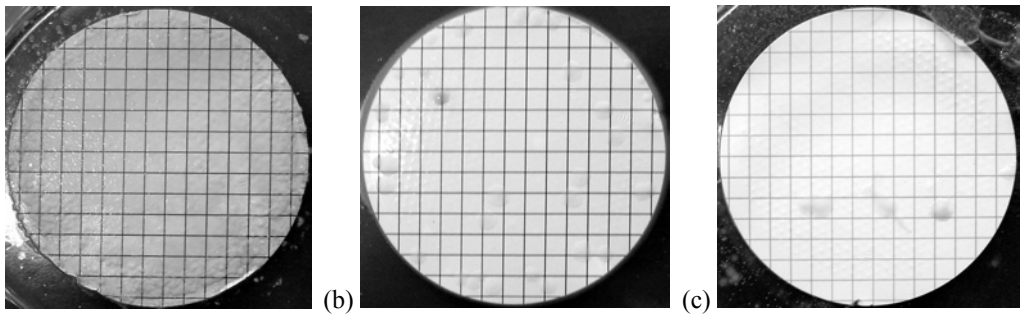


Figure 12. (a) Sample of the residual serum (cheese production) without filtration; (b) and (c). Permeate of residual serum after 20 and 50minutes. Bilayer tube without silver impregnation (sinterized at 1450º).

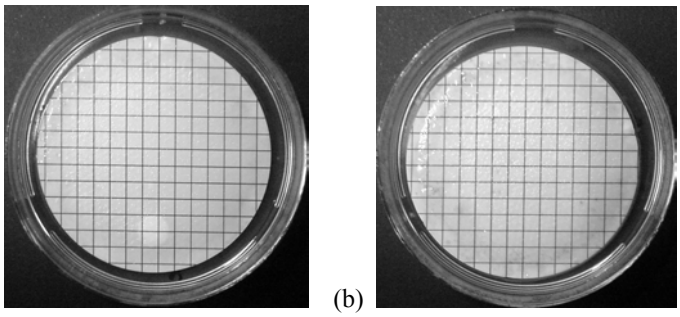


Figure 13. Permeate flux of emulsion residual serum (cheese production) after: (a) 20minutes, (b) 50minutes with commercial membrane (0,8µm).

The Figure 11b and 12b indicates the permeate after 20 minutes of filtration for the monolayer tube and bilayer tube. In these plates are observes what there are many bacteria indicated with gray tone in figures 11b and 12b. The same occured with the permeate after 50 minutes (figure 11c and 12c).

The Figure 13a and 13b shows with gray tone, the presence of bacteria in the permeate after 20 minutes and 50 minutes of filtration with commercial membrane. It indicates that the crossflow filtration is not satisfactory for bacteria reduction, although its nominal size (cut-of of 0,8µm) is for the retention and it should obtain the complete retention of bacteria of residual serum.

5. Conclusion

The following conclusions can be drawn from the results of this study:

i) It was possible to establish with this methodology the impregnation of silver´s nanoparticles in the micro-porous tube. The influences of silver in the micro-porous tube can have meant for tubes with larger pores than 1μm.

ii) The process of tangential filtration was efficient in the reduction of found bacteria of the coliform group in the buffalo´s milk, with the tube of 0,5μm (porous size) .

iii) The tube with pores size large what 1,0μm (sinterized at 1450º)without silver impregnation showed unsatisfactory results in the retention of bacteria.

iv) The commercial membrane tubular with nominal size pores 0,8μm did not retain bacteria in this process.

6. Acknowledgements

The authors are gratefully to the Cooperative of Laticínios of São Carlos; the Eng. Luis Fernando Port (Managing of the Tecnicer-Cetebra Ltda); the Prof. Dr. Luis Daniel, for the using the Laboratory of Sanitary. EESC-USP; and Mario Godinho Júnior and Luis P. S. Santos (students of the Federal University of the São Carlos).

7. References

Carić, M. D., Milanović, S.D., Krstić, D.M., Tekić, M.N., "Fouling of inorganic membranes by adsoption of whey proteins" – J. Membr. Sci., nº165, p.83

Cheryan, M., 1998, "Ultrafiltration and Microfiltration". Tech. Publish. Comp., Lancaster, USA.

Guerra, A., Jonsson, G., Rasmussen, A., Waagner Nielsen, E., Edelsten, D., 1998 "*Low Cross-flow Velocity Microfiltration of Skim Milk for Removal of Bacterial Spores*", International Dairy Journal, nº 7, pp. 849-861.

Kourai, H., Manabe, Y., Yamada, Y., 1994, "Mode of bactericidal action of zirconium phosphate ceramics containing silver íons in the crystal structure", Journal Antibact. Antifung. Agents, nº22, pp.595-601

Krstić, D. M., Tekić, M. N., Carić, M. D., Milanović, S. D., 2002 "*The effect of turbulence promoter on cross-flow microfiltration of skim milk*" – Journal of Membrane Science, nº 208, pp. 303-314.

Lightfood, E. N., 1974 "Transport Phenomena and Living system" – J Wiley & Sons.

Matsunaga, T., Tomoda, R., Nakajima, T., Wake, H., 1985 "Photoelectrochemical sterilization of microbial cells by semiconductor powders", FEMS Microbiol. Lett., nº 29, pp.211-214.

Ripperger, S., Altmann, J., 2002 " *Crossflow microfiltration – state of the art*" – Journal of Membrane Science, nº 26, pp. 19- 31.

Savello, P., Carić, M., Mahmoud, P., "Fouling of ceramic membrane by milk proteins during microfiltration – Aust. J. Dairy Technol., nº52, p.60.

Sondi, I., Salopek-Sondi, B., 2004, "*Silver nanoparticles as antimicrobial agent: a case study on E. coli as a model for Gram-negative bactéria*" – Journal of Colloid and Interface Science, nº 275, pp. 177-182.

Suchecka, T., Biernacka, E., Piatkiewicz, W., 2003, *"Microorganism Retention on Microfiltration Membranes"* – Elsevier Science Ltd., ISSN 0015-1882-2003, pp. 51-55.

Surel, O. M. H., Famelart, 1995, "*Ability of ceramic membranes to reject lipids of dairy products*" – Aust. Journal Dairy Techonology, nº 50, p36.

Silver Healthy...Louisville, KY (2004). Eletronic Publication available in: <http://home.aol.com/silverhealthy/agresearch.htm.

Vetier, C., Bennasar, M., Torado de la Fuente, B., 1988, "Study of the fouling of a mineral microfiltration membrane using scanning electron microscopy and physicochemical analyses in the processing of milk – J. Dairy Res., nº55, p.381

Zeman, L.J. and Zydney, A.L., 1996, "Microfiltration and Ultrafiltration - Principles and Applications", Marcel Dekker, N.Y.

8. Responsibility notice

The authors are the only responsible for the printed material included in this paper.