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Performing a Three-Step Process for Conversion of Chitosan to Its Oligomers Using a Unique Bipolar Membrane Electrodialysis System

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Chitosan, a linear polysaccharide composed of β-1,4 linked D-glucosamine residues, can be depolymerized into oligomers by enzymatic reaction with chitosanase. Recently, bipolar membrane electrodialysis (BMED) has been used for chitosan solubilization and for terminating the enzymatic reaction by action of electrogenerated acid and base, respectively. The aim of the present study was to test a complete "3-in-1" process using a three-compartment BMED configuration to perform simultaneously the solubilization of chitosan, the inactivation of chitosanase, and the demineralization of the oligomers. In addition, the BMED process was compared to a conventional process using chemical acid and base. The BMED method was found to be as effective as the conventional method for solubilizing the chitosan and for inactivating the chitosanase. Furthermore, the use of BMED allowed a demineralization rate of 53% of the chito-oligomer solution in the diluate compartment. A global process of chitosan hydrolysis into its oligomers using a BMED system was proposed. This technology has great potential for industrial application in chitosan oligomer preparation, because it is convenient and ecological and it produces chito-oligomers with a lower mineral content compared with the conventional method.

KEYWORDS: Chitosan oligomers; chitosanase; bipolar membranes; demineralization

INTRODUCTION

Chitosan is a linear polysaccharide composed of β-1,4 linked D-glucosamine residues extracted from shellfish exoskeletons. This polymer is considered to be a totally or partially deacetylated derivative of chitin. Much attention has been paid to the conversion of chitosan to functional chito-oligomers, because these low molecular weight saccharides exhibit biological and pharmaceutical properties, including inhibition of the growth of fungi and bacteria (*1, 2*), antitumor and immunoenhancing effects (*3*), and eliciting phytoalexin production in plants (*4*). Chito-oligomers can be produced by enzymatic hydrolysis of chitosan by chitosanase. Chitosanase (EC 3.2.1.132) catalyzes the hydrolysis of the glycosidic bonds of chitosan and has been found in bacteria, fungi, and plants (*5*). The traditional method of producing chito-oligomers consists of solubilizing chitosan by chemical acidification, incubating the chitosan solution with chitosanase enzyme, and terminating the enzymatic reaction by heating at 100 °C (*6*). However, addition of salts occurs with chitosan acidification because of the use of chemical acid, resulting in a decrease of purity of the oligomers.

Recently, bipolar membrane electrodialysis (BMED) has been used for chitosan solubilization by electroacidification (*7, 8*) and for terminating the enzymatic reaction of chitosan with chitosanase by electrobasification (*9*). BMED is an innovative technology for acid and base generation using protons and hydroxyl ions produced by water dissociation at the interface of the bipolar membrane (*10*). According to results obtained by Lin Teng Shee et al. (*7, 8*), solubilization of chitosan was most effective using a bipolar/anionic membrane configuration, whereas basification of chitosanase was successfully achieved with a bipolar/cationic membrane configuration. However, because electroacidification and electrobasification steps led to an increase in minerals in the final product, a further demineralization process would be necessary to purify the chito-oligomers.

The aim of the study was to test a complete process for chitosan transformation into its oligomers using a three-compartment BMED configuration (bipolar/anionic−cationic/bipolar). Each compartment will be used to carry out simultaneously chitosan solubilization, chitosanase inactivation, and chito-oligomer demineralization. The BMED process will be compared to a traditional process using chemical acid and base. Physicochemical parameters and chemical composition of chitosan and chito-oligomers products will be presented for the BMED or chemical preparation method.

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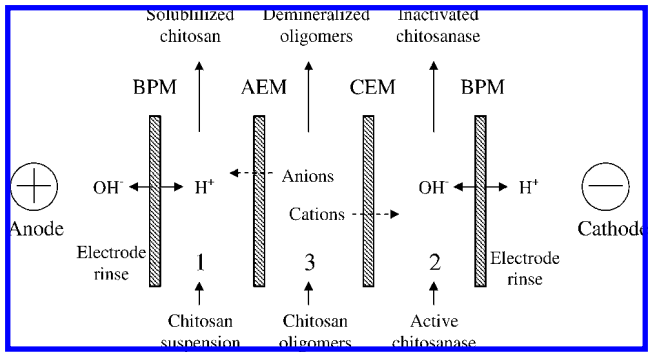

Figure 1. Elementary unit used in the three-compartment bipolar membrane electrodialysis cell configuration for production of chito-oligomers: (1) electroacidified compartment; (2) electrobasified compartment; (3) demineralized compartment. BPM, bipolar membrane; AEM, anion-exchange membrane; CEM, cation-exchange membrane.

MATERIALS AND METHODS

Chitosan and Chitosanase. Chitosan and chitosanase were kindly provided by ISM Biopolymer (Granby, QC, Canada). Chitosan (96% deacetylation) had a moisture content of 7.1 ± 0.1% (w/w on wet basis) and an ash content of 0.40 ± 0.01% (w/w on dry basis). According to the supplier, the chitosanase, isolated and purified from *Streptomyces* sp. N174, had an enzyme activity of 9.6 units/mL.

Preparation of Chito-oligomers Using BMED. *BMED Configuration.* The BMED configuration consisted of a three-compartment electrodialysis cell (**Figure 1**). The module was an MP type cell (100 cm^2 of effective surface) equipped with a dimensionally stable anode (DSA) and a 316 SS cathode from ElectroCell AB (Täby, Sweden). The ionic membranes were three bipolar membranes (model Neosepta BP-1), two anionic membranes (model AMX-SB), and two cationic membranes (model CMX-SB) from Tokuyama Soda Ltd. (Tokyo, Japan) and purchased from Ameridia (Somerset, NJ). The electrodialysis stack was composed of two elementary cells (**Figure 1**). The electrode rinse was a 20 g/L NaCl solution (6 L). The other reservoir tanks were filled with 1.5 L of the following solutions: (1) a chitosan suspension in the acidified compartment, (2) an enzymatic solution of chitosanase and chitosan oligomers in the basified compartment, and (3) a solution of chito-oligomers in the diluate compartment (**Figure 1**). During the BMED treatment, a constant current density of 10 mA/cm^2 was applied during 60 min. The flow rates were maintained constant at 4 L/min.

BMED Protocol. The preparation of chito-oligomers using BMED starts with chitosan solubilization, then continues with chitosanase inactivation, and ends with oligomer demineralization (**Figure 2**). However, before the three BMED steps were performed simultaneously, preliminary chitosan acidification and chito-oligomer basification were carried out to provide the solutions for all compartments. The specific procedure for each BMED compartment was as shown in **Figure 2**.

(a) Electroacidification. Chitosan (product 1) was solubilized in the acidified compartment by a stepwise feeding mode, according to previous results of Lin Teng Shee et al. (*7*). Six aliquots of 1.15 g of chitosan powder were added every 10 min in 1.5 L of 0.05 M NaCl solution. At the end of the acidification process, the cumulative addition of chitosan corresponded to a content of 4.6 g/L and led to product 2 (acidified chitosan solution).

(b) First Electrobasification. Chitosan solution recovered from electroacidification (product 2) had a pH of 2 and was adjusted at pH 5.5 using the base produced by BMED to give product 2 preadjusted at pH 5.5.

(c) Enzymatic Hydrolysis. Product 2 preadjusted was collected in a beaker and incubated with chitosanase during 12 h at 25 °C under agitation, using a ratio of 1 unit of chitosanase for 1 g of chitosan, producing a chitosan hydrolysate (product 2 preadjusted and hydrolyzed).

(d) Second Electrobasification. Enzymatic reaction of chitosan with chitosanase was terminated by circulating the enzymatic solution (product 2 preadjusted and hydrolyzed) in the basified compartment.

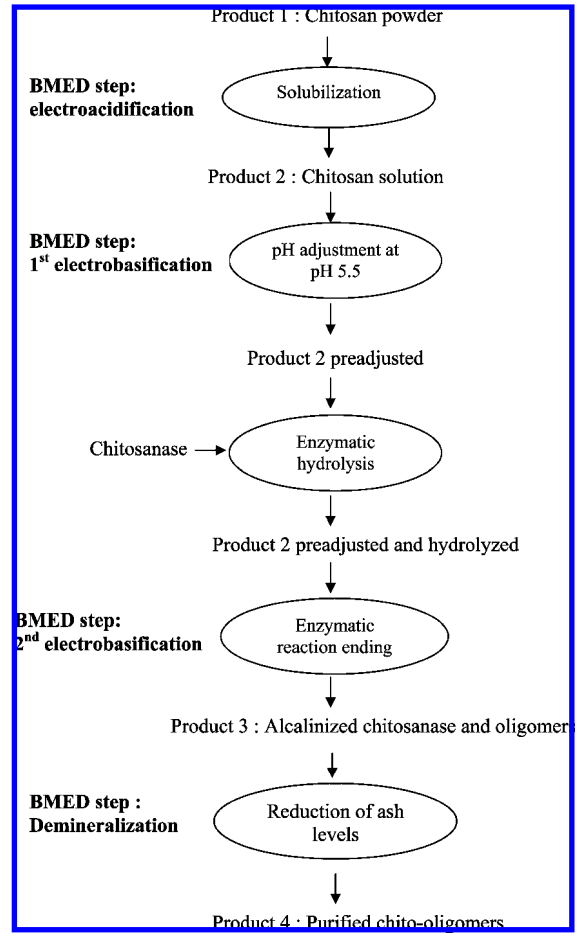

Figure 2. Process scheme for chitosan transformation into oligomers using a bipolar membrane electrodialysis (BMED) system.

The initial pH of 5.5 was adjusted at alkaline value up to pH 11 and led to product 3.

(e) Demineralization. The previous alkaline chito-oligomer solution (product 3) was demineralized in the diluate compartment to give product 4. Samples of each compartment (15 mL) were collected every 10 min from 0 to 60 min for chemical analysis.

Physicochemical Parameters and Chemical Analysis. Physicochemical parameters were followed along the BMED process by measuring the pH and conductivity of the solutions in the reservoirs corresponding to the three compartments. In addition, the voltage and current intensity data were recorded. Chemical analysis of the samples consisted of determining their chitosan content, reducing sugar content, and ash level. Finally, chito-oligomers were characterized qualitatively by thin-layer chromatography in the final product.

(a) pH. pH in the three compartments was measured with a pH-meter (SP 20 model, ThermoOrion, purchased from VWR International, Montreal, Canada) equipped with an automatic temperature compensated electrode (no. 14002-778).

(b) Conductivity. Electrical conductivity of the solutions in the three compartments was measured with a YSI conductivity meter (model 3100, Yellow Springs, OH) equipped with an YSI automatic temperature compensated probe (model 3252) with a cell constant K of 1.0 cm^{-1}.

(c) Relative Energy Consumption and Electrical Resistance. Relative energy consumption was calculated to measure the efficiency of the BMED process as in a previous study by Lin Teng Shee et al. (*7*). Electrical resistance was calculated according to Ohm's law ($R = U/I$). The voltage difference (U) was measured by a Mastercraft digital multimeter purchased from Canadian Tire (QC, Canada). The current intensity (I) was read on control panel and maintained constant at 1.0 A.

(d) Limiting Current Density. Limiting current density was determined for monopolar membranes at the beginning and at the end of

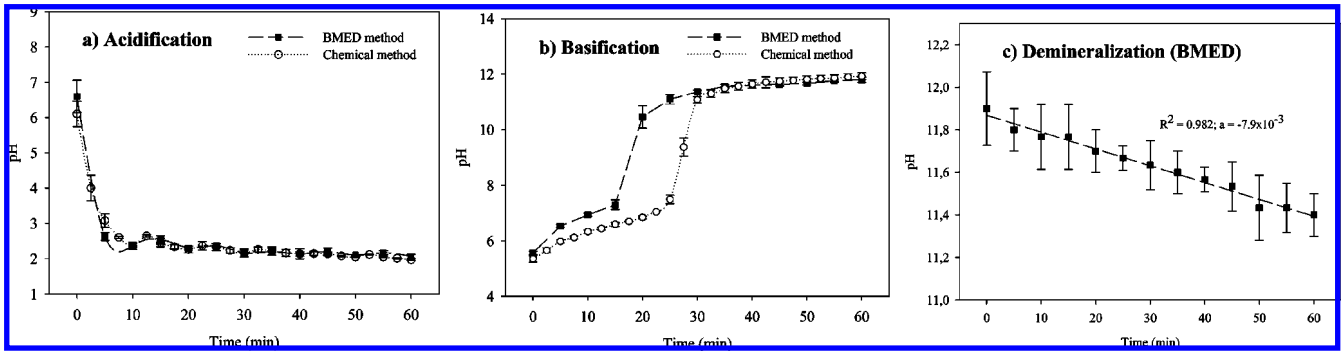

Figure 3. Change of pH during chito-oligomer preparation by BMED or chemical method: (**a**) chitosan acidification; (**b**) chitosanase basification; (**c**) chito-oligomers demineralization. R^2 is the correlation coefficient, and *a* is the slope coefficient for linear regression.

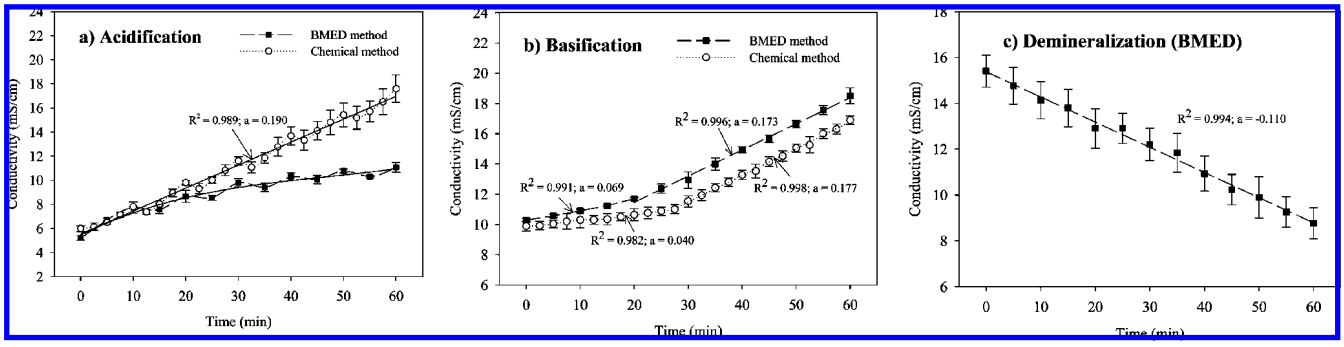

Figure 4. Change of conductivity during chito-oligomer preparation by BMED or chemical method: (**a**) chitosan acidification; (**b**) chitosanase basification; (**c**) chito-oligomer demineralization. R^2 is the correlation coefficient, and *a* is the slope coefficient for linear regression.

BMED using the method of Aritomi et al. (*11*). The current density was adjusted from 0 to 50 mA/cm^2, and the voltage was measured by a Mastercraft digital multimeter connected to two chloridized silver wire electrodes placed on each side of anionic or cationic membranes. The flow rates of the solutions were 4 L/min, and the temperature was 25 °C.

(e) Soluble Chitosan. Soluble chitosan content was measured on samples collected in the acidified compartment using a colorimetric method adapted from Muzzarelli (*12*) with modifications (*7*).

(f) Reducing Sugars. Reducing sugars content was measured on chitosan hydrolysate samples using a colorimetric method (*13*) with modifications (*9*).

(g) Ash Content. Crucibles were washed beforehand in 3 N nitric acid, rinsed with deionized water, dried at 100 °C, and cooled in a desiccator. About 5 g of chitosan solution samples was dried in an oven and ashed at 500 °C for a minimum of 18 h. The samples were cooled in a desiccator and weighed.

$$\text{ash (\% w/w)} = [(\text{wt of residue, g})/(\text{sample wt, g})] \times 100 \quad (1)$$

(h) Thin-Layer Chromatography (TLC). Chitosan oligomers were qualitatively analyzed by TLC using a silica gel plate (Kieselgel 60 F254, Merck). After the oligomers were developed with a solvent system of *n*-propanol/30% ammonium hydroxide/acetonitrile (2:2:1, v/v), the detection was made by spraying 0.1% ninhydrin in *n*-butanol and by heating in an oven at 110 °C during 10 min. Standards were D-glucosamine (Sigma, Ontario, Canada), and dimers, trimers, and tetramers (ISM Biopolymer, Granby, Canada).

Preparation of Chito-oligomers Using the Conventional Method. Chito-oligomers were also prepared using the traditional chemical method. The chemical method was carried out by adding 5.4×10^{-2} mol/L of HCl or NaOH under agitation in a glass beaker. The concentration of chemical reagents to be added was determined by titration of acid and base produced by the BMED system at specific conditions (current density of 10 mA/cm^2 and two elementary cells). Acidification of chitosan was carried out by addition of 112 μL of 12.1 M HCl every minute during 60 min to a 0.05 M NaCl solution (1.5 L), while chitosan was added stepwise as in the BMED method. After chitosan hydrolysis with chitosanase, chemical basification of chito-oligomers was carried out by addition of 135 μL of 10 M NaOH every minute during 60 min to the chitosan hydrolysate (1.5 L). Measurements of pH, conductivity, and ash levels were made on samples for comparison of the chemical and BMED methods.

Statistical Analysis. Data from the BMED and chemical methods were submitted to multivariate analysis of variance (MANOVA) using SAS software (version 8.2, SAS Institute, Cary, NC). Results of MANOVA were used to evaluate the effects of acidification, basification, and demineralization time, as well as the effect of preparation method (BMED or chemical) on the physicochemical variables during the process.

RESULTS AND DISCUSSION

Chitosan Acidification. Chitosan was solubilized by acidification using a BMED system or a chemical acid (HCl). The pH of the resulting solution was measured along the process and expressed as a function of acidification time (**Figure 3a**). Statistical analysis showed that acidification time ($P < 0.0037$) had a significant effect on the pH, whereas acidification method ($P > 0.72$) had no effect on the pH change. The kinetic of chitosan acidification was similar for BMED or chemical method, with a pH decrease of about 4.3 units, from pH 6.3 to 2.0, due to protons from water dissociation or HCl. The rapid decrease in pH was consistent with previous observations (*8*) and was made possible by the chitosan stepwise feeding mode and by the excess of protons in the medium. Estimations made using a chitosan pK_a value of 6.5 (*14*) showed that the fraction of protonated chitosan was 99.9% at pH 2.0. These conditions of extreme acidic pH values allowed full protonation of chitosan amine groups resulting in the conversion of chitosan base into its polyelectrolyte soluble form (*15*).

The conductivity of the solution was measured for BMED and chemical method as a function of time (**Figure 4a**). Conductivity was influenced by the acidification time ($P <$

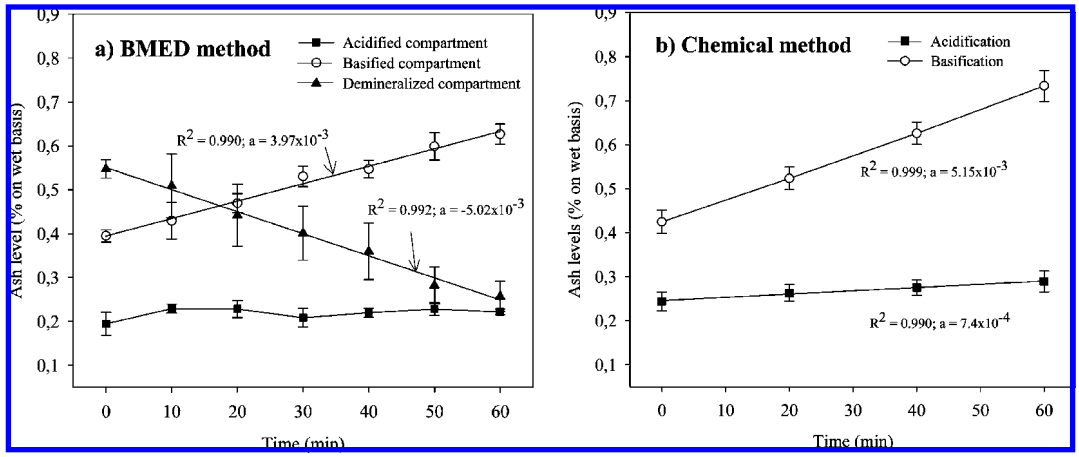

Figure 5. Change of ash contents during chito-oligomer preparation by (**a**) BMED method and (**b**) chemical method. R^2 is the correlation coefficient, and *a* is the slope coefficient for linear regression.

0.0014) and by the acidification method ($P < 0.0213$). At the end of acidification, conductivity increased by 2.1 times for the BMED method and by 2.9 times for the chemical method. Conductivity increased linearly for the chemical method ($R^2 = 0.989$), whereas its change was curvilinear for the BMED method ($R^2 = 0.962$, with a third-order polynomial regression). The linear change of conductivity for the chemical method was due to the progressive addition of HCl into the medium, with a constant increase of H^+ and Cl^- concentration. The lower increase of conductivity for the BMED method, represented by a curvilinear pattern, can be explained by the migration of OH^- ions from the diluate compartment (pH >11) to neutralize partially the H^+ protons generated by the bipolar membrane (**Figure 1**).

For the ash content of the solution, statistical analysis of data showed that the acidification time ($P > 0.0733$) and the acidification method ($P > 0.5291$) had no effect on the ash levels (**Figure 5**). Indeed, the average ash content was initially 0.22% (w/w on wet basis) and did not increase significantly during the process for the BMED and chemical methods. Although mineralization occurred for both methods because of the addition or migration of Cl^- anions, the results of ash contents did not show evidence of this phenomenom. This is because the calcination method at 500 °C poorly preserved the Cl^- ions, resulting in a low Cl^- content in the ashes (*16*). However, it is possible to estimate the theoretical mineralization by Cl^- ions in the case of the chemical method, because the amount of HCl added was precisely known: the increase of mineralization was 45% due to the addition of Cl^- ions from HCl. As for the BMED method, the mineralization rate should be lower than that of the chemical method, because the anions migrating in the acidified compartment were not only mineral ions (Cl^-) but also organic ions (OH^-). The lower mineralization of the solutions treated by BMED would also explain the lower conductivity values found for BMED in comparison with the chemical method.

Basification of Chitosan Hydrolysate Solution. Enzymatic reaction of chitosan with chitosanase was terminated by basifying the solution using the BMED or chemical method. Change of pH as a function of basification time is presented in **Figure 3b**. Statistical analysis indicated that basification time ($P < 0.0026$) and method of basification ($P < 0.0126$) had a significant effect on the pH variation. The increase in pH from 5.5 to 12 was due to hydroxyl ions from BMED or NaOH. The rate of basification was characterized by a sigmoidal curve, which is typical of a titration curve of chitosan by a strong base (*17*). Indeed, chitosan and its depolymerized products can be considered as weak acids due to their protonated amine groups. As the basification progresses, the amine groups are deprotonated, until all NH_3^+ groups are neutralized, corresponding to the equivalence point on the sigmoidal curve. Although the equivalence point was the same for the BMED and the chemical method, the increase of pH was faster for the BMED method. This can be explained by the lower concentration in chitosan material in the BMED system because chitosan precipitation occurred during the first electrobasification consisting of adjusting the pH from pH 2 to pH 5.5 by BMED (**Figure 2**).

The conductivity of the solution was measured for the BMED and chemical methods as a function of basification time (**Figure 4b**). The conductivity was influenced by the basification time ($P < 0.0001$) and by the basification method ($P < 0.0025$). The conductivity increased by 80% from 10.3 to 18.5 mS/cm for BMED method, whereas it increased by 71% from 9.9 to 16.9 mS/cm for chemical basification. At the beginning of the process, the increase in conductivity was faster for the BMED method in comparison with the chemical method with slopes of 0.069 and 0.040, respectively, considering data from 0 to 20 min. As previously explained, the increasing rate of conductivity is different for the two methods because the chitosan content was lower in the BMED method, resulting in a higher rate for the BMED conductivity. After the equivalence point, where all amino groups were neutralized, the increase in conductivity was the same for both methods, with an average slope of 0.175. In the second part of the curve, the change of conductivity is not dependent on the chitosan content, but depends only on the ions addition, mainly OH^- and Na^+ ions.

Mineralization of the basified solution was measured by total ash measurement for the BMED (**Figure 5a**) and chemical methods (**Figure 5b**) as a function of time. The ash content increased by 66% on average for the BMED and chemical methods due to alcalinization. With regard to the chemical method, the mineralization was due to Na^+ cations from NaOH. As for the BMED method, the cationization was done by Na^+ and K^+ from NaCl and KCl salts, which were added initially to conduct electricity. The mineral contents were similar at the beginning of the basification process (0.4% in average). Then, the slopes of the linear regression showed that the mineralization rate was faster for the chemical method than for the BMED method, with slopes of 5.15×10^{-3} and 3.97×10^{-3} for the two methods, respectively, resulting in a superior final ash level (0.73%) for the chemical method in comparion with BMED (0.62%). In the case of basification, the change of ash content

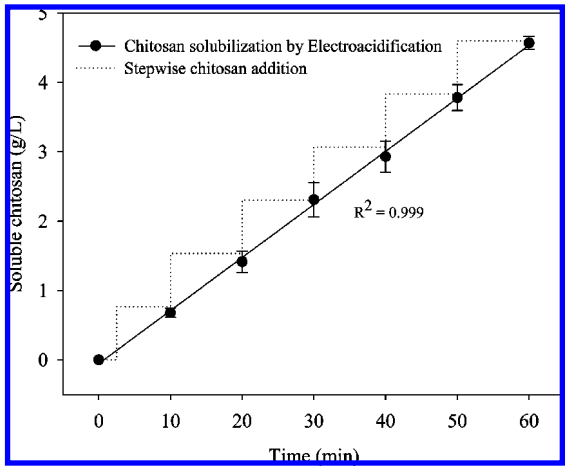

Figure 6. Change of soluble chitosan content as a function of time using a bipolar membrane electrodialysis system.

was clearly established in comparison with the electroacidification step, because the method of calcination at 500 °C preserved better the cations than the anions (*16*).

Demineralization of Chitosan Hydrolysate Solution by BMED. After basification of the chitosan hydrolysate, the solution was demineralized in compartment 3 of the BMED system (**Figure 1**). The pH and conductivity of the solution were measured as a function of electrodialysis time. Statistical analysis indicated that the electrodialysis time ($P < 0.0001$) had a significant effect on pH variation. The pH value decreased by 0.5 pH unit from pH 11.9 to pH 11.4 (**Figure 3c**). The decrease of pH can be explained by partial dealkalinization of the solution, because part of the hydroxyl ions left the diluate compartment to migrate to the adjacent acidified compartment by crossing the anionic membrane (**Figure 1**). The difference of 0.5 pH unit from 11.9 to 11.4 corresponds to a decrease in hydroxyl ions of 5×10^{-3} mol/L in the diluate compartment, based on pH calculations. However, the migration of OH^- ions from the diluate to the acidified compartment did not influence the solubilization of chitosan because the amount of protons generated in the acidified solution (5×10^{-2} mol/L) was about 10 times higher than the amount of OH^- ions originating from the desalting compartment.

The conductivity decreased linearly ($R^2 = 0.994$) by 43% from 15.4 to 8.8 mS/cm (**Figure 4c**). The linear decrease of conductivity is typical of a desalting compartment in electrodialysis (*18*) and is due to the migrations of anions and cations across the anionic and cationic membranes, respectively (**Figure 1**). The cations removed from the diluate were Na^+ and K^+ ions from NaCl and KCl salts initially added, whereas the anions removed were Cl^- and OH^- from the ions added initially and consecutively to basification. Moreover, we observed that the slope for linear regression of conductivity ($a = -0.110$) was inferior in absolute value to the slope calculated for conductivity in the basified compartment ($a = 0.173$). Indeed, ions removed from the diluate compartment (mostly Na^+, K^+ and Cl^-) are less conductive than the OH^- produced in the basified compartment (*19*), resulting in a lower conductivity variation for the diluate compartment.

The demineralization level in the diluate compartment was measured by ash determination. The ash level decreased linearly as a function of electrodialysis time ($R^2 = 0.992$) (**Figure 5a**). The mineral content was reduced by 53% from 0.55 to 0.26% (w/v). The decrease of mineral content was higher than the decrease of conductivity (43%). In general, the conductivity is used as an indicator for demineralization rate (*18*). However,

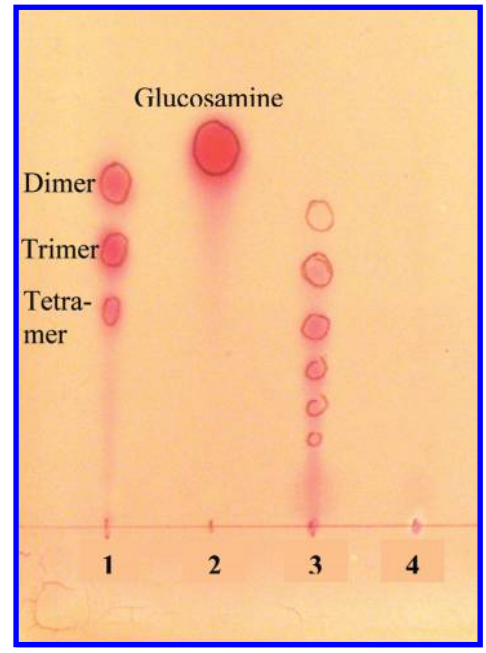

Figure 7. Thin-layer chromatography of chitosan oligomers. Lanes: (1) dimer, trimer, and tetramer standards; (2) glucosamine standard; (3) chitooligomers produced and demineralized by BMED; (4) original chitosan.

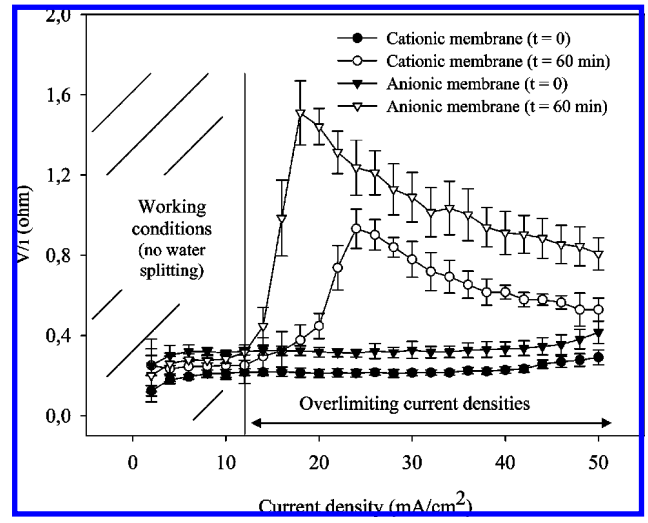

Figure 8. Monopolar membrane resistance as a function of current density, at initial time ($t = 0$) and at the end of electrodialysis ($t = 60$ min).

in this case the demineralization rate (53%) was underestimated because of the presence of OH^- ions. This phenomenom has been previously demonstrated for demineralization of cheese whey by bipolar electroacidification (*20*). Because OH^- and H^+ are highly conductive ions, the demineralization rate cannot be calculated directly from conductivity values and has to be determined by ash content measurements. The demineralization of 53% led to a final ash content of 0.26% (w/w on wet basis), which is close to the initial ash content before the BMED treatment. This result suggests that, after the initial mineral addition to start the BMED system, the three-compartment electrodialysis does not require further salt addition, as the salts necessary for conducting the electricity were provided by the desalting compartment.

Performance of the Proposed BMED System. One of the advatanges of the BMED over the chemical method is the possibility to remove the salts previously added during acidi-

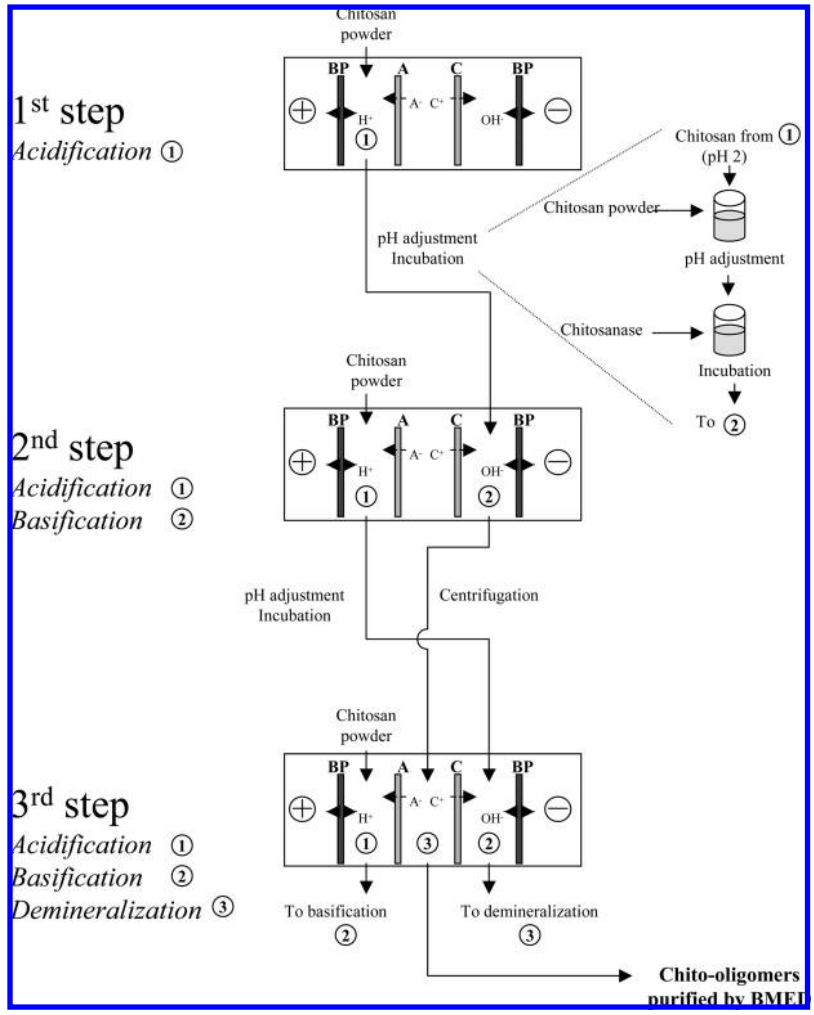

Figure 9. Process flow for chitosan hydrolysis into oligomers using a three-compartment bipolar membrane electrodialysis system.

fication and basification, so that at the end of a 1 h treatment, the ash content returns to its initial value (about 0.26%). However, the production of chito-oligomers purified by electrodialysis with bipolar membrane can be effective only if the following conditions are respected: (a) a high chitosan solubilization rate during acidification; (b) a complete inhibition of enzymatic reaction; (c) an effective demineralization; (d) a good membrane integrity; and (e) a low energy consumption.

Chitosan Solubilization Rate. A complete solubilization of chitosan was achievable using the BMED system, as shown by the linear increase in soluble chitosan content as a function of time ($R^2 = 0.999$) to reach a final solubilization rate of 4.6 g/L (**Figure 6**). Because the final product contained an excess of protons, an additional amount of chitosan can be solubilized, which would permit the adjustment of the pH to the optimum pH for the enzyme activity. Thus, this operation should be carried out outside the cell to prevent chitosan overloading in the electrodialysis system (**Figure 9**).

Inhibition of Enzymatic Reaction. Complete inhibition of the enzymatic reaction was ensured by the extreme basic pH of solution (pH >11). As shown by Lin Teng Shee et al. (*9*), inhibition of reaction occurs at pH 7, but higher pH values are required if denaturation of the enzyme is required. Apart from denaturation of chitosanase, Li et al. (*21*) showed that the alkaline pH values have also an effect on chitosan, by precipitating the high molecular weight chitosan, whereas the low molecular weight material remains soluble. Thin-layer analysis of the chito-oligomers end-products confirmed the presence of low molecular weight chitosan as dimers, trimers, and tetramers, with small amounts of higher molecular weight (**Figure 7**). In addition, indirect assay of chito-oligomers was done by measuring the reducing sugar content. After chitosan hydrolysis, the reducing sugar content was 0.36 μmol/mL, and no significant loss was observed after BMED treatment of the chito-oligomer solution.

Demineralization Rate. Increase of minerals in chito-oligomers was due to initial salt addition and to successive acidification and basification of the solution using BMED. The diluate compartment allowed the demineralization of the chito-oligomers solution up to 53%. The final ash content [0.26% (w/w) on wet basis] is 2-fold lower than that of chemical method. The conventional method of chitosan acidification and chitosanase inactivation gave products somewhat similar to the electrodialytic method in terms of mineral contents, but did not allow the subsequent reduction of ash content as possible by the BMED method.

Membrane Integrity and Limiting Current Density. Membrane integrity can be affected by fouling during BMED. Previous work showed that monopolar membranes can be fouled by a chitosan deposit (*7, 8*). The chitosan precipitate was due to OH^- ions produced by water spliting at the interface of the monopolar membranes. In the present work, the limiting current density was determined at the interface of the monopolar membranes and was found to be 45 and 12 mA/cm^2, repectively, at the

beginning and at the end of the BMED treatment (**Figure 8**). Because the current density used in this study was 10 mA/cm^2, water dissociation at the interface of monopolar membranes was prevented, and a good intergrity of bipolar and monopolar membranes was maintained with no chitosan deposit. It is also interesting to note that cationic membranes showed lower tendency to water splitting than anionic membranes, because their limiting current density was found to be higher (about 18 mA/cm^2) than that of the anionic membranes (about 12 mA/cm^2) at the end of the treatment (**Figure 8**). These results confirmed previous observations by Simons (*22*) on the higher water splitting ability of anionic membranes in comparison with cationic membranes.

Energy Consumption. The relative energy consumption, calculated from voltage, intensity, and duration, was estimated to be 0.02 kWh/L of solution. This energy consumption has been improved in comparison with previous study by Lin Teng Shee et al. (*7*), who reported a 0.05 kWh/L energy consumption. The lower energy consumption of the present study can be explained by a higher chitosan solubilization rate and by a low overall electrical resistance ($12 \pm 0.5\ \Omega$). In addition, the energy was used for running three treatments simultaneously, allowing efficient utilization of the electrical current.

An integrated process for chitosan hydrolysis into oligomers using a BMED system is proposed as follows (**Figure 9**):

• In the first step, the acidification compartment is used to solubilize the chitosan powder. The resulting chitosan solution (pH 2) is taken out of the BMED system, and the solution is adjusted at the pH of incubation of chitosanase by adding chitosan powder. This method of pH adjustment consists of the use of chitosan instead of electrobasification to avoid chitosan loss by precipitation, as noted in the present work, when the pH was preadjusted from 2 to 5.5. After the pH adjustment, the chitosan solution is incubated with chitosanase under controlled conditions of pH and temperature.

• In the second step, the basification compartment is used to terminate the chitosan hydrolyis by elevating the pH of the solution (pH >7). Simultaneously, chitosan is being solubilized in the acidified compartment. The resulting acidified solution is treated as described before, by adjusting the pH and incubating with chitosanase. As for the basified solution, a centrifugation step allows the separation of insoluble chitosan and soluble chito-oligomers.

• In the third step, the three compartments are running simultaneously. The acidification and basification compartments are used for chitosan solubilization and chitosanase inactivation, respectively. Meanwhile, the diluate compartment situated between the acidification and basification compartments is used for demineralization of the basified solution to produce purified chitosan oligomers.

This work showed that BMED can perform three operations in a single operating unit, including chitosan solubilization, chitosanase inactivation, and chito-oligomers demineralization. This innovative "3-in-1" process for chitosan transformation into oligomers has great potential for industrial application, because it is convenient and ecological and produces oligomers with a lower mineral content in comparison with the conventional method.

The BMED for the preparation of chitosan oligomers carries advantages from the standpoint of purity of the product, operation, and ecology. Whereas conventional methods produce oligomers with considerable salt content, BMED is a cost-effective process that allows the simultaneous conversion of chitosan in its soluble form, the termination of enzymatic hydrolysis of chitosan, and the preparation of low-ash oligomers. Moreover, no storage and handling of chemical acids and bases are required because the protons and hydroxyl ions are produced in situ in the BMED system, thus creating a safe working environment. Finally, the BMED system is considered to be a promising process with ecological benefits, because no effluents are generated and all streams in the three compartments can be valorized to produce the purified oligomers.

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