

The aim of the dissertation “Multistage membrane separation of selected proteins and coupled with bioreactor process” was to develop a process of whey waste management. The object of the research was waste stream after goat cheese production with high turbidity, fat content and some curd residues.

The first step was developing effective whey proteins separation process. Some of them (e.g. lactoferrin or serum albumin) have health promoting properties and they are used widely in the pharmacological industry.

This process provides a lot of difficulties, because the results of experiments do not coincide with the assumption based on membrane separation theory, especially on sieving separation mechanism. The cut-off coefficient for ultrafiltration membranes or pore size in case of microfiltration membranes indicates what size of particles should be transmitted through the membrane. Proteins smaller than this value should freely pass through the pores and their retention coefficient should be nearly zero. Many of the experiments show, that the whey proteins separation does not apply to this theory. Protein with molar mass significantly smaller than cut-off coefficient was retained by membrane. It was impossible to isolate one pure fraction of whey protein.

High retention of whey proteins according to a literature could be decreased by addition of salt (increase of ionic strength) and pH modification. Influence of NaCl addition and pH change on quality and quantity of ultra- and microfiltration has been investigated. Influence of proteins concentration on retention degree has also been determined.

During experiments higher retention than cut-off coefficient indicated by the membrane manufacturer was observed. High diversity and strong molecular interactions could be an explanation of this fact.

Second step of research was to develop hydrolysis of whey protein – albumin, in order to obtain active peptides.

To create an appropriate mathematical description of proteolysis reaction with thermolysin, there were carried out a series of experiments in batch reactor. Based on obtained results, there was observed that reaction slow down soon after its beginning. It was a consequence of the creation of an inhibitor, which is also a product of hydrolysis. Based on graphic simulation there was non-competitive inhibition specified and proteolysis was described by a Michaelis – Menten’s equation. In range of used concentration (under 25[g l⁻¹])

model was simplified to first order reaction. The kinetic constants was $K_i = 250.0$ [$\text{g}_{\text{products}} \text{g}_{\text{enzyme}}^{-1}$] and $k/K_m = 13.3$ [$\text{l g}^{-1} \text{min}^{-1}$].

Based on these constants there was examined applicability to continuous proteolysis. The analysis of match errors was satisfactory and developed model could successfully have described a process carried out for different concentration of enzyme and substrate.

For active peptides recovery from enzymatic hydrolysis the membrane was selected. There were three membranes tested with cut-off coefficient from 1-15[kDa] range and ionic strength and pH influence on separation was investigated. The biggest influence on separation selectivity had cut-off coefficient. The influence of NaCl addition and pH changing were negligible.

To membrane reactor two of these membranes was selected: polyethersulphone flat membrane with cut-off coefficient 1-3[kDa] and ceramic tubular membrane with 15[kDa] cut-off coefficient. Both of them provided a total enzyme retention in reaction zone and small peptides recovery, among which were potential inhibitors, resulted an increase of hydrolysis degree.

Last steps of research was to develop biodegradation waste stream process. The study started from checking microbial activity of native strains, which was very low. Then there was selected strain to biodegradation process and it was bacterial strain – *Bacillus licheniformis*. This microorganism effectively metabolizes lactose present in whey and on the other hand it has an ability to nitrate-respiration and it results little decrease of protein content.

Equation, which describes a growth rate of *B.licheniformis* on whey ground was developed. Above 5.4 [g l^{-1}] lactose concentration there was very long duration of lag-phase observed. Kinetics of growth in batch breeding was described by Loung's equation with substrate inhibition with constants: $K_i = 6.03$ [g l^{-1}], $n = 1.10$, $K_M = 4.79$ [g l^{-1}] and $\mu_{\text{max}} = 2.29 \cdot 10^{-4}$ [s^{-1}].

Performing biodegradation on continuous stirred tank reactor, points were obtained on the rising side of the graph and they could be expressed by Monod's equation as it was in periodic breeding, but with another constants: $K_m = 0.45 \text{ g} \cdot \text{dm}^3$, $\mu_{\text{max}} = 8.52 \cdot 10^{-5} \text{ s}^{-1}$ in range of application below the lactose concentration 2.25 g dm^{-3} .

Based on obtained data there was developed relation describing the rate of lactose conversion and proteins with respect to their and biomass concentration. The rate of protein conversion was significantly lower than the lactose, but their concentration after recycling (e.g. membrane separation) is negligible waste streams.

Experiments carried out in continuous system confirmed the ability *B.licheniformis* strain to effectively reducing of lactose content and slower reducing of nitrogen sources (proteins). Applying residence times above 37 [h] resulted of COD index reduction to the value required by the Minister of the Environment. Calculated COD index – based on concentration of organic matter – in exiting from bioreactor stream after this time was 7.6 [mgO₂ l⁻¹].