SERICIN PROTEIN RECOVERY FROM SILK DEGUMMING WASTEWATER IN PILOT SCALE VIA MEMBRANE HYBRID PROCESSES

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ABSTRACT

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The conventional degumming method that consists of alkaline washing of silk yarn with Marseille soap at high temperatures, dissolves sericin protein into the water and produces so-called silk degumming wastewater (SDW) which is rich in sericin protein. In this study, the separation of sericin protein from the SDW was studied. Firstly, the separation of sericin containing phase from the SDW was optimized. SDW samples were prepared with the application of different soap to silk yarn ratios (FO) and tested for phase separation performance. It was determined that the best phase separation occurs when the ratio was 0.40. Pilot-scale membrane filtration tests were applied for further separation of sericin with the use of Ultrafiltration (UF) and Nanofiltration (NF) membranes at 2 and 4 bar, respectively. UF and NF membranes were also tested in a sequential mode to increase sericin recovery. NF membrane rejection was around 90-100% while UF membrane had only 10-30% sericin rejection. NF membrane feed tank sericin concentration reached 37000 mg/L whereas UF membrane feed tank concentration was around 6000 mg/L.When further purification of sericin from the retantate of the NF was tested by the application of ethanol-induced precipitation and lyophilization, powder sericin was obtained.

Keywords: Silk Degumming Wastewater, Resource Recovery, Sericin Protein, Ultrafiltration,Nanofiltration

ÖZ

MEMBRAN HİBRİT PROSESLER İLE PİLOT ÖLÇEKTE İPEK YUMUŞATMA ATIK SUYUNDAN SERİSİN PROTEİNİ GERİ KAZANIMI

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İpek ipliğinin, yüksek sıcaklıkta, Marsilya sabunu ile yıkandığı konvansiyonel ipek yumuşatma yönteminde, serisin proteini suda çözünür ve serisin proteini açısından zengin olan ipek yumuşatma atıksuyu (İYA) oluşur. Bu çalışmada, serisin proteininin İYA'dan ayrılması incelenmiştir. İlk olarak, serisin içeren fazın İYA'dan ayrılması optimize edilmiştir. Sabunun ipliğine olan oranı (FO) farklı olacak şekilde İYA numuneleri hazırlanmış ve faz ayırma performansı açısından test edilmiştir. Oran 0,40 olduğunda en iyi faz ayrımının gerçekleştiği belirlenmiştir. Serisinin geri kazanımının sağlanması için pilot ölçekli membran filtrasyon testleri, UF ve NF membranlarına sırasıyla 2 ve 4 bar basınçta uygulanmıştır. Serisin geri kazanımını artırmak için UF ve NF membranları ardışık düzende test edilmiştir. NF membranında %90-100 oranında serisin tutulurken, UF membran için sericin proteini tutma oranı %10-30 değerindedir. NF membran besleme tankı konsantrasyonu 37000 mg/L değerine kadar çıkarken, UF membran besleme tankı konsantrasyonu 6000 mg/L olarak tespit edilmiştir. NF membranda konsantre hale gelen serisin proteinine, etanol ile çökeltme ve liyofilizasyon uygulandığında, toz serisin elde edilmiştir.

Anahtar Kelimeler: İpek Yumuşatma Atıksuyu, Kaynak Geri Kazanımı, Serisin Proteini, Ultrafiltrasyon, Nanofiltrasyon

Dedicated to my family

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ABBREVIATIONS

CMF	: Concentration Mode of Filtration
COD	: Chemical Oxygen Demand
CCW	: Cocoon Cooking Wastewater
Da	: Dalton
EPA	: Environmental Protection Agency
FO	: Soap to Silk Yarn Ratio
HPLC	: High Performance Liquid Chromatography
J_{cw}	: Clean Water Flux
J_{ww}	: Wastewater Flux
MF	: Microfiltration
MW	: Molecular Weight
MWCO	: Molecular Weight Cut-Off
UF	: Ultrafiltration
NF	: Nanofiltration
rpm	: Revolution Per Minute
SDS-PAGE	: Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis

SDW	: Silk Degumming Wastewater
SEM	: Scanning Electron Microscopy
TFC	: Thin Film Composite
TRMF	: Total Recycle Mode of Filtration

CHAPTER 1

INTRODUCTION

The textile industry has an important place in the whole world and Turkey. There are many sub-sectors of textile for natural (wool, cotton, silk) and synthetic yarns and fabrics with different methods for business purposes. This industrial activity has a high water demand and discharges a large amount of wastewater. Main environmental problems caused by the textile industry are generally resulting from the complex characteristics and high volumes of wastewaters. The textile wastewaters have a broad range of organic chemicals with low biodegradability, color, and salinity. The textile industry wastewater discharges may have 115– 175 kg of COD/ton of finished textile (Nistor et al., 2008).

Silk production is one of the sub-sectors in the textile industry. Sericulture is a process to cultivate silkworms to produce silk. There are four types of silkworm used in sericulture. The most cultivated one is Bombyx Mori L. This silkworm feed on Mulberry leaves for silk production. The silkworm spins a protective cocoon around itself so that it can safely transform into a chrysalis, and it generates two types of protein. One of these proteins is fibroin, i.e., fiber itself, and the other one is sericin, which works as a piece of gum to bind fibroin filaments to each other. Most of the commercial varieties of silkworm cocoons are composed of approximately 75% silk fibroin and 25% sericin, which is a globular glue protein (Cao & Zhang, 2016). Elimination of sericin protein from the silk yarn enables it a clearer and shiny look, which makes it colorable and soft (Vyas ve Shukla, 2015). Thus, for industrial purposes, sericin is degummed from the silk yarn.

In silk production steps, two types of wastewaters are generated. These are cocoon coking wastewater (CCW) and silk degumming wastewater (SDW). While CCW is produced during taking fibers out of the cocoon with boiling, SDW is generated at the stage of final degumming of the silk yarn with marseille soap under at 100˚C and at pH 10-12. Both wastewaters include high amounts of sericin because of the solubility of sericin protein in water. Thus, SDW includes both organic waste content coming from dissolved sericin protein and inorganic waste content results from the salt formed by HCl and NaOH addition at acidification and neutralization stage.

Just like other sectors, in the textile industry, in order to reduce environmental pollution or keep it under control, the traditional method of end-of-pipe treatment is used by taking wastewater having different characteristics and treating it with different methods before discharge into the receiving environment. However, with the increasing population and decreasing natural resources, it is not possible to continue with the traditional end-of-pipe treatment approach. A new and more sustainable approach is necessary to change the linear economy model to a circular one. As evidenced from the emerging terminologies like ‘resource recovery’, ‘sustainable development’ and ‘integrated water management’, a sustainable way of production has to be achieved. Therefore, instead of treatment and discharge processes, adopting technologies that will minimize waste generation and recycle valuable raw materials in industrial production processes is required for protecting the environment. To this end, recovery and reuse of the resources in waste streams represent an economic and ecological challenge for the overall sector.

Silk production wastewater can be considered as a source for resource recovery since it includes a high amount of sericin, which is a useful protein for many industries such as cosmetics, pharmaceutical and textile industries, as well as a potential biomaterial (Cho et al., 2003; Zhang, 2002). In 2006, 100 tons of silk yarn were processed. Since the sericin content is around 25% by weight of the silk

yarn, it can be argued that 25 tons of sericin was discarded as a waste in a year (Capar et al., 2008). Instead of wasting this material, it can be recovered from silk production wastewaters.

In order to remove sericin protein from SDW, degumming process is necessary. For degumming, there are several methods such as boling, alkaline washing of silk yarn by using soap, using organic solvents (tartaric acid, citric acid), high-temperature high pressure (HTHP) and exposing enzymatic reactions (Gupta, Agrawal, Chaudhary, Gulrajani, & Gupta, 2013). All methods have some advantages and limitations. In this study, wastewater (SDW) from alkaline degumming process, which is a simple and most widely applied method (Gupta et al., 2013), has been used for sericin recovery.

Sericin recovery from silk effluents can be done by separating the protein via ion-exchange chromatography, gel filtration, electrophoresis, ultrafiltration (UF) and foam fractionation (Li et al., 2018). Membrane technology is one of the protein recovery options. Membrane filtration has both advantages and disadvantages. It has high operational cost. Membrane fouling is another critical issue in the application of membrane technology for protein purification (Kwon et al., 2008). However, recovery with membrane filtration not only enables high removal efficiencies, but also allows recovery of water and some valuable waste constituents for possible end uses (Fersi et al., 2005). Thus, membrane filtration was applied for sericin recovery in this study.

Membrane technology applications have been increasing rapidly in recent years. Just like other recovery methods, membrane technology has advantages and some limitations. Their basic disadvantage is the fouling problem. However membranes provide unique solutions for separation and recovery of substances. In this study, pressure-driven UF and nanofiltration (NF) membranes were used in order to concentrate the sericin solution separated from SDW, and the most suitable

membrane hybrid processes covering pre-treatment and recovery stages has been determined. Flux reductions were evaluated, and the restoration of fluxes was investigated by chemical washing. Finally, for recovering silk sericin, a process train was developed, including pretreatment, neutralization, membrane hybrid process (NF), ethanol-induced precipitation and lyophilization.

In literature, membrane filtration was applied both for CCW and SDW in laboratory scale. As an example, by using CCW, sericin was separated from other impurities via centrifugation followed by microfiltration in the pre-treatment stage. In this study, partial recovery of sericin was achieved by UF (20kDa), UF (5kDa) and UF (1kDa) membranes, where the rejection performances were as low as 37%, 52% and 60%, respectively. NF membranes provided much higher sericin recovery ratio of 94–95%, where the recovery of high MW sericin was achieved (Capar, Aygun, and Gecit 2008). In this study, sericin recovery from SDW by membrane filtration was done with UF (10kDa), UF (5kDa) and UF (1kDa) membranes, where the rejection performances were 69-78%, 77% and 68% respectively. Also NF membrane was applied after UF membrane and its rejection rate was 90% in laboratory scale studies. As given, technical feasibility studies for sericin recovery were conducted with laboratory-scale experiments. However, the pilot scale experiments bring us closer to the real situation. The pretreatment, rejection and flux of the membranes in pilot scale are closer to the conditions in the real systems and giving more realistic results. Thus, in this thesis, pilot scale experiments were conducted for sericin recovery from SDW.

1.1 Aim and Scope of the Study

This study was carried out within the framework of the TÜBİTAK 114Y461 Project entitled “Sericin Protein Recovery and Prototype Series Production with Membrane Hybrid Processes from Silk Processing Wastewater” (Capar et al. 2018).

In literature, sericin recovery has not been studied at a pilot scale. Since the pilot-scale results are more representative of the real filtration process, the aim of the study is to study the recovery of sericin protein from silk production processes using membrane hybrid processes at a pilot scale. The effect of soap to silk yarn ratio on the phase separation was also investigated in the pretreatment stage.

This study covers the experimental results of sericin recovery from SDW in pilot scale. Pilot scale membrane system was established in Bursa Kirman İplik Factory. Since the conventional degumming method (boiling of silk yarn with soap) was used, it was needed to separate sericin, dissolved in SDW and soap. Thus, pretreatment stage consisted of the acidification with HCl. At that point, pH was observed and amount of added HCl was noted. As similar, in neutralization stage, amount of added NaOH was noted and pH was controlled. In membrane filtration flux of UF and NF membranes were monitored and sericin concentrations were analysed in Ankara University Water Management Institute. Also, due to the problems with phase seperation in pilot scalee studies, reasons for failed pretreatment experiments were searched and pretreatment process was simulated with different soap to silk yarn ratio and pH. Addition of HCl, temperature and pH was recorded. Samples were analyzed for zeta potential.

In the thesis, introduction and aim of this study is given in Chapter 1. The litrature information is presented in Chapter 2. Materials and methods are given in Chapter 3. Experimental results and discussion are given in Chapter 4 and finally in Chapter 5, conclusion is given.

CHAPTER 2

LITERATURE REVIEW

2.1 Sericulture

Silk has come a long way since the Silk Road. It is still a highly prized commodity. It is a popular and valuable material in a variety of industries. The textile industry is one of them. It's a popular fabric for high-end clothing like wedding gowns and blouses. Accessories such as handbags, headbands, and scarves typically feature it. Furthermore, silk, particularly Bombyx mori silk, has a long history of use in biomedical applications. Because of its biocompatibility, slow degradability, and outstanding mechanical qualities, silk fibroin is being intensively investigated for potential biomedical uses. Silk from the silkworm B. mori has been used as a biomedical suture material for centuries. The unique mechanical properties of these fibers provide important clinical repair options (Hakimi et al., 2007). Also, silk is a fiber with remarkable mechanical properties. This unique characteristic of silk has led to its use in fiber-reinforced composites for various applications. Silk yarn is easily available as the waste product of the textile industry, so the composite is cost-effective and the perfect utilization of a waste product (Babu, 2012). Though silk has long been valued as a valuable textile material, it has recently gained popularity as a reinforcing material for epoxy and other biodegradable biopolymeric resin composites. The organization of the silk fibres can contribute significantly to impact resistance by ensuring either or both a sufficient strength of the composite and good deformability of the composite. Furthermore, silk nonwoven fabrics can be developed from silk reeling waste and hard waste generated during twisting and weaving. Only in India, over 4000 metric tons of silk waste in various forms is generated annually during the conversion of cocoons to

fabric. This waste is now utilized to make spun silk yarn, noil yarn, throw-ster yarn, and carpet yarn, as well as hand-spun yarn. This waste can be better exploited in the manufacture of silk nonwoven fabrics for a variety of applications. (Babu, 2012).

Sericulture or silk farming is to cultivate silkworms to produce silk. Major activity is to cultivate food-plant for silkworms so that they can spin silk cocoon. Sericulture is important to improve the rural economy as a secondary agricultural activity. However, it has a challenging part in itself. The addition to high cost of labor and heavy industrialization in these countries climatically changes relapse the cultivation of mulberry and mulberry leaf availability as well. For these reasons, in temperate- zone countries like Japan, South Korea, silk production is declining. On the other hand, in China and India, sericulture is a very popular agroindustry. They meet 60% of the annual silk demand (Babu, 2012).

In Turkey, Bursa city is known for the sericulture activity. However, in recent years, thanks to some economic strategy plans, not only in Bursa but also in Diyarbakır city, a high amount of silk production and sericulture activity take place. “Silk Production Facility Project” was started to be implemented where 60 tons of fresh cocoons are expected. In this project, 7 tons of yarn obtained with 60 tons of wet cocoons will turn into silk carpets, shawls, ties, and scarves (TRT, 2019).

The most frequent silkworm species used in sericulture is Bombyx mori. The silkworm spins ceaselessly, rotating its head from side to side, until it has completed the construction of a fully formed cocoon. During the cocoon spinning process, the silkworm is left undisturbed because even the least interference could cause the silkworm to perish. Silkworm larvae complete the cocoon spinning process 4-6 days after it begins, and the larvae inside pupate after another 4-6 days. It takes about 8-10 days from the time it starts to pupate until it is ready to be harvested. Preliminary sorting of cocoons takes place during the harvesting process. Healthy seed cocoons are used for reeling, while unreelable cocoons such

as fragile, perforated, pinhole, doubly deformed cocoons, sliced cocoons, and others are used to make spun yarn.

There are four ways of sericulture, and each uses different silkworm as Mulberry, Tasar, Muga, and Eri. Mulberry is the most used one in Turkey and all over the world. According to Table 2.1, it is seen that maximum sericin concentration exists in *Bombyx Mori* silk. Also, this type of silk yarn has fewer minerals, ash, and other impurities. This fact may support the case of sericin recovery from Mulberry silk effluents.

Table 2.1. Composition of Silk Fibers (%)

Component	**Mulberry**	**Tasar**	**Muga**	**Eri**
Fibroin	66-72	78-85	80-86	82-88
Sericin	25-32	14-17	12-16	11-13
Wax	0.3-0.4	1-2	.5-1	1.5-2.2
Minerals, ash and others	0.7-0.8	3-4	2-3	2-3

2.2 Structure of Silk Yarn

Silk yarn is obtained from a thick, soft and cream-colored cocoon that the silkworm (Bombyx mori) knits around itself after maturation to protect it in the pupal stage. The cocoon is knitted from a shiny and very thin silk thread, secreted by the silk glands of the developing insect. This liquid silk hardens when it comes into contact with air. Sericin, which is a second secretion and makes up about 25-30% of the silk protein, surrounds fibroin as shown in Figure 2.1, the main protein that forms the thread, with its adhesive feature and binds the threads together.

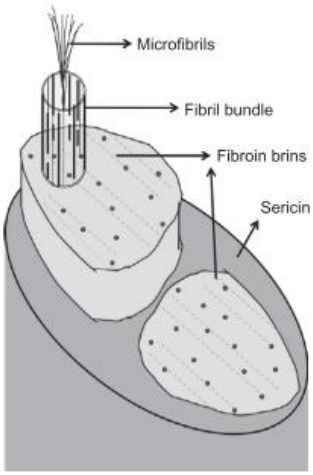

Figure 2.1. Structure of Silk Fiber (Padaki, Das, and Basu 2015)

A cocoon gives around 500-1500 m of silk yarn. It is important to obtain this yarn without breaking it in terms of winding the yarn. For this reason, in the textile industry, the silkworm is usually boiled to death before leaving the cocoon. This prevents the butterfly from piercing the cocoon and the thread splintering (Padaki, Das, and Basu 2015).

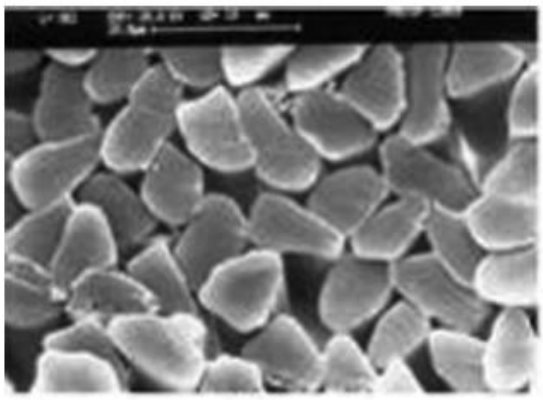

Figure 2.2. Side Section of Silk Yard (Padaki, Das, and Basu 2015)

Silk is made up of various amino acids because it is a protein fiber. Protein qualities are essentially determined by the reactive groups of their constituent amino acids, as well as the features related with the protein's size. Amino acids are bifunctional, meaning that their chemical structure contains both acidic carboxyl

(COO) and basic amino (NH_3^+) groups. Silk has good resistance to acids, but hot concentrated acids break the peptide bonds of the amino acid molecular chains, thereby damaging the fiber (Babu 2012).

Silk is a textile fiber with a high hygroscopicity. Mulberry raw silk fiber has an 11% moisture recovery, which drops to roughly 9% after degumming (at standard atmospheric conditions, 27 °C and 65% RH). This is due to removing much hygroscopic sericin from the raw silk fiber during the degumming process. The wild silk fibers display higher moisture regain values compared to the degummed mulberry silk fiber (Padaki et al., 2015).

2.3 Formation of Silk Yarn

Silk thread production is another sub-sector of the textile industry, which is an addition to wool, cotton and synthetic production. Silk, which is a natural yarn, has more moisture retention properties compared to many synthetic yarns. Silk has been a very valuable textile raw material throughout history due to its softness and durability (Keskin ve Çeliker, 2003).

The first step in silk thread production is to pull the strands out of the cocoon. For this, the cocoons are softened by soaking them in hot water and the ends of the silk strand are exposed. These strands are put together, pulled out, twisted and the yarn is obtained. During the boiling and softening of the cocoons, some of the sericin protein surrounding then the strands is dissolved in water and separated from the cocoon.

2.4 Silk Degumming Methods

For sericin recovery from silk fiber, the degumming stage is necessary. For degumming, there are several methods such as boiling, at alkaline conditions using Marseille soap, using organic solvents (tartaric acid, citric acid), high-temperature

high-pressure (HTHP), and exposing to enzymatic reactions (Gupta et al. 2013). Advantages and limitations were given in Table 2.2.

Table 2.2. Conventional Silk Degumming Methods (Gupta et al. 2013)

Degumming Method	**Advantages**	**Limitations**
Boiling	Simple process	Time-consuming Degrades sericin Damage fibroin
Soap and alkali	Simple process Most widely used	Effluent problem Difficulty in recovering sericin
Organic solvents (tartaric acid, citric acid)	Milder in action compared to alkali	Effluent problems Not efficient
High-temperature high pressure (HTHP)	Does not result in any impurity	Causes fumes odor Damage fibroin
Enzymes	Saves water, energy and chemicals	Expensive Degrades sericin extensively

When these degumming methods are applied, sericin is dissolved and the threads are untied and the surrounding protein is removed as shown in Figure 2.3.

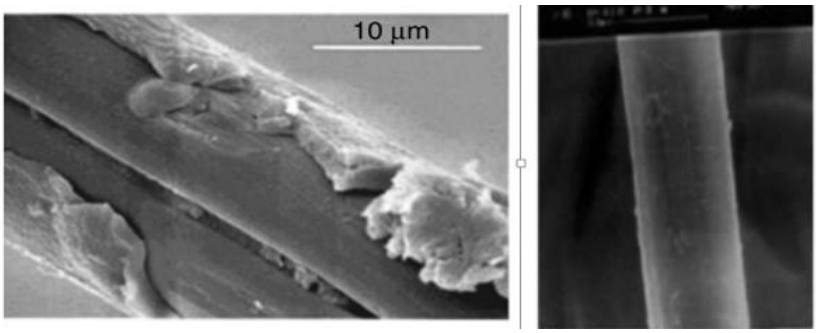

Figure 2.3. Before and After Degumming SEM Pictures (Padaki, Das, and Basu 2015)

2.5 Sercin Recovery via Membrane Filtration

There are many application of membrane filtration in recovery of proteins. It has been proven that membrane technology is efficient for the concentration of proteins from waste sources. The integration of MF and UF results in reduction in the volume of waste generated. Membrane technology is developed to recover valuable substances from different wastewaters. Different membrane processes are applied with respect to wastewater type and recovering materials (Shahid, et.al., 2021).

In sericin recovery, membrane technology is also actively used. According to the study, sericin solution from SDW in Thailand has 4840 mg/L BOD, 8870 mg/L COD. This shows that the protein solution causes high treatment cost. In order to desrease this cost and regain the protein from wastewater membrane filtration and enzymatic hydrolysis were studied. After membrane filtration, wastewater had 158 mg/L BOD and 260 mg/L COD (Vaithanomsat & Kitpreechavanich, 2008). Another application of membrane technology in sericin recovery were conducted as a combined process of acidulation precipitation–ultrafiltration (UF)–nanofiltration (NF). This study shows that recover high-purity sericin protein from SDW. Inner-pressure hollow fiber polyamide NF composite membrane was prepared through the interfacial polymerization. Results shows that the rejection rate of sericin was obtained as 72.1% when using the UF membrane (MWCO 6kDa) and NF membrane process(Li et al. 2015).

2.6 Characteristics of Sericin Protein

Sericin and fibroin are two distinct families of proteins. Sericin is the second protein present in raw silk. Its structure is essentially the same as that of fibroin. Sericin is a family of glycoproteins generated by alternative splicing of sericin genes and comprises 25 to 30% of the cocoon weight. Sericin provides silk a strong attitude by bonding the silk threads taken from the cocoon to each other. At the same time, due of its yellowish tone, it obscures silk's natural whiteness. It's a

coating that protects fibroin, the fiber's basic ingredient, and it dissolves in water, acidic, and basic solutions. When it is removed from raw silk by a process called cooking or degumming, the silk gets a soft and shiny appearance (Babu 2012). The most significant distinction between sericin and fibroin is that fibroin has a crystalline structure whereas sericin has an amorphous structure. Because of its amorphous form and hydroxyl and carboxyl groups, sericin dissolves in hot water, especially slightly basic hot water. The iso-ionic point of sericin is pH 4.1, which is much lower than fibroin and keratin.

The structure of silk fibers is hydrophilic. Silk fibers, unlike wool fibers, do not have a cuticle layer on their surface, which makes water absorption difficult. Although fibroin does not dissolve in water, when silk fibers are exposed to boiling water for an extended period of time, macromolecules in the fibers are broken down. As a result of the ability of water to break hydrogen bridges in amorphous regions, the tensile strength of wet silk fibers is 5-25% lower than the tensile strength of dry fibers (Babu 2012).

Macromolecule of hydrophilic character is composed of 18 amino acids with strong polar groups such as hydroxyl, carboxyl, and amino groups, capable of forming crosslinks, copolymerization’s, and combinations with other polymers. Its organic composition is given by 46.5% carbon, 31% oxygen, 16.5% nitrogen, and 6% hydrogen (Kunz et al. 2016).

2.7 End-uses of Sericin Protein

Sericin is a water-soluble protein. Factors such as temperature, pH, and processing time applied during dissolution in a polar solvent in an acidic or alkaline solution affect the molecular weight of the solute sericin. The molecular weight of sericin changes from 10 kDa to 200 kDa (Capar et al., 2008; Capar et al., 2009). Generally, sericin peptides with a molecular weight of less than 20 kDa are used in cosmetics, skin and hair care products, and other health-related products. Sericin

peptides with a molecular weight greater than 20 kDa are mostly used in medical biomaterials, hydrogel, functional ropes, and fabrics.

In literature, many end-use areas are given such as cosmetics, pharmaceutical and textile industries and it is also recommended to use as a biomaterial (Cho et al. 2003; Mori and Tsukada 2000; Panilaitis et al. 2003; Siritienthong, Ratanavaraporn, and Aramwit 2012; Zhang 2002). In another study, it was determined that nanofibers prepared from chitosan and sericin mixture were successful in the biomedical application and had antibacterial effect (Wang et al. 2014).

Anionic nanoparticles prepared with sericin have been tried and patented in cosmetic hair care products (Santana vd., 2010). The nanoparticles were tested in hair care products due to their positive effects such as hair gloss, volume reduction, softness, ease of scanning, repair, and color protection. Sericin is especially useful in the development of artificial polymers such as polyester and polyamide. It is also used as a coating or blending material in natural and artificial rope and fabrics. In order to test the antibacterial effect of sericin, the cotton fabric was modified with sericin /nano-TiO2 nanocomposites (Gokce, et.al., 2020). At the end of the contact time of 3 h, the antibacterial effect was evaluated, and complete sterilization was performed against the bacteria tested. This method was found to be more effective on S. aureus than E. coli among hospital pathogens. Sericin alone was less effective than nanocomposite (Doakhan et al., 2013). Another application area of sericin is membrane production (Gimenes, Liu, and Feng 2007).

The sericin in silk processing wastewaters is a valuable protein. However, it is currently discarded as a waste. Sericin can be used in different areas such as food, cosmetics and pharmaceutical products as well as for manufacturing biomaterials because of its unique properties such as moisture absorption/desorption, antibacterial and antioxidant properties, and UV resistance (Fabiani et al., 1996;

Rigueiro et al., 2001; Shen et al., 1998; Wu et al., 2007). The commercial value of sericin is high, as evidenced from the price of about 159 € per gram on the market (Sigma Aldrich Catalog, 2021). The cocoon production in the world is about 1 million tons, and dry cocoon equivalent is 400000 tons, and processing of the raw silk produces about 50000 tons of sericin (Zhang, 2002). Therefore, the recovery of sericin from cocoon cooking wastewaters would provide economic benefits and also reduce the environmental impact of silk production processes (Fersi et al., 2005) and help sustainable development.

CHAPTER 3

MATERIALS AND METHODS

In this chapter, methodology of sericin recovery experiments in pilot scale and lab experiments done for pretreatment optimization were given. In pilot scale studies, pretreatment methods including acidification and neutralization, and methodology of membrane hybrid processes i.e. UF and NF were given. Mainly two different wastewater was used for this study. For pilot scale studies, real SDW generated from the factory was used, and for pretreatment optimization experiments, simulated SDW was used. The method of obtaining SDW in lab scale was explained in this chapter. Also, analytical methods were given.

3.1 Pilot-scale experiments

3.1.1 Silk Degumming Wastewater

SDW was obtained from the Kirman İplik Factory located in Bursa, Turkey. At that factory, SDW is generated conventionally by boiling 80 kg of silk yarn with 25 kg of Marseille soap (75% olive oil) in a water tank of 4 m^3 as shown in Figure 3.1.

Figure 3.1 Conventional Silk Degumming Process

3.1.2 Sampling

Pilot-scale studies have been carried out in Bursa, and SDW was directly used for sericin recovery experiments. However, for the laboratory-scale studies, SDW was brought to the Water Management Institute of Ankara University and kept at +4 °C.

The composition of SDW is given in Table 3.1.

Table 3.1. Composition of SDW Collected from the Plant at Varying Times

Sample No	Sericin Concentration (mg/L)	COD Concentartion (mg/L)	TSS (mg/L)
1	9681	20862	12550
2	5743	15850	8550
3	9400	23811	13803
4	6888	17434	9268

3.1.3 Sericin Recovery Experiments

Sericin recovery experiments cover the pretreatment and the membrane hybrid processes. Pretreatment consists of acidification and neutralization. In membrane filtration UF and NF membranes were used since previous lab experiments had shown that 70-78% and 91% rejections occurred by these membranes respectively (TUBITAK114Y461, 2018). The purpose of the pretreatment is to separate sericin from soap and to make sericin solution ready for filtration. Also, the aim of the membrane filtration was to concentrate the sericin in SDW. Thus, SDW was exposed to pretreatment stage, i.e. acidification and neutralization processes, then

membrane filtration was performed in three cases as single UF, UF+NF and single NF as shown in

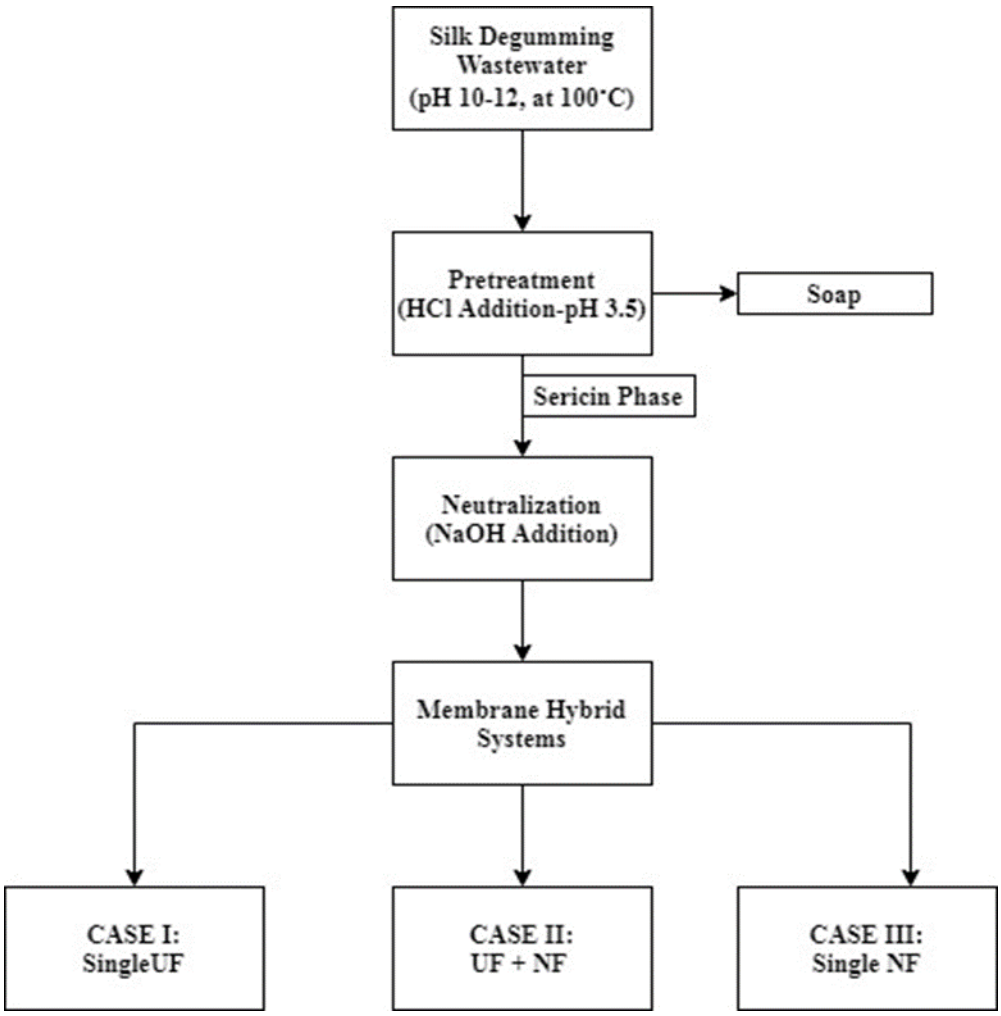

Figure 3.2. Treatment Scheme for Sericin Recovery Experiments

3.1.3.1 Pretreatment

Pretreatment was applied for the separation of soap and the dissolved sericin in SDW. Acidification was done with the addition of HCl until the pH becomes 3.5. After acid addition, flocculation and neutralization was applied.

3.1.3.1.1 Flocculation

The conventional silk degumming method results in raw wastewater rich in sericin and fatty acids originated from Marseille soap at almost 100˚C. This wastewater was cooled down to 60˚C to save the pump between the degumming tank and phase separation tank, which had 1 m^3 volume. In order to separate soap and sericin, pretreatment of SDW was done in a conical phase separation tank as shown in Figure 3.3, by adding HCl to get pH 3.5. Average HCl addition was 1.5 – 2.5 L/m^3 of wastewater. For better contact of acid and SDW, 100-120 rpm mixing was applied for 20 min. Phase separation was reached in 1 day at room temperature. Its final temperature was about 30 – 35 ˚C. At that temperature, soap was floating, so water- rich in sericin concentration was collected from the bottom part of the phase separation tank.

Figure 3.3. Conical Mixing and Phase Separation Tank

Wastewater, rich in sericin concentration, was drawn by the valve located at the bottom of the conical tank and taken into a neutralization tank with 2 m^3 volume. Since the wastewater at pH 3.5 might damage the membrane system and membranes' lifetime might get shorter, wastewater with pH 7 was preferable.

3.1.3.1.2 Neutralization

Neutralization of wastewater was done by adding NaOH manually, and mixing was done with a submerged pump since it has no mixing system, as shown in Figure 3.4. In both stages, pretreatment, and neutralization, pH was monitored regularly.

Figure 3.4. Neutralization Tank

3.1.3.2 Membrane Hybrid System

The membrane hybrid sistem includes UF membrane module connected the UF feed and permeate tank, NF membrane module and NF permeate tank. UF feed tank was used as NF feed tank. Both UF and NF had a 200 L feed and a permeate tank, as shown in Figure 3.6. The pilot system was provided by İstanbul Technical University National Research Center on Membrane Technology (ITU MEM-TEK).

The membrane experiments was based on three different scenarios as single UF, UF + NF, and single NF. Since the tank volume was relatively low, with the help of the submerged pump, UF feed tank was filled continuously with wastewater in neutralization tank.

Figure 3.5. Ultrafiltration Module

Figure 3.6. Nanofiltration Module

The membrane system shown in Figure 3.5 and Figure 3.6 has a backwash system for NF membrane, whereas it does not have a backwash system for UF membrane. NF membrane backwash was done with 8 L filtered water in permeate tank automatically in 30 min time intervals.

3.1.3.2.1 Membrane Characteristics

After pretreatment, in order to recover sericin, the hybrid membrane system, including UF and NF membranes, was used. The system included Alfa Laval ETNA10PP model 10 kDa UF and Alfa Laval NF membranes. The NF system was with 3838 housing and 30 mL spacer. Detailed information is given in Table 3.2.

Table 3.2. Properties of Membranes Tested at Pilot Scale Experiments

Membrane Type	**Membrane**	**Size**	**Characteristics**	**MWCO**	**Effective Area (m^2)**
Spiral Element	UF ETNA10PP	6338	Thin-film composite (TFC)	10 kDa	6.34
	NF	3838	Composite fluoropolymer on polypropylene	>99% Rejection of MgSO4 (9 bar, 25ºC)	7.61

3.1.3.2.2 Ultrafiltration and Nanofiltration

The UF and NF membranes were operated in cross-flow and concentration mode of filtration (CMF). Retantate was recycled back to the feed tank, and thus, in the feed tank, sericin was concentrated as shown in Figure 3.7.

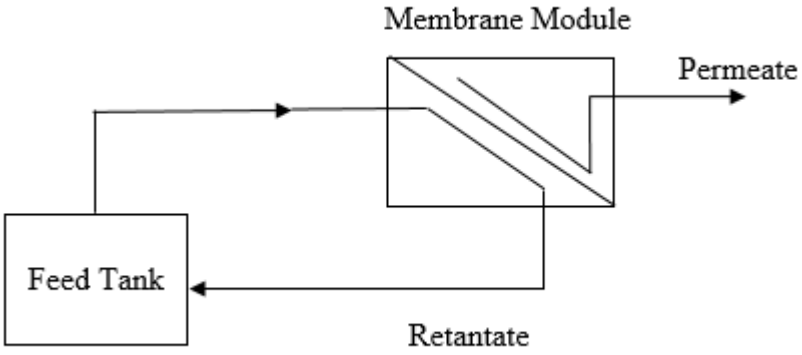

Figure 3.7. Schematic Representation of UF and NF Systems

Single UF membrane filtration flow chart is given in Figure 3.8.

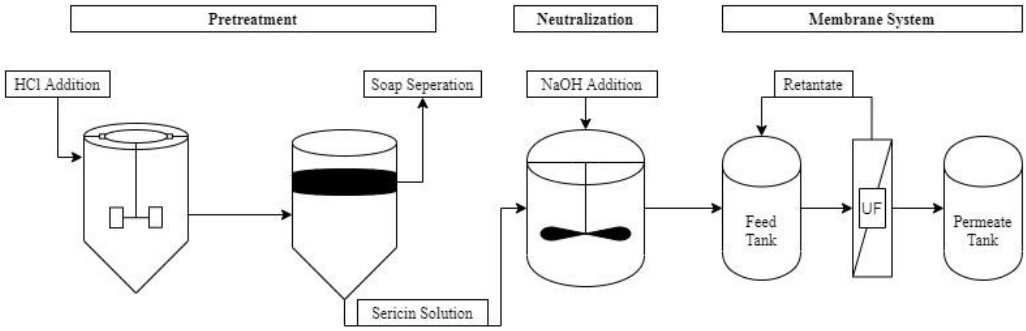

Figure 3.8. Single UF Membrane Filtration Flow Chart

Single UF+NF membrane filtration flow chart is given in Figure 3.12. In the UF+NF membrane system, pretreated wastewater was taken to the UF feeding tank with the help of a submerged pump. At the beginning of the filtration process, wastewater was transferred from the neutralization tank to the UF feed tank; then, wastewater was continued to be added in certain intervals to keep concentration at a certain level in the feed tank. Initially, treatment with UF was initiated. In the UF filter tank, when enough water has been accumulated in the NF feed tank, NF was was performed. In that way, UF and NF were run together

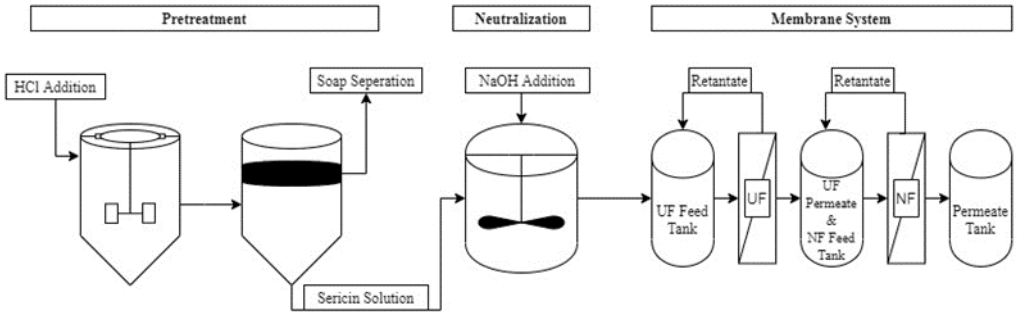

Figure 3.9. UF + NF Membrane Filtration

Single NF membrane filtration flow chart is given in Figure 3.10.

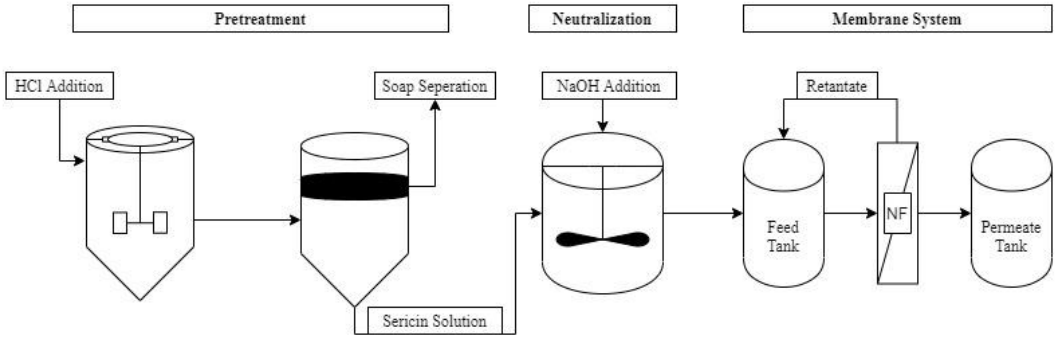

Figure 3.10. Single NF Membrane Filtration

Note that the pretreatment was done in a single tank, i.e. both mixing and phase separation are in the same tank and in Figure 3.8, Figure 3.9 and Figure 3.10 , two different tanks were drawn to shows the process.

For the experiments carried out in the laboratory, UF and NF inlet pressures were set to 2 bars and 5 bars, respectively. In pilot phase studies, while the same pressure value was used in UF filtration process since the pressure upper limit of the existing NF Membrane system was not sufficient, the NF inlet pressure was set to 4 bars.

3.1.4 Membrane Filtration Performance

3.1.4.1 Volume Reduction Factor (VRF)

The membrane system is used to concentrate sericin solution since the more concentrated retentate means less volume for further applications. VRF is calculated as

$$\mathrm{VRF} = \frac{\mathrm{Vf}}{\mathrm{Vr}}$$

where V_f is the initial feed volume of feed (L) and V_{is} the final volume of retentate (L).

3.1.4.2 Flux Decline

After neutralization, wastewater was ready to be fed to the membrane system. In the membrane system, it was possible to use single UF, UF+NF, and single NF. In all cases, permeate volume was measured at 30 min time interval. Then the flux, J (L/m^2h) was calculated as

$$J = \frac{V}{A\Delta t}$$

where V is the amount of water collected from permeate stream (L), A is the effective membrane area (m^2), and Δt is the filtration time interval (h).

3.1.4.3 Sericin Rejection

In order to achieve a sericin rejection ratio, sericin concentration analysis was done in HPLC (High-Performance Liquid Chromatography) at Water Management Institute in Ankara University. For both feed and permeate streams, sericin rejection, SR (%) was calculated as

$$SR\ (\%) = (1 - \frac{Cp}{Cf}) \times 100$$

where C_p is the permeate sericin concentration, and C_f is the feed sericin concentration in mg/L.

3.1.4.4 Membrane Cleaning

In the pilot-scale system, since the spiral element membranes were used, mechanical membrane cleaning was not possible, unlike the flat sheet membrane experiments in the laboratory. Also, softening process water was used in the factory for membrane cleaning, whereas distilled water was used for that purpose in laboratory experiments.

Chemical cleaning was done for UF and NF membranes. In chemical cleaning, water was heated up to 35ºC and mixed with NaOH to get pH 10-10.5, and Cl was added to adjust Cl concentration to 200 ppm. With this cleaning, the water membrane was run for about 20 min. After chemical cleaning, clean water flux (Jcc) was measured.

3.2 Lab-scale Experiments

3.2.1 Further Purification of Sericin

After membrane filtration in pilot scale, samples taken from retantate are brought to Ankara University Water Management Institude, Water Laboratory for further purification of sericin. Ethanol precipitation and centrifugation was applied here. The centrifugated samples sent to lyophilization to get powdered sericin.

3.2.1.1 Ethanol Precipitation

Retantate taken from the UF and NF membrane was sent to ethanol precipitation. At that stage, cold ethanol was added with a ratio of 3:1 (ethanol:retentate) and left at +4˚C for a while. Precipitated sericin was separated and ethanol was used for the process of fractional distillation for ethanol recovery.

3.2.1.2 Centrifugation

Centrifugation was applied to precipitated and concentrated sericin at ethanol precipitation in order to increase the solid ratio in sericin solution and eliminate the water inside. This process was done at 3000 rpm for 10 minutes with Hettich Universal 320 model centrifugation device.

3.2.1.3 Lyophilization

Lyophilization was done at METU MERLAB Molecular Biology and Biotechnology R&D Center. In this process, samples were frozen at -115 °C and then vacuumed until all ethanol was removed and powder sericin was left. It wasthen dried at room temperature.

3.2.2 Pretreatment Optimization

In pilot-scale experiments, during pretreatment, phase separation was not observed in some cases in Bursa Kirman İplik Factory. In order to understand why the phase separation did not occur properly, the effect of the ratio between soap and silk yarn was studied. In conventional degumming, for 80 kg silk yarn, 25 kg soap is added to the 4-ton tank. Based on this data, the soap to silk ratio was calculated as 0.31.

In this factory, water amount is constant for all degumming processes, but silk yarn and accordingly the amount of soap may vary as shown in Table 3.3. This change is caused by the silk yarn amount needed to be processed. If this amount is high, it means more than one degumming cycle was performed in the same degumming water. Also, the shape and size of the silk yarn affected how much soap was added. If it is thick silk yarn, more degumming time and soap are needed.

Table 3.3. Change of Content in Degumming Process

Silk Processing Content	Soap to Silk Yarn Ratio	Phase Separation
100 kg silk yarn 30 kg soap	0.3	Yes
60 kg silk yarn 20 kg soap	0.33	Yes
75 kg silk yarn 30 kg soap	0.4	No
150 kg silk yarn 40 kg soap	0.27	No
70 kg silk yarn 30 kg soap	0.43	No

The reason why the phase separation does not occur in pretreatment has been associated with these changes in the ratio between soap and silk yarn. For this reason, SDW having different amount of soap and silk yarn ratio was simulated in lab, and acidification was applied for each one. Also, acidification was done at six different pH values between pH 3-4. Then, the effect of soap to silk yarn ratio on phase separation was observed at different pH values.

3.2.2.1 SDW Formation in Laboratory

For simulation of the silk degumming process, silk yarn and Marseille soap were provided and brought to Ankara University Water Management Institute Laboratory. Using these materials, SDW samples were prepared in different silk yarn to soap ratios.

Silk yarn was weighted as 160 g for each soap to silk yarn ratio, and the soap amount was changed. Soap amount was determined as 40, 48, 56, 64 and 72 g. After all, components were prepared, the degumming process was started.

Figure 3.11. Marseille Soap and Silk Yarn

In the degumming stage, for each set of experiments, 8 L of water was boiled with related soap amount and silk yarn was added and degummed for 3 h. Then, degumming water was drained and filled into five different beakers. Cooled, degummed wastewaters were sent to the jar test for pH adjustment.

Figure 3.12. Silk Degumming in Laboratory Conditions

3.2.2.2 Effect of 'Soap to Silk Yarn Ratio' on Pretreatment

In this part of the study, pretreatment optimization was done with respect to both soap to silk yarn ratio and pH. Phase separation was observed according to soap to silk yarn ratio (FO) as 0.25, 0.30, 0.35, 0.40 and 0.45. For each ratio, acidification was done at different pH values. Since at pH 3.5 free fatty acids are formed and fatty acids generated by soap is removed (Capar, Aygun, and Gecit 2009), during optimization pH range was detected to cover this value. Thus, pH values were set as 3.0, 3.2, 3.4, 3.6, 3.8 and 4.0.

For each experimental setup (SetI & SetII), 30 samples and totally 60 samples were sent to zeta potential measurement at the METU MERLAB. The results are given in Chapter 4.

3.3 Analytical Methods

In pretreatment, optimization experiment, pH was measured regularly. Also, to see the phase separation performance in pretreatment, the zeta potential was measured. In membrane filtration experiments, sericin and COD concentrations were measured.

3.3.1 Molecular Weight Analysis of Sericin

It is important to learn the MW of sericin since sericin with different molecular weights are used for different purposes. Generally, sericin peptides with a molecular weight of less than 20 kDa are used in cosmetics, skin and hair care products, and other health-related products. Sericin peptides with a molecular weight greater than 20 kDa are mostly used in medical biomaterials, hydrogels, functional threads and fabrics (TUBITAK 114Y461, 2018). In this study, MW analysis like SDS-PAGE (Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis) and HPLC were done to detect the MW of recovered sericin. For

HPLC analysis, calibration curve has been created for MW analysis of sericin. For this calibration curve, standard protein solution (Calbiochem) including five different proteins with known MW was used. This standard protein solution consists of cytochrome c monomer (12.4 kDa), myokinase (32 kDa), enolase (67 kDa), lactate dehydrogenase (142 kDa) and glutamate dehydrogenase (290 kDa) proteins. HPLC chromotagram of the proteins in this solution was given in Fig. 3.15.

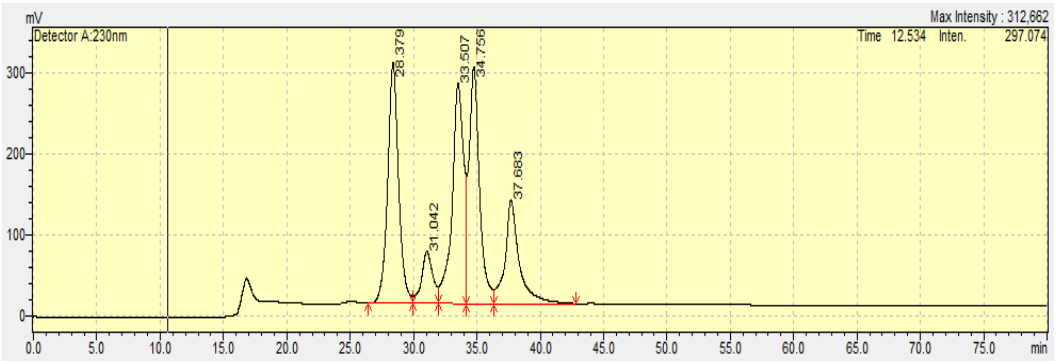

Figure 3.13. HPLC Chromotogram of Standard Protein Solution (TUBITAK 114Y461, 2018)

Regarding retention time of these proteins, a linear regression curve is formed, and given below.

log MW = - 0,15t + 6,7704 (R^2=0,9878)

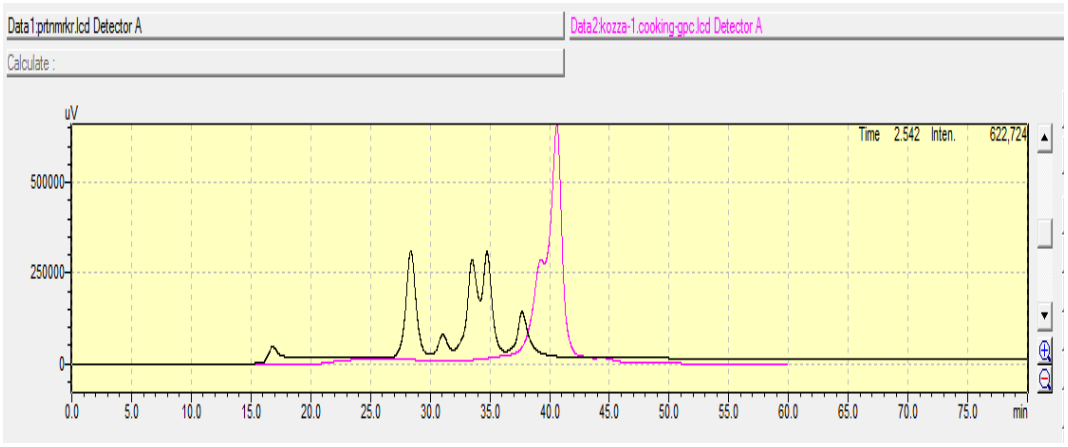

Figure 3.14. Comparison of Sericin and Standard Protein Solution (TUBITAK 114Y461, 2018)

When the chromatograms given in Figure 3.13 and Figure 3.14 were compared, sericin protein was detected around 2 min later than the smallest standard protein, that is cytochrome c monomer (12.4 kDa). In Figure 3.14, delayed retention time of sericin compared to standard proteins was seen clearly. Pink color was representing sericin and black color was representing the marker proteins. The delay in sericin elution time made it questionable to calculate its MW with the calibration equation as it corresponds to a MW of 2.16 kDa, which is quite low. Additionally, sericin solutions taken from pilot scale membrane filtration were sent to the SDS-PAGE to obtain the MW of the sericin that was concentrated. This analysis also showed that the molecular weight of sericin was only about 10 kDa. Finally, LC-MS/MS analysis were carried out by using recovered sericin from UF and NF membrane filtration at Acıbadem Univercity. In that analysis, sericin protein could not be identified.

When HPLC, SDS-PAGE and LC-MS/MS analysis were taken into consideration, it was said that the recovered sericin protein could be degraded into smaller MW during the recovery processes since the previous study said that SDW sericin has MW of 110-120 kDa (Aygün, 2008). However, recovered sericin has a program, it performs as well as the Elidor shampoo (with conditioner) when tested in shampoo. Again, it gave positive results in nanocomposite synthesis.

3.3.2 Sericin Concentration

Quantitative analysis of sericin and molecular weight distribution was determined with a Shimadzu, Prominence LC-20AT model HPLC. Gel separation chromatography column (PSS PROTEEMA Analytical 300^{o}A) with a mobile phase including 0.3 M NaCl and 0.05 M KH_2PO_4 was used. Concentration analysis was done at 30 ^{o}C column temperature and at 230 nm UV absorbance. The flow was adjusted as 1 mL/min, and injected volume was selected as 20 µL. All samples were filtered with 0.45 µm (Millipore Millex-HV) before HPLC. For the formation

of sericin calibration curve, commercial sericin was used, and its peak was observed between 7.5 to 17.5 min.

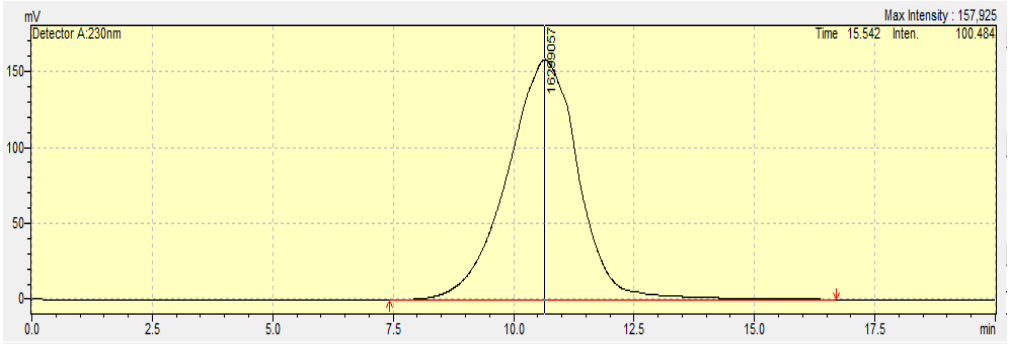

Figure 3.15. HPLC chromatogram of the standard sericin solution (6 g/L)

3.3.3 COD

Chemical Oxygen Demand (COD) analysis of sericin samples was made according to the standard method: 5220D using the spectrophotometer (HACH DR-2000 model) shown in Figure 3.16. Hach-Lange COD kits were selected in the range appropriate to the concentration of organic matter expected to be present in the sample, and the relevant procedure was applied.

Figure 3.16. Spectrophotometer

3.3.4 Conductivity and pH

Conductivity and pH readings were made by a pH meter (HACH brand HQ40d model) applying Standard Method 2510B for pH analysis and Standard Method 4500H+ for conductivity analysis. The conductivity and pH probes were calibrated using reference solutions.

3.3.5 Zeta Potential

Zeta potential is a measurement of charge repulsion/attraction between particles. The zeta potential is used to evaluate the charge stability of the system. When the charge neutralization is provided, particles do not stay in he suspended form and phase separation occurs. In order to see charge stability of the system the at different pH and FO ratio, zeta potential analysis was conducted. Zeta potential and particle size analysis were done in the METU MERLAB with a Malvern Nano ZS90 device. With this device shown inFigure 3.17, a certain electric field is applied during analysis, and the velocity of the particle is found by applying the Doppler principle.

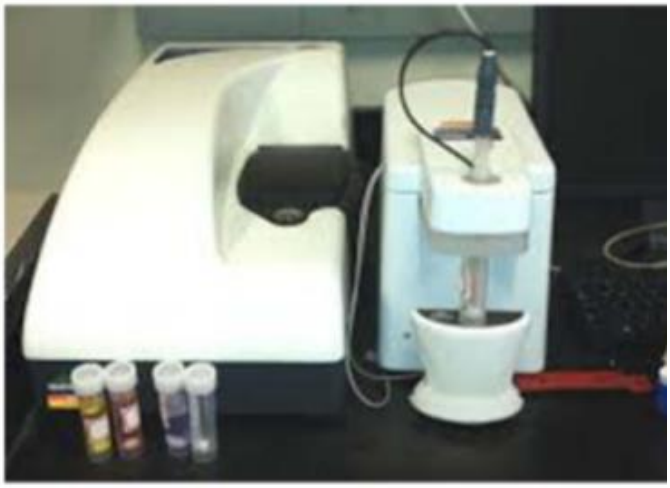

Figure 3.17. Malvern Nano ZS90

CHAPTER 4

RESULTS AND DISCUSSION

This chapter presents the results of experiments on pretreatment performance, optimization of pretreatment by changing soap to silk yarn ratio and membrane filtration performance, mainly depending on the observation of flux, and sericin rejection.

4.1 Pretreatment

Pretreatment is the first step of the sericin recovery. Due to the use of soap in conventional degumming, SDW is rich in both sericin and fatty acids originating from soap. Fatty acids need to be separated from sericin, and this is possible by decreasing pH from around 9 to 3.5 (Capar, Aygun, and Gecit 2009). After acidification, neutralization was applied with NaOH to protect membrane system. Eliminating of this impurity from sericin is also necessary to prevent clogging of the membranes.

4.1.1 Effect of 'Soap to Silk Yarn Ratio' to Pretreatment

In some of the pilot scale experiments, sericin could not be seperated from soap at at pretreatment stage although the acidification was applied at pH 3.5. In order to find out the reason for insufficient pretreatment results, optimization experiments were performed. It was observed that phase separation performance changed with respect to soap to silk yarn ratio at different acidification pH values.

4.1.1.1 Soap to Silk Ratio: 0.25

160 g silk yarn and 40 g of soap were boiled in 8 L of water for 3 h. After the yarn was drained, degumming water was kept for cooling down to 60˚C. Then, 800 mL of wastewater was filled into each of the six beakers, and a different amount of acid was added to each to adjust different pHs. After mixing for 5 min at 150 rpm, samples were left for phase separation at room temperature for a day. The pH values of the beakers from left to right were 4.0, 3.8, 3.6, 3.4, 3.2, and 3.0, shown in Figure 4.1.

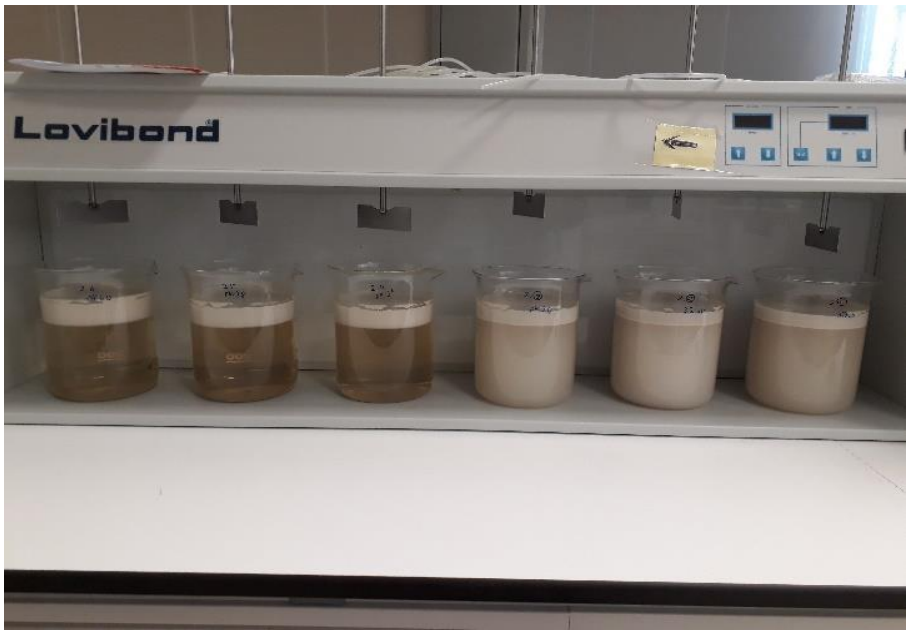

Figure 4.1. Phase Separation at pH 3-4 (FO Ratio: 0.25)

After the acidification process, as seen in Figure 4.1, in samples with pH 3.4, 3.2, and 3.0, phase separation was not sufficient, but at higher pH values, phase separation was observed clearly, where soap was accumulated at the top of the beakers. The amount of HCl added, and physical conditions are given in Table 4.1.

Table 4.1. Amount of Acid Added and Temperature (FO Ratio: 0.25)

pH_i	pH_f	HCl (mL)	T_i (˚C)
9.23	3.0	1.7	60
9.23	3.2	1.6	60
9.23	3.4	1.6	60
9.23	3.6	1.6	60
9.23	3.8	1.6	60
9.23	4.0	1.6	60

Figure 4.2. Phase Separation at pH 3-4 (FO Ratio: 0.25- Replication)

It is seen that in Figure 4.2, like the first experiment, higher pH values gave a clear sericin phase. However, in this case, the soap was accumulated at the bottom. Accumulation of soap at the bottom can be associated with room temperature. In Figure 4.1, the room temperature was about 22-23˚C, while in Figure 4.2, it is around 17-18˚C.

4.1.1.2 Soap to Silk Ratio: 0.30

160 g silk yarn and 48 g of soap were boiled in 8 L of water for 3 h. After the yarn was drained, it was waited for the received wastewater to cool down to 60˚C. 800 ml of wastewater was filled into each of the six beakers, and a different amount of acid was added to each. After mixing for 5 min at 150 rpm, the samples were left for phase separation at room temperature during a day. The pH values of the beakers from left to right were 4.0, 3.8, 3.6, 3.4, 3.2 and 3.0 as shown in Figure 4.3 and Figure 4.4.

Figure 4.3. Phase Separation at pH 3-4 (FO Ratio: 0.30)

After the acidification process, as seen in Figure 4.3, just like in the case of Soap to Silk Ratio: 0.25, for pH 3.4, 3.2 and 3.0, clear phase separation could not be observed. However, at higher pH values such as pH 4.0, 3.8, 3.6, phase separation was observed clearly, and soap was accumulated at the top of the beakers. The amounts of HCl added and physical conditions of phase separation were given in Table 4.2 for each beaker.

Table 4.2. Amount of Acid Added and Temperature (FO Ratio: 0.30)

pH_i	pH_f	HCl (mL)	T_i (˚C)
9.20	3.0	2.4	60
9.20	3.2	2.2	60
9.20	3.4	2.1	60
9.20	3.6	2.0	60
9.20	3.8	1.9	60
9.20	4.0	1.8	60

Figure 4.4. Phase Separation at pH 3-4 (FO Ratio: 0.30- Replication)

When it was compared with the first experiment, repetition of the experiment gave the unclear phase separation, and the same result could not be seen in Figure 4.3 and Figure 4.4.

4.1.1.3 Soap to Silk Ratio: 0.35

160 g silk yarn and 48 g of soap were boiled in 8 L of water for 3 h. After the yarn was drained, it was waited for the received wastewater to cool down to 60˚C. 800 mL of wastewater was filled into each of the six beakers, and a different amount of acid was added to each. After mixing for 5 min at 150 rpm, samples were left for phase separation at room temperature during a day. The pH values of the beakers from left to right were 4.0, 3.8, 3.6, 3.4, 3.2, and 3.0, as shown in Figure 4.5 and Figure 4.6.

Figure 4.5. Phase Separation at pH 3-4 (FO Ratio: 0.30)

After the acidification process, just like in the case of Soap to Silk Ratio: 0.25, for pH 3.4, 3.2 and 3.0, clear phase separation could not be observed. However, at higher pH values such as pH 4.0, 3.8, 3.6, 3.4 and 3.2, phase separation was observed clearly, and soap was accumulated at the top of the beakers. The amount of HCl added, and physical conditions of phase separation were given in Table 4.2 for each beaker.

Table 4.3. Amount of Acid Added and Temperature (FO Ratio: 0.30)

pH_i	pH_f	HCl (mL)	T_i (˚C)
9.20	3.0	2.4	60
9.20	3.2	2.2	60
9.20	3.4	2.1	60
9.20	3.6	2.0	60
9.20	3.8	1.9	60
9.20	4.0	1.8	60

Figure 4.6. Phase Separation at pH 3-4 (FO Ratio: 0.30- Replication)

When it was compared with the first experiment, repetition of the experiment gave the unclear phase separation, and the same result could not be seen. Only the conditions at pH 4.0 and pH 3.8 provided clear phase separation for both experiments.

4.1.1.4 Soap to Silk Ratio: 0.40

160 g silk yarn and 64 g of soap were boiled in 8 L of water for 3 h. After the yarn was drained, it was waited for the wastewater received to be 60˚C. 800 ml of wastewater was filled into each of the six beakers and a different amount of acid was added to each. After mixing for 5 min at 150 rpm, samples were left for phase separation at room temperature during a day. The pH values of the beakers from left to right were 4.0, 3.8, 3.6, 3.4, 3.2 and 3.0 as shown in Figure 4.7.

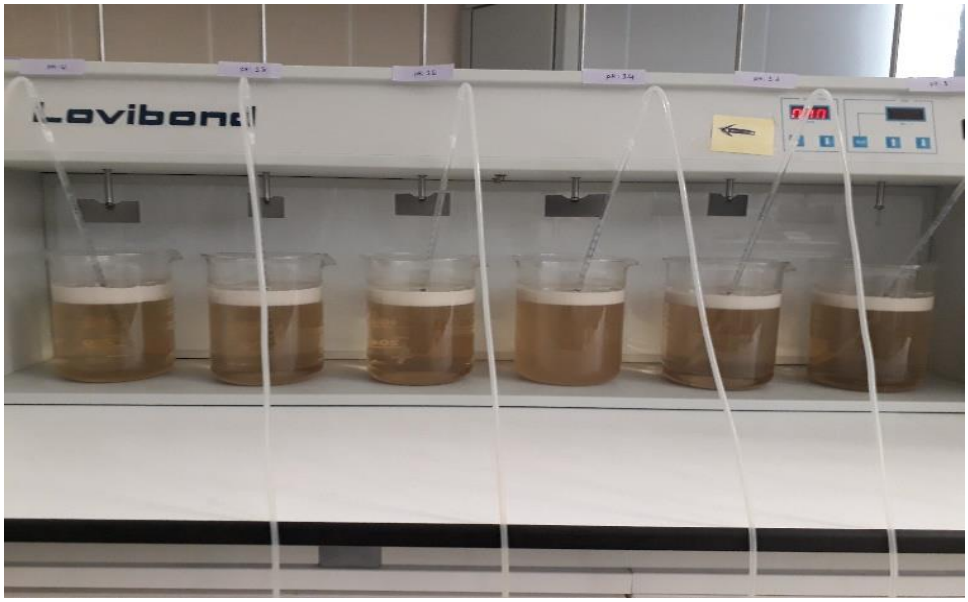

Figure 4.7. Phase Separation at pH 3-4 (FO Ratio: 0.40)

As shown in Figure 4.7 for all pH values, it was observed that phase separation occurred and soap was collected at the top. The amount of HCl added and physical conditions of phase separation were given in Table 4.4 for each beaker.

Table 4.4. Amount of Acid Added and Temperature (FO Ratio: 0.40)

pH_i	pH_f	HCl (mL)	T_i (˚C)
9.30	3.0	2.5	60
9.30	3.2	2.4	60
9.30	3.4	2.3	60
9.30	3.6	2.2	60
9.30	3.8	2.1	60
9.30	4.0	2.0	60

Figure 4.8. Phase Separation at pH 3-4 (FO Ratio: 0.40- Replication)

4.1.1.5 Soap to Silk Ratio:0.45

160 g silk yarn and 72 g of soap were boiled in 8 L of water for 3 h. After the yarn was drained, it was waited for the wastewater received to be 60˚C. 800 ml of wastewater was filled into each of the six beakers and a different amount of acid was added to each. After mixing for 5 min at 150 rpm the samples were left for

phase separation at room temperature during a day. The pH values of the beakers from left to right were 4.0, 3.8, 3.6, 3.4, 3.2 and 3.0, as shown in Figure 4.9.

Figure 4.9. Phase Separation at pH 3-4 (FO Ratio: 0.45)

Although the previous ratio gave the clear phase separation for all pH values, in this case, again, the lower pH values did not enable sufficient phase separation, but through the higher pH values, clarity of the sericin solution improved. For this experiment, the amount of HCl added and physical conditions of phase separation are given in Table 4.5.

Table 4.5. Amount of Acid Added and Temperature (FO Ratio: 0.45)

pHi	pHf	HCl (mL)	Ti (˚C)
9.25	3.0	2.6	60
9.25	3.2	2.6	60
9.25	3.4	2.5	60
9.25	3.6	2.5	60
9.25	3.8	2.4	60
9.25	4.0	2.3	60

4.2 Zeta Potential Results of Pretreatment Optimization Sample

In this part of the study, samples prepared in the laboratory by imitating the real silk degumming processes were collected and sent for the zeta potential analysis. Zeta potential analysis was done dor For SDW samples having 6 different FO ratio, acidification was applied at 5 different pH values in the range of pH 3-4. This set of experiments was repeated and, results were given as Set I and Set II.

4.2.1 Set I

The zeta potential results obtained are given in Figure 4.10 and Figure 4.11. As can be seen, the zeta potential values of the degumming wastewater in the range of pH 9 – 9.5 are between -37.2 mV and -62.7 mV.

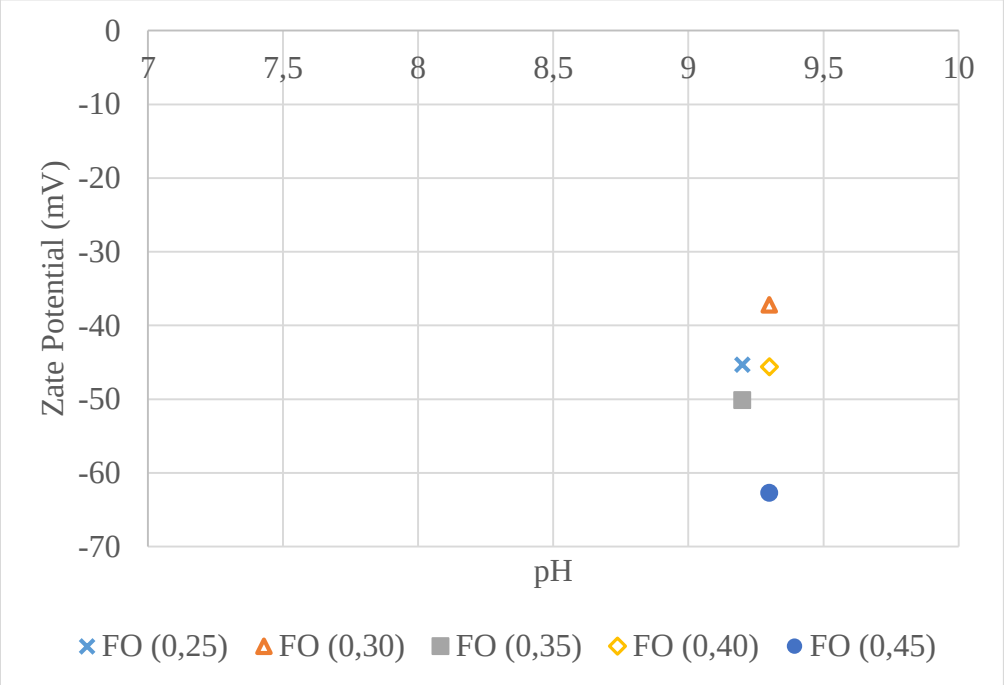

Figure 4.10. Change of Zeta Potential with FO Ratio

When acid was added to the samples, zeta potential values were measured positively in the pH range of 3-3.4, and zeta potential was measured generally

negatively in the pH range of 3.6-4. It was observed that zeta potential decreased as pH increased from 3 to 4.

Table 4.6. Zeta Potential After Acidification for Each 'Silk to Soap Ratio'

FO	Zeta Potential (mV)					
	pH 3	pH 3.2	pH 3.4	pH 3.6	pH 3.8	pH 4
0.25	11.9	11.6	7.8	**-2.3**	**0.1**	**-8.0**
0.30	12.1	9.6	4.9	**-7.3**	**-10.2**	**-6.0**
0.35	14.3	**11.3**	**8.2**	**4.7**	**0.1**	**-2.3**
0.40	**5.7**	**3.6**	**4.5**	**1.2**	**0.1**	**-1.7**
0.45	8.7	**2.5**	**2.1**	**-0.5**	**-2.0**	**-5.7**

For the occurrence of phase separation, the experimental results were highlighted in bold in Table 4.6. These results show that when the FO ratio was around 0.4, phase separation was observed at all pH values. This ratio is shown as yellow line in Figure 4.11, its all zeta potential is between -5mV and +5mV. This improves that surface tension was around 0, phase separation occurs. Also, at pH 4 for all FO values phase separation was observed.

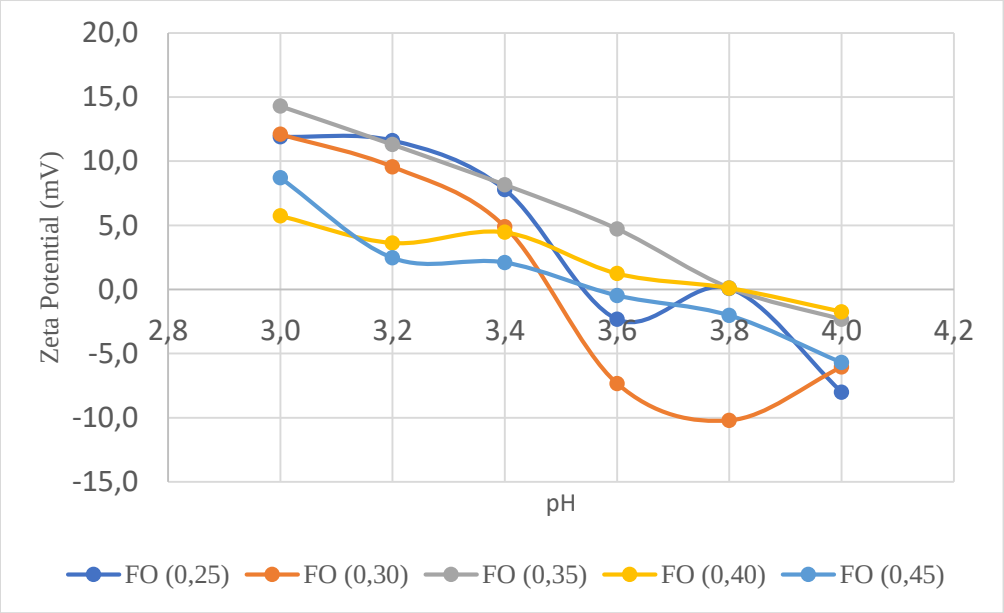

Figure 4.11. Zeta Potential After Acidification for Each 'Silk to Soap Ratio'

When all the figures were examined, it was determined that there was no phase separation between pH 3.0 and pH 3.4 in the samples where the silk to soap ratio is 0.25 and 0.30. This shows that both pH and FO decreases, pretreatment was not successfully done. When this ratio was 0.35 and 0.45, only phase separation at pH 3.0 still did not occur. At pH 3, soap and sericin separation was observed only at FO 0.40. In the sample with the FO 0.40, phase separation occurred at all pH values. Also, all FO values gives successfully separation results at pH 4. Therefore, for successful phase separation, pH must be kept at 4. This is also a good result for real scale application since phase separation at higher pH would lower the acid addition at pretreatment, which in turn would lower the operational cost.

4.2.2 Set II

The zeta potential results obtained are given in Figure 4.12 and Figure 4.13. As can be seen, the zeta potential values of the degumming wastewater in the range of pH 9 – 9.5 are between -37.4 mV and -60.6 mV.

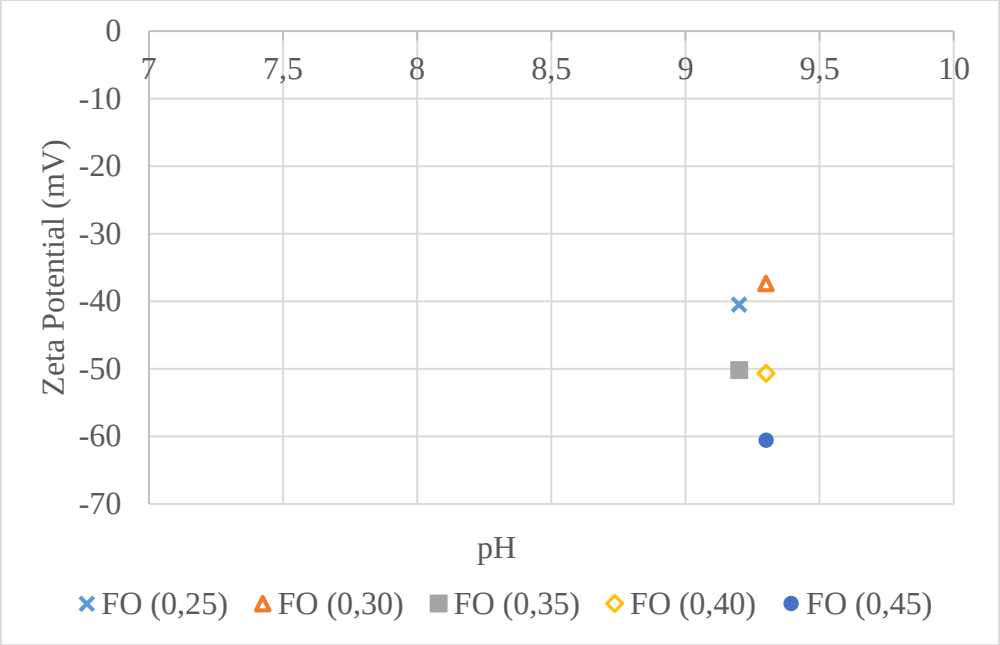

Figure 4.12. Zeta Potential of SDW Formed in the Laboratory (Set II)

When acid was added to the samples, zeta potential values were measured positively in the pH range of 3.0 - 3.8 and measured negatively at pH 4.0. It was observed that zeta potential decreased when pH increased from 3 to pH 4.

Table 4.7. Zeta Potential After Acidification for Each 'Silk to Soap Ratio' (Set II)

FO	Zeta Potential (mV) for each pH					
	pH 3	pH 3.2	pH 3.4	pH 3.6	pH 3.8	pH 4
0.25	13.8	11.0	8.9	5.8	**2.7**	**-0.3**
0.30	11.4	10.3	8.7	5.6	**2.9**	**-0.2**
0.35	13.3	10.1	8.2	6.4	**2.7**	**-1.1**
0.40	**13.2**	**11.5**	**4.5**	**5.8**	**3.0**	**-1.4**
0.45	12.4	**12.4**	**7.7**	**4.6**	**1.8**	**-0.3**

Similar to Table 4.6, Table 4.7 shows numbers in bold to indicate clear phase separation experiments. When the results in these tables are compared, it can be said that when the soap to silk ratio is small as 0.25 and 0.30, lower pH values such

as 3 and 3.2 have no clear phase separation as same as Set I results. However, this ratio gets higher as 0.35, 0.40 and 0.45, phase separation can be seen at all pH values easily. Also, when Figure 4.11 and Figure 4.13 are examined, zeta potential is in the range of 5 mV and -5 mV for all FO values between pH 3.6 and pH 4. Zeta potential of successful phase separation was again very close to -5mV/+5mV range. This also supports the information that the surface tension was around 0, phase separation was observed. When we look at the Set I and Set II results, common judgment is that at higher pH values and at higher FO ratios, it is possible to reach successful phase separation since their surface tension was near to 0. Also, the zeta potential variance according to addition of acid and changing of FO ratio showed that the pH value of wastewater significantly affected the phase separation between soap and sericin.

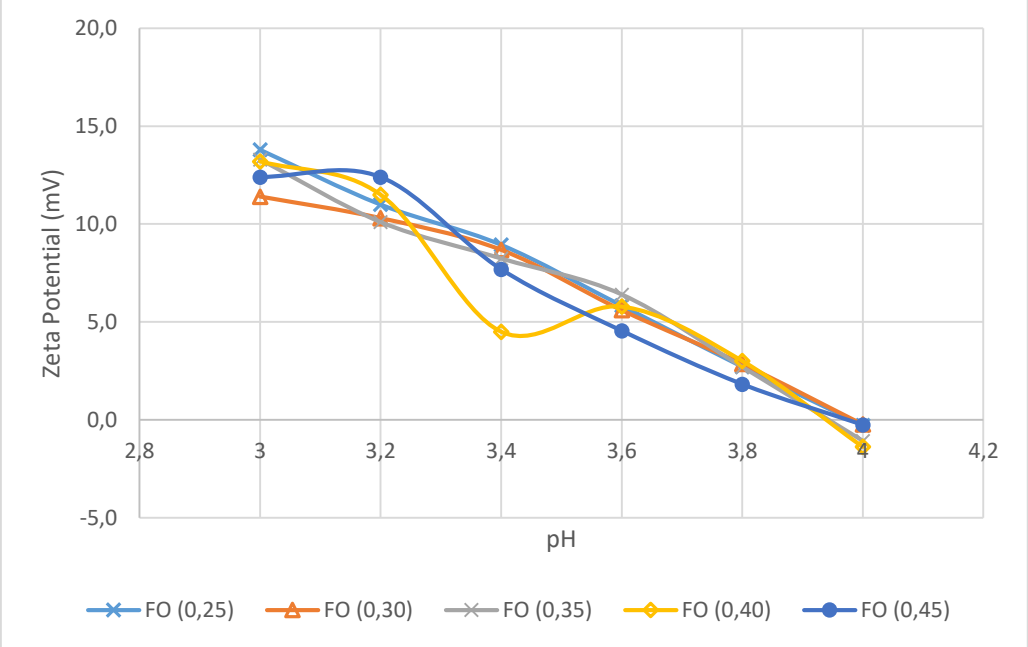

Figure 4.13. Zeta Potential After Acidification for Each 'Silk to Soap Ratio'(Set II)

4.3 Membrane Hybrid System Flux and Sericin Recovery Results

In this part, three different membrane systems were set up. These cases were single UF, UF prior to NF and finally single NF. Also, each membrane operation was repeated and results for repetition were given as Set I and Set II. For each membrane operation, change in flux, temperature and sericin concentration were monitored and these results were visualized on their graphs. Moreover, the first experiment's results include the change in COD concentrations. This step was eliminated in further experiments after it was seen that the sericin concentration and COD concentration were parallel to each other, and the major aim was to get concentrated sericin and observe its concentration. Sericin is separated from fatty acids after pretreatment stage. In membrane filtration, concentrating of sericin protein and elimination of other impurities from sericin were done. In permeate COD results from the salts forming by acidification and neutralization processes.

4.3.1 Results for Single UF Membrane

In this part, a UF membrane module was used, and operated at 2 bar. This experiment was done twice and results was given as Set I and Set II. Different volume of wastewater was filtered in these two operation. Flux and rejection rates were given for each operation.

4.3.1.1 Single UF (Set I)

This part of the study was based on the recovery of sericin by UF membrane of the hybrid membrane system. 500 L of wastewater was taken into the process. After pretreatment, 180 L of wastewater was discarded as the soap phase. UF module was run for 6 h in order to filter 320 L feed volume. After 6 h, while 35 L of feed volume was concentrated as retentate into the feed tank, 285 L was filtered and

collected into permeate tank. Then VRF was calculated as 9 by the following calculation:

$$VRF = \frac{Vf}{Vr} = \frac{320}{35} \sim 9$$

Throughout filtration, measurements were done at 15 min time intervals during the first 3 h and in 30 min time intervals during the last 3 h.

Initial wastewater flux was 31.53 L/m^{2}h at 20˚C, while final flux was observed as 5.56 L/m^{2}h at 25.5 ˚C. When there was no longer significant change among the last three flux data, the filtration was stopped. It is seen that wastewater flux had a sudden decrease at the first 15 min in Figure 4.14. Then the rate of flux decline was getting slower, and a more gradual decrease was observed for the rest of filtration. Although filtration was done at room temperature, due to lack of temperature control of the membrane system, it was not possible to keep wastewater temperature constant. Thus, along with the decrease in flux, the temperature of wastewater increased evenly. After UF operation, chemical cleaning was performed for 30 min with clean water at pH 11 and 35 ˚C. Then the clean water flux was measured as 14.95 L/m^{2}h at 17.5˚C.

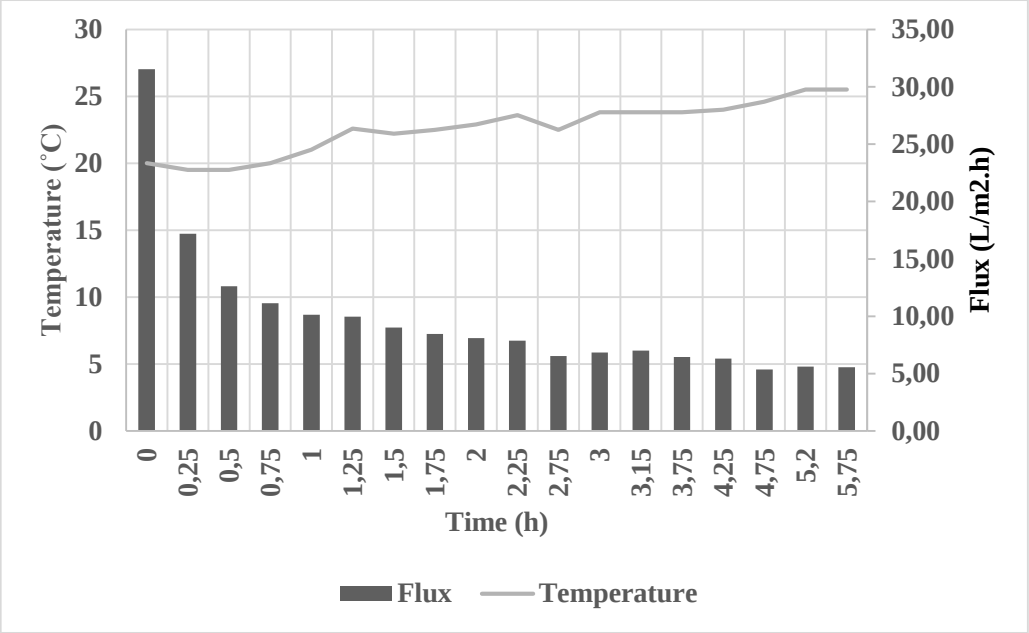

Figure 4.14. Changes in Flux and Temperature for Single UF Membrane Filtration (Set I)

During UF, the initial feed sericin concentration was 8040 mg/L; after 6 h this concentration was 12841 mg/L. While the rate of sericin retained in the membrane was 5% at the beginning, rejection of sericin reached 26-30% with the increasing concentration at the end of 6 h.

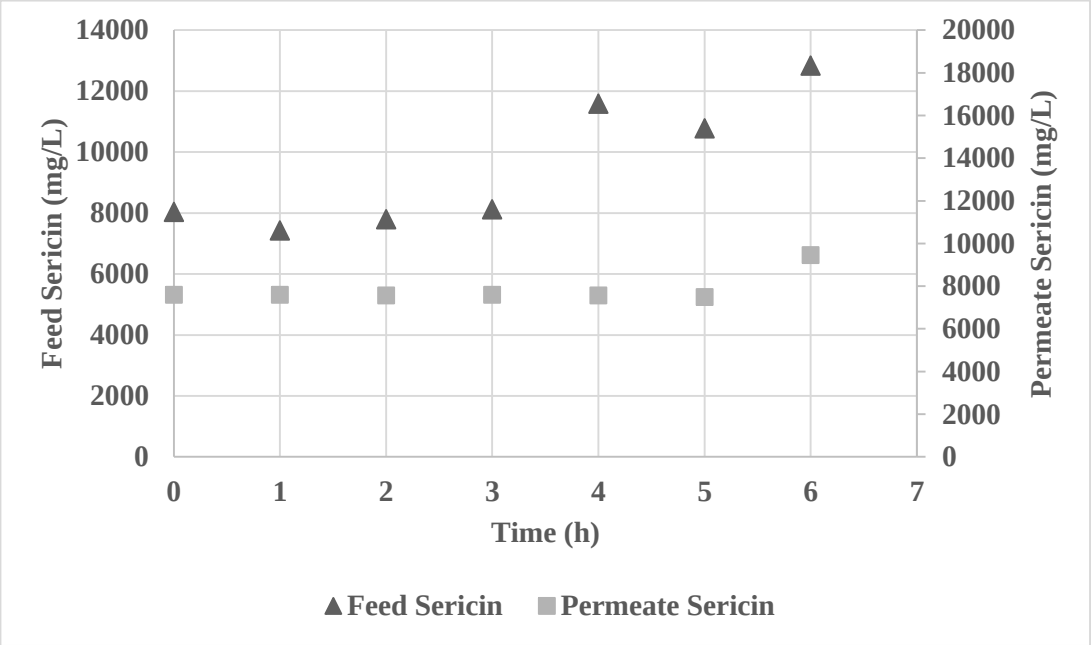

Figure 4.15. Sigle UF Sericin Concentration Graph (Set I)

COD feed and permeate values follow a parallel trend with sericin feed and permeate concentrations. As with the sericin concentration, the retention rate for COD is low for UF. Likewise, with the effect of fouling and the accumulation on the surface over time, COD retention rates increased.

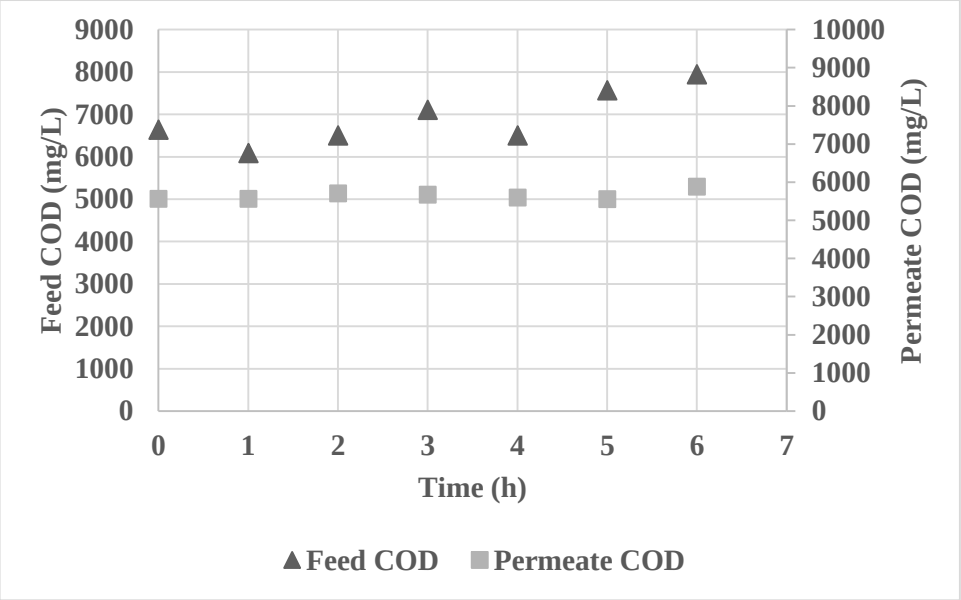

Figure 4.16. Single UF COD Concentration Graph (Set I)

4.3.1.2 Single UF (Set II)

UF module was run for 10 h in order to filter 540 L feed volume. After 10 h, while 54 L of feed volume was concentrated as retentate into the feed tank, 486 L was collected into the permeate tank. Then VRF was calculated as 10 by the following calculation:

$$VRF = \frac{Vf}{Vr} = \frac{540}{54} = 10$$

Measurements were done at 30 min intervals and results are given in Figure 4.17.

After UF, chemical cleaning was performed with water at 35˚C at a concentration of 200 ppm Cl, pH 10-11 for 20-25 min. After chemical cleaning, clean water flux was obtained as 19.57 L / m^2h at 23˚C.

In order to monitor the decrease in flux with respect to time through the filtration, these flux data were visualized in Figure 4.17.

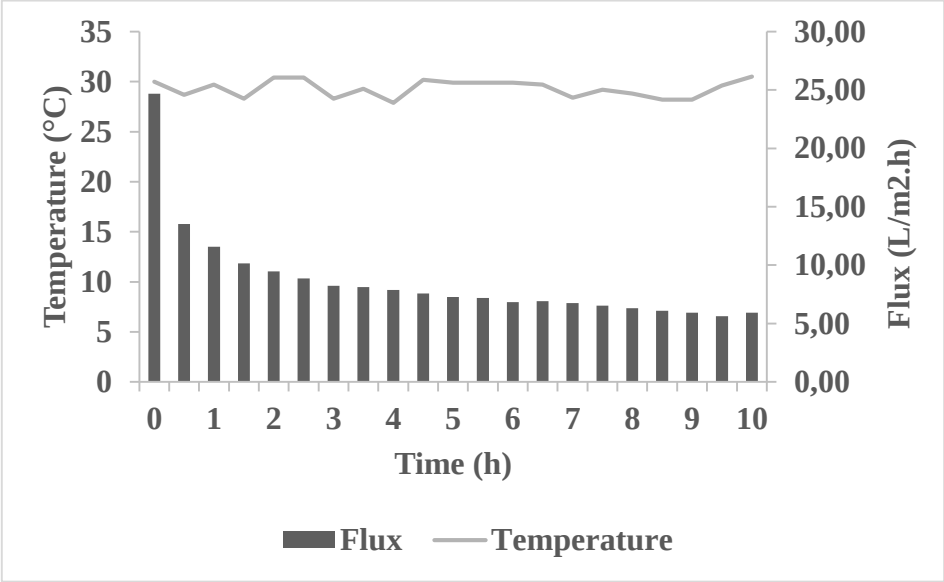

Figure 4.17. Changes in Flux and Temperature for Single UF (Set II)

In Figure 4.17, like Figure 4.14, at the beginning of the filtration, severe flux decline has been observed, then it decreased slowly till the end of filtration.

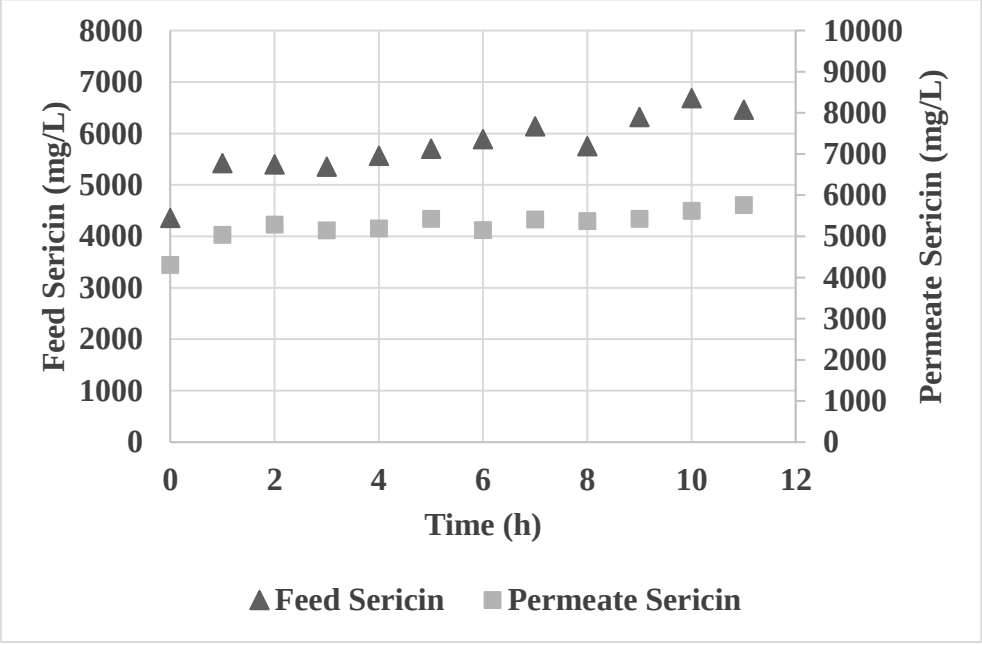

Figure 4.18. Single UF Sericin Concentration (Set II)

During UF, the initial feed sericin concentration was 4361 mg/L. After 11 h this concentration was 6466 mg/L. While the rate of sericin retained in the membrane was 1% at the beginning, after 11 h, rejection of sericin reached 10-15% with increasing concentration and fouling and cake formation.

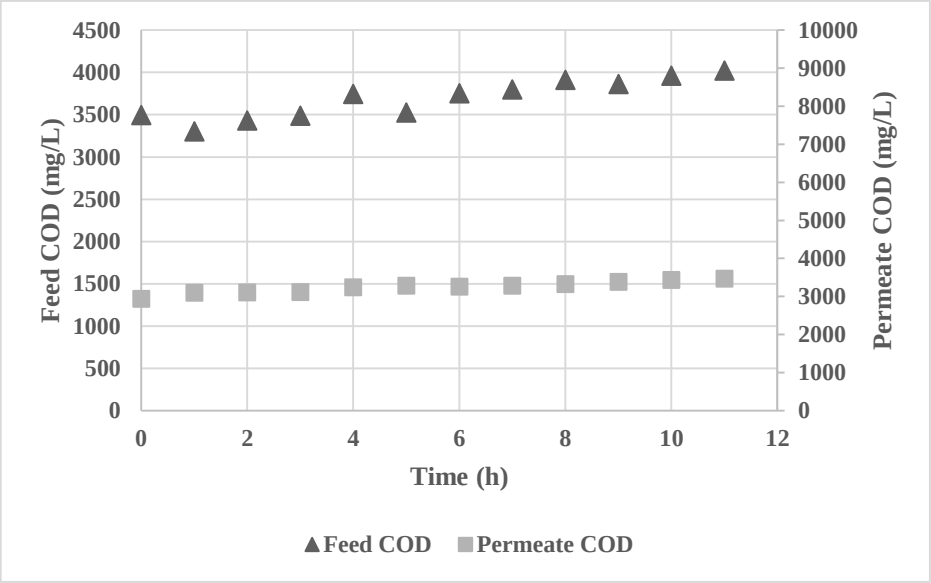

Figure 4.19. Single UF COD Concentration (Set II)

4.3.2 Results for UF+NF

In this part of the study, UF followed by NF was adopted. As for the previous case, experiments were repeated, and the results are given as Set I and Set II. Different volume of wastewater was filtered at different time intervals. Flux and temperature data was given separately for UF and NF in each operation while concentration data was given in tha same graph. Also, UF membrane filtration results of this part are same with the single UF operation.

4.3.2.1 UF+NF (Set I)

In this set of experiments, by using the permeate of UF as the feed of NF, sericin recovery was studied. The results belonging to UF in this part is the same as the single UF operation. UF plus NF membranes were used simultaneously, and they were run for almost 6 h and 3.5 h, respectively. While collecting UF flux data, measurements were done at 15 min time intervals during the first 3 h, and 30 min time intervals during the last 3 h. NF flux measurements were done at 15 min time intervals during the first 1 hour and continued with 30 min measurements.

UF was run for 6 h in order to filter 320 L feed solution. 285 L of this volume was filtered while 35 L remained as retentate. At this point, the Volume Reduction Factor (VRF) of the UF membrane was determined as 9.

NF started 3 h after starting of UF operation. Since the feed volume of the NF membrane was the permeate of the UF membrane, the NF membrane feed volume was 285 L. While 250 L of the feed was filtered, again 35 L of concentrated wastewater was obtained. VRF value was calculated as 8 for NF by the following formula:

$$VRF = \frac{Vf}{Vr} = \frac{285}{35} \sim 8$$

As shown in Figure 4.15, initial wastewater flux was 31.53 L/m^{2}h at 20˚C, while final flux was observed as 5.56 L/m^{2}h at 25.5 ˚C.

Figure 4.20 shows that initial wastewater flux is 12.45 L/m^{2}h at 22˚C while final flux was observed as 7.88 L/m^{2}h at 25.1 ˚C during NF operation. It is seen that wastewater flux decreases slightly opposite to the UF flux pattern. When the final and first wastewater flux is compared, 38% of the initial wastewater flux is lost. As same as UF, due to flux decline, wastewater temperature gradually increased.

After NF operation, chemical cleaning was done for 30 min with the clean water at pH 11 and 35 ˚C, then the clean water flux is measured as 14,82 L/m^{2}h at 17,4˚C.

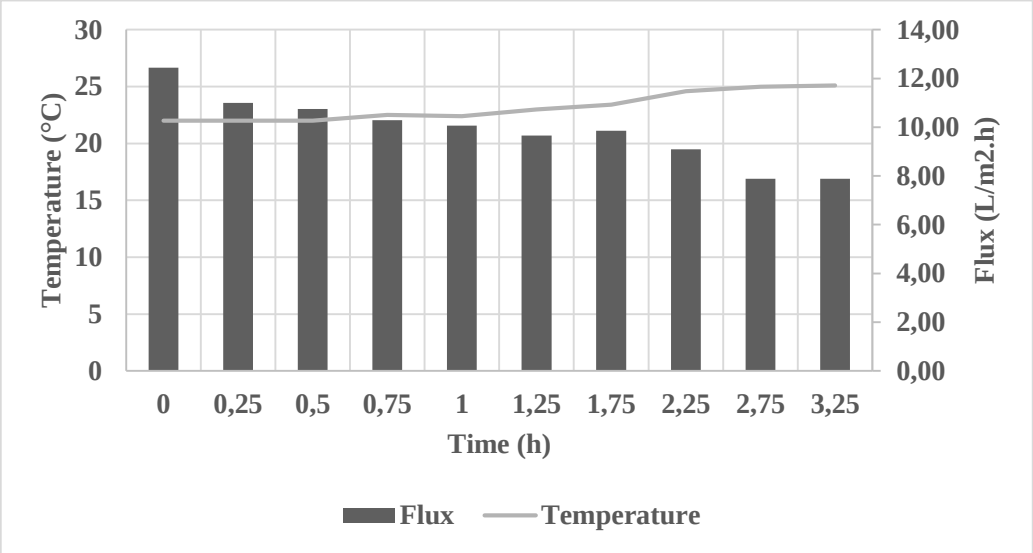

Figure 4.20. Changes in Flux and Temperature for NF Membrane Filtration (UF+NF Set I)

When UF and NF are compared, flux decline of NF membrane had more slightly decrease than UF membrane. Also, while UF membrane final wastewater flux dropped by 82% of the first wastewater flux, NF membrane final wastewater flux dropped only by 38% of the first wastewater flux. This shows that the UF membrane was clogged faster.

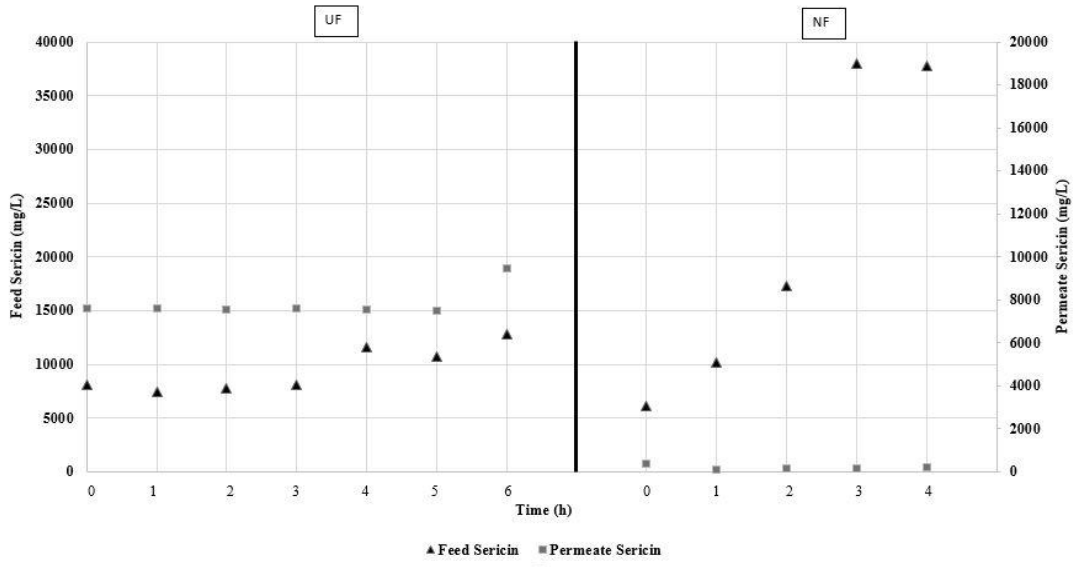

Figure 4.21. Change of Feed and Permeate Sericin Concentration (UF+NF Set I)

During UF, the initial feed sericin concentration was 8040 mg/L. After 6h this concentration was 12841 mg/L. While the rate of sericin retained in the membrane was 5% at the beginning, the rate of retention of sericin in the membrane reached 26-30%, with the increasing concentration at the end of 6 h.

During NF, the initial feed sericin concentration was 6171 mg/L; after 4 h this concentration was 37752 mg/L. While the rate of sericin retained in the membrane was 94% at the beginning, with the increased concentration at the end of 4 h, rejection of sericin in the membrane reached 99.5%.

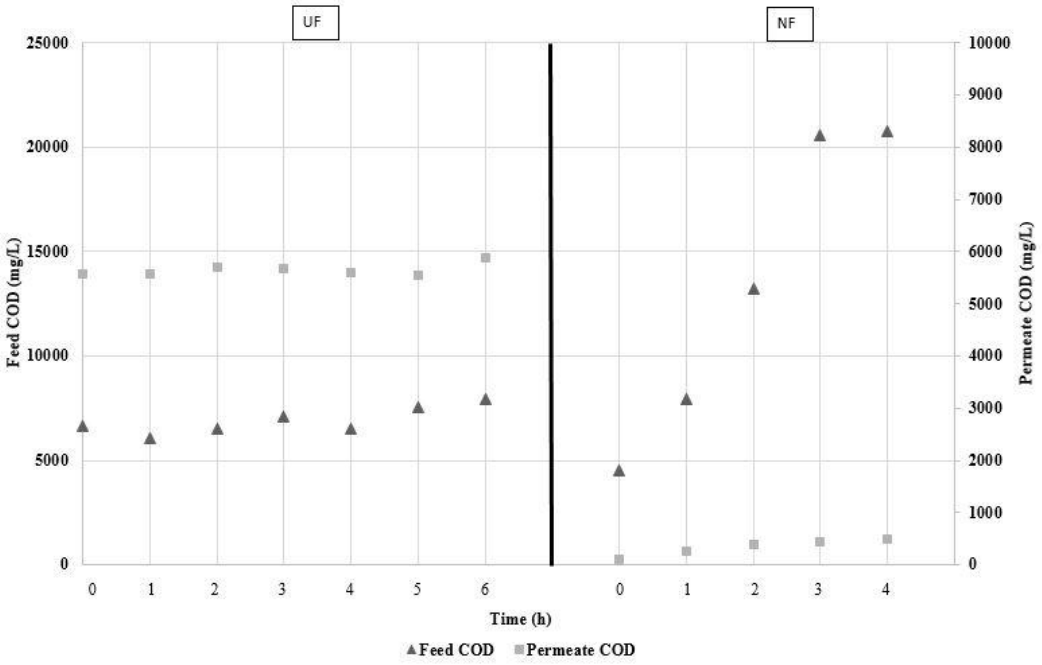

Figure 4.22. Change of Feed and Permeate COD Concentrations (UF+NF Set I)

COD feed and permeate values follow a parallel trend with sericin feed and permeate concentrations. As with the sericin concentration, the retention rate for COD was low for UF and high for NF. Likewise, due to fouling and cake formation over time, COD retention rates increased, including UF and NF.

4.3.2.2 UF+NF (Set II)

In this part of the study, UF was operated for approximately 10 h and NF was operated for 4.5 h. In total, 486 L were filtered through the UF membrane, while 54 L of concentrated wastewater was obtained (Volume reduction factor 10; 90% concentrate). A total of 436 L of wastewater was filtered through the NF membrane, while 50 L of concentrated wastewater were obtained (VRF of approximately 10; 89.7% concentrate). Measurements were made at 30 min intervals.

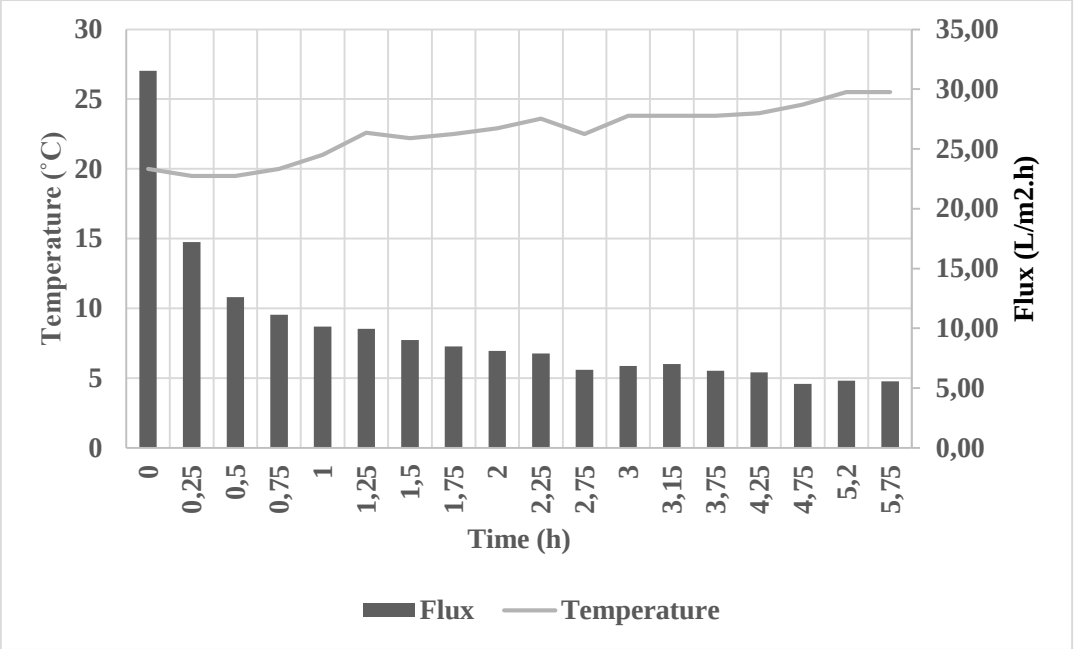

Figure 4.23. Pilot NF Flux and Temperature Graph for UF Membrane Filtration (UF+NF Set II)

After UF, chemical cleaning was done with water at 35 ˚C at a concentration of 200 ppm Cl, pH 10-11 for 20-25 min. After chemical washing, clean water flux was obtained as 19.57 L / m^2h at 33 ˚C.

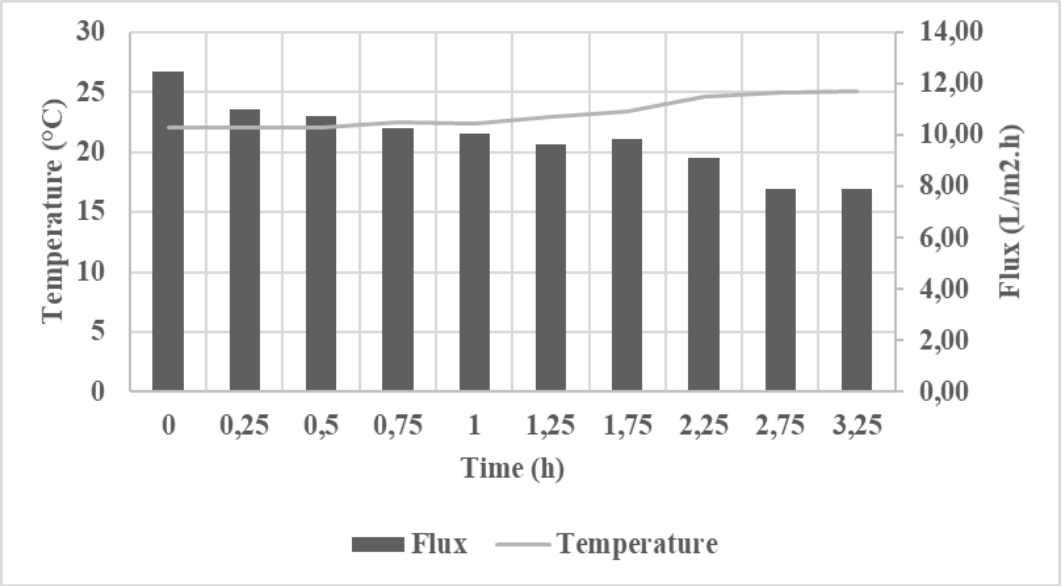

Figure 4.24. Figure 4.25. Change of NF Flux and Temperature (UF+NF Set II)

After NF, chemical cleaning was done with water at 35 ˚C at a concentration of 200 ppm Cl, pH 10-11 for 20-25 min. After chemical washing, NF clean water flux was obtained as 24.90 L / m^2h at 33 ˚C.

During UF, the initial feed sericin concentration was 4361 mg/L, After 11 h this concentration was 6466 mg/L. While the rate of sericin retained in the membrane was 1% at the beginning, after 11 h, the rejection of sericin in the membrane reached 10-15% due to increasing concentration and fouling.

During NF, the initial feed sericin concentration was 4220 mg/L, after 4 h this concentration increased to 14242 mg/L. While the rate of sericin retained in the membrane at the beginning was 90%, with the increased concentration at the end of 4 h, the rate of retention of sericin in the membrane reached 97%.

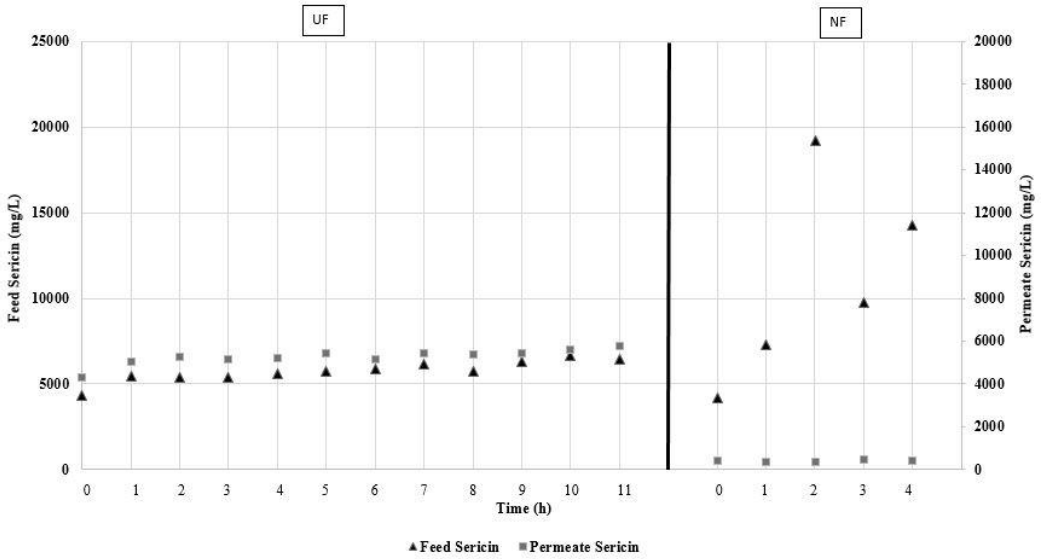

Figure 4.26. Change of Sericin Concentration (UF+NF SetII)

COD feed and permeate values follow a parallel path with the feed and permeate sericin concentrations such that the retention rate of COD was low for UF and high for NF. Likewise, with the effect of fouling and cake formation on the surface over time, COD retention rates increased due to the fouling of membrane over time an cake formation on the membrane surface, including UF and NF. UF permeate water tank was also used as NF feed tank. Not enough water had accumulated in the NF feed tank because the UF filtration rate was slower than the NF filtration rate. Therefore, NF had been paused for a while. Meanwhile, since the UF membrane continued to produce permeate, 19210 mg/L sericin remained in the NF feed tank, and the permeate water up to 11520 mg/L COD concentration was diluted with lower UF permeate water. This is reflected in the results.

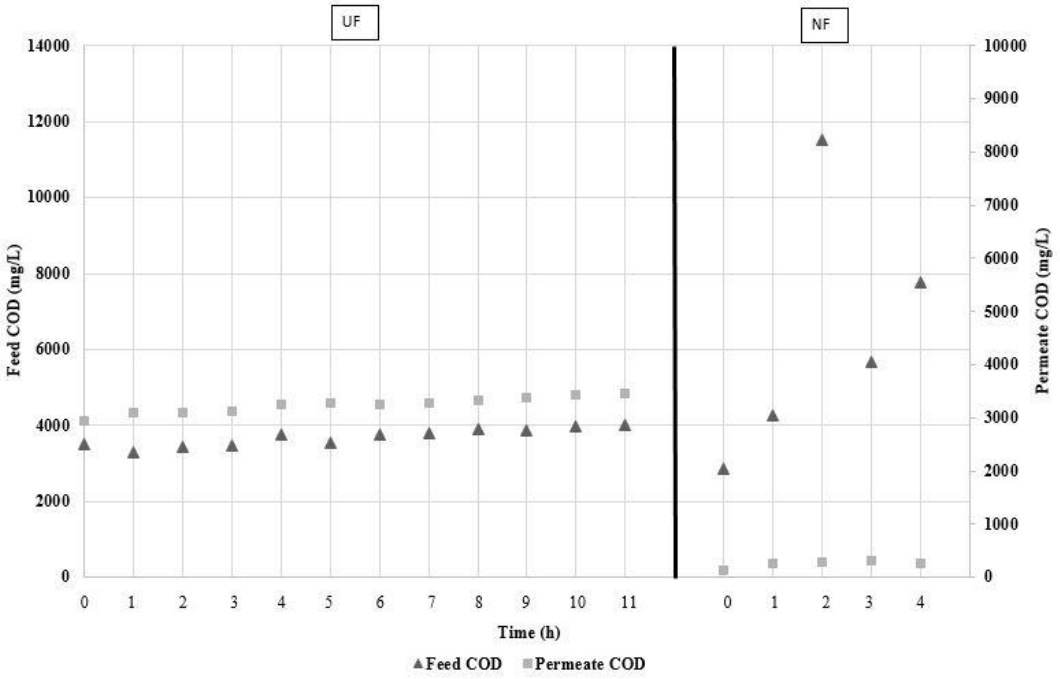

Figure 4.27. UF+NF Membrane Filtration COD Concentration (SetII)

4.3.3 Results for Single NF Membrane

In this part of the study, single NF membrane filtration was performed at 4 bar. The results were given as SetI, SetII and SetIII. Flux and rejection data were given.

4.3.3.1 Single NF (Set I)

In this study, single-stage NF was run for 5 h. In total, 374 L were filtered through the NF membrane, while 41 L of concentrated wastewater was obtained (90% concentrated). Measurements were made at 30 minutes intervals.

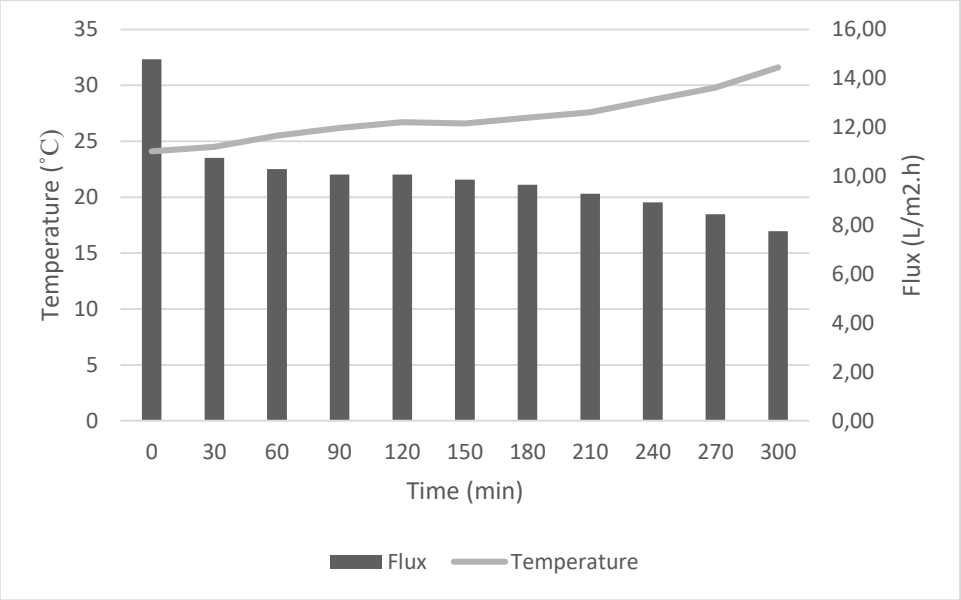

Figure 4.28. Change of NF Flux and Temperature (Set I)

After NF, chemical cleaning was done with water at 35˚C at a concentration of 200 ppm Cl, pH 10-11 for 20-25 min. After chemical cleaning, NF clean water flux was obtained as 15.26 L/m2h at 25.8˚C.

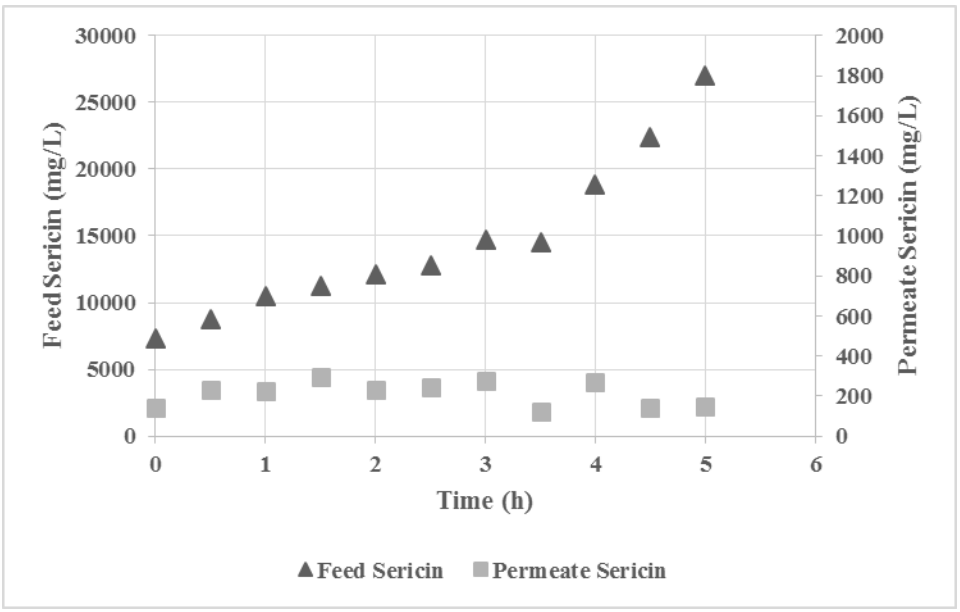

Figure 4.29. NF Feed and Permeate Sericin Concentrations (Set I)

During NF, the initial feed sericin concentration was 7288 mg/L, after 5 h this concentration increased to 27017 mg/L. While the rate of sericin retained in the membrane at the beginning was 98%, the rate of retention of sericin in the membrane reached 99.4% with increasing concentration after 5 h.

4.3.3.2 Single NF (Set II)

In this run, single-stage NF was operated around 6h, and a total of 380 L of wastewater was filtered through the NF membrane, while 40 L of concentrated wastewater was obtained (90.5% concentrated). Measurements were made at 30 minutes intervals.

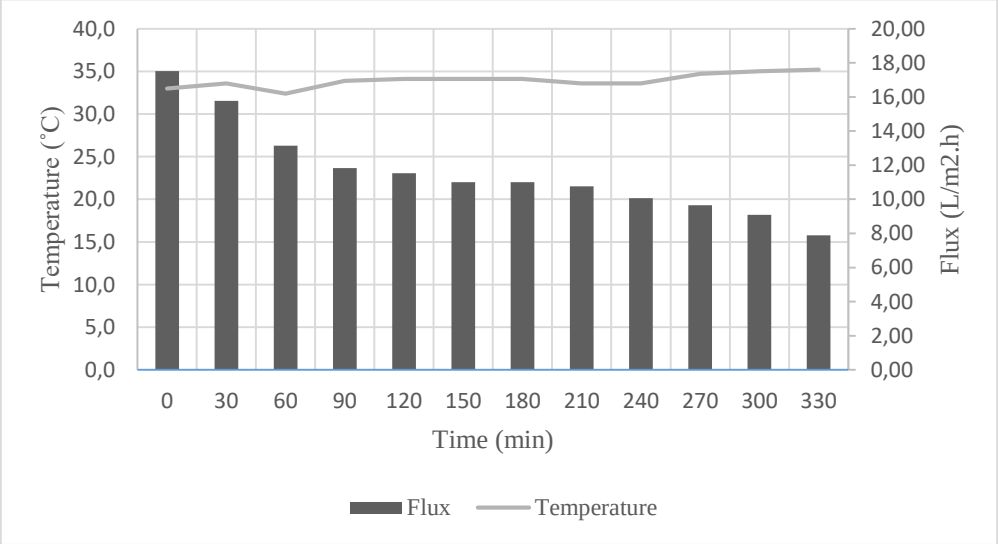

Figure 4.30. Change of NF Flux and Temperature (Set II)

After NF, chemical cleaning was done with water at 35˚C at a concentration of 200 ppm Cl, pH 10-11 for 20-25 min. After chemical cleaning, NF clean water flux at 33.7˚C was obtained as 21.50 L/m^{2}h.

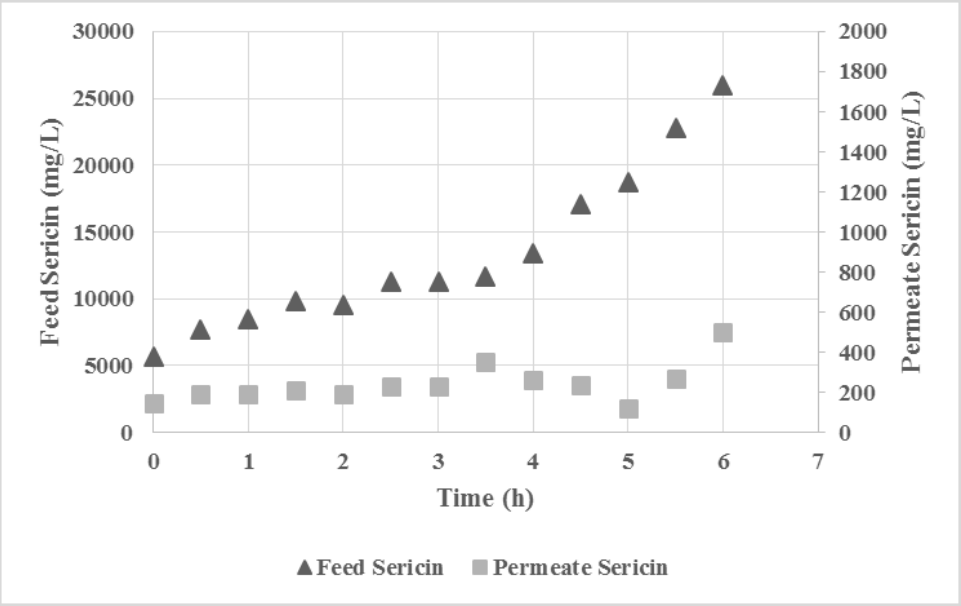

Figure 4.31. NF Feed and Permeate Sericin Concentrations (Set II)

During NF, the initial feed sericin concentration was 5679 mg/L, after 6 h this concentration increased to 25932 mg/L. While the rate of sericin retained in the membrane at the beginning was 97%, with the increased concentration at the end of 6 h, the rate of retention of sericin in the membrane reached 98%.

4.3.3.3 Single NF (Set III)

In this part of the study, single-stage NF was run for 15 h. In total, 900 L of wastewater was filtered through the NF membrane, while 90 L of concentrated wastewater was obtained (90% concentrated). Flux measurement was made at 30 minutes intervals.

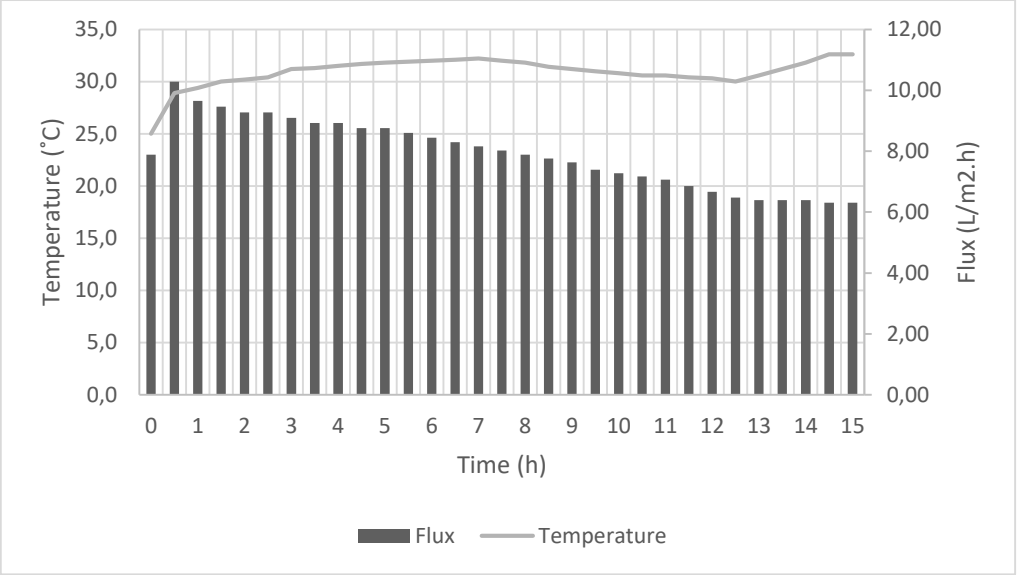

Figure 4.32. Change of NF Flux and Temperature (Set III)

After NF, chemical cleaning was done with water at 35˚C at a concentration of 200 ppm Cl, pH 10-11 for 20-25 min. After chemical cleaning, NF clean water flux was obtained as 16 L/m2h at 26 ˚C.

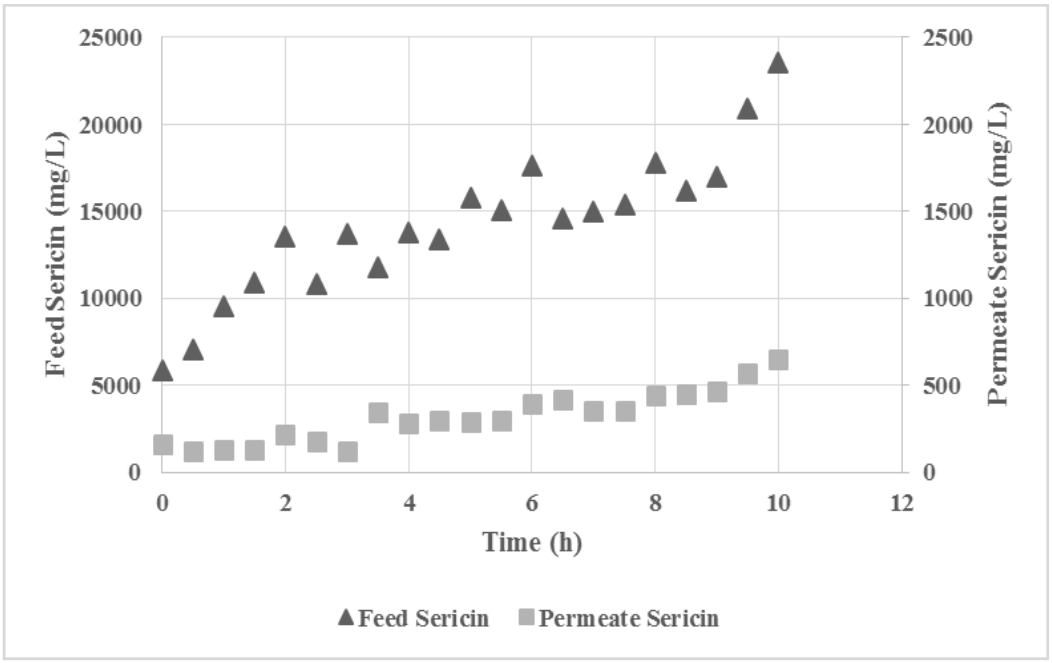

Figure 4.33. NF Feed and Permeate Sericin Concentrations (Set III)

During NF, the initial feed sericin concentration was 5839 mg/L, after 15 h this concentration increased to 23537 mg/L. While the rate of sericin retained in the membrane at the beginning was 97%, with the increased concentration at the end of 15 h, the rate of sericin retention reached 93%. This decrease in rejection rate can be caused by the opening of the pore size of the membrane after a long time operation.

4.4 Comparison of Laboratory and Pilot Scale Experiments

Table 4.8 is given to show a summary of sericin rejection values for each set of experiments. This table shows that, while rejection values of UF were not enough for sericin recovery, NF had a high rejection, so it is more suitable to use NF to recover a high amount of sericin and to minimize loss of sericin.

Table 4.8. Membrane Filtration Results

Filtration	**Operation Time**	**VRF**	**Sericin Rejection (%)**	**Sericin Rejection in Lab Scale (%)**
Single UF	6	9	30	70 (Ufx10pHt)
	10	10	15	78 (GR80PP)
UF+NF	6 + 3.5	9/8	30/99.5	91 (NF, after UF)
	10 + 4.5	10/10	15/97	
Single NF	5	10	99.4	-
	6	10	97	
	15	10	93	

Since the lab scale studies showed that the UF (10 kDa) membranes had good rejection rates. Then, pilot scale experiments were conducted with UF membrane as well. Also, it was proved that NF membrane increased the the rejection rates at a high level in lab experiments. Thus, it was decided that UF and NF membranes could be used as hybrid system to recover the sericin protein having different molecular weight and rejection rates could be increased by using NF prior to UF. However, the material of lab and pilot scale UF membranes were different, they showed different rejection behavior on lab and pilot scale studies. While the rejection rate of UF membrane at lab scale was around 70-78%, this rate was as low as 15-30%. This difference may be due to the change in the membrane characterization, as well as the inability to retain the low molecular weight sericin if the sericin has been degraded. Hence, in pilot scale studies, since the rejection of sericin was very low for UF membrane, it was not possible to recover significant amount of sericin having different MW. However, good rejection rates were obtained by using of NF membrane in pilot scale experiments, and this was the reason of continuning with single NF membrane filtration for long term operation.

When the pilot scale UF and NF membrane fluxes were compered, final wastewater fluxes of UF and NF membrane values were very near to each other. Although UF membrane has higher pore size than the NF membrane, UF membrane has lower final wastewater flux.UF membrane wastewater fluxes were between 5-6 L/m^2h while final wastewater fluxes of NF membrane were generally between 7-8 L/m^2h. This difference can be caused by two reason. One of them is that the NF membrane has backwash system and it cleans itself otomotically, but UF membrane module can not do that. Thus, at the end of the filtration process, NF has a higher wastewater flux as it gets rid of the substances that cause clogging on the membrane. Also, this wastewater flux values can be associated with the membrane characteristics. Membrane materials of UF and NF membranes were different than each other. This can be result in more clogging on UF membrane surface due to cake formation, concentration polarization etc. since each membrane material has its own filtration resistance.

In most of the cases, clean water flux was higher than the wastewater flux for both UF membrane and NF membrane filtration. This can be caused by the opening of pore size of membranes after cleaning since the chemical cleaning was done at 35˚C and with 200 ppm Cl.

In laboratory-scale experiments, the operation mode of filtration was total recycle mood of filtration (TRMF) and all mechanical and chemical cleaning procedures had been applied in the previous work packages of the project (Capar et al., 2018). Thus, resistances of membranes were calculated as shown in Table 4.9. However, in pilot scale, spiral membranes were not cleaned mechanically, and only chemical cleaning was applied. Also, operation of pilot-scale experiments was done as CMF, so the resistance of pilot-scale studies could not be calculated.

Table 4.9. UF 10 kDa Membrane Resistance in Lab-Scale Experiments

Resistance	**UFx10pHt (10^13/m)**	**GR80PP Resistance (10^13/m)**
R_m	0.1026	0.1636
R_t	0.2866	0.3017
R_m+R_p	0.1126	0.1540
R_p	0.0100	-0.0096
R_c	0.1739	0.1477

Resistance on membrane can be due to the membrane itself and fouling of membrane. Fouling of membrane could be originated from cake formation on membrane surface and pore clogging.

Resistances in Table 4.9 can be explained as:

R_m: Resistance of membrane

R_t: Total resistance, $R_t = R_m+R_f$

R_f: Resistance due to fouling of membrane, $R_f = R_p+R_c$

R_m+R_p: Resistance of membrane and resistance due to the pore clogging

R_p: Resistance due to the pore clogging

R_c: Resistance due to cake formation

In Table 4.10, Distribution of tatal resistance of 10 kDa UF membranes used in lab scale experiments was given. Highest resistance of Ufx10pHt membrane was due to the cake formation on membrane surface while that of GR80PP membrane was caused by membrane itself. Moreover, with these results, it was obvious that considerable clogging of pore did not occur for UF 10 kDa membranes used in lab scale experiments. Also, GR80PP membrane pore clogging resistance gave the negative result, and this could be due to the pore size openning after filtration.

Table 4.10. Distribution of Total Resistance

Resistance	**UFx10pHt (%)**	**GR80PP Resistance (%)**
Rm/Rt	35.8	54.2
Rp/Rt	3.5	-3.2
Rc/Rt	60.7	49

4.5 Ethanol-induced Precipitation and Lyophilization

In the pilot plant, 500-1000 L of wastewater was filtered through UF and NF membranes, and the wastewater was concentrated by 90%. A portion of concentrate between 50 and 100 L was brought to the laboratory and precipitation was done in ethanol. Subsequently, centrifugation and lyophilization were applied, approximately 150 grams of prototype powder sericin was obtained, as shown in Figure 4.35, from each 20 L of NF concentrate. The amount of powder sericin obtained was lower as the efficacy of the sericin was lower with UF. HPLC

chromatograms of sericin samples obtained from both UF and NF membranes were determined.

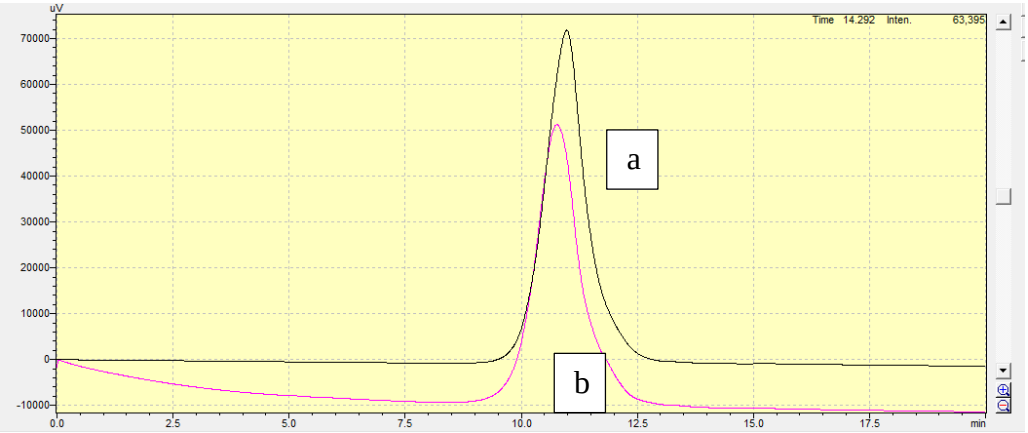

Figure 4.34. HPLC Chromatogram of Sericin Obtained from UF and NF Membranes in the Pilot Phase a) NF Sericin b) UF Sericin

Recovered sericin solutions and CCW sericin were sent to LC-MS/MS analysis at Acıbadem Labmed. The results show that sericin protein was detected from CCW, but it was not detected at recovered sericin from SDW. This difference between sericin from CCW and SDW can be caused by the SDW sericin was faced with more heat and pH fluctuation in recovery and degumming process. The more hydrophilic the proteins are, the more hydrolytic degradation is expected to take place. Sericin has hydrophilic properties due to the presence of several hydroxyl groups (Akturk et al. 2011). Thus, the proteins in SDW can be damaged during these processes. However, this SDW recovered proteins still protected their functionality. They were sent to functionality test to UNILEVER to be used in Elidor Shampoo with conditioner and recovered sericin gave a good result as much as the origins. Also, recovered sericin was used in synthesis of nanocomposite material and it was successful for this end-use as well (TUBITAK 114Y461, 2018).

This result can be explained as; sericin cocoon wastewater is only heat treated. In other wastewater, soap is removed from the thread and longer heat work is also

contained in alkali. It has also been subjected to many processes for recovery. Therefore, protein structure may be greatly altered / degraded. However, it has a program, it performs as well as the Elidor shampoo (with conditioner) when tested in shampoo. Again, it gave positive results in nanocomposite synthesis. The recovered samples are shown in Figure 4.38.

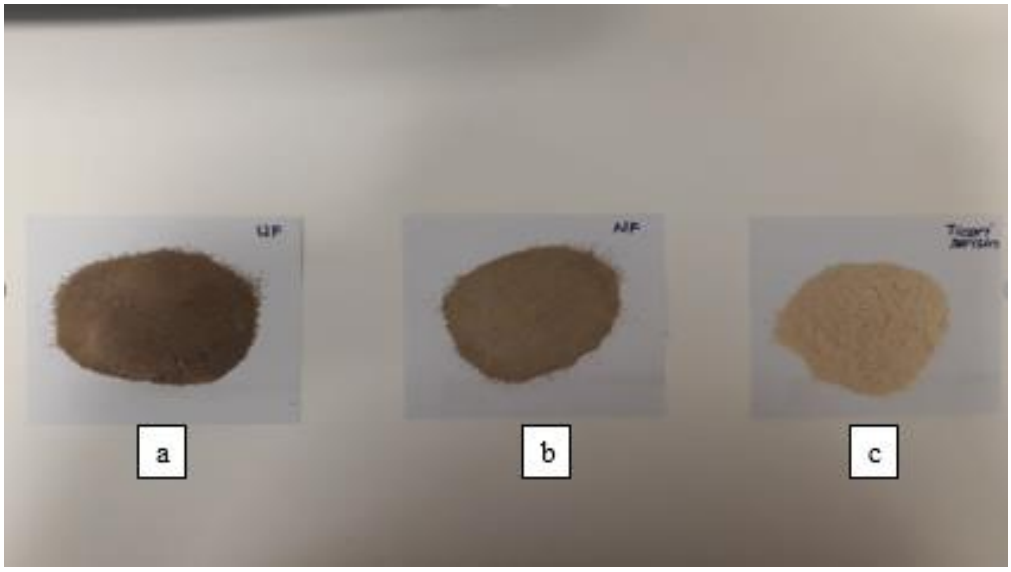

Figure 4.35. Recovered Sericin from a) UF, b) NF Membrane Filtration and c) Commercial Sericin

CHAPTER 5

CONCLUSION

Conventional silk degumming wastewater was used for sericin protein recovery. SDW was taken from Kirman İplik Factory in Bursa. Also, pilot scale studies were done in that factory by using wastewater directly in pretreatment and membrane filtration. Approximately 4 tons of wastewater is produced in the facility where pilot tests are applied.

It was concluded that when the soap to silk yarn ratio was 0.40, soap and sericin were separated in all values tested in the pH 3-4 range.

Recovery stages can be explained as pretreatment (pH adjustment, coagulation and flocculation) membrane filtration (single UF, UF+NF and single NF), ethanol-induced precipitation and lyophilization. In operation, UF membrane was rapidly clogged and NF membrane flux decreased slowly. Thus, NF membrane flux performance were better than the UF membrane and it was clear that for long time operation NF membrane works better, so 15 h operation was done with NF membrane only.

In terms of the sericin rejection NF membrane performance was better than UF membrane performance. While NF membrane rejection was around 90-100%, UF membrane had only 10-30% sericin rejection. Thus, sericin solution was more concentrated in NF feed. NF membrane feed tank sericin concentration reached 37000 mg/L whereas UF membrane feed tank concentration was around 6000 mg/L. This shows that sericin recovery is more feasible with NF.

The concentrate sericin was obtained in powder form via applying ethanol-induced precipitation and lyophilization.

CHAPTER 6

FUTURE STUDIES

The recovered powder sericin protein should be further characterized in terms of MW and amino acids. Since the flux and rejection rates of UF membrane used in pilot scale experiments were not high enough, filtration and rejection of sericin with another UF membrane can be conducted. Moreover, according to the results of pretreatment optimization trials, acidification can be done at pH 4 because at pH 4 all FO ratios between 0.25 and 0.40 provide phase separation.

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