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Conversion of Whey into Value-Added Products through Fermentation and Membrane Fractionation

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Abstract: The cheese whey (95% composed of water) is an effluent produced in the cheese industry, of which more than 1.5 million tons are generated in Spain, constituting a serious environmental problem. The process starts with a new fermentative/enzymatic technology that totally converts whey, mainly composed by lactose, proteins, and salts, into a fermented product with higher added value. This new product is mainly composed by lactic acid bacteria biomass, ammonium lactate, and a protein hydrolysate. To separate valuable fractions, this fermented product is processed by a two-stage membrane system, which is a very innovative process in this type of fermented product. The first stage consists of ultrafiltration to separate all suspended solids. As a result of this stage, a product mainly constituted by lactic acid bacteria that have both agronomic applications, mainly as a biocontrol and biofertilizer/bio-stimulant, and applications in animal feeding as a probiotic, is obtained. The second stage consists of reverse osmosis used to concentrate the ultrafiltered permeate obtained earlier, leading to a microbiologically stable product and reducing transport costs. The concentrate is mainly composed of ammonium lactate and a protein hydrolysate, constituted by peptides and free amino acids, which has application both in agriculture as a bio-stimulant and in animal feeding, and the permeate is water, reusable in other industrial processes. This work demonstrates the technical feasibility of this valorization process to achieve the objective of "Waste 0" from a problematic by-product, while obtaining products with commercial utility.

Keywords: fermentation; membrane fractionation; ultrafiltration; reverse osmosis

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1. Introduction

The cheese whey originates after the coagulation of milk in the manufacturing process of cheese, after the separation of casein and fat [1]. Whey represents 80% to 90% of the total volume of the raw milk in cheese production, generating around 9 L of whey for each kg of cheese produced. It is basically constituted by water (93–95%), organic matter (around 50% of the nutrients in the original milk, mainly lactose and proteins), and mineral salts [2].

However, this by-product is underused, since it is only reused in 50% of cases [3,4], and only the large cheese factories implement reuse and minimization processes of this by-product, while small and medium-sized cheese makers do not have adequate systems for treating this type of effluent due to the high treatment costs. Currently, the main uses of whey are for animal feed in liquid form and without practically any type of processing, or transformed by concentration and/or separation of its main components (lactose and protein) for use as excipients in the food industry, pharmaceuticals, sports nutrition, etc. The other 50% of whey must be eliminated using conventional purification techniques [3,4].

This 50% of non-reused whey is the cause of serious environmental problems, raising the cheese industry to the top of the most polluting industries in the agri-food sector [5]. This is due, on one hand, to the large volumes of whey produced (around 180 million tons generated worldwide), and, on the other hand, to its own characteristics, since for every 1000 L of whey, approximately 27–60 kg of biochemical oxygen demand (BOD) and 50–102 kg of chemical oxygen demand (COD) are generated [6]. This causes serious cases of eutrophication in aquifer courses (rivers, lakes, etc.), overload problems in wastewater treatment plants, etc.

Filtration processes are widely used in the dairy industry, being involved directly or indirectly in all common processes. This technology is mainly used for improving the value of dairy products and to reuse water, as well as in the manufacture of fermented products and in the recovery of whey protein. In some studies, membrane technology, such as micro- and ultra-filtration, have been used to concentrate milk or raw whey [7,8]. Chen et al. [9] carried out an in-depth study of different types of nanofiltration membranes after a pre-treatment based on isoelectric precipitation and ultrafiltration, obtaining promising results in the concentration and fractionation of dairy wastewater. Nevertheless, the composition of the whey has not been biologically nor chemically modified prior to membrane concentration processes in any case.

The objective of this study is the design and development of a sustainable technology for the valorization of whey and the obtaining of several products for environmental/nutritional applications, with the aim of transferring this technology as an alternative with low installation costs and minimum operating costs, feasible for small and medium cheese producers, being linearly scalable at all levels.

2. Materials and Methods

2.1. Chemicals and Microorganisms

Whey was obtained from Quesos Los Vázquez SL, Castilleja del Campo (Seville, Spain), a medium-sized cheesemaker. Bioprotease L-450, obtained from *Bacillus licheniformis*, was supplied by Biocon (Barcelona, Spain). MRS broth was prepared according to de Man, Rogosa, and Sharpe's (MRS) indications [10]. Other chemicals and reagents employed in the study were of an analytical grade and used with no further purification. The microorganism used to carry out whey fermentations was *Lactobacillus rhamnosus*, that was previously isolated from the whey microbial consortium, identified by 16S rDNA gene sequencing and stored at −80 °C.

2.2. Analytical Techniques

Dry matter was determined after drying samples at 105 °C until constant weight. The soluble and insoluble dry matter was determined after centrifuging the samples for 30 min at 12,000× *g* and 4 °C, then separating the soluble and insoluble fractions and drying at 105 °C.

2.3. Whey Fermentations

In order to obtain a starter culture for the fermentations, a previously sterilized flask with 100 mL of MRS medium was inoculated with the *Lactobacillus rhamnosus* strain previously isolated and identified by the research group AGR-212 (Universidad de Sevilla). Whey fermentations were carried out in stages to reach a final volume of 1000 L. Sequential volumes were 2, 50, and 1000 L, using the previous volume to inoculate the next fermentation. Fermentations were performed as described by Caballero et al. [11] in 3 pilot scale bioreactors under previously optimized controlled conditions of pH (pH 5.5, using ammonia as base), temperature (40 °C), and stirring (300 rpm). Prior to fermentation, whey was pasteurized to ensure a microbiologically pure final product, and the initial fermentation was inoculated with 2% *v*/*v* exponential-phase culture of *L. rhamnosus* grown in MRS medium. To increase whey protein bioavailability, 0.1% *v*/*v* of protease (Bioproteasa L-450) was added to fermentations as an inductor.

2.4. Filtration Test

The fermented whey was processed out sequentially with two tangential filtration systems:

(1) Cross-flow ultrafiltration (UF) with a 0.1 μm pore size membrane, model M-7P1940 manufactured by Pall Corporation; made in ceramic material, the pilot system was configured with 7 membranes with a total filtration surface of 1.68 m^2. Through this technique, the lactic bacteria will be retained in the concentrate. The use of ceramic UF membranes allowed for separating biomass from fermented whey, maintaining steady stable process parameters, reducing the fouling effect in comparison with conventional polymeric UF membranes. Feed pressure and concentrate pressure increased through the test duration, varying from 4 to 4.5 bar and from 2 to 2.5 bar, respectively. These values are determined by the characteristics of the fermented whey to be filtered, mainly by the concentration of solid particles contained in it. On the contrary, permeate pressure decreased from 2.25 to 1.2 bar. Evaluating the pressure values as a whole makes it possible to obtain a calculation of the transmembrane pressure, which increased from 0.75 to 2.30 bar, as a consequence of the increase of concentration factor. The increase of pressure is directly linked to the increase of fluid temperature, ranging from 20 to 34 °C. The permeate flow rate also experienced a decrease over the test time, from 240 to 110 L/h, which means that the flux (flow rate/membrane surface) decreased accordingly. The UF concentrate was reserved for subsequent binding to the concentrate resulting from reverse osmosis (Figure 1).

(2) Reverse osmosis (RO) of the filtrate resulting from the UF, using RO membranes manufactured by Filmtec. Model RO-3840/30FF with a 200 Da pore size, made in propylene. The pilot system was configured with 2 membranes connected in series, with a total filtration surface of 15.6 m^2 (Figure 2). Reverse osmosis membranes allow for recovering water from the permeate of the ultrafiltrate and obtaining a high-value concentrate. With respect to the operational parameters, they have the same behavior as in the ultrafiltration stage. Feed pressure and concentrate pressure increased throughout the test duration, varying from 18 to 30 bar and from 16.5 to 28.5 bar, respectively. These values are determined by the characteristics of the fermented whey to be filtered, mainly by the concentration of dissolved solids contained in it. Evaluating the pressure values and considering that in this case, permeate discharge pressure is considered as 0 bar, makes it possible to obtain a calculation of the transmembrane pressure, which increased from 17.25 to 29.25 bar, as a consequence of the increase of concentration factor. The permeate flow rate also experienced a decrease over the test time, from 22 to 12 L/h, which means that the flux (flow rate/membrane surface) decreased too.

The filtration tests have been carried out according to this protocol:

(1) 707 kg of fermented whey was processed.
(2) Before starting the filtration process, fermented whey material was stirred, due to the presence of small precipitates at the base of the feeding tank.

Both UF and RO steps were configured as full recirculation processes to achieve the maximum volumetric concentration factor (VCF). This factor is calculated as a relation between whey concentrated volume and initial whey volume.

Pressure was monitored using an electronic pressure transmitter. Flow was measured and monitored by means of an electromagnetic flow meter. Whey volume was determined by graduated containers, where the different fractions of processed whey were collected and stored.

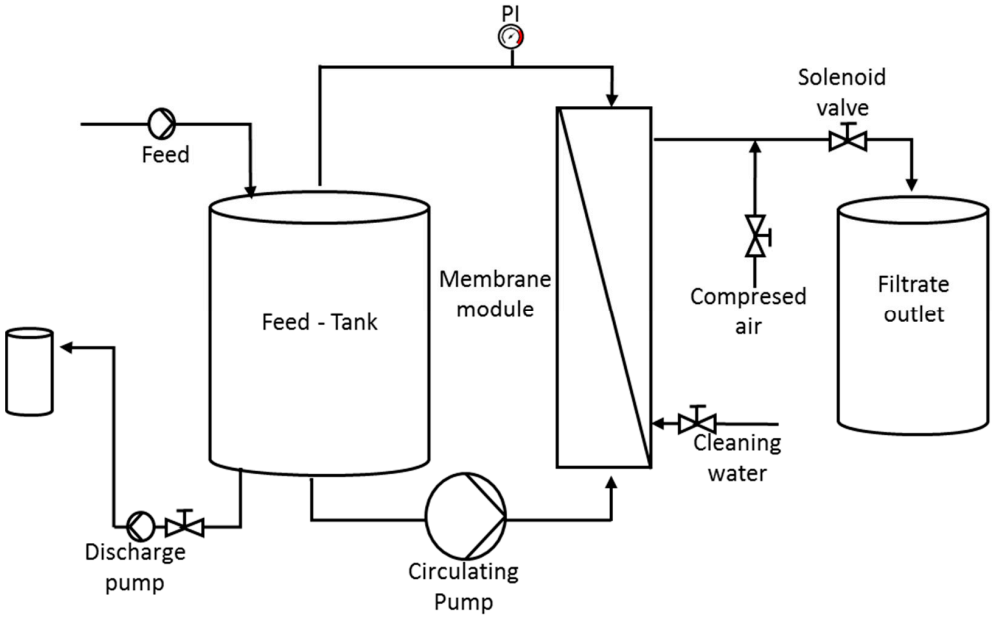

Figure 1. UF pilot plant scheme (Source: Atech Innovations gmbh).

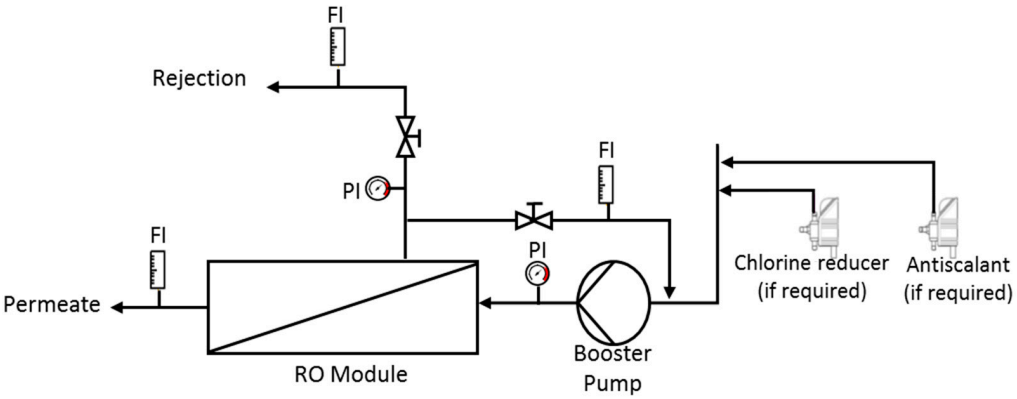

Figure 2. RO pilot plant scheme (Source: Bioazul S.L.).

2.5. Dry Matter Determination

The total, soluble, and insoluble dry matter content of filtration products were determined as follows. For the determination of the total dry matter, the samples were dried in an oven at 105 °C for 24 h, and the dry weight was referred to the fresh weight. For the determination of soluble dry matter, the samples were centrifuged (12,000× *g*, 30 min, 4 °C), the pellet was discarded, and the supernatant was dried in an oven at 105 °C for 24 h, and the dry weight was referred to the fresh weight of said supernatant. Insoluble dry matter was determined as the difference between total and soluble dry matter.

3. Results and Discussion

As described by Caballero et al. [11], the whey transformation process consists of a fermentation, with a bacterial inoculum, to obtain a product rich in lactic acid bacteria (biofertilizer/bio-stimulant and animal probiotic) and bio-stimulant compounds, such as ammonium lactate and a highly assimilable protein hydrolysate (whey and fermented whey compositions in Table 1).

During fermentation, *Lactobacillus rhamnosus* metabolizes whey lactose, giving rise to lactic acid. This fermentative metabolism is limited by the acidification of the medium [12]. To avoid this acidification, the pH of the medium is kept constant by adding ammonia,

which complexes with lactic acid, giving rise to ammonium lactate. *L. rhamnosus* is a probiotic bacterium that can exert beneficial effects as a supplement for animal feeding, providing both immune protection and therapeutic benefits to infected/inflamed animals [13].

Table 1. Composition of both initial whey and fermented whey. Data are the means of three samples [11].

	Whey (g/L)	Fermented Whey (g/L)
Bacteria	0.07 ± 0.01	3.2 ± 0.7
Nitrogen	10 ± 2 (Proteins)	9.16 ± 1.8 (Hydrolyzed proteins)
Lactic acid	5.2 ± 0.6	42 ± 2.7
Minerals	2.4 ± 0.2	2.4 ± 0.2
Lactose	50 ± 3.9	1.7 ± 0.2

In order to improve the fermentation process and achieve almost total conversions of lactose into lactic acid, a proteolytic process has been coupled to fermentation, as described by Caballero et al. [11]. Thus, a 0.1% v/v protease has been added during fermentation, leading to the breakdown of whey proteins into peptides and free amino acids, which are more easily assimilated by *L. rhamnosus*, favoring the fermentative process.

However, although resulted fermented whey presents lower contaminating parameters than the initial whey, its discharge parameters (BOD and COD) continue above the limits, therefore not allowing its discharge into the environment.

As a novelty, in this work, the use of membrane technology has been proposed, firstly for the separation of the useful components present in the fermented whey, and secondly for the reduction of the total volume through the removal of water from the whey. Other advantages related to the process are the reduction of transport costs and the microbiological stabilization of obtained products, all of which lead to a substantial improvement in the livestock industry. Thus, fermented whey was submitted to a two-stage filtration process consisting of ultrafiltration and reverse osmosis (Figure 3).

The membrane process allows to separate the insoluble matter of fermented whey in the form of a pasty-liquid fraction that is mainly composed by the biomass of *L. rhamnosus* that can be used directly both for agronomic purposes as a biofertilizer/bio-stimulant and in animal feeding, as it is rich in proteins and in active probiotic biomass.

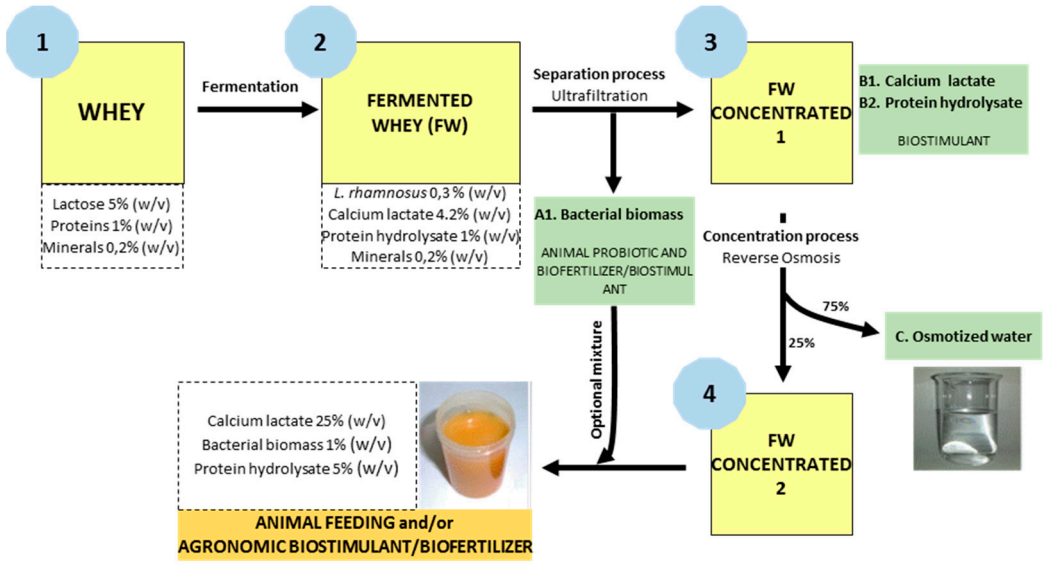

Figure 3. Scheme of the whey treatment process. Detailed steps are shown as well as each valuable fraction obtained.

Next, soluble matter of fermented whey has been concentrated approximately 3 times, until reaching a value close to 11% w/v on a dry matter basis (DMB) (Figure 3). This fraction has the appearance of a slightly dense amber-colored liquid and is rich in ammonium lactate, a protein hydrolysate, and mineral salts, presenting bio-stimulant capacity [11]. Although compared to the non-concentered product it already presents a considerable improvement, to optimize its transport and livestock application, it would be necessary to increase its concentration to values up to nearly 20–25% DMB.

While both fractions can be used separately, they can also be mixed, obtaining a product with a higher dry matter content as well as nutritionally and functionally more complete, mainly for agronomic purposes.

Pilot Filtration Test

The pilot test developed provides information in two different areas:

(1) Behavior of the filtration steps processing fermented whey.
(2) Quantitative analysis of the different fractions obtained from the fermented whey.

This information is valuable in order to apply improvements that optimize the process of obtaining valuable products from the fermentation process explained above. In order to obtain data that visually provide information on the performance of the concentration and separation processes, the data are represented in various graphs that are shown and discussed below.

It can be seen how the retentate of the reverse osmosis stage presents a certain content of insoluble matter (1.21% w/v, Figure 4), despite the fact that the starting material of this stage (ultrafiltration permeate) did not contain any insoluble matter (Figure 4). This can be explained by the fact that while concentering, the salt content (mainly ammonium lactate) precipitates when exceeding solubility limits. This precipitate can be recovered by ultrafiltration or remain in the retentate fraction, which can lead to problems related to membrane clogging associated with the presence of suspended particles.

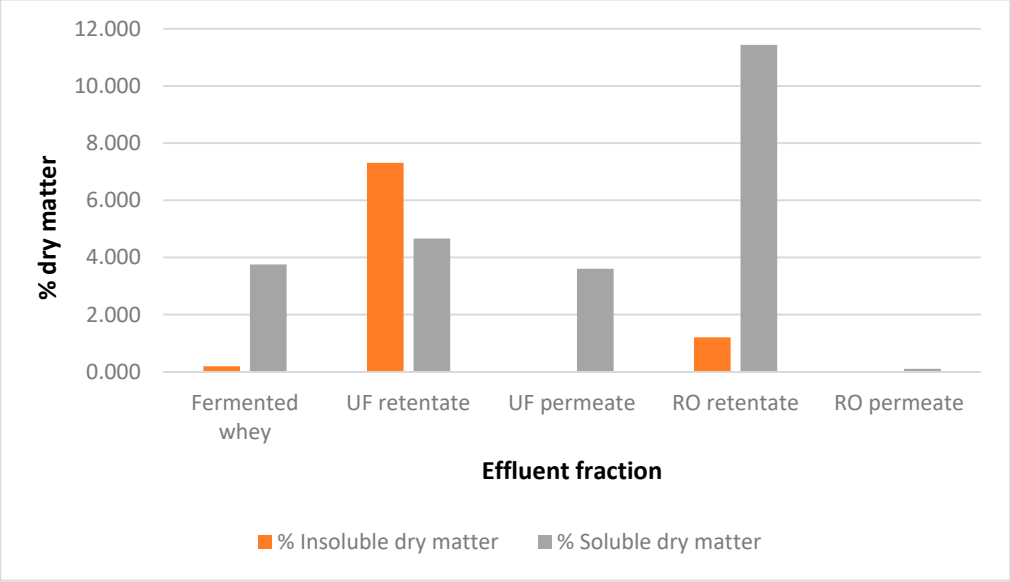

Figure 4. Percentages of dry matter (insoluble and soluble) in different fractions of the tested whey.

Due to technical limitations, the filtration process has been carried out with a total recirculation of the rejection in each stage. Due to this, the conversion rates are relatively high, with a detriment of membrane flux during the duration of the tests.

Volumetric concentration factor (VCF) and flux have been evaluated in both processes, ultrafiltration and reverse osmosis (Figures 5 and 6). As previously indicated, due to the configuration of the processes during the tests developed, there is a clear decreasing trend

in flux, resulting in a 54% reduction in UF and a 94% reduction in RO. In the test with UF membranes, a stabilization of this parameter has been achieved, being able to maintain a sustained production independently of VCD values' increase, however in the RO system, there is a drastic reduction as the concentration factor increases, leading to a practically complete loss of flow per membrane surface unit.

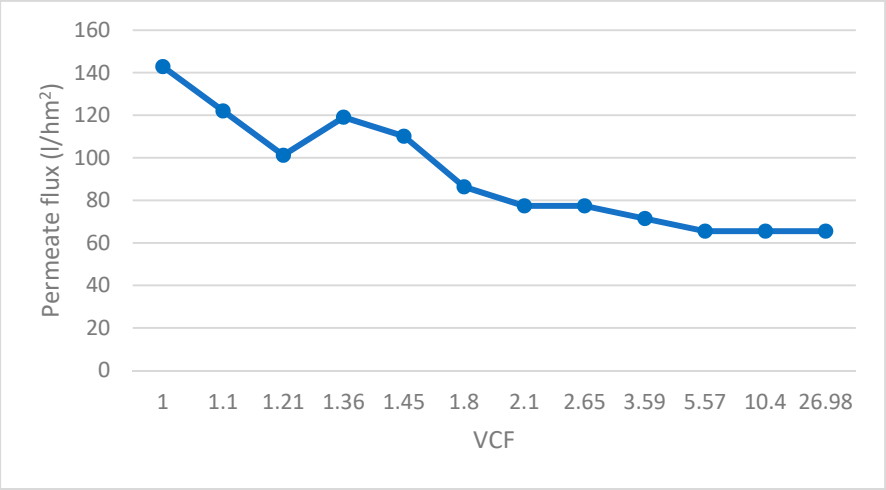

Figure 5. Permeate flux vs. VCF over the duration of the ultrafiltration pilot test.

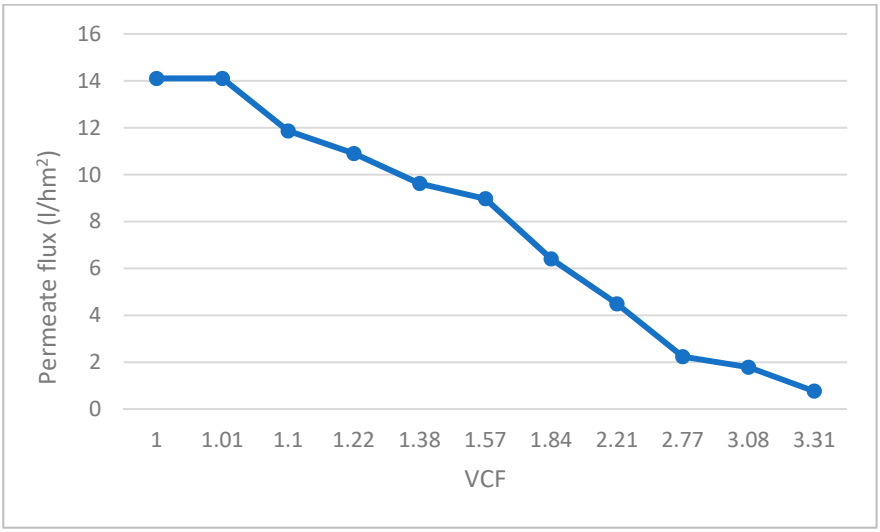

Figure 6. Permeate flux vs. VCF over the duration of the reverse osmosis filtration pilot test.

Similar information is shown in Figures 7 and 8, where transmembrane pressure related to VCF variations are shown from both the ultrafiltration and reverse osmosis filtration pilot tests. Likewise, there is a direct and evident relationship between both parameters and in both processes, increasing the transmembrane pressure throughout the test with an increase of the VCF value.

The tests carried out have provided results that allow the development of parametric adjustments in the operating conditions of the filtration systems. They have also made it possible to redesign the filtration stages to obtain greater specificity in revalued products.

Tests with new configurations will be performed to provide continuity to the previous tests that formed the basis of these developments. Therefore, for a real and practical application of this technology, the system must perform steadily to prevent membrane silting and unnecessary cleaning stops, which is a main objective of the tests and operational

commissioning of the filtration units. It must be considered that it is not only a development with technical feasibility, but that it must offer economic viability that provides this technology with functional capacity in the industry.

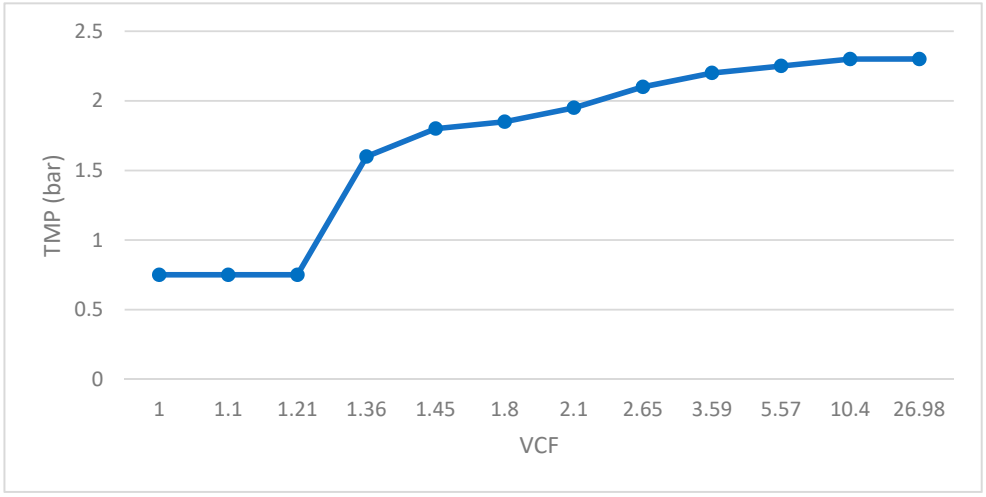

Figure 7. Changes in TMP related to variations of VCF during ultrafiltration test development.

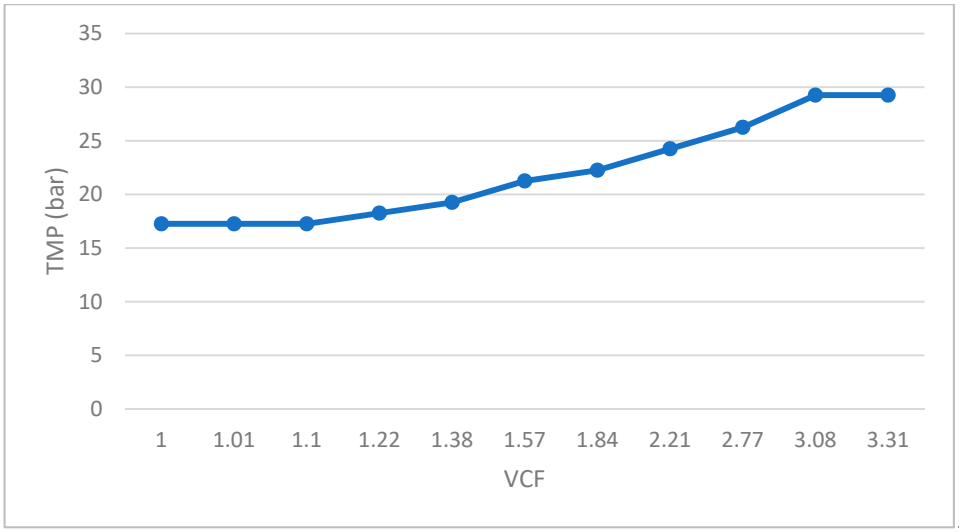

Figure 8. Changes in TMP related to variations of VCF over the duration of the reverse osmosis filtration pilot test.

4. Conclusions

Filtration technology has been coupled with the fermentative process of converting whey into animal probiotics and/or agronomic bio-stimulants/biofertilizers. The process concentrates the whey, improving its stability, livestock application, and transport.

The pilot tests performed provide valuable information for further study:

(1) The filtration tests carried out must be considered as a proof of concept. A full recirculation process has led to a severe reduction of the flux and a fast increase in the TMP, clear indicators of membrane silting, both in UF and RO steps. Industrial processes to perform a filtration step in continuous mode should be re-designed to reject a convenient part of the brine.

(2) The rejection of the ultrafiltration stage is mainly constituted by bacterial biomass (*Lactobacillus rhamnosus*). On the other hand, the concentrate obtained in the reverse osmosis stage is rich in ammonium lactate as well as peptides and free amino acids. Both products are excellent food additives for the livestock industry as probiotics in

animal feeding and as bio-stimulants/biofertilizers for agronomic purposes. They can be applied separately or mixed into a single, more complete product.

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