"Science Stays True Here"
Biological and Chemical Research, Volume 2014, 67-75 | Science Signpost Publishing

Photoinactivation of Gram-Negative Bacteria in Circulating Water Using Chitosan Membranes Containing Porphyrin

Cintia Ramos Camargo[1], Virginia da Conceição Amaro Martins[2], Ana Maria de Guzzi Plepis[1,2] and Janice Rodrigues Perussi[1,2*]

1. Programa de Pós-Graduação Interunidades Bioengenharia - EESC/FMRP/IQSC, Universidade São Paulo - São Carlos - SP, Brazil

2. Instituto de Química de São Carlos, Universidade de São Paulo -São Carlos – SP, Brazil.

Received: September 30, 2014 / Accepted: October 26, 2014 / Published: December 25, 2014

Abstract: Two different porphyrins were incorporated to chitosan membranes aiming photoinactivation of the Gram-negative bacteria *Escherichia coli*, which can be present in drinking water due to contamination. The incorporated porphyrin were 5,10,15,20-tetrakis(*p*-aminophenyl)-porphyrin (p-TAPP) and *meso*-tetrakis (4-N-metylpyridyl)-porphyrin (TMPyP). To verify the photoactivity of the membranes were employed static and dynamic (water flow system) arrangements containing *E. coli* exposed to an apparatus containing white LEDs. Evaluation of the static setup showed that both incorporated porphyrins have caused a considerable bactericidal effect under irradiation (3 log reduction in 180 min, for p-TAPP and 5 log reduction in 120 min for TMPyP). Therefore, the photoinactivation was most effective for TMPyP leading to a greater reduction in cell proliferation with shorter irradiation time. The results showed that the dynamic setup, using chitosan membranes containing TMPyP, was effective showing 3 log reduction at 80 min irradiation. These results suggest that the process is valuable and has potential to eliminate microbial contaminants in water.

Keywords: Bacteria, water, photoinactivation, quitosan, photosensitizer

1. Introduction

The ever-increasing demand for drinkable and clean water represents a challenge, and this is mainly due to an environmental contamination from domestic and industrial waste discharges, which has become a serious public health problem [1,2]. Methods of water disinfection for human use aim inactivation of pathogenic microorganisms in order to minimize the risk of waterborne diseases. Treatments of water with chemicals such as chlorination are the most used due to its effectiveness and low cost [3]. However, implementation of these disinfectants can form toxic and potentially carcinogenic by-products (organo-chlorinated), suggesting the inadequacy of this method [3-5]. Furthermore water treatments do not completely eliminate the pathogenic microorganisms and other toxic substances, since these agents can be present in the treatment effluents [2,6]. Therefore it is important to develop new methods that does not endanger human health and have high efficacy of decontamination.

Corresponding author:

Janice Rodrigues Perussi, Instituto de Química de São Carlos, Universidade de São Paulo -São Carlos – SP, Brazil. E-mail: janice@iqsc.usp.br.

The Photodynamic inactivation (PDI) seems to be a very promising possibility to inactivate microorganisms in water without formation of potencially hazardous by-products [3,7-9]. PDI is a process that utilizes photosensitizers and light in the presence of the oxygen to promote a phototoxic response, normally oxidant, which is capable to damage biomolecules and cellular structures and thus killing microorganisms. The photoinactivation has the advantage of having a broad spectrum of action, in general bacteria [10], fungi [11], protozoa [12] and viruses [13] are efficiently photoinactivated [14]. Regarding bacteria, both Gram-positive and Gram-negative are susceptible to photoinactivation under certain conditions [9,10,15] with the Gram-positive bacteria being more easily damaged [10,16-19]. This is attributed to differences in the structure and organization of the cell wall of these bacteria. In Gram-positive bacteria, the outer portion of the cell wall is relatively more porous, allowing neutral or anionic photosensitizers to bind efficiently and to spread to sensitive sites of the cell. In Gram-negative bacteria, the structure of the outer membrane is more complex, forming a physical and functional barrier between the cell and the environment, thus making difficult the penetration of the photosensitizer [18-20].

There are studies in the literature suggesting the use of PDI for microbiological water disinfection, but the application of this process in water treatment is still under investigation. The majority of studies aimed at the inactivation of bacteria have been performed using photosensitizers in solution [3,11,13,17,21-28]. However, this is not suitable for application in water treatment processes, where the presence of residual traces of photosensitizer is not acceptable. In this kind of purpose, the procedure requires removal of the photosensitizer after its photodynamic action [29] since photosensitizer cannot remain free as a contaminant [8,26,29-31]. Therefore, in the present paper, chitosan membranes containing photosensitizers were developed and tested against Gram-negative bacteria *Escherichia coli*, commonly present in drinking water. In order to verify the possibility of membranes application to microbiological disinfection of water, a circulatory flow system was used.

2. Materials and Methods

Photosensitizers and Membrane Components

The meso-tetra(N-methyl-4-pyridyl)-porphyrin (TMPyP, Midcentury Chemicals-USA) and 5,10,15,20-tetrakis (p-aminophenyl)-porphyrin (p-TAPP, Sigma Aldrich-USA) were a gift of Prof. Marcel Tabak and were used without further purification. The chemical structures are represented in Fig. 1. The other reagents used in this study were of pure analytical grade. A stock solution of the TMPyP and p-TAPP were prepared at a concentration of 1 mg mL^{-1} in ethanol and maintained at 4°C.

Chitosan was obtained by deacetylation of β-chitin from pens of *Loligo sp* [34]. Chitin is one of the most abundant natural polymers in Earth. The degree of acetylation of chitosan was determined by conductimetric measurements Molar mass was determined by viscometric measurements [35].

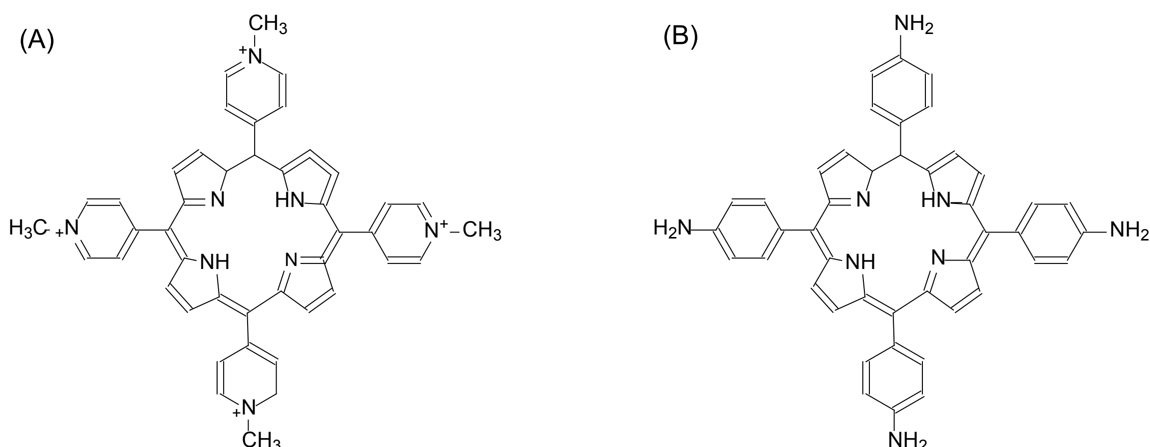

Fig. 1 Chemical structure of TMPyP (A) and p-TAPP (B)

Preparation of Chitosan Membranes

The stock of chitosan gel was prepared at a concentration of 1% (w/w) in acetic acid. Ten grams of chitosan gel was placed in Teflon® molds (4.7 x 4.7 x 0.7 cm). The molds were kept in a chamber with air flow for three days. After drought the membranes were treated in solution 0.5% (w/w) of sodium tripolyphosphate (TPP) in 2 mol L^{-1}NaOH for 30 min, in order to give the material a greater stability against fragmentation and less swelling. The membranes were then washed, dried and stored at room temperature (25°C).

The chitosan membrane prepared for use in the water circulatory system was made by incorporating a nylon net (thickness 0.45 ± 0.01 mm and mesh 2.38 ± 0.01 mm), in order to strengthen the membranes[8]. The process of preparing chitosan membranes was done as described previously, except that a fragment of a nylon mesh (4.5 x 4.5 cm) was placed in the bottom of the Teflon®molds before the evaporation step.

Preparation of Chitosan Membranes with Incorporated Photosensitizer

One mL of each photosensitizer solution was added to 15 g of chitosan gel 1% (w/w) in acetic acid. The mixture was homogenized and then treated as described under "preparation of the chitosan membranes". After drought the *in vitro* release of the photosensitizer not immobilized in chitosan membranes was performed. Membranes (22 cm^2) containing photosensitizer were placed in 200 mL of distilled water under mechanical agitation for three days or until no more photosensitizer was observed by spectroscopy (416 or 425 nm, for p-TAPP and TMPyP, respectively). The membranes were then air-dried. The concentration of photosensitizer in chitosan membrane was estimated by UV-Visible Spectroscopy at the above wavelength [8].

Characterization

FT-IR spectra were recorded in silicon support, after samples of chitosan gel 1% (w/w) in acetic acid, with and without photosensitizer, were oven dried in vacuum. FT-IR spectra were obtained in a spectrophotometer Bomen MB-102 at 400 to 4000 cm^{-1} with 32 scans.

Microorganisms and Growth Conditions

Escherichia coli (ATCC 25922), a Gram-negative bacterium, was kindly provided by Prof. José Francisco Hoffling, Department of Oral Diagnosis, UNICAMP. *E. coli* was grown aerobically for 18 h at 37 °C in Luria-Bertani (LB, Broth-Oxoid, São Paulo, Brazil) in an orbital shaker (Marconi MA 410, Piracicaba, Brazil) at 100 rpm. The bacteria were harvested by centrifugation of broth cultures (1300 rpm for 10 minutes) and the pellet suspended in 10 mL of sterile saline. This procedure was repeated two more times, yielding a bacterial concentration of $1x10^9$ cells mL^{-1} Counting of cells in suspension was performed using a spectrophotometer at 590 nm (Hitachi U2800, Japan).

Irradiation Conditions

An apparatus similar to a reactor was constructed in order to reproduce a water tank. A water reservoir (30cm large, 10 cm deep) made of acrylic was placed into a metal box (50 x 50 cm), made of steel sheet, lined with aluminum foil containing a system of three white LEDs (400-700 nm, $3x10^3$ mW cm^{-2}, Empalux, model IE33001), disposed on each side and on top of the water container. The membrane containing the photosensitizer was placed inside of the reservoir. A small pump was responsible for the water circulation from one side of the membrane to another. The device was used in experiments on dynamic photoinactivation setup.

Static Photoinactivation Setup

In the static system the photodynamic effect was assessed by incubating the membrane under study (surface area 1 cm^2) with 1.2 mL of bacterial suspension ($1x10^9$ cells mL^{-1}), placed in a polystyrene plate with 24 wells (Corning Costar) and irradiated with LED during different time intervals (20-180 min). Aliquots of 0.1 ml of suspension were removed and the number of colony-forming units per milliliter (CFU mL^{-1}) was determined. Control experiments were performed with chitosan membrane without sensitizer under irradiation and chitosan membrane with photosensitizer in the dark. Three independent experiments were performed for each sample of microbiological analysis and the results presented are the average of the individual results of each assay.

Dynamic Photoinactivation Setup

For the experiments conducted in a dynamic setup, the water circulatory system was employed. The acrylic reservoir was filled with 280 mL of bacteria suspension (1×10^8 cells). A silicone rubber was used to connect the tank with a submerged pump (Sarlo Better). The chitosan membranes reinforced with nylon and containing photosensitizers were fixed to a plastic frame (4.5 cm diameter) in order to prevent bending of the membranes. The frames were then coupled to the acrylic plates fixed to the acrylic box. Six chitosan membranes were placed in parallel at a distance of 3 cm from each other. The flow rate employed was 25 mL min^{-1}, corresponding to a cycle time of 11.32 minutes. At appropriate intervals aliquots (3 mL) were taken for determination of CFU mL^{-1}. The experiments were repeated three times and the results presented are the average of the individual results of each one.

3. Results and Discussion

Chitosan Membranes

The degree of chitosan acetylation of 9% was determined by conductmetric measurements. Viscometric measurements were used to estimate the chitosan molar mass as 1.248×10^5 g mol^{-1}.

Two different porphyrins were incorporated into chitosan. Chitosan membranes prepared without (CH) and with photosensitizer were translucent with thicknesses of 60.0 µm and 120 µm for reinforced membrane with a nylon net.

The chitosan membranes with p-TAPP (CHpTAPP) and TMPyP (CHTMPyP) were light brown and light yellow, respectively.

The characterization of chitosan gel with and without photosensitizer was performed by FT-IR. These FT-IR spectra (Fig 2) exhibit characteristic absorption bands between 1600 and 1670 cm^{-1} - C=O stretch of amide I, since chitosan is not completely deacetylated; at 1550 cm^{-1} - angular deformation of N–H (amide II); at 1150 cm^{-1} - axial deformation of O–H in the hydrogen bond; between 800 and 1200 cm^{-1} - pyranoside ring [36-39]. In all cases the FT-IR spectra presented similar behavior to those shown for chitosan and chitosan with p-TAPP or TMPyP, but was observed that the peaks of amino groups (amide I) of chitosan with porphyrins were shifted to lower wavenumbers, suggesting an interaction with the photosensitizers.

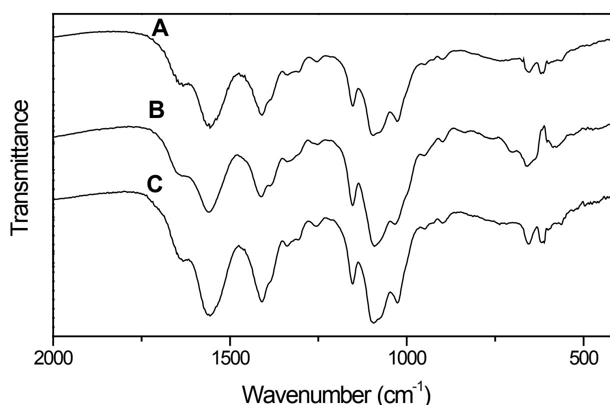

Fig. 2 Infrared spectra of chitosan membrane without photosensitizer (A), containing p-TAPP (B) and TMPyP (C) incorporated.

Before experiments of photoinactivation, the release of photosensitizer that were not immobilized into chitosan membranes was performed to ensure that during PDI experiments the photosensitizer would not be released from the chitosan membranes. After these procedures, part of the membranes was used in photoinactivation assays and part was used to determine the final concentration of photosensitizer that was incorporated to the membrane. There is, however, a problem with their quantitative characterization. Due to the fact that photosensitizers are immobilized into chitosan membranes, which are insoluble in water, one cannot make a direct comparison of their spectroscopic properties with free photosensitizer molecules. For this purpose, the absorption spectrum of the chitosan membranes with photosensitizer was obtained by dissolving it in acetic acid 1% (w/w) in order to compare with the absorption spectrum of the photosensitizer in 1% acetic acid solution.

The Fig. 3 presents p-TAPP and TMPyP spectra obtained from the dissolution of the chitosan membrane with photosensitizer (CHpTAPP or CHTMPyP) and of the photosensitizer (p-TAPP or TMPyP) in 1% (w/w) acetic acid exhibiting a Soret band at 416 and 425 nm, respectively. In spectra obtained of CHpTAPP and CHTMPyP dissolution it was observed a small red shift of the Q-band (500-600 nm), when compared with spectrum of p-TAPP and TMPyP solution, respectively. From the absorbance at 416 nm for p-TAPP and 425 nm for TMpYP a rough estimate may be given that p-TAPP or TMPyP content in the membrane were about 1 µmol L^{-1} p-TAPP and 2 µmol L^{-1}TMPyP in 1 g of chitosan membrane. The estimated value to the photosensitizer attached to the chitosan membranes was rough because the molar extinction coefficient is dependent on the composition of the solvent [40]. Bonnett et al (2006) [9] showed that incorporation efficiency of p-TAPP in chitosan membranes were approximately 67%, much higher than that obtained in this study (1.5 %). To date, there is no reported studies in the literature using TMPyP incorporated in polymeric support.

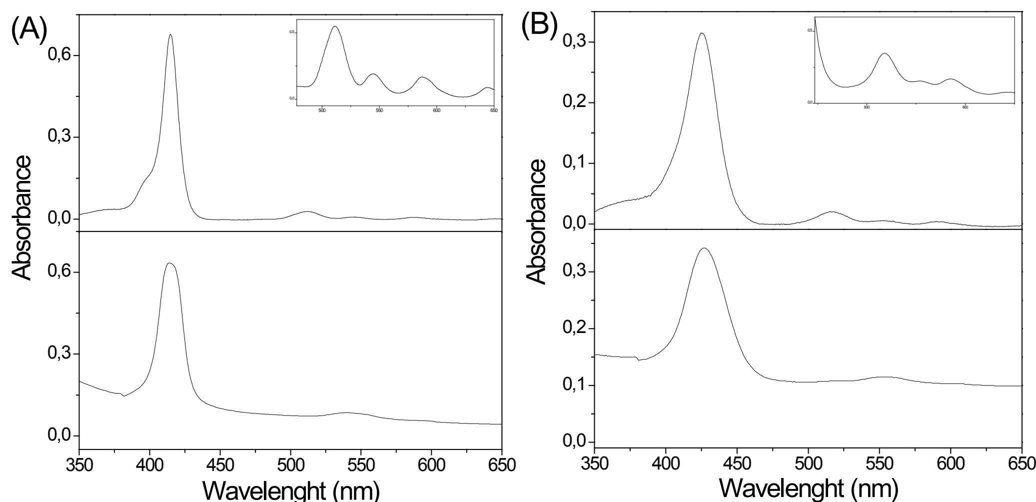

Fig. 3 Absorption spectrum of p-TAPP (A) and TMPyP (B) obtained from photosensitizers solution in 1% acetic acid (upper graph) and dissolution of chitosan membrane with photosensitizer in 1% acetic acid (bottom graph). The spectrum shown in the inserts in graphs refer to the expansion of the porphyrin Q-band in solution.

Static Photoinactivation of *Escherichia coli*

The results of light and dark control experiments showed that the viability of *E. coli* is neither significantly affected by chitosan membrane under irradiation (light control) nor by chitosan membrane with photosensitizer in the dark (dark control) (Fig. 4), indicating that the reduction on cell survival obtained with chitosan membrane with photosensitizer under irradiation was due to photosensitizing effect of the CHpTAPP or CHTMPyP.

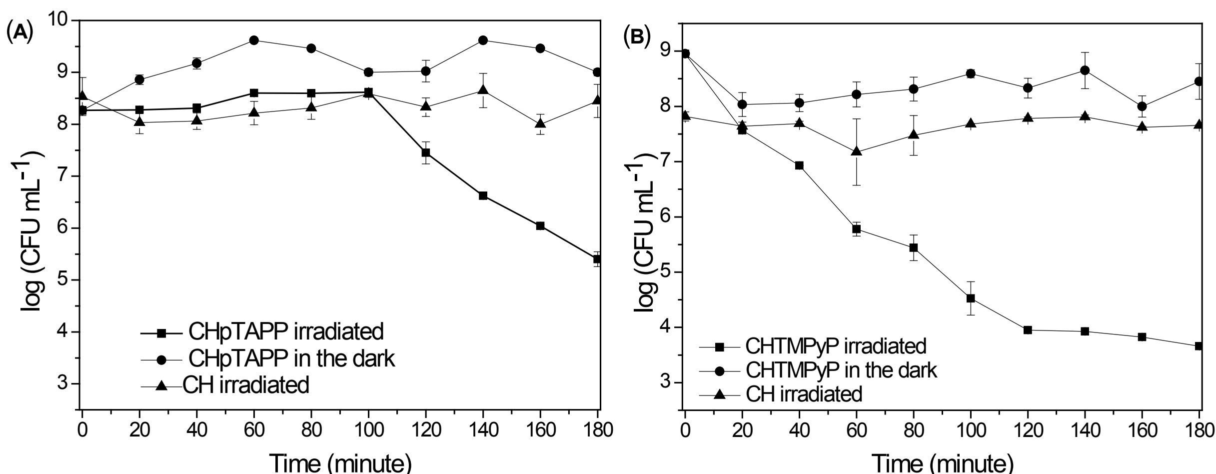

Fig. 4 Variation on viability of *Escherichia coli* in the presence of CHpTAPP (A) and CHTMPyP (B) exposed to the apparatus containing white LEDs and control trials (membrane without photosensitizer irradiated and membrane with photosensitizer in the dark). The points presented in the graph refer to the average of three independent experiments.

The CHTMPyP demonstrated a rapid decrease on cell survival (about 5 log reductions after 120 min of exposure to the light) and CHpTAPP showed about 3 log reduction after 180 min irradiation. These results suggest that p-TAPP and TMPyP incorporated in heterogeneous phase (chitosan membrane) have significant photodynamic activity when using high initial concentration (1×10^9 cells ml^{-1}) of bacterial suspension of which would be considered a huge bacterial

contamination. It should be noted that similar study in the literature with chitosan membrane with incorporated photosensitizer [8] showed a significant reduction when using higher concentration of phosensitizer (p-TAPP) and lower initial concentration of bacterial suspension 3.5 x 10^3 cells mL^{-1}.

It can also be observed that the more efficient system was CHTMPyP, since it caused higher cell reduction in a shorter time of irradiation. This may be due to the larger concentration of TMPyP incorporated in the chitosan membrane than the p-TAPP (2 µmol L^{-1} and 1 µmol L^{-1} per gram of chitosan membrane, respectively). Furthermore TMPyP is tetra-cationic, while p-TAPP has no ionic charge, and TMPyP has a quantum yield of singlet oxygen greater than the p-TAPP (0.74 and 0.53, respectively) [41,42]. Results found in the literature suggest that photoinactivation depends on the concentration, quantum yield of singlet oxygen and ionic charge of photosensitizers, furthermore these also suggest that Gram-negative bacteria (such as *E. coli*) are more easily photoinactivated by cationic photosensitizer.

These experiments allowed us to select the CHTMPyP for the next phase, consisting in simulate a real situation of photoinactivation of the bacteria *E. coli* present in the water supply system, using a circulatory water flow.

Dynamic photoinactivation of *Escherichia coli*

The results show that no significant cell death occurred in the light and dark controls (Fig 5). In contrast, it was observed around 3 log reduction of *E. coli* in 80 min irradiation in experiments using CHTMPyP reinforced membranes. Results found in the literature had approximately 2 log reduction in assays employing photoinactivation circulatory system of water and chitosan membranes with p-TAPP [8]. Therefore, one can consider this as a satisfactory result, which shows that the concept of photoinactivation using an incorporated photosensitizer in a polymeric membrane in a dynamic system is effective. However, the process of the dynamic system needs to be optimized by manipulating parameters such as: amount of photosensitizer immobilized on the polymeric support and/or number of membranes used, the initial concentration of bacterial cells; power of the light employed and the amount of oxygen in the medium.

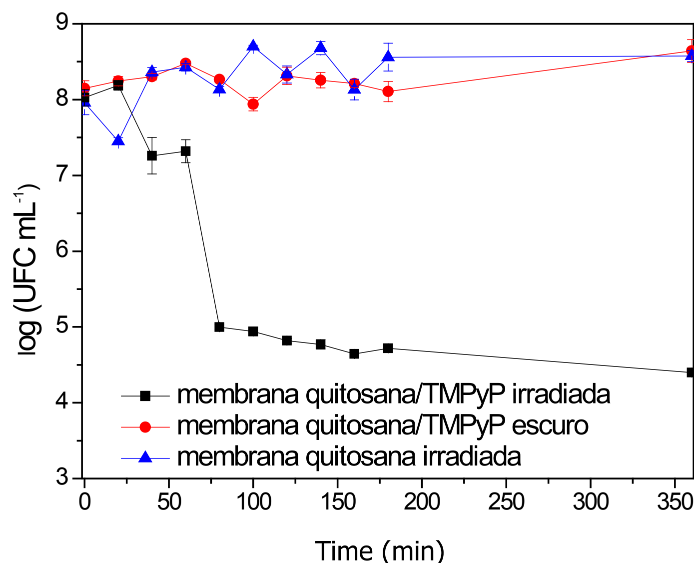

Fig. 5 Variation on viability of *Escherichia coli* using the water circulatory system in the presence of chitosan membranes with TMPyP incorporated exposed to the apparatus containing white LEDs as well as control trials (membrane without photosensitizer irradiated and membrane with photosensitizer in the dark). The points presented in the graph refer to the average of two independent experiments.

4. Conclusion

The photoinactivation of the bacteria *Escherichia coli* employed as a model organism was tested using chitosan membrane with photosensitizer incorporated in a static and dynamic system. The results of static setup show a significant cell reduction for both porphyrins (p-TAPP or TMPyP) incorporated in chitosan membrane when irradiated with a white LED system. Furthermore, the results suggest that the photodynamic inactivation process employing photosensitizer incorporated into the chitosan membranes depends on the concentration, ionic charge and photophysical properties of the photosensitizer. In the dynamic setup employing a circulatory system of water and chitosan membranes with TMPyP reinforced with nylon, the process of bacteria photoinactivation showed a significant bactericidal effect suggesting to be effective to inactivate potential bacterial water contaminants.

Acknowledgments

We are grateful to FAPESP, CEPOF and CAPES for the financial support. We thank Prof. Marcel Tabak for kindly provide the porphyrins and Prof. José F. Hoffling from the Department of Oral Diagnosis, UNICAMP for kindly provide the microorganisms used in this study and to Wanessa C. M. A. Melo for her able assistance.

References

[1]. V. O. Domingues, G. D. Tavares, F. Stüker, T. M. Michelot, L. G. B. Reetz, C. M. Bertoncheli, R. Höner, Saúde, 2007, 33, 15-19.

[2]. U. Szewzyk, R. Szewzyk, W. Manz, K. H. Schleifer, Annu. Rev. Microbiol., 2000, 54, 81-127.

[3]. M. Jemli, Z. Alouini, S. Sabbahi, M. Guerddari, J. Environ. Monit., 2002, 4, 511-516.

[4]. A. Dabrowska, J. Nawrocki, Water Res., 2009, 43, 2201-220.

[5]. S. D. Richardson; Anal. Chem., 2009, 79, 4295-4324.

[6]. S. D. Richardson; Trends. Anal. Chem., 2003, 22, 666-684.

[7]. L. Costa,C. M. B. Carvalho,M. A. F. Faustino, M. G. P. M. S. Neves,J. P. C. Tomé, A. C. Tome´,J. A. S. Cavaleiro, A. Cunha, A. Almeida, Photochem. Photobiol. Sci., 2010, 9, 1126-1133.

[8]. R. Bonnett, M. A. Krysteva, I. G. Lalov, S. V. Artarsky, Water Res., 2006, 40, 1269-1275.

[9]. L. Costa, E. Alves, C. M. B. Carvalho, J. P. C. Tomé, A. M. Faustino, M. G. P. S. Neves, A. C. Tomé, J. A. S. Cavaleiro, A. Cunha, A. Almeida, Photochem. Photobiol. Sci., 2008, 7, 415-422.

[10]. M. R. Hamblin, T. Hasan, Photochem. Photobiol. Sci., 2004, 3, 436-450.

[11]. L. N. Dovigo, A. C. Pavarina, D. G. Ribeiro, C. S. Ariano, V. S. Bagnaton, Brazilian J. Microbil., 2010, 41, 42-49.

[12]. Z. Alouini, M. Jemli, J. Environ. Monit., 2001, 3, 548-551.

[13]. M. Wainwright, Photochem. Photobiol. Sci., 2004, 3, 406-4011.

[14]. G. Jori, S. B. Brown; Photochem. Photobil. Sci., 2004, 3, 403-405.

[15]. M. Merchat, G. Bertolini, P. Giacomini, A. Villaneuva, G. Jori. J. Photochem. Photobiol. B., 1996, 32, 153-157.

[16]. A. Minnock, D. I. Vernon, J. Schofield, J. Griffiths, J. H. Parish, S. B. Brown, J. Photochem. Photobiol. B, 1996, 32, 159-164.

[17]. E. Alves, L. Costa, C. M. B. Carvalho, J. P. C. Tomé, M. A. Faustino, M. G. P. M. S. Neves, A. C. Tomé, J. A. S. Cavaleiro, A. Cunha, A. Almeida, BMC Microbiology, 2009, 9, 70-83.

[18]. T. A. Maisch, Mini Rev. Med. Chem., 2009, 9, 947-983.

[19]. H. M. Tang, M. R. Hamblin, C. M. N. Yow, J. Infect. Chemother., 2007, 13, 87-91.

[20]. K. Ergaieg, M. Chevanne, J. Cillard, R. Seux; Solar Energy, 2008, 82, 1107-1117.

[21]. A. Acher, E. Fischer, R. Zellingher, Y. Manor, Water Res., 1990, 24, 837-843.

[22]. A. Acher, E. Fischer, R. Turnheim, Y. Manor, Wat. Res., 1997, 31, 1398-1404.

[23]. E. Alves, C. M. B. Carvalho, J. P. C. Tomé, M. A. F. Faustino, M. G. P. M. S. Neves, A. C. Tomé, J. A. S. Cavaleiro, A. Cunha, S. Mendo, A. Almeida, J. Ind. Microbiol. Biotechnol.; 2008, 35, 1447-1454.

[24]. A. M. B. Carvalho, A. T. P. C. Gomes, S. C. D. Fernandes, A. C. B. Prata, M. A. Almeida, M. A. Cunha, J. P. C. Tomé, M. A. F. Faustino, C. G. P. M. S. Neves, A. C. Tomé, J. A. S. Cavaleiro, Z. Lin, J. P. Rainho, J.Photochem. Photobiol., B, 2007, 88, 112-118.

[25]. T. Cooper, D. Y. Goswami, J. Sol. Energy Eng., 2002, 124, 305-309.

[26]. K. Ergaieg, R. A. Seux, Desalination, 2009, 246, 353-362.

[27]. N.A. Kuznetsova, D.A. Makarov, O.L. Kaliyaand G.N. Vorozhtsov, J. Hazard. Mat., 2007, 146, 487-491.

[28]. F. Manjón, M. Santana-Magaña, D. García-Fresnadillo, G. Orellana, Photochem. Photobil. Sci., 2010, 9, 838-845.

[29]. M. E. Jiménez-Hernández, F. Maanjón, D. García-Fresnadillo, G. Orellana, Solar Energy, 2006, 80, 1382-1387.

[30]. K. Benabbou, C. Guillard, S. Pigeot-Rémy, C. Cantau; T. Pigot, P. Lejeune, Z. Derriche, S. Lacombe, J. Photochem. Photobiol., A., 2011, 219, 101-108.

[31]. N. Todorova, M. Krysteva, K. Maneva, D. Todorov, J. Bioact. Compat. Polym., 1999, 14, 178-184.

[32]. L. Villén, F. Manjon, D. García-Fresnadillo, G. Orellana; Appl. Catal. B: Environ., 2006, 69, 1-9.

[33]. P. K. Dutta, J. Dutta, V. S. Tripathi, J. Sci. Ind. Res., 2004, 63, 20-31.

[34]. M. M. Horn, V. C. A. Martins, A. M. G. Plepis, Carbohydr. Polym., 2009, 77, 239-243.

[35]. J. E. Santos, J. P. Soares, E. R. Dockal, S. P. Campana-Filho, E. T. G. Cavalheiro, Polymer, 2003, 44, 6521-6530.

[36]. Y. X. Xu, K. M. Kim, M. A. Hanna, D. Nag, Ind. Crops Prod., 2005, 21, 185-192.

[37]. J. Brugnerotto, J. Lizardi, F. M. Goycoolea, W. Argüelles-Monal, J. Desbrières, M. Rinaudo, Polymer, 2001, 42, 3569-3580.

[38]. F. A. A. Tirkistani, Polym. DegradStab., 1998, 60, 67-70.

[39]. A. Josué, M. C. M. Laranjeira, V. Fávere, I. Y. Kimura, Polímeros, 2000, 03, 116-121.

[40]. Moczek, M. Nowakowska, Biomacromolecules, 2007, 8, 433-438.

[41]. E. Reddi, M. Ceccon, G. Valduga, G. Jori, J. C. Bommer, F. Elisei, L. Latterini, U. Mazzucato, Photochem. Photobiol., 2002, 75, 462-470.

[42]. J. Bhaumik, R. Weissleder, J. R. McCarthy, J. Org. Chem., 2009, 74, 5894-5901.