Vol. 24 No.2 (Juli 2023) 258-263
e-ISSN 2548-6101 p-ISSN 1411-318X

Jurnal Teknologi Lingkungan

Journal Homepage: ejurnal.bppt.go.id/index.php/JTL

Protein Recovery from Tofu Whey Wastewater Using a Column Reactor: Preliminary Results

Pemulihan Protein dari Limbah Tahu Pekat Menggunakan Reaktor Kolom: Hasil Pendahuluan

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INFORMASI ARTIKEL

Histori artikel:
Diterima 28 April 2023
Disetujui 13 Juli 2023
Diterbitkan 31 Juli 2023

Kata kunci:
Protein
Tofu whey
Foam fractionation
Enrichment ratio
Column reactor

ABSTRAK

Industri tahu menghasilkan air limbah pekat atau whey (dadih), yang memiliki pH rendah dan bahan organik yang tinggi, sehingga dapat menyebabkan pencemaran lingkungan jika tidak diolah dengan baik. Di sisi lain, salah satu kandungan limbah tahu pekat yaitu protein dapat dimanfaatkan kembali untuk mengurangi potensi pencemaran lingkungan dan diolah lebih lanjut menjadi produk yang bernilai ekonomis. Penelitian ini bertujuan untuk mendapatkan data awal untuk menilai kelayakan penggunaan metode fraksinasi busa untuk pemulihan protein dari limbah tahu pekat. Limbah tahu pekat dalam penelitian kami memiliki konsentrasi protein awal 3.115 mg/l. Percobaan dilakukan dengan menggunakan reaktor fraksinasi busa berbentuk kolom dalam mode semi-curah. Kombinasi tiga laju alir udara (10, 30, dan 60 l/jam) dan tiga konsentrasi limbah tahu pekat (10, 50, dan 100%) diuji. Hasil percobaan menunjukkan bahwa konsentrasi protein awal mempengaruhi pemulihan protein, sehingga diperlukan konsentrasi protein awal sekitar 3.000 mg/l. Rasio pengayaan tertinggi 1,0 ± 0,5, setara dengan pemulihan protein 6 ± 4%, dicapai pada laju alir 10 l/jam dan konsentrasi limbah tahu pekat 100%. Peningkatan laju alir hingga 30 l/jam tidak mengubah rasio pengayaan (0,9 ± 0,2), tetapi meningkatkan pemulihan protein hingga 71 ± 14%. Penelitian lebih lanjut diperlukan untuk parameter-parameter lain seperti konsentrasi protein awal, komposisi limbah tahu pekat, laju aliran udara, ukuran gelembung, holdup cairan, dan desain reaktor.

ARTICLE INFO

Article history:
Received 28 April 2023
Accepted 13 Juli 2023
Published 31 Juli 2023

Keywords:
Protein
Limbah tahu pekat
Fraksinasi busa
Rasio pengayaan
Reaktor kolom

ABSTRACT

The tofu industry generates highly-polluted wastewater called whey, which has a low pH and high organic matter, therefore causing environmental pollution when not treated properly. On the other hand, one of the whey constituents, namely protein, can be recovered from the wastewater to reduce its environmental potency and to be further processed as a product with an economic value. This study aimed to obtain preliminary data to assess the feasibility of using the foam fractionation method to recover protein from tofu whey wastewater. Tofu whey in our study had an initial protein concentration of 3,115 mg/l. The experiment was performed using a column foam fractionation reactor in a semi-batch mode. Combinations of three air flow rates (10, 30, and 60 l/h) and three tofu whey concentrations (10, 50, and 100%) were tested. Our results show that initial protein concentration influenced protein recovery, with an initial protein concentration of approximately 3,000 mg/l required. The highest enrichment ratio of 1.0 ± 0.5, which corresponded to 6 ± 4% protein recovery, was achieved at a flow rate of 10 l/h and 100% whey concentration. Increasing the flow rate to 30 l/h did not change the enrichment ratio (0.9 ± 0.2), but increased the protein recovery to 71 ± 14%. Further investigation is needed for other parameters such as initial protein concentration, whey composition, air flow rate, bubble size, liquid holdup, and reactor design.

1. INTRODUCTION

1.1 Background

Soybean tofu is one of the most popular protein sources in Indonesia, with weekly consumption of 0.16 kg per capita and annual consumption growth of 3% (Karnadi, 2022). Tofu processing, as is common in the food industry, requires a large volume of water, in which up to six tons of water is required to produce a ton of tofu (Sintawardani et al., 2022). In the meantime, approximately 90% of the water ends up as wastewater.

There are two types of wastewater from the tofu industry: lightly-polluted wastewater from the soaking and washing processes and whey from the coagulation and pressing processes (Sintawardani et al., 2022). The whey has a low pH between 3.5 and 5.5 (Sintawardani et al., 2022; Sriwuryandari et al., 2019). In addition, whey contains 5–16 g/l organic matter as chemical oxygen demand (COD) (Faisal et al., 2014; Sriwuryandari et al., 2019), of which approximately 60% is easily biodegradable (Sintawardani et al., 2022). Therefore, tofu wastewater has a high potential for environmental pollution.

Of the organic content, tofu whey contains 8.5–11.3 g/l carbohydrate, 1.3–8.2 g/l protein, and 3.9–10.0 g/l lipid (Chua & Liu, 2019). Protein, as one of whey major constituents, is a major pollutant. Protein in wastewater gives an unfavorable carbon/nitrogen ratio for biological wastewater treatment, and its degradation results in the foul-smelling from ammonia and hydrogen sulfide gases (Chen et al., 2016). On the other hand, protein can be recovered from the wastewater. Recovery of protein from wastewater can provide two benefits, namely as a pretreatment before biological wastewater treatment and to be further processed as a product that has an economic value, e.g., emulsifier, foaming agent, adhesives, and bioplastics (Kumar et al., 2002; Sobral et al., 2018; Sorgentini & Wagner, 2002; Widyarani et al., 2019). Several methods have been investigated to separate and recover protein from wastewater, including foam fractionation, centrifugation, acid-base precipitation, coagulation-flocculation, ultrafiltration, electrochemical process, and adsorption chromatography (Gong et al., 2016). The main challenge of the process is the dilute stream, which requires the proteins to be concentrated or their solubility properties altered to enable separation from the liquid phase. Foam fractionation or adsorptive bubble separation is a method to concentrate protein and separate it from the liquid phase by adsorption to bubbles. The main mechanism underlying foam fractionation is that surface-active agents, such as proteins, are adsorbed on the surface of the bubbles generated by gas bubbles through the solution. The surface-active agent is carried upward by rising bubbles, which accumulate above the surface of the solution. Consequently, when the foam phase collapse, the protein solution remains and is recovered from the foam phase with a low liquid content (Du et al., 2000). This method is effective in recovering protein from solution due to the hydrophobic and hydrophilic properties of proteins. Foam fractionation had been applied to recover protein in whey wastewater from soybean protein isolate production at concentrations of 0.2–2.0 g-protein/l (Wang et al., 2013). A protein enrichment ratio of 10.5 and 82% protein recovery was achieved at the optimum condition of pH 7.0, 25 °C, 150 ml/min air flow rate, and a gas distributor of 0.180 mm diameter (Liu et al., 2017). On the other hand, the application of this process for tofu whey treatment is still limited.

1.2 Aims

This study aimed to obtain preliminary data to assess the feasibility of using the foam fractionation method for the recovery of protein from tofu whey wastewater. An experimental setup and semi-batch mode were applied. The results were compared with a similar process applied to other soy-processing and protein-containing wastewater in general to establish parameters that need further investigation.

2. METHOD

2.1 Materials

Tofu whey wastewater sample was collected from a small-scale tofu factory in Garut, West Java, Indonesia. The sample was stored at 4 °C until used in the experiment.

2.2 Experimental Setup

The experiment was performed in an acrylic cylindrical column reactor (Liu et al., 2017), as shown in Figure 1. The column (1,000 mm height, 46 mm inner diameter) was divided into two parts: the lower part for the tofu whey and the upper part for foam formation. The upper part was divided into four parts parallel to the column length using a 2 mm acrylic partition. The air was supplied with an air pump (Resun LP100 aerator) and the flow rate was regulated with a flowmeter. A 30 mm cylindrical airstone was used as a gas distributor.

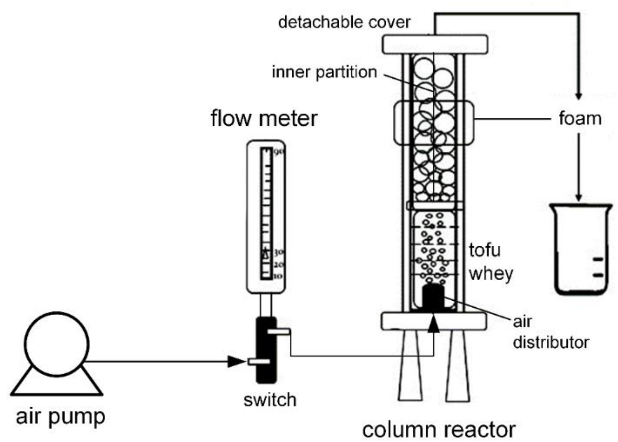


Figure 1. Reactor setup.

The experiment was performed in a semi-batch mode. Combinations of three flow rates (10, 30, and 60 l/h) and three tofu whey concentrations (10, 50, and 100%) were tested. Each process condition was performed in three replicates.

The lower part of the reactor was filled with tofu whey (620 ml). Air was channeled into the reactor for ten minutes; the time was determined in a preliminary experiment as the minimum time to form a stable foam (unpublished result). During the aeration, the foam was formed and expanded along the column until it overflowed and came out of the reactor outlet. After ten minutes, the aeration was stopped and the overflowed foam would collapse and be collected as the foamate (foam fraction). The foam that could not come out of the reactor also collapsed and was collected with the remaining whey left in the reactor as the residue fraction.

2.3 Analysis

The pH and total dissolved solids (TDS) were measured by the electrochemical method (EZ9908 multimeter). Turbidity was measured using the nephelometric method (Orbeco-Hellige 965). Chemical oxygen demand (COD) was measured using the closed reflux method (APHA, 2005). Protein concentration was measured using the modified Lowry method (Widyarani et al., 2019).

2.4 Calculations

The process performance was evaluated by volume recovery, enrichment ratio, and protein recovery. Each parameter was respectively calculated using equations (1), (2), and (3).

$$Volume\ recovery = \frac{V_f}{V_w} \times 100\% \quad (1)$$

$$Enrichment\ ratio = \frac{C_{foam}}{C_w} \quad (2)$$

$$Protein\ recovery = \frac{V_f \times C_f}{V_w \times C_w} \times 100\% \quad (3)$$

Note :
V_f = volume of fraction (foam or residue) after the aeration
V_w = initial volume of tofu whey
C_{foam} = protein concentration in the foam
C_w = initial protein concentration in the tofu whey
C_f = protein concentration of fraction (foam or residue) after the aeration

3. RESULT AND DISCUSSION

3.1 Tofu Whey Characteristics

The characteristics of tofu whey used in the experiment are presented in Table 1. The whey had an acidic pH (4.0), a general characteristic of tofu whey in Indonesia due to the use of fermented whey as a coagulant. Previous research reported TDS and turbidity of 1,184 mg/l and 680 NTU, respectively (Sintawardani et al., 2022), which were in the same order of magnitude as our findings. COD concentration was higher than the previously reported values of 7,676 mg/l and 7,000 mg/l (Sintawardani et al., 2022; Widyarani et al., 2019), but was still within the range reported in previous research.

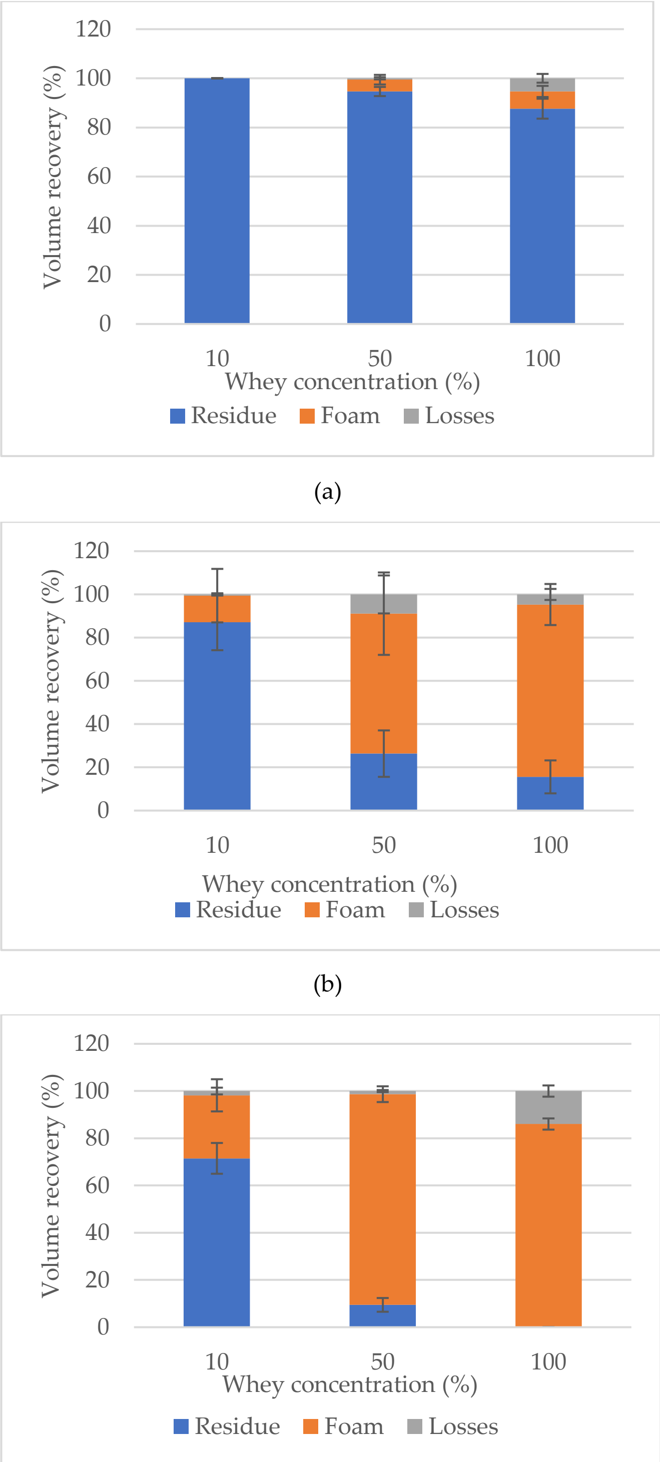
Table 1. Tofu whey characteristics.

Parameter	Unit	Concentration
pH	-	4.0
TDS	mg/l	1,420
Turbidity	NTU	640
COD	mg/l	8,640
Protein	mg/l	3,115

The protein concentration of tofu whey used in this study was 3,115 mg/l, similar to previous research. Assuming a conversion factor of 1.4 g-COD/g-protein (Widyarani et al., 2019), this concentration was equivalent to 4,361 mg-COD/l, which means that 50% of the organic matter in the tofu whey consisted of proteins.

3.2 Protein Recovery

In this study, protein recovery from tofu whey was tested using a column foaming reactor on three whey concentrations and flow rates. The results are presented in Figure 2, Figure 3, and Table 2.



(a)

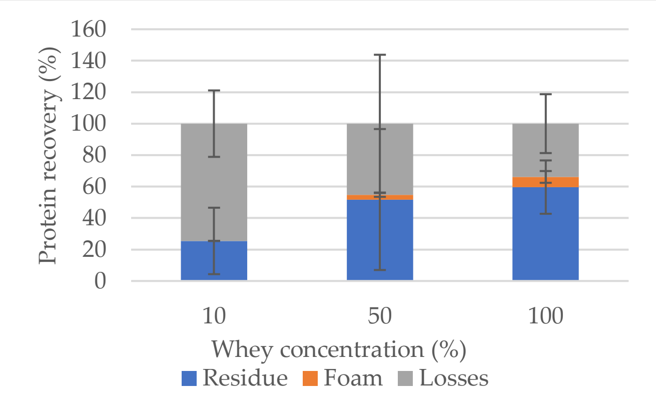
(b)

(c)

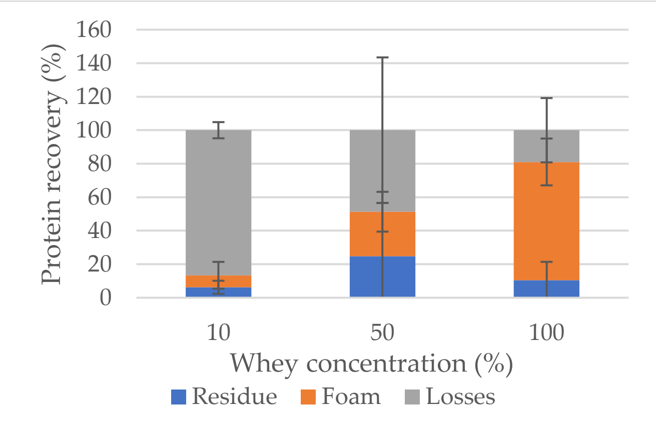
Figure 2. Volume recovery in residue and foam fractions at flowrates of (a) 10 l/h, (b) 30 l/h, and (c) 60 l/h.

Table 2. Protein concentrations in different fractions.

Flowrate (l/h)	Whey concentration (%)	Protein concentration (mg/l)			Enrichment ratio
		Initial	Residue	Foam	
10	10	344	87 ± 73	0	-
	50	1,587	859 ± 727	990 ± 213	0.6 ± 0.1
	100	3,115	2,107 ± 525	3,019 ± 1,605	1.0 ± 0.5
30	10	344	25 ± 17	300 ± 193	0.9 ± 0.6
	50	1,587	1,245 ± 1,307	745 ± 552	0.6 ± 0.3
	100	3,115	1,754 ± 1,066	2,795 ± 682	0.9 ± 0.2
60	10	344	14 ± 4	132 ± 100	0.4 ± 0.3
	50	1,587	1,211 ± 1,128	776 ± 383	0.5 ± 0.2
	100	3,115	0	2,069 ± 1,159	0.7 ± 0.4



(a)



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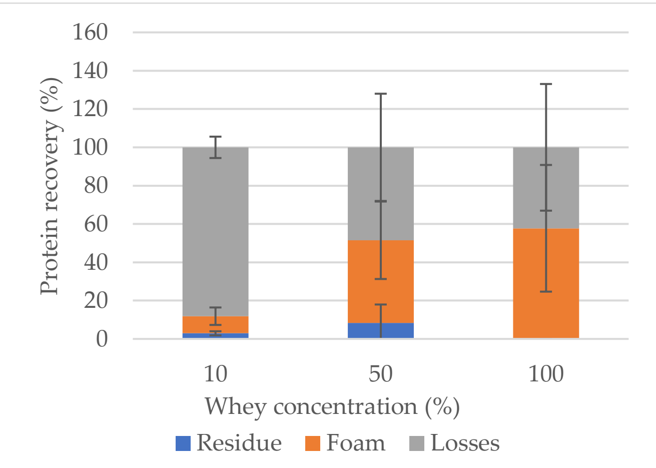

(c)

Figure 3. Protein recovery in residue and foam fractions at flowrates of (a) 10 l/h, (b) 30 l/h, and (c) 60 l/h.

The three whey concentrations used in this study were 10%, 50%, and 100%, which corresponded to protein concentrations of 344, 1,587, and 3,115 mg/l, respectively (Table 2). Figure 2 shows the volume percentage of tofu whey recovered in the foam and residue fractions. Foam formation increased with the increase of both flow rate and concentration. At the lowest flow rate (10 l/h) and concentration (10%), the whey did not form any foam, while at the highest flow rate (60 l/h) and concentration (100%), all whey went to the foam fraction and left no residue.

Foaming of the whey wastewater was expected to concentrate the protein in the foam fraction. As shown in Table 2, however, this could not be achieved with the current setup. The highest enrichment ratio of 1.0 ± 0.5, which corresponded to 6 ± 4% protein recovery (Figure 3a), was achieved at 10 l/h flow rate and 100% whey concentration. Increasing the flow rate to 30 l/h did not change the enrichment ratio (0.9 ± 0.2, Table 2) but increased the protein recovery to 71 ± 14% (Figure 3b). Even though volume losses were relatively low (Figure 2), Figure 3 shows large and varying protein losses of up to 88%. After the aeration, foam that could not come from the reactor should lose the trapped air and return to liquid form (residue). However, we observed that the retained foam was stable and stayed as foam for more than 30 minutes. The retained foam might be accounted for the protein losses.

3.3 Process Evaluation

Our results show that protein recovery in foamate increased with initial protein concentration. This is in line with previous research on whey from soybean protein isolate production, which reported that within initial protein concentrations of 200–2,000 mg-protein/l, protein recovery was proportional to initial protein concentration (Wang et al., 2013). Our results suggest that an even higher protein concentration of approximately 3,000 mg/l is required in foam fractionation. Lower concentrations yielded lower enrichment ratios and protein recovery. Unfortunately, we could not test tofu whey with higher concentration, therefore, our conclusion on the influence of protein concentration was limited.

Unlike the concentration, we tested three flowrates and observed that both 10 l/h and 30 l/h flowrates might be considered the optimum. Foaming of 100% whey concentration at 30 l/h yielded the highest protein recovery but also the highest foam volume, which was not ideal as this lowered the protein concentration and residue volume. On the other hand, even though foaming at 10 l/h yielded only 6%

protein recovery, the concentration factor was similar to 30 l/h. In addition, 34% protein was lost, presumably in the retained foam. Previous research indicated that increasing the air flow rate from 6 to 21 l/h increased the enrichment ratio but decreased protein recovery (Liu et al., 2017), suggesting that the flow rate should be optimized.

Proteins are surface-active molecules due to having both hydrophilic heads and hydrophilic tails. Proteins in tofu whey were identified as predominantly glycinin, basic 7S globulin, γ-conglycinin, and β-conglycinin (Stanojević et al., 2023), where the more abundant glycinin had more hydrophobic groups on the surface that contributed to higher adsorption of oil/protein on the interface (Riblett et al., 2001). The configuration of hydrophobic and hydrophilic groups contributes to protein agglomeration and drainage during foam fractionation (Li et al., 2017). On the other hand, foam drainage and holdup time are also influenced by the interaction between the foam-protein system and reactor surface, therefore, the use of a hydrophobic surface is envisaged (Liu et al., 2017).

Apart from protein, tofu whey also contains lipids (Chua & Liu, 2019). During foam fractionation, lipids will also interact and be adsorbed to the bubble surface. Therefore, high content of lipids might compete with protein and decrease protein adsorption (Du et al., 2000). One of the factors that might regulate lipid-protein adsorption is bubble size. In this study, we did not vary the pore diameter of the gas distributor, which should influence the bubble size (Liu et al., 2017). Further study is required to investigate the influence of tofu whey composition and bubble size on protein recovery.

Our experiment was performed in semi-batch mode, which resulted in unaccounted proteins (losses) that were not recovered in either foamate or residue. The continuous mode can also be applied for protein recovery (Li et al., 2018; Merz et al., 2011), and might be more suitable for field application.

4. CONCLUSION

In our experiment using a column foam fractionation reactor in semi-batch mode, the highest enrichment ratio of 1.0 ± 0.5 (6 ± 4% protein recovery) was achieved at 10 l/h flow rate and 100% whey concentration (3,115 mg-protein/l). Increasing the flow rate to 30 l/h increased the protein recovery to 71 ± 14%, but resulted in an enrichment ratio of 0.9 ± 0.2. Our results show that initial protein concentration influenced protein recovery, with an initial protein concentration of approximately 3,000 mg/l was required. Further investigation is needed for parameters such as initial protein concentration, whey composition, air flow rate, bubble size, liquid holdup, and reactor design.

ACKNOWLEDGEMENT

This research was funded by Riset dan Inovasi untuk Indonesia Maju (RIIM), National Research and Innovation Agency (No. 12/II.7/HK/2023) and Lembaga Pengelola Dana Pendidikan (LPDP) Republic of Indonesia. The authors acknowledge the facilities and scientific support from Advanced Characterization Laboratories of the National Research and Innovation Agency, Indonesia through E-Layanan Sains (ELSA).

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