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Effects of Enzyme Types on Cleaning of Coconut Milk Foulants and Properties of the Cleaning Effluents

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Abstract

Cleaning of foulants with enzymes has been considered more environmentally friendly compared to alkali cleaning. Cellulase was used to clean coconut milk foulants but the cleaning had to be done in two stages: cleaning by a surfactant followed by the enzyme. To simplify the enzymatic cleaning of coconut milk foulants, this work aimed to study effects of types of cellulase enzymes used on the cleaning efficiency. In addition, the work aimed to explore valuable components found in enzymatic cleaning effluents. Moreover, properties of the effluents, namely, TS, TSS, TDS, COD and pH were studied. The effluents from enzymatic cleaning had smaller TSS but significantly larger TS, TDS and COD compared to that from the cleaning using sodium hydroxide. However, the effluent from the cleaning by sodium hydroxide may be more difficult to treat, particularly when considering the pH of the effluent that was as high as pH 13. It was also discovered that all enzymatic cleaning effluents contained coconut oil, which could account for the high organic compounds in the effluents.

Keywords
coconut milk; enzymatic cleaning; clean-in-place; foulant; wastewater

Introduction
Coconut milk is a major food ingredient in Southeast Asia. Pasteurization of coconut milk is one process in coconut milk processing. During the pasteurization, coconut milk fouling, which is the accumulation of unwanted deposits on heat transfer surfaces, occurs. Issues with fouling include reduction of heat transfer, increased energy consumption, costs involved in cleaning, and possible contamination of the product. Cleaning costs do not only involve costs of chemicals, energy, and water used during the cleaning but also include loss of production during the cleaning downtime. Hence, an efficient cleaning method of foulants is vital. Coconut milk foulants is typically cleaned by alkali (sodium hydroxide: NaOH). NaOH is efficient in cleaning coconut milk foulants and cheap. However, wastewater of alkali cleaning is not environmentally friendly (Paul et al. 2014, Guerrero-Navarro et al. 2022). Recently, it has been shown that Cleaning-in-Place (CIP) of coconut milk foulants can be done by 2-staged cleaning using a surfactant followed by cellulase (Chutrakul et al. 2019). The cleaning efficiency was comparable to that obtained using NaOH. As the surfactant and the enzyme used in the work were biodegradable, the 2-staged cleaning proposed was claimed to be environmentally friendly. However, the properties of the cleaning effluents were not studied.

As cellulase cleaned the foulants via digestion of the foulants (Chutrakul et al. 2019), there could also be valuable components in the cleaning effluent, e.g., oil and proteins, which could be made into useful byproducts.

1.1 Objectives
This work aimed to study effects of cellulase enzyme types on cleaning of coconut milk foulants and components obtained in the cleaning effluents. Moreover, the qualities of effluents related to wastewater treatment from both the enzymatic cleaning and the cleaning using NaOH were investigated.

2. Literature Review
Coconut milk foulants contain 23wt% protein, 62wt% fat and 15wt% carbohydrate (Saikhwan et al. 2015). Because of the large content of oil, cleaning of coconut milk foulants using NaOH solution required large concentration of the solution (Saikhwan et al. 2015). Cleaning using NaOH is cheap but not environmentally friendly. There has been various research on cleaning milk foulants using enzymes (Pottchoff et al. 1997; Grasshoff et al. 2002; Paul et al. 2014). Cleaning of dairy fouling under real cleaning conditions has been studied and enzymatic cleaning could save water and energy and reduce the use of chemicals (Guerrero-Navarro et al. 2022). Cleaning of coconut milk foulants using enzyme has also been studied (Chutrakul et al. 2015). However, the cleaning efficiency obtained with using only enzyme in the cleaning was lower than that achieved using NaOH. This was because of the large fat content found in the foulants and hence a 2-staged cleaning using a surfactant followed by cellulase was adopted. This cleaning protocol showed slightly higher cleaning efficiency compared to the cleaning by NaOH at a lower temperature (50ºC vs. 70ºC). Although a single staged cleaning is preferable, the first stage using a surfactant was required to remove fat layers at the top of coconut milk foulants making carbohydrates in the foulants more accessible by the enzymes (Saikhwan et al.2022). To simplify the enzymatic cleaning of coconut milk foulants, an enzyme with higher performance or other ways to remove the fat layers should be considered.

Recently, many industries are adopting green production (GP) and green technology (GT) to reduce waste. Waste from the enzymatic cleaning of coconut milk foulants could also be reduced by recovering valuable products from the cleaning effluents. Based on the components of the foulants, oil and proteins are expected to be presence in the cleaning effluents. Oil from coconut milk foulants has been reported to appear like virgin coconut oil (VCO) which has a high value (Saikhwan et al. 2022). Proteins in coconut milk have been used extensively as a plant-based protein (Ng et al. 2015) and emulsifiers (Nor et al. 2017). Plant hormones (kinetin and zeatin) have also been found in skimmed coconut milk, the waste of VCO production (Ng et al. 2015).

3. Methods
3.1 Preparation of coconut milk foulants
Fresh grated coconut meat was purchased from local markets and coconut milk was extracted from 500 g grated coconut meat using 200 ml distilled water. This milk was preheated (in a beaker immersed in hot water at 50ºC) before being fed into a lab-scaled heat exchanger (Figure 1) to generate coconut milk foulants as reported by Saikhwan et al. (2015). The milk flow rate was 0.048 m/s. The heat exchanger operated co-currently with water as heating fluid (95ºC). At steady state, coconut milk temperature was at 70ºC and the process was continued for 20 minutes after that. At the end of pasteurization, coconut milk was drained out. Then sample plates with coconut milk deposits were collected and rinsed with RO water to remove any weakly bound deposits. In each fouling run, three sample plates with coconut milk deposits were generated and each plate was labelled by their positions in the heat exchanger (Figure 1(b)).

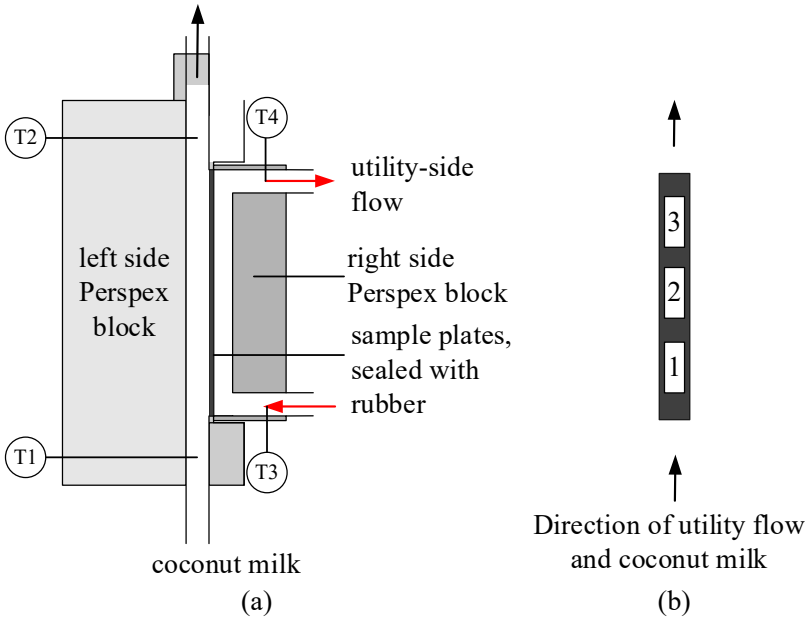


Figure 1. (a) Schematic of apparatus used in formation of coconut milk foulants (T1-T4 are thermocouples used for temperature monitoring); (b) positions of three sample plates placed between utility low and coconut milk flow.

3.2 Cleaning of coconut milk foulants

A cleaning experiment was conducted following the protocols reported by Paul et al. (2014). A sample plate with coconut milk foulants was fixed using an adhesive tape at the bottom of a glass bottle with a cover. 150 ml of cleaning solution was added to the bottle. Then the bottle was placed in an orbital shaker at 50 rpm for 4 hours. The plate was observed for cleanliness and the cleaning effluents were collected for further studies. Cleaning conditions used in this study are shown in Table 1. Two types of cellulase enzyme were used in this study, Cellic® Ctec2 and Celluclast® to investigate effects of the enzyme type on the cleaning efficiency. Using Celluclast® alone to clean coconut milk foulants in CIP was reported to be not efficient (Chutrakul et al. 2019). Hence, Cellic® Ctec2 which has a higher digestibility power than Cellulast® was used in this study. Concentrations of Cellic® Ctec2 were selected based on the manufacturer's instructions whereas concentrations of Celluclast® and a mixture of Celluclast® and Acalase were within the optimized concentrations reported by Chutrakul et al. (2019). All enzymes were obtained from Novozyme, Thailand and all chemicals were of analytical grades. It should be noted that a surfactant was not used in this study to avoid cleaning in two stages. It was expected that the flow patterns occurred in this set-up would be different from the flow patterns occurred in CIP and this could improve cleaning efficiency.

Table 1. Cleaning conditions used in this study.

Cleaning agent	Concentration (wt%)	Temperature (°C)
NaOH	1	70
Cellic® Ctec2	1, 2	50
Celluclast®	2, 3	50
Celluclast® mixed with Protease (Acalase)	4 : 2	45

3.3 Major components in cleaning effluents

The enzymatic cleaning effluents were tested for sugar protein and plant hormones. According to Ng et al (2015), kinetin and zeatin could be found in waste of coconut milk processing. These two plant hormones are high value products because they can be employed to stimulate the cell division in the human body. The test for sugar and protein were conducted using Benedict's solution and biuret. The test for plant hormones was by extracting the non-aqueous phase of the cleaning effluents in chloroform. Water in the extract was removed using magnesium sulphate. The

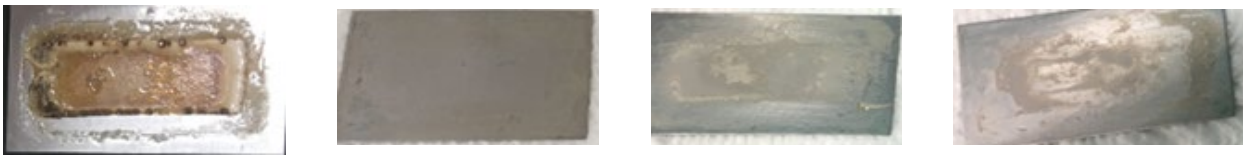
chloroform in the extract was then removed using a rotary evaporator and chloroform-D was added. Then NMR spectra of the extracts were obtained.

3.4 Properties of cleaning effluents

To investigate impacts on environment of the cleaning effluents both from enzymatic cleaning and the cleaning using NaOH, qualities of both cleaning effluents were determined in terms of TS, TSS, TDS, COD (closed reflux method) and pH. As from the cleaning studies, using Celluclast® mixed with protease gave the highest cleaning efficiency, only the cleaning effluent under this condition was studied for the wastewater properties.

4. Results and Discussion

4.1 Cleaning of coconut milk foulants



(a) (b) (c) (d)

Figure 2. Coconut milk foulants (a) before cleaning and after cleaning by (b) NaOH and by a mixture of Celluclast® and Protease (Acalase) at (c) 50 rpm for 4 hr and (d) 55 rpm for 2 hr.

Figure. 2 shows sample plates before cleaning and after cleaning. As the condition of the enzymatic cleaning that gave the best cleaning efficiency was when cellulase was mixed with protease (Celluclast® mixed with Acalase), only the sample plates after cleaning under this condition are shown in Figure 2. It can be seen clearly from the figure that the cleaning efficiency obtained from the alkali cleaning (Figure 2(b)) was higher than those obtained using enzymatic cleaning (Figure 2(c) and (d)). Nevertheless, the enzymatic cleaning efficiency could be improved by increasing fluid velocity used while cleaning; Comparing Figure 2(c) and (d), increasing the rotational speed used during the cleaning experiment slightly, cleaning efficiency increased significantly.

In addition, shorter cleaning time could be used. The results showed the possibility of using a single-stage enzymatic cleaning without the use of a surfactant. This will not only simplify the cleaning process but also decrease the amount of chemicals used. Moreover, oil in the cleaning effluents could be recovered more easily without the use of a surfactant. It should also be noted that with the set-up used in this experiment, the deposits could be sheared by the cleaning fluids in various directions. The set-up used in the work by Chutrakul et al. (2019), however, the deposits were sheared only in the direction along the plate's length parallel to the plate. Hence, the results in this work suggested that direction of cleaning fluids while cleaning could affect the cleaning efficiency. As the effects of the flow regime could affect the strength of a deposit (Zhou and Mattsson 2019), coconut milk foulants' strength along the direction of fluid used during pasteurization could be larger than in other directions. Further work must be conducted to confirm this.

4.2 Components found in cleaning effluents

Table 2 summarizes the test results for sugar and protein found in cleaning effluents obtained from various cleaning conditions. It was found that the enzymatic cleaning effluent Cellic® Ctec2 enzyme contained sugar whereas the effluents obtained by using Celluclast® had no sugar. Although both enzymes are cellulase, Cellic® Ctec2 is designed to digest cellulose to sugar. Celluclast®, however, is used in digesting cellulose to polysugar. It was also observed that there was more digesting of foulants when Cellic®Ctec2 was used. Nevertheless, the polysugar obtained when using Celluclast® might be used as a useful product because it could be polysaccharide which has been reported to have many uses, e.g. emulsifier, antibacterial, antioxidant (Nor et al. 2017; Bin 2010). Comparing benedict's solution mixed with the effluents from using Celluclast® (C2, C3) and Celluclast® mixed with Acalase (CP), it was found that the solutions associated with C3 and CP were clear blue whereas that from C2 was cloudy turquoise (Data not shown). This suggested that if low concentration of Celluclast® was used, there would be some suspended white particles not

digested by the enzyme.

Table 2. Summary of results of testing for sugar and protein in cleaning effluents

Sample code	Cleaning agent	Concentration (wt%)	Sugar	Protein
CC1	Cellic® Ctec2	1	✓	X
CC2	Cellic® Ctec2	2	✓	✓
C2	Celluclast®	2	X	✓
C3	Celluclast®	3	X	✓
CP	Celluclast® mixed with Acalase	4 : 2	X	✓

When extracting the non-aqueous phase of enzymatic effluents, it was found that the effluents obtained from using Celluclast® formed stable emulsions (no phase separation at room temperature of 30°C in 1 week) with chloroform. This is in accord with the assumption that there were polysaccharides in the effluents obtained from using Celluclast®. The observation also suggests possible use of the polysaccharides as an emulsifier. The use of polysaccharides have been reported in literature (Dickinson 1995; Dickinson 2009).

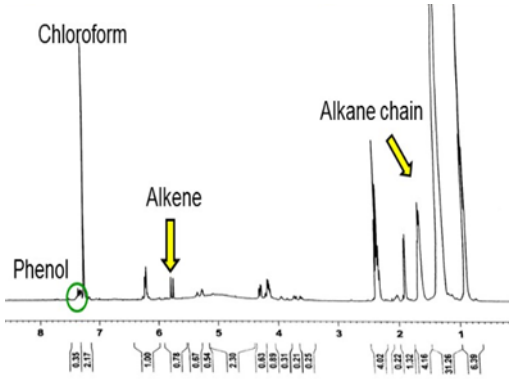


Figure 3. NMR spectroscopy (1-Dimension) of the non-aqueous phase of one sample of the enzymatic effluent

Plant hormones (kinetin and zeatin) that have been found in the waste of VCO production (Ng, et aL2015), were not discovered in any enzymatic cleaning effluents (Figure 3). However, a peak corresponding to phenol was observed from the NMR results (Figure 3) which suggests the presence of phenolic compound generally found in VCO (Marina et al. 2009; Naczk and Shahidi 2004).

4.3 Properties of cleaning effluents

Table 3 shows properties of wastewater samples obtained from the enzymatic cleaning (Celluclast® mixed with protease) and the alkali cleaning. According to the table, TS, TDS and COD of the effluent from the enzymatic cleaning were larger than those observed with the effluent from the alkali cleaning. TSS, however, was lower in the wastewater from the enzymatic cleaning. This could be explained by the mechanisms involved in the enzymatic cleaning where enzymes digest cellulase to polysugar and amino acid which both can dissolve in water. The differences in cleaning mechanisms may also explain why TDS was larger in the effluent from the enzymatic cleaning. The larger TDS was also due to the presence of fat in the enzymatic cleaning effluent; the fat was observed as oil during the evaporation of water during the TS measurement.

Table 3. Properties of wastewaters from enzymatic cleaning (Celluclast® mixed with protease) vs. from alkali cleaning

Properties	Enzymatic cleaning	Alkali cleaning
TS (mg/L)	31,831	15,493
TSS (mg/L)	104	186
TDS (mg/L)	31,727	15,307
COD (mg/L)	29,600	837
pH	4.53	13.40

The presence of fat was observed before when the effluent was measured for plant hormones and this fat could dissolve in the effluent due to the emulsifying property of the effluent. It should also be noted that due to the emulsifying property of the enzymatic cleaning effluent, it was not possible to centrifuge to separate the fat out before the measurement of TS.

The larger value of COD observed with the enzymatic cleaning effluent was not unexpected as there would be more organic compounds, e.g. fat, protein and polysaccharides, in the enzymatic cleaning effluent than in the alkali cleaning effluent. However, it might be more difficult to treat the alkali cleaning effluent due to its high pH and a large amount of inorganic salts. The latter was reported to cause high salt load on the environment affecting aquatic life (Grasshoff, 2002; Paul et al. 2014). If the organic compounds found in the enzymatic cleaning effluent are removed to make useful products, COD of the effluent could also be lowered. Further work should be conducted to explore this possibility.

5. Conclusion

The two cellulase types, Cellic® Ctec2 and Celluclast® gave similar cleaning efficiencies. However, they resulted in different compounds in the effluents. Sugar was found when Cellic® Ctec2 was used whereas polysaccharides were found with Celluclast®. From the results, polysaccharides could be used as an emulsifier. There was no plant hormones found in any effluents studied. However, the presence of phenolic compounds normally associated with VCO, was observed in all the enzymatic cleaning effluents. Since there have been reported several uses of polysaccharides and benefits of phenolic compounds found in VCO, the enzymatic effluent could be converted into useful products.

Using a mixture of Celluclast® and Acalase gave the highest cleaning efficiency. Cleaning results also suggested the possibility of using a single stage using this mixture of enzymes to clean coconut milk foulants by increasing the cleaning fluid speed. Direction of flows could also play an important role in the cleaning efficiency; this work suggested that having various directions of shear forces could improve cleaning performance.

Considering water properties of the effluent from the enzymatic cleaning (Celluclast® mixed with protease) and the effluent from the alkali cleaning, TS, TDS and COD of the enzymatic cleaning were larger. This could be due to the presence of coconut oil and polysaccharides in the enzymatic cleaning effluent. Hence, a method to remove the oil and/or polysaccharides from the effluent should be considered in the treatment of the water from the enzymatic cleaning effluent. Nevertheless, the lower pH and smaller concentrations of salts in the enzymatic cleaning effluent suggested that the effluent could be treated and could have less effects on the environment.

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Biography

Thamonwan Noppirun received a bachelor's degree in chemical engineering from Thammasat English of Engineering, Thammasat University. This paper is based on her senior project. She currently works for Standard Chartered, Thailand.

Phanida Saikhwan graduated from Cambridge University. She has been a lecturer at department of Chemical Engineering, Thammasat School of Engineering, Thammasat University since 2008. She is currently associate professor of chemical engineer. Her work focusses on fouling and cleaning and extraction of bioactive compounds, particularly from agricultural waste and byproducts.