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Investigations on separation properties of ceramic membranes in ultrafiltration process of model myoglobin solutions

Introduction

The results of investigations on ultrafiltration process of model myoglobin solutions with use of ceramic membranes have been presented in this work. Myoglobin is a water-soluble globular protein present in muscle tissues of animals, also found in fish muscles, and among other fish proteins occurring in wastewaters from fish processing. Application of ultrafiltration process for waste brine purification has several advantages [*Afonso and Bórquez, 2002*]. It enables elimination of undesirable components as well as recovering of valuable products. The presented studies have been undertaken in order to investigate possibilities of applying ultrafiltration for treatment and fractionation of salted water produced in fish processing industry. The results could be a basis for elaboration of a treatment technology enabling recycling of both regenerated salted water and fractionated proteins, thus decreasing water use and wastewater discharge by closure of water loops. Development of membrane processes for waste brine purification requires studies on model solutions of individual proteins including analysis of the membrane selectivity and performance.

The aim of the study was assessment of the impact of myoglobin ultrafiltration process parameters on rejection coefficient and permeate flux. For further clarification of protein rejection mechanisms, the studies were supplemented with molecular modeling experiments in order to define the size, shape and electrostatic properties of the analyzed protein.

Experimental

The measurements were carried out in laboratory-scale using installation equipped with cross-flow module with flat ceramic TiO_2/ZrO_2 membranes, feed tank of 2 dm^3 volume, heat exchanger and pressure pump. During the studies membranes of 0,0056 m^2 filtration surface with a cut-off of 50 kDa were used. The ultrafiltration tests were performed under the following conditions: temperature 20°C, cross-flow velocity (CFV) 60 dm^3/h, transmembrane pressure (TMP) 0.05-0.2 MPa, *pH* 3,3÷8,9 and protein concentration 0.005%. The myoglobin from equine skeletal muscle (95÷100%, essentially salt-free, lyophilized powder) was used for preparing the myoglobin solutions. HCl or NaOH was used to adjust the *pH* of investigated solutions. The experiments were conducted in a system with continuous permeate and retentate recycling. The procedure of membrane cleaning (acid base washing) was conducted according to the producer's recommendations.

For determination of protein concentration in the feed and permeate samples UV-VIS absorbance measurements were performed on spectrofotometer with 190 to 1100 nm wavelength range using quartz cuvettes of 10 mm path-length.

The calculations on optimal myoglobin structure by molecular modelling methods were performed using the *HyperChem* software (release 8.0.9 for *Windows*) while the further analysis of geometrical parameters was carried out using *VEGA ZZ* software [*Pedretti et al., 2002*]. To predict the *pH* dependent protein stability its electrostatic properties were determined basing on *pKa* calculations for the ionizable residues. The *PropKa 3.1 Web Interface* [*Li et al., 2005*] was used for this purpose.

Ultrafiltration experiments

During the ultrafiltration tests of model myoglobin solutions membrane permeability expressed by permeate flux, *Jv*, m^3/m^2s, and membrane selectivity expressed by rejection, *R*, were measured versus transmembrane pressure and *pH*.

The rejection of myoglobin was calculated using the formula:

$$R = 1 - C_P/C_F \quad (1)$$

where C_P and C_F are myoglobin contents in permeate and feed respectively, expressed in g/dm^3.

The protein concentration C_P and C_F were determined on the basis of UV-VIS absorbance measurements using the following equation [*Sikorski, 2007*]:

$$C = 1{,}55\, A_{280nm} - 0{,}76\, A_{260nm} \quad (2)$$

where A_{280nm} and A_{260nm} are the absorbance values measured at wavelengths of 280 and 260 nm respectively.

The results of performed experiments are presented in Fig. 1.

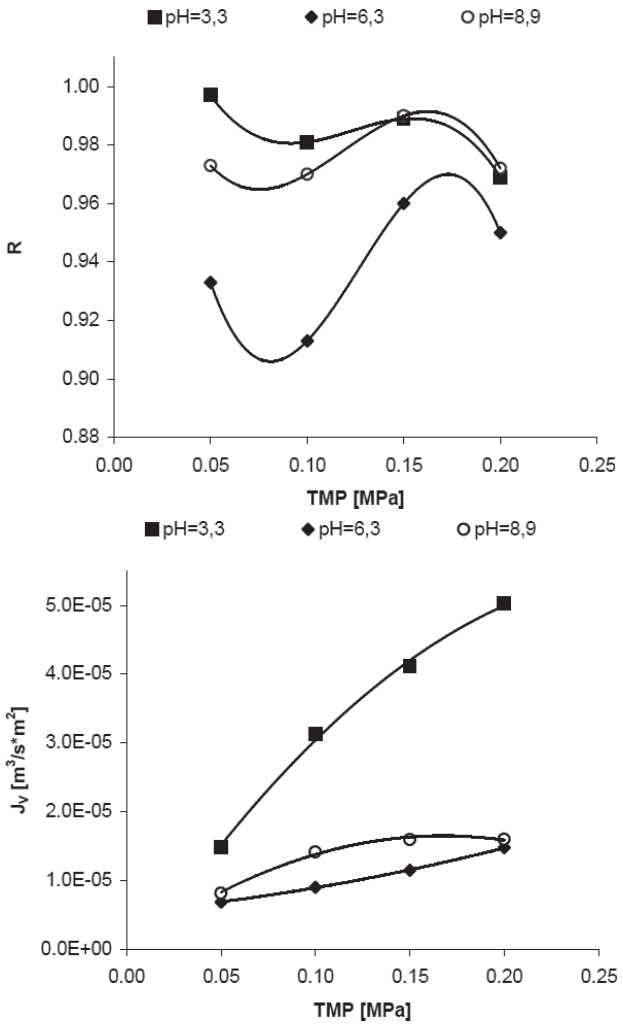

Fig. 1. Dependence of rejection and permeate flux on TMP and *pH* determined from ultrafiltration tests

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The highest permeate flux values were observed for *pH* values of 3.3 and TMP of 0.2 MPa, while the rejection coefficient reached its maximum for *pH* 3.3 and TMP of 0.05 MPa.

Molecular modelling of myoglobin

The starting structure of myoglobin used in the simulations was taken from the pdb file (entry code: 4DC8) deposited at *RCSB Protein Data Bank*. The file was obtained for a crystal structure of equine myoglobin by X-ray diffraction with resolution of 1.50 Å [*Kissick et al., 2013*].

The myoglobin structure was first geometrically optimized (GO) *in vacuo* using molecular mechanics methods with AMBER force field and applying *Polak-Ribiere* minimization algorithm. Then the structure was geometrically optimised in aqueous solution, at the same molecular mechanics settings, applying the periodic boundary conditions with explicit solvent. The cubic periodic box of 56.104 Å with 4940 water molecules was used.

After the geometrical optimization the system of solvated protein structure was annealed at temperature of 300 K with use of molecular dynamics (MD) to obtain lower energy minimum.

The optimised structure was analysed in *VEGA ZZ* software to determine approximate dimensions, molecular weight, radius of gyration and ovality.

The results of molecular modeling calculations are presented in Tab. 1.

Tab. 1. The results of calculations by molecular modelling

Calculated parameter	Myoglobin in vacuo	Myoglobin in solution (after MD and GO)
Minimum energy [kcal/mol]	-2216.86	-77278.69
Molecular weight [Daltons]	16905.29	
Approximate dimensions [Å]	47.826 45.266 46.976	48.976 31.656 49.646
Radius of gyration [Å]	15.202	15.159
Ovality	5.920	5.895

The free energy of folding (kcal/mol) as a function of *pH* was determined for the optimized myoglobin structure using *PropKa Web Interface*. Comparison of myoglobin stability (free energy of folding) to myoglobin rejection by ultrafiltration membrane is presented in Fig. 2.

According to the *PropKa* calculations the *pH* of optimum stability of analyzed myoglobin structure is 6.4 for which the free energy is -33.9 kcal/mol at 298 K.

Conclusions

The results of performed ultrafitration experiments on model myoglobin solutions showed that for the 50 kDa membrane permeate flux took highest values for *pH* of 3.3 and increased with increasing TMP. The lowest values of myoglobin rejection (0.91 to 0.96) were observed for *pH* of 6.3 what coincides with the minimum of free energy of folding corresponding to maximum stability of myoglobin (more compact structure). Higher rejection values for *pH* 3.3 and 8.9 might be connected with higher tendency to unfolding corresponding to higher free energy of folding.

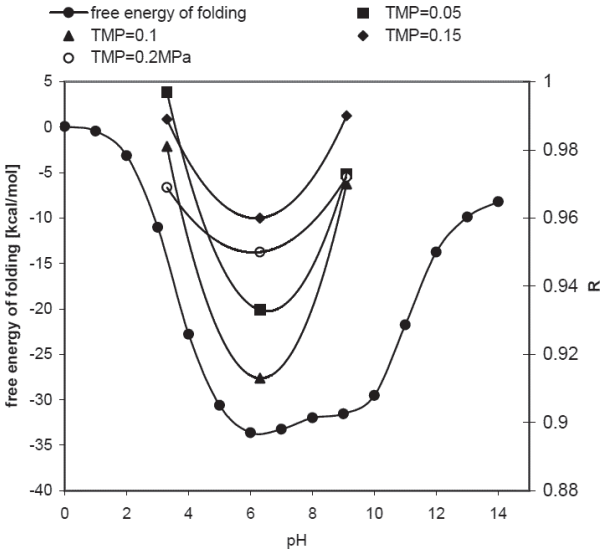

Fig. 2. The free energy of folding (kcal/mol) as a function of pH determined by PropKa for optimised myoglobin structure compared to rejection dependence on *pH* determined from ultrafiltration tests

The calculated values of molecular weight and approximate dimensions show that myoglobin molecules are smaller from the cut-off value of investigated membrane (50 kDa). Thus except the size and shape of protein also the membrane-protein charge interactions and fouling effects must be considered while explaining the rejection mechanisms.

The results of performed experiments showed that further research should be focused on fouling and membrane-protein interactions in investigated ultrafiltration process. The studies on membrane selectivity, and its dependence on size, shape and geometrical parameters of protein for lower cut-off values should be included. The research with use of molecular modeling techniques should be continued for the protein structures at various *pH* values.

LITERATURE

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