



Development of A Multi-stages Membrane Filtration Process for Separation and Purification of Protease from the Wastes of Fishery Industry

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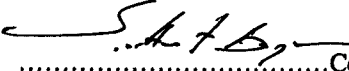

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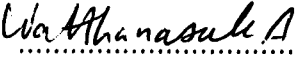
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ABSTRACT

In order to reuse the waste of fishery industry, a multi-stage membrane filtration process was developed to separate and partially purify protease from yellowfin tuna spleen which is the waste from tuna canning industry.

For selection of membrane size of microfiltration, the dead-end microfiltrations with membrane pore size 0.10, 0.22 and 0.45 μm were tested to remove suspended solids from yellowfin tuna spleen extract. It was found that 0.10 μm membrane provided the best performance. Almost all trypsin and chymotrypsin were recovered from the permeate of microfiltration while most large particles and half amount of other soluble proteins were rejected by a 0.10 μm membrane at transmembrane pressure 4 psi. The transmissions were about 1 and 0.5 for enzymes and soluble protein, respectively. The pore blocking resistance-limited was the major fouling mechanism for permeate flux decline while cake resistance-limited dominated the most duration of microfiltration process. Increasing both membrane pore size and transmembrane pressure caused partially loss of enzyme activity. Centrifugation and simple pre-filtration changed the particle size distribution in the extract and resulted in the change of fouling mechanism during microfiltration.

Crossflow microfiltration was applied to remove suspended particles from tuna spleen extract. For the condition of sustainable operation with low fouling in batch concentration crossflow microfiltration, the relation between critical ratio of flux (J) to wall shear stress (τ_w) and volume concentration factor (VCF) was found as $J/\tau_w = 3.29 (\text{VCF})^{-0.74}$ at a given transmembrane pressure of 0.15 bar. The present study revealed a simple method to predict low fouling condition in batch concentration operation during membrane separation process.

Based on total recycle and single-batch concentration crossflow microfiltration, a continuous-batch concentration crossflow microfiltration (CBC-CFMF) with 0.10 μm hollow fiber membrane, crossflow velocity of 0.2 m/s, transmembrane pressure of 0.15 bar and gas injection factor of 0.38 was designed and applied successfully to remove suspended solids from tuna spleen extract. Transmissions of about 1 for both trypsin and chymotrypsin were attained in this study. The optimal gas injection factor (r) of 0.38 resulted in a 300% improvement in flux comparing to the process without gas injection. A clear permeate with slight yellow colour was obtained after CBC-CFMF.

Ultrafiltration was applied for recovery of protease from microfiltration pretreated yellowfin tuna spleen extract. Effect of hydrodynamics and gas sparging on flux enhancement and selectivity was studied by a total recycle mode using a hollow fiber membrane with the molecular weight cutoff 30 kDa. The critical flux varied from 28.8 to 44.2 $\text{l/m}^2\cdot\text{h}$ and limiting flux varied from 34.3 to 52.4 $\text{l/m}^2\cdot\text{h}$ while crossflow rate increased from 17.55 to 69.98 l/h without gas sparging. A low gas injection factor of 0.15 could improve critical and limiting flux significantly.. Higher gas injection factors varied from 0.30 to 0.61 did not give remarkable improvement of both critical and limiting flux. The benefit of increasing crossflow rate to enhance critical and limiting flux was great when gas sparging did not applied. Selectivity was increased with increasing permeate flux at sub-critical condition and critical flux condition. It became insensitive to the flux and crossflow rate at limiting flux condition when gas sparging was not applied. Gas sparging gave negative effect on soluble protein and peptide transmission and resulted in the decay of selectivity at sub-critical condition and critical flux condition. The selectivity at limiting flux condition was not sensitive to gas injection factor.

Protease from tuna spleen extract was finally purified by an ultrafiltration with diafiltration mode. Severe fouling was avoid while 12-fold purification of protease was achieved in mode 1 (pre-diafiltration followed by post-concentration) with a critical flux condition operation. A conventional operation, i.e. mode 2 (pre-concentration followed by post-diafiltration) provided 2-fold purification of protease. Fouling was much severer in mode 2 than mode 1. Consequently low flux caused by fouling led to a long operational time in mode 2. The difference of resistance between mode 1 and mode 2 was mainly due to concentration polarization and external membrane fouling.

The purified protease from tuna spleen was compared with two commercial proteases based on degree of hydrolysis of protein. Degree of hydrolysis of 43% were reached after hydrolysis of casein by Alcalase and protease from tuna spleen, respectively while Delvo-Pro showed lower degree of hydrolysis at the same condition. In the case of soybean protein isolate as substrate, protease from tuna spleen provided lowest degree of hydrolysis which was due to the residual activity of soybean protein inhibitor in soybean protein isolate.

The presented work showed that a trypsin-like serine protease with low-cost and qualified hydrolysis efficiency could be obtained from tuna canning waste by membrane filtration.