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Continuous hydrolysis of milk proteins in membrane reactors of various configurations

Ksenia A. Ryazantseva*, **Evgeniya Yu. Agarkova**, **Olga B. Fedotova**

All-Russian Scientific Research Institute of the Dairy Industry, Moscow, Russia

* e-mail: k_riazantseva@vnimi.org

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Abstract:
Introduction. The article provides a review of technologies for membrane fractionation of various hydrolyzed food substrates in membrane bioreactors (MBR). In food industry, MBRs are popular in functional food production, especially in the processing of whey, which is a very promising raw material due to its physicochemical composition.
Study objects and methods. The research was based on a direct validated analysis of scientific publications and featured domestic and foreign experience in MBR hydrolysis of protein raw material.
Results and discussion. The MBR hydrolysis of proteins combines various biocatalytic and membrane processes. This technology makes it possible to intensify the biocatalysis, optimize the use of the enzyme preparation, and regulate the molecular composition of hydrolysis products. The paper reviews MBRs based on batch or continuous stirring, gradient dilution, ceramic capillary, immobilized enzyme, etc. Immobilized enzymes reduce losses that occur during the production of fractionated peptides. Continuous MBRs are the most economically profitable type, as they are based on the difference in molecular weight between the enzyme and the hydrolysis products.
Conclusion. Continuous stirred tank membrane reactors have obvious advantages over other whey processing reactors. They provide prompt separation of hydrolysates with the required biological activity and make it possible to reuse enzymes.

Keywords: Milk proteins, whey proteins, hydrolysis, membranes, enzymes, membrane reactor, substrate

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INTRODUCTION

Balanced diet and natural food quality are the most important issues of contemporary food science [1–4]. Environmental pollution and such diet-related diseases as hypertension, diabetes, allergies, etc., require new types of diet and functional products [5–8]. Modified milk and whey proteins can serve as basic components of functional foods [9–13]. Enzymatic hydrolysis of dairy proteins is the most popular method of whey modification, which makes it possible to impart additional functional and technological properties, e.g. emulsifying, foaming, antioxidant, antihypertensive, immunomodulatory, etc. [14, 15].

Whey proteins and their hydrolysates possess high nutritional value, which makes them the most promising components for diet therapy products. Whey proteins owe their useful functional properties to bioactive peptides [16, 17]. Bioactive peptides are amino acid sequences, encoded in the primary structure of native proteins. A protein hydrolyzate contains a mix of biologically active and inactive peptides, in addition to non-hydrolyzed proteins. Fractioning can isolate certain biologically active peptide fractions from hydrolysates. Fractioning relies on such membrane separation processes as ultrafiltration and microfiltration [18–22].

Membrane separation means that two or more components are separated through a membrane that acts as a selective semipermeable barrier that partially or completely stops one or more substances. The retained components produce retentate, while those that pass through the membrane form permeate [23, 24]. Membrane processes have several advantages over other separation methods. First of all, they require less energy than evaporation or distillation. Second, they demonstrate high selectivity and are easy to scale. Finally, they are material friendly, which is a very important factor for food industry [24].

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Development and design of new membrane bioreactors (MBR) is one of the most promising and dynamic areas of industrial biotechnology. MBR technology combines various membrane and biochemical separation processes, the latter being induced by a catalyst of biological origin, i.e. an enzyme. The main advantage of MBR enzymatic hydrolysis is that it saves expensive enzyme preparations and regulates the molecular composition of hydrolysis products by combining membranes with a recommended molecular weight cut-off [18].

Unfortunately, contemporary food industry uses only about 50% of the whey produced worldwide, which means that the task of whey recycling is yet to be solved. This issue remains controversial and requires comprehensive research. The present review describes how various whey processing MBRs can increase the value of whey components [25].

STUDY OBJECTS AND METHODS

The present research was based on a direct validated analysis and featured the most recent domestic and foreign publications on protein hydrolysis in various membrane reactors.

RESULTS AND DISCUSSION

Figure 1 illustrates two most common membrane reactors (MBR). In the first type, the membrane controls the mass transfer of the substrate and enzyme preparation to and from the reactor module, thus producing an indirect effect on the hydrolytic degradation of the substrate (Fig. 1a). In the other type, the reaction occurs at the membrane level and complements the regulation of substrate and enzyme mass transfer [26, 27]. Complex as it is, MBRs of the second type makes it possible to control proteolysis at the cellular level (Fig. 2b) [26, 27].

Such MBRs are called biocatalytic because the membrane itself acts as a catalyst. They are based on continuous stirring: the product either passes through the membrane, which retains the enzyme and returns it to the reactor, or remains in the membrane module. The biocatalyst is immobilized and separated by a membrane in the reaction vessel [26, 28]. As a rule, the membrane immobilizes the enzymes on membranes because biomolecules are covalently attached to the surface of the carrier. As a result, the system is more stable, and the microreactor can be reused while the enzyme is no longer active. The covalent attachment of enzymes to solid substrates is very strong and increases the service life of the microreactor and immobilized enzymes [29].

The numerous advantages of these MBRs make them an alternative to simple bioreactors. The most important advantage is that the catalyst (enzyme) can be recovered and reused in a continuous system, which increases the efficiency of the process. The yield rises, while the expensive enzyme preparation is spared, which lowers the cost of the final product. In addition, the selective removal from the reaction medium is continuous, and the supply of the reagent to the catalytic reaction medium is easy to control [26].

Ultrafiltration is the most common separation process used in this type of MBR. Unfortunately, polarization remains its main disadvantage: eventually, the membrane pores get clogged. Nearly all membrane filtration processes gradually decrease, as trapped particles accumulate on the surface of the

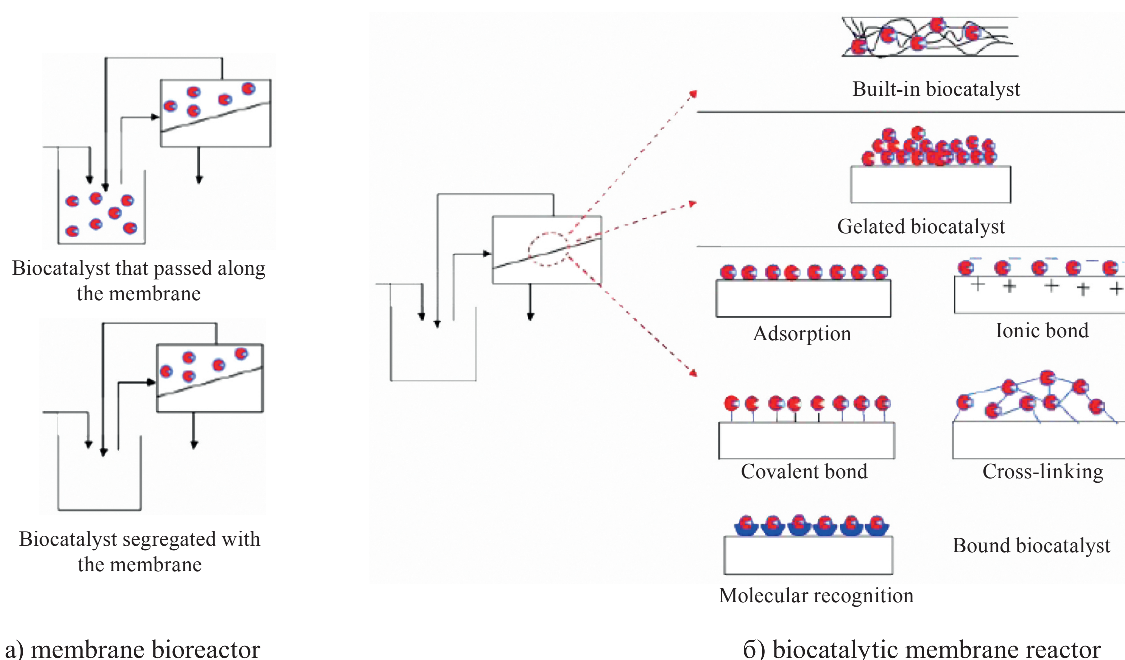


Figure 1 Schematic illustration of membrane reactors

membrane. The rate depends on the operation type of the membrane, the nature of the flow, the pore size, and charge of the membrane. The flow decreases because of certain physical or chemical interactions that occur between the interface of the membrane and the components of the feed stream. The formation rate of the surface layer has to be controlled, as it keeps accumulating on the side of the membrane that experiences excess pressure. No pre-treatment can prevent clogging, and the membrane has to be cleaned regularly [26].

In a biocatalytic MBR, the membrane not only separates but also catalyzes. The enzyme enters the membrane matrix and is immobilized there (Fig. 1b), increasing its stability, which is another advantage of this type of MBR [30]. Immobilization increases the stability of enzymes during storage, namely, their resistance to changes in temperature and pH [31].

In their study of continuous MBRs, Wang *et al.* focused on transglutaminase, which was covalently bound to the surface of the polyethersulfone membrane. The enzyme cross-linked α-lactalbumin and β-lactoglobulin, thereby retaining them on the membrane [32]. Using transglutaminase for enzymatic modification of milk protein can prevent protein loss during whey processing and increase the biological value of the product [33]. During whey ultrafiltration, α-lactalbumin and β-lactoglobulin can pass through the membrane under transmembrane pressure, in which case they block the pores or penetrate into the filtered solution. As a result, β-lactoglobulin is the main cause of membrane clogging during whey filtration [34–36].

Wang *et al.* studied an enzymatic MBR with transglutaminase, its efficiency, the catalysis of protein crosslinking, and its separation from whey. The protein recovery rate reached 85%, but it decreased over time, as did the relative membrane flow, probably, following the decrease in enzymatic activity on the membrane surface after 1365 min of continuous operation. The overall specific performance of the enzyme bound membrane was about 50% less than that of the pure polyethersulfone membrane. Wang *et al.* concluded that the efficiency failed because of the repulsion forces that appeared between the cross-linked proteins and the membrane [32].

Vasileva *et al.* studied β-galactosidase that was covalently bound by glutaraldehyde to the surface of the modified polypropylene membrane. They determined the optimal hydrolysis conditions for lactose in a batch MBR: enzyme activity 13.6, temperature 40°C, pH 6.8, time 10 h. The scientists compared the resulting degree of hydrolysis with that obtained by a free non-immobilized enzyme. The immobilized enzyme method proved 1.6 times more effective than the one based on a free enzyme, as the immobilized enzyme itself was twice as stable as the free enzyme. The resulting immobilized β-galactosidase/polypropylene membrane system was used to obtain glucose-galactose syrup from whey waste. Vasileva *et al.* carried out hydrolysis of whey lactose in a MBR using an immobilized enzyme and a spiral membrane. The optimal membrane surface and the whey flow rate were 100 cm^2 and 1.0 mL/min, respectively. After 10 h, the lactose hydrolysis reached 91%. After cycle 20, the yield was 69.7% [37].

Sen *et al.* focused on skim milk hydrolysis in a batch MBR using β-galactosidase immobilized on a polyethersulfone membrane with a pore diameter of 30 kDa. The study featured aqueous solutions of skim milk in the concentration range of 30–80 kg/m^3. The solutions underwent deproteinization through two membrane ultrafiltration modules with pore sizes 30 kDa and 5 kDa. As a result, 95–97% of lactose became permeate. The permeates obtained were subjected to hydrolysis in a batch MBR equipped with an enzyme-immobilized membrane. The enzyme was immobilized by cross-linking on an ultrafiltration membrane using 3 and 4% glutaraldehyde. The 4% glutaraldehyde solution provided a greater enzyme activity retention (94.2%) and enzyme loading (98%). The final conversion of lactose was 45.2 and 21.4% when β-galactosidase was immobilized with 4 and 3% glutaraldehyde, respectively. The control experiment with an immobilized enzyme showed a significant decrease in the flow of pure water: 27.5 for 3% glutaraldehyde and 67.5 for 4% glutaraldehyde [38]

When the biocatalyst is confined to the membrane module, not the reservoir with the reagents, it is not recirculated into the outlet flow; with that, low molecular weight products and inhibitors leave the system directly through the membrane. This type of MBR finds application in bio-artificial pancreas or extracorporeal detoxification devices [26].

Biocatalytic MBRs are undoubtedly more efficient, since both the reaction and the separation occur in the same membrane module. However, current knowledge about the nanoscale processes within the microenvironment of the membrane remains insufficient. Equally lacking is the knowledge about the control of continuous hydrolysis at the macroscopic level. As a result, biocatalytic MBRs cannot be used for commercial production [39–41].

Biocatalytic MBRs, or bioreactors, are integrated with such membrane processes as microfiltration, ultrafiltration, reverse osmosis, membrane extraction, etc. They are especially effective for food and beverage production, e.g. wine, fruit juices, milk, etc. [42, 43]. In the dairy industry, MBRs were first used to produce low lactose milk [43]. Such MBRs are still widely used to produce functional products for patients with lactase deficiency. However, lactose is not the only substance that causes milk intolerance: some people cannot absorb high molecular proteins ($\geq$ 5 kDa) due to inadequate immune response. MBRs are also used to produce low-allergenic milk [44].

MBRs are getting more popular in food industry as a result of industrial demand for functional foods, e.g. hypoallergenic, nutraceutical, or alternative foods, ingredients that are part of dietary and preventive

menus, etc. MBRs are actively used in whey processing. The physicochemical composition of whey makes it a very promising raw material for functional food production. Whey contains 0.4–0.8% of protein and 4.4–5.5% of lactose. Whey proteins possess a good latent potential of biofunctional properties [43].

Batch MBRs are simple enough to gain extensive use in the production of protein hydrolysates. However, they need a lot of enzyme, energy, and labor, which makes it expensive [19]. American scientists from the Department of Food Science (Pennsylvania, USA) attempted to process food substrates using batch-type enzyme reactors with an immobilized enzyme. They identified a number of additional disadvantages, e.g. high losses in the activity of the biocatalyst, the expensive enzyme immobilization, etc. [44].

Continuous stirred tank membrane reactors (CSTMR) are an alternative to batch MBRs. They are based on the difference in molecular weight between the enzyme and the hydrolysis products. CSTMRs can separate products from the reaction medium to increase the yield. The soluble enzyme is confined to the retentate side of the membrane, where it comes in contact with the substrate. CSTMRs make it possible to reuse the enzyme and select a suitable membrane pore size, which facilitates the control of the molecular weight of the final product [44].

Ewert *et al.* used a two-stage enzymatic membrane bioreactor (EMBR) to obtain sodium caseinate hydrolyzate with improved antioxidant capacity and reduced bitterness (Fig. 2) [44]. At the first stage, sodium caseinate was hydrolyzed at 65°C and pH 6.7 using endopeptidase Sternzym BP 25201. The stage took 12 h and involved hydrolysis and filtration through a ceramic ultrafiltration membrane made of hollow fiber with a molecular weight cut-off of 10 kDa. The antioxidant activity of the resulting permeate increased by 33%, compared to sodium caseinate. The volume of permeate that left EMBR-1 was automatically compensated for by adding a new substrate to the reactor vessel.

At the second stage, the main objective was to remove bitterness. The hydrolysis was carried out in EMBR-2 using Flavorzyme at 50°C and pH 6.7. After 12 h of hydrolysis, it was filtered through a UV polyethersulfone membrane with a molecular weight cut-off of 10 kDa. EMBR-2 also increased the antioxidant capacity of the permeate to its half-maximal inhibition concentration (IC_{50}) of 13.8 μg/mL, which was 39% more than that of sodium caseinate. The experiment made it possible to avoid the mutual effect of peptidases by separating endo- and exopeptidases at the two stages of hydrolysis. The selected conditions proved optimal and ensured a stable production for three days. The research featured the degree of hydrolysis of biocatalysis products. The hydrolyzate obtained in EMBR-1 had the following parameters: degree of hydrolysis – 8.0 ± 0.2%, permeate – 8.7 ± 0.4%, sediment fraction – 2.9 ± 0.3%. The permeate hydrolyzed in EMBR-2 had a degree of hydrolysis of 21.8 ± 0.8%. The loss of enzymatic activity in both reactor vessels was compensated by the daily addition of the corresponding enzyme. The whole process took 110 h [45].

Due to the applied temperature, the relative activity of peptidase in EMBR-1 decreased to 82 ± 6.9% of its initial value during the preliminary hydrolysis. As for EMBR-2, its initial activity remained the same during the preliminary hydrolysis (26–38 h) and decreased to 82% after 24 h of filtration (38–62 h). The two reactors maintained stable conditions because the activities were adjusted every 24 h. The experiment proved that CSTMRs can be used for commercial production of functional antioxidant ingredients based on sodium caseinate [45].

Guadix *et al.* studied hydrolyzate production of hypoallergenic whey [44]. The research objective was

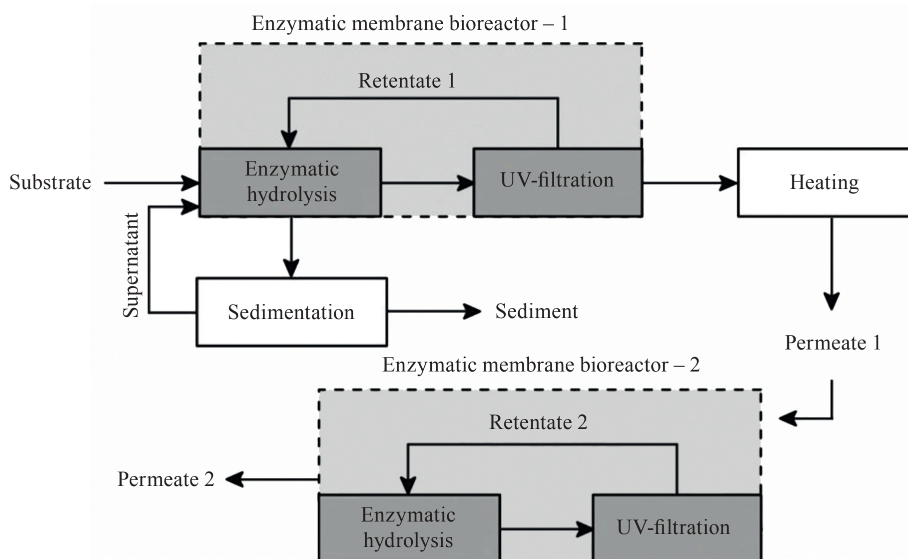


Figure 2 Block diagram of a two-stage installation of a two-stage enzymatic membrane bioreactor with continuous hydrolysis

to create a stable long-term process for the production of whey protein hydrolysates with low antigenicity. The study was based on other scientific schools of continuous hydrolysis. For instance, specialists from the University of Illinois (USA) studied continuous hydrolysis of soy protein from Promin-D in a CSTMR with hollow membrane fibers. At the initial stage, the conversion rate was 90%, which dropped to 60% after 10 h because of the leakage of the enzyme through the membrane and thermal deactivation. The Illinois team also studied milk protein hydrolysis. They hydrolyzed casein with alkalase, also in a CSTMR with hollow fibers. Their experiments determined the efficiency of the reactor at 50 and 37°C. After a 15-h fermentative treatment, the degree of conversion dropped from 96 to 62% at 50°C and from 75 to 51% at 37°C. Like in the first case, the efficiency fell down because of enzyme leakage, thermal deactivation, and enzyme-membrane interactions.

French scientists studied the effect of operating variables on the performance of hollow fiber CSTMRs for hydrolysis of blood plasma proteins using alcalase. After 35 h of operation, the permeate flow dropped due to membrane clogging, which occurred as a result of the polarizing layer that accumulated on the membrane surface. Spanish and Colombian biochemists hydrolyzed whey proteins with alcalase using the same CSTMRs with hollow fibers. They managed to maintain an uninterrupted process only for 7 h because of the rapid clogging and the inactivation of enzymes. Both the proteolysis regimes and the design features of the membranes obviously needed correction.

A team from Taiwan managed to maintain uninterrupted operation for 16 h. In addition to alcaslase, they also included Flavuerzyme into the enzyme preparation. The Laboratory of New Dairy Technologies (France) used CSTMRs to obtain specific bioactive peptides by hydrolysis of casein-macropeptide.

Cow's milk whey is not the only type of whey in such studies. Cambridge specialists studied hydrolysates of goat whey from the point of view of the formation of biologically active peptide compounds. Goat whey was hydrolyzed with pepsin in an enzymatic reactor. The ultrafiltration polymer membrane was combined with a mineral membrane with a cut-off of 30 kDa. Peptides in the permeate were separated by reversed-phase HPLC, which is the most common method for separating milk peptides [46, 47]. As β-lactoglobulin is resistant to pepsin, most opioid and antihypertensive peptides were derived from α-lactalbumin. Pepsin exhibited a considerable substrate specificity; the molecular weights of the obtained peptides ranged from dipeptides to very large peptides with disulfide bridges (150–6900 Da). As a result of the α-lactalbumin hydrolysis, the amount of peptides with a molecular weight of $\leq$ 600 Da was 36%, 600–2000 Da – 24%, and $\geq$ 2000 Da – 40%.

Guadix *et al.* hydrolyzed diluted milk whey concentrate (50 g protein/L) in a CSTMR at 50°C and pH 8.5 using Protex 6L bacterial protease obtained from *Bacillus licheniformis*. The design of the membrane reactor included a 3-L vessel, an automatic controller of pH and temperature, a recirculation pump, and a frame membrane ultrafiltration module with a polyethersulfone plate with an effective area of 0.07 m^2 and a molecular weight cut-off of 3 kDa. The reaction mix was continuously recirculated at a rate of 1.5 L/min with a pump at a rate of 0–15 L/min. The pump was installed between the reaction vessel and the inlet of the membrane module.

As a result of membrane clogging, the permeate flow dropped from 10 mL/min to 6.3 mL/min after 16 h. After 10 h of operation, the degree of hydrolysis stabilized at about 80%, while the permeate flow stabilized after 13 h. As the permeate flow decreased during the first 13 h, the enzymes demonstrated signs of thermal inactivation. The resulting hydrolyzate contained peptides that consisted of four amino acids. The content of antigenic whey protein decreased by 99.97% in the final product, which means that it can be used in hypoallergenic diets, baby food, and enteral feeding. However, the authors had to compensate for the loss of enzymatic activity by feeding small amounts of fresh enzyme [44].

O'Halloran *et al.* developed an EMBR in which the whey protein isolate was subjected to enzymatic hydrolysis to obtain antidiabetic peptides that inhibit dipeptidyl peptidase-IV (DPP-IV). The efficiency grew

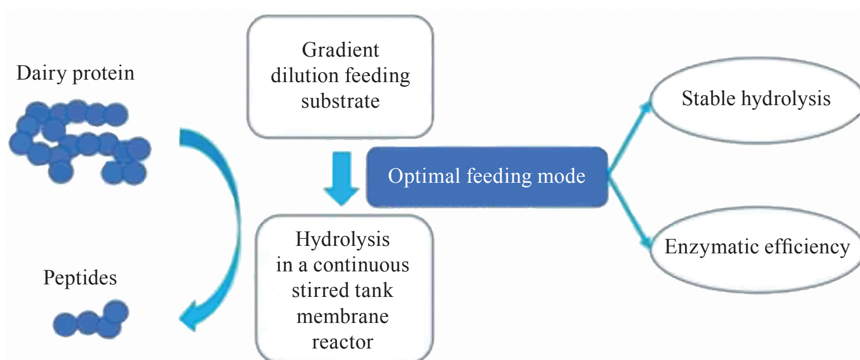


Figure 3 Method of gradient dilution feeding substrate in an enzymatic membrane reactor

by 7.2 and 8.7% when using Protamex and Korolase 2TS, respectively, compared to the standard method of batch processing. Previously, neither of the enzymes was considered effective for obtaining peptides with antidiabetic activity. Protamex and Korolaza 2TS proved capable of producing peptides that inhibit DPP-IV. The permeate hydrolyzate obtained with Protamex showed a 33.7% higher DPP-IV inhibition value compared to the hydrolyzate obtained using Korolase 2TS. J. O'Halloran and colleagues proved that Protamex can be used to produce protein substrates with antidiabetic activity [48].

Huang *et al*. used a CSTMR to improve the yield of peptides that inhibit angiotensin-converting enzyme from milk protein. The research employed a new method of gradient dilution feeding substrate (GDFS) (Fig. 3) [49]. The scientists compared the stability of the hydrolysis process, enzymatic efficiency, and kinetics of the method with the traditional modes of feeding, when adding water after feeding the substrate, or feeding the substrate with a constant concentration. The GDFS method showed the highest membrane flow rate and the lowest fluctuations in the protein concentration in the reactor. GDFS also had a higher rate of protein hydrolysis, which increased by 67.58%. The yield of peptides reached 138.51 g/g neutrase, and the angiotensin-converting enzyme inhibitory activity of hydrolysates was 0.74 mg/mL. The optimal operating time was 720 min. The GDFS method can serve as an alternative method for obtaining highly efficient bioactive peptides [49].

German researchers developed a stable process for obtaining specific hydrolysates with selected biological properties. They developed and tested a continuous reactor system with a ceramic capillary module with various combinations of enzymes and protein substrates (Fig. 4) [49]. Alcalase was immobilized on the surface of capillaries modified with aminosilane with a pore size of 1.5 μm. The loading capacity was 0.3 μg of enzyme per 1 mg of capillary with a residual enzyme activity of 43%. They tested controlled hydrolysis of casein, sunflower, and lupine isolates. Casein hydrolysates proved to possess the largest amount of peptides with enhanced biological properties [50].

A continuous reactor consists of a ceramic capillary with one enzymatic filler. The filler is made of yttrium-stabilized zirconium oxide. It is fixed in a special stainless steel casing (Fig. 4). In a way, this system is a plug flow reactor system. The protein solution is pumped through the capillary module with a peristaltic pump. The capillary module is part of the column oven, which makes it possible to keep the temperature at 37°C. The end of the capillary is sealed with cyanoacrylate cement to inject the flow from the intracapillary space into the extracapillary space. The enzyme is immobilized on the activated surface of the ceramic capillary with an APTES linker. The protein moves through ceramic capillaries by forced convective flow. The immobilization makes it possible to use the entire available capillary surface. As a result, enzymes can be immobilized on the inner and outer surfaces, as well as on the pore walls. One capillary is 10 cm long and has an outer diameter of 1.8 mm, an inner diameter of 1 mm, and an average pore size of 1.5 μm. The ceramic capillary was replaced with a new immobilized enzyme to prevent protein contamination. The residence time of the substrate appeared to be inversely proportional to the flow rate: the longer the residence time of the substrate in the capillary filled with the enzyme, the higher the continuous yield. These continuous reactors produced specific peptides with the desired biologically active properties [50].

New combined hypoallergenic functional products need new methods of gluten reduction. For example, MBRs can be used for wheat processing to create dairy products fortified with vegetable protein, but with hypoallergenic proteins and a low content of lactose and gluten.

Merz *et al*. developed a 96-h continuous hydrolysis of wheat gluten with flavurzim in an EMBR [51].

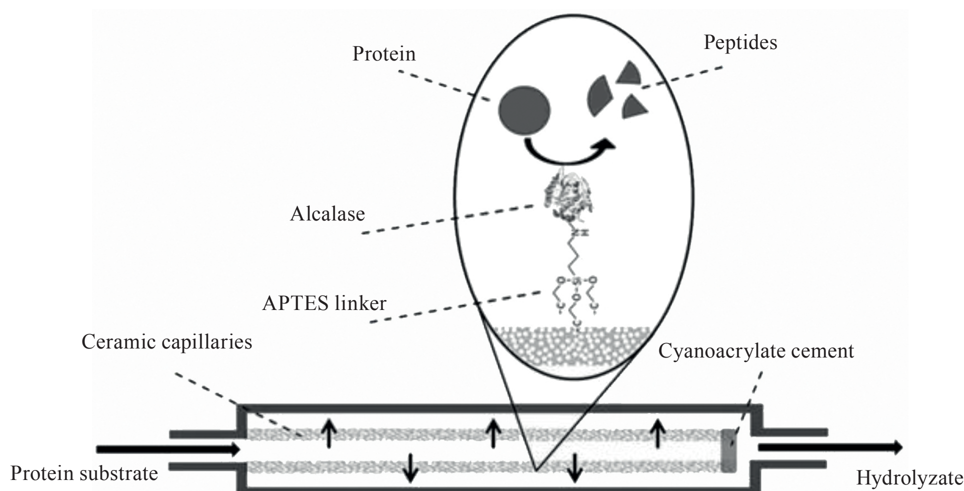
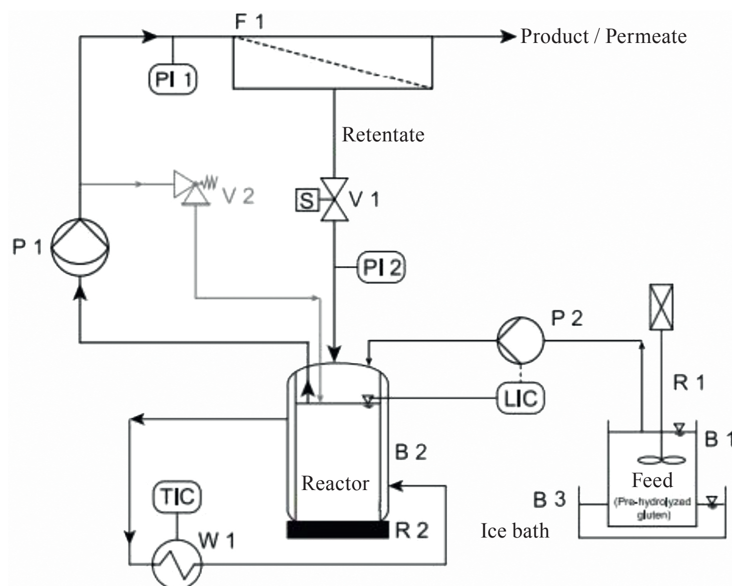


Figure 4 Capillary module that immobilizes enzymes on a ceramic substrate APTES



* – the gray line indicates a membrane restart, which is activated if the pressure exceeds 6 bar

Figure 5 Enzymatic membrane reactor with two stirred reactors (B 1, B 2), a water bath (W 1) with a thermostat (TIC), a membrane pump (P 1), a feed pump (P 2), a transverse filtration unit flow (F 1), two barometers (PI 1, PI 2), level indicator (LIC), and valves (V 1, V 2)

Temperature, pump load, and enzyme flow through the membrane were the main criteria for hydrolysis stability and direction. The scientists optimized the hydrolysis to maximize the space-time yield. For microbial stability, they included 8% ethanol with a substrate concentration of 100 g/L at 37°C and pH 7.5 for 96 h (Fig. 5) [51].

A diaphragm pump (P 1) circulated one liter of substrate. The flow rate was 3.3 L/min. Hollow fiber ceramic membranes were 45 mm in length, 6 mm in diameter, and 0.0085 m^2 in surface area. They performed cross-flow ultrafiltration of hydrolysates (F 1) on a membrane with a pore size of 1, 5, or 10 kDa. The hydrolyzate inside the reactor was stirred using a magnetic stirrer (R 2). A constant transmembrane pressure of 2 Bar was adjusted with a ball valve (V 1) and measured with barometers (PI 1, PI 2). The substrate was fed continuously using a tubular pump (P 2). The feed container was kept in an ice bath during the entire test [51]. This EMBR hydrolysis scheme can be cost-effective in the industrial production of hydrolysates from grain proteins.

Russian specialists also developed a CSTMR that produced a hydrolyzate of whey proteins with low residual antigenicity. The installation was based on enzyme preparation alcalase 2.4 L (Fig. 6) [52]. Hydrolysis products were accumulated in an enzymatic medium, which was followed by membrane separation into a purified hydrolyzate (permeate) and an insoluble residue (retentate). The experiment aimed at complete separation of the enzyme to keep it active inside the reactor core.

The scientists reproduced the process described in foreign publications, i.e. protein hydrolysis, combined with the separation of hydrolysis products on ultrafiltration membranes. The resulting hydrolyzate had a low solids content (1.5%). The technology proved commercially unprofitable and expensive. The low solids content resulted from the low cut-off of membranes (5 and 10 kDa). In this case, a portion of hydrolysis products was retained by the elective membranes and remained in the concentrate. Another disadvantage of membranes with a low molecular weight cut-off (≤ 10 kDa) was the low filtration rate and high transmembrane pressure. The latter triggered the formation of a polarization layer and, eventually, membrane clogging [52].

The molecular weight of the enzyme used for protein biocatalysis is the most important parameter for determining the cut-off threshold of membranes. Alcalase, which we used for hydrolysis of whey proteins in our research, has a molecular weight of 24–27 kDa. Membranes with a cut-off threshold of 20 kDa could easily separate an enzyme with such a molecular weight [22]. Such membranes could significantly reduce the transmembrane pressure, thus minimizing the formation of a polarization layer and subsequent membrane clogging.

Separate hydrolysis and filtration made it possible to provide optimal conditions for each of the processes (Fig. 6).

The hydrolysis was carried out under the previously established conditions: substrate concentration – 4.5%; enzyme concentration – 0.5%, hydrolysis temperature –

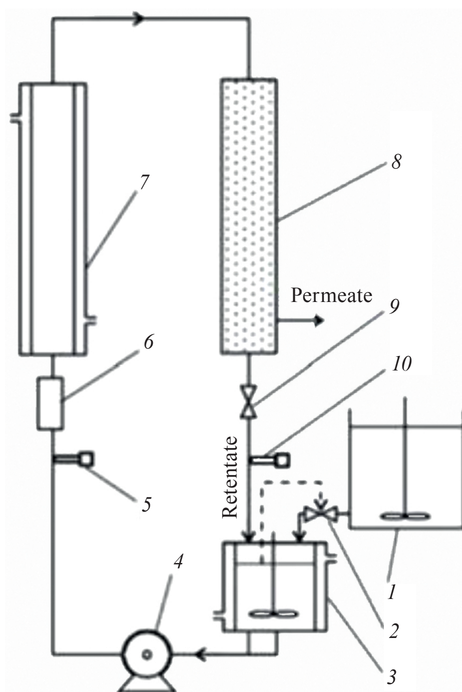


Figure 6 Enzymatic membrane reactor: *1* – container with a substrate for hydrolysis; *2* – tap with an overflow valve, *3* – fermentation reactor, *4* – recirculation pump; *5*, *10* – manometers; *6* – flow meter; *7* – heat exchanger; *8* – membrane module; *9* – retentate dump valve

65°C; hydrolysis time – 1 h. The proteolysis did not include pH-statisation. The initial active acidity of the reaction mix was 10. As for the molecular weight distribution of hydrolysis products, the residues of unhydrolyzed protein were retained during fractionation, which decreased the hydrolyzate yield. However, a double filtration made it possible to increase the yield of the finished product by an average of 6%.

The whey protein hydrolyzate had the following parameters: degree of hydrolysis – 18–25%; mass fraction of ash – 6.5–6.9%; osmolality of a 10% solution – 280–300 mmol/L of water; residual antigenicity – $\leq 2\times10^{-5}$ of the protein mass. The resulting hydrolyzate in the form of a 10% aqueous solution had a clear, moderately bitter taste, without off-flavors. Its antigenic properties make it possible to use it in therapeutic and prophylactic functional foods based on enzymatic protein hydrolysates [30].

CONCLUSION

In addition to batch enzymatic reactors, bioactive peptides are obtained by a semi-continuous reaction or a continuous reaction in an enzymatic membrane reactor (EMBR) [31, 39, 40, 42–45].

Considering the enzymatic efficiency and cost of enzymatic hydrolysis, continuous reaction has obvious advantages. Hydrolysates can promptly be separated from the substrate, the yield of biological peptides can be significantly increased, and enzymes can be used more than once. In addition, the production process is quite simple, which reduces labor costs [47, 48]. As a result, this method is popular in food industry.

Membrane reactors can process a variety of protein food media of plant and animal origin. They have good prospects for whey processing in functional food production. Bioreactors can also be used for the proteolysis of whey proteins with maximal antigenic, antihypertensive, and antidiabetic properties.

Protein hydrolysis in continuous EMBRs is attracting scientific attention because it can simplify the technological process and reduce the cost of the final product while increasing the yield, despite high operating costs. Therefore, the need to improve and develop these technologies is obvious.

CONTRIBUTION

K.A. Ryazantseva supervised the project. E.Yu. Agarkova and O.B. Fedotova conducted the theoretical research, processed the data, and prepared the manuscript.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this article.

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ORCID IDs

Ksenia A. Ryazantseva https://orcid.org/0000-0003-3207-2837

Evgeniya Yu. Agarkova https://orcid.org/0000-0001-8967-7074

Olga B. Fedotova https://orcid.org/0000-0002-7348-6019