Lactic acid fermentation of waste milk

Hideaki NAGARE[1)], Teppei MORITA[2)], Satoshi AKAO[3)], Pham Thi Minh Duc[4)]

1) *Graduate school of Environmental and Life Science, Okayama University*
2) *Faculty of Environmental Science and Technology, Okayama University*
3) *Graduate School of Engineering, Tottori University, Japan*
4) *Da Nang Environmental Technology Center, Institute of Environmental Technology, VAST, Vietnam*

ABSTRACT

We have been developing a treatment process for waste milk with a concept of resource recovery. In this process, casein, the main protein in milk, is to be precipitated by coagulation, and lactose, the main carbohydrate in milk, is to be fermented to lactic acid. Since a prototype of the process showed lower lactate yield of 26% than we expected last year, we studied the fermentation reactions to improve the yield. Although the reason for the low yield was not clearly specified, possible fermentation products such as amino sugars and monosaccharides were found.

KEYWORDS

Waste milk, resource recovery, lactic acid fermentation, capillary electrophoresis

INTRODUCTION

Wastewater from milking parlor is one of the pollutant sources in agricultural area because of its high organic content. It usually contains washing effluent from milker, the milking system, and also wasted milk which is disposed because it was taken from diseased cows and containing medicine for treatment. The wasted milk has extremely high organic content (BOD concentration bout 120 g/L) which is about 600 times higher than domestic sewage, so that the discharge of the milk gives severe environmental impact even with small amount. However, treatment facility does not widely used especially to small farmers because of cost.

We have been developing a new treatment process of waste milk with a concept of resource recovery, so that farmers can obtain benefit from the materials they recovered from the process. Last year, a prototype of the process was developed, and treatment efficiency was studied. However, the lactic acid yield was only 26% that was much lower than we expected. Akao et al. obtained nearly 80% of yield from both glucose and galactose, the monosaccharides composing lactose, by *Bacillus* *coagulans* JCM2258 (Akao et al., 2010). Then, we studied the fermentation reactions to improve the lactic acid yield.

MATERIALS AND METHODS

1. L-lactic acid fermentation

Commercial milk was diluted 10 times with distilled water and then supplied for coagulation. pH of the diluted milk was adjusted to 4.5 by adding HCl so that casein in milk precipitated to be removed. A 100 mL of the supernatant after coagulation was supplied to L-lactic acid fermentation (LAF). After adding 2 g of east extract as the nitrogen source and 1 mL of *Bacillus coagulans* JCM2258, the supernatant in a grass reactor was incubated at pH5.5 and 55 degree for four to five days (Maeda *et al*., 2011).

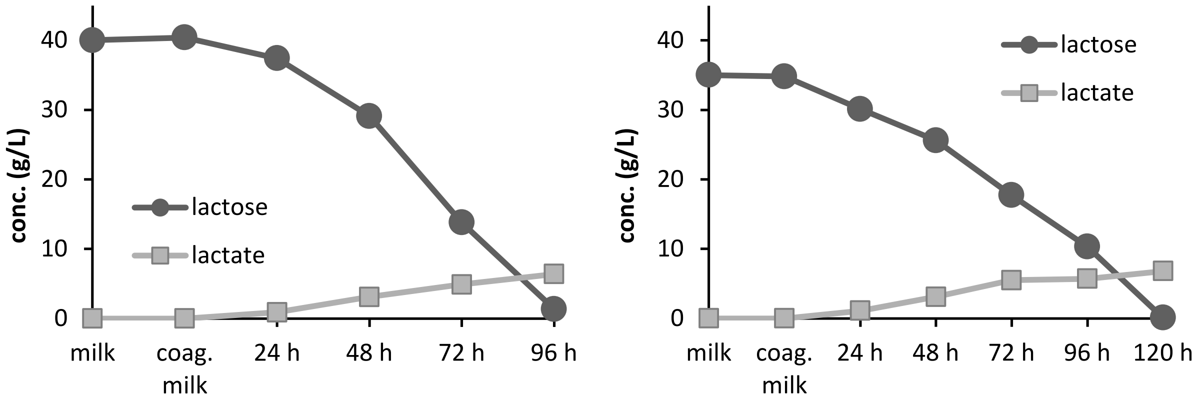
2. Analysis of contents

Coagulated milk or fermented broth was filtered through 0.45 μm syringe filter (Sartorius Minisart RC25) and then used for analysis. Dissolved organic carbon (DOC) was measured by TOC analyser (Shimadzu TOC-5000A). Concentrations of lactose, lactate, glucose and galactose were measured with an enzymatic analysis kit by R-Biopharm. Capillary electrophoresis was applied for the comprehensive analysis of compounds in broth. In the analysis was used the method developed by Soga and Ross (1999), in which simultaneous determination of inorganic anions, organic acids, amino acids and carbohydrates can be performed. CE experiments were performed using a Beckman Coulter P/ACE MDQ system which comprises a CE unit with built-in diode-array detector and and software for system control, data collection and data analysis.

RESULTS AND DISCUSSION

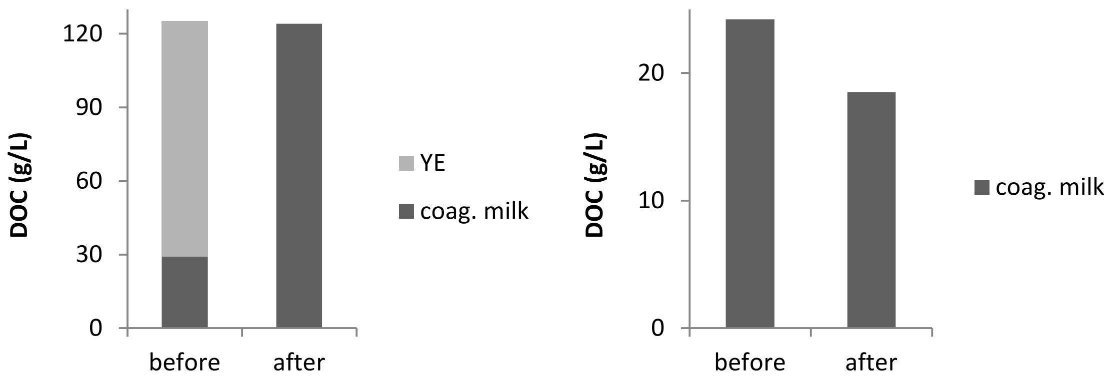
During the lactic acid fermentation, lactose was transformed into lactate as shown in Fig. 1 in both cases of with and without yeast extract. However, the yields of lactate were 16 and 19%, respectively.

Since yeast extract supplied as nitrogen source had large DOC as shown in Fig. 2(a), DOC balance during the fermentation was discussed in the case of without YE shown in Fig. 2(b). DOC of coagulated mil, the fermentation substrate, was 24.2 g/L of DOC, while it decreased to 18.5 g/L after fermentation. The difference accounted for 25% of the initial DOC, indicating that a portion of lactose was used for respiration and cell production, but the amount was not significant.



(a) with YE (b) without YE

Fig. 1 Concentration changes of lactose and lactate during LAFs. Two cases of fermentation were studied: with and without yeast extract (YE) as nitrogen source.



(a) with YE (b) without YE

Fig. 2 Changes in DOC during LAFs.

Since 75% of DOC was conserved in the reactor, we continued the investigation of the fermentation products other than lactate. Since lactose is a disaccharide from D-glucose and D-galactose, those monosaccharides might have been accumulated as the enzymatic hydrolysis products. However, neither of them was found throughout the fermentation as shown in Fig. 3.

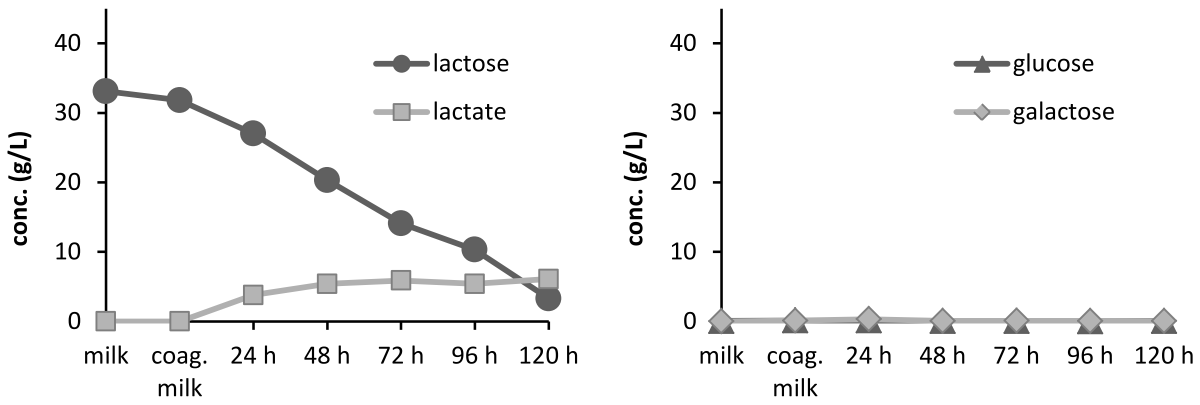


Fig. 3 Concentration changes of carbohydrates during LAFs.

Then, the components in fermentation broth were comprehensively analyzed by capillary electrophoresis (CE) based on Soga and Ross (1999). With this method, large number of substances including inorganic anions, organic acids, amino acids and carbohydrates can be measured in one analysis.

The electropherograms obtained in CE analysis were shown in Fig. 4. There were no obvious peaks after 20 minutes, although sugars including lactose, glucose and galactose were to be observed in this span based on Soga and Ross (1999). This indicated that lactose was decomposed into other organics other than glucose and galactose. Lactate should have shown the peak around 15.5 minutes. There were other two peaks at 10 and 17 minutes in the figure: from Soga and Ross (1999) organic acids appear between 9 to 13 minutes, while free amino acids have peaks around 13 to 20 minutes. This suggests that an organic acid and a free amino acid might have produced during the fermentation.

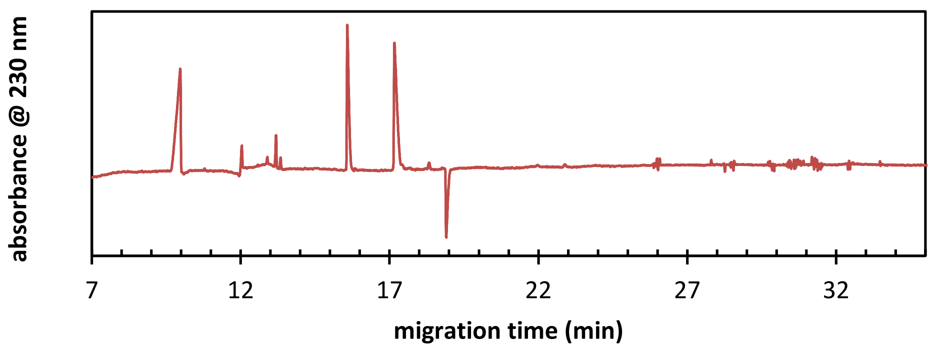


Fig. 4 Electropherograms of filtered fermentation broth.

CONCLUSIONS AND PERSPECTIVES

In this research, lactic acid fermentation from wasted milk was studied for improvement of lactate yield. Although the reason for the low yield was not specified, possible fermentation products such as an organic acid and a free amino acid were found. We hope that through the identification of those compounds the lactate yield can be improved.

REFERENCES

Akao, S., Sakae, Y., Hosoi, Y., Nagare, H., Maeda, M., Fujiwara, T.: Fermentation of Lignocellulosic Biomass Derived Sugars to L-Lactate by Thermophilic Bacillus coagulans, 12th World Congress on Anaerobic Digestion, 31 October-4 November, 2010, Guadalajara, Mexico.

Maeda, K., Akao, S., Hosoi, Y., Nagare, H., Maeda, M. and Fujiwara, T.: Comparative Evaluation of Different Pretreatment Methods for Enzymatic Saccharification of Lignocellulose, *Journal of Japan Society of Civil Engineering*, Ser. G, 67(7), III_433-III_440 (2011).

Soga, T. and Ross, G.A.: Simultaneous determination of inorganic anions, organic acids, amino acids and carbohydrates by capillary electrophoresis, *Journal of Chromatography A*, 837, 231–239 (1999).

Lactic acid fermentation of waste milk

Hideaki NAGARE[1)], Teppei MORITA[2)]
Satoshi AKAO[3)], Pham Thi Minh Duc[4)]

1) Graduate school of Environmental and Life Science, Okayama University
2) Faculty of Environmental Science and Technology, Okayama University
3) Graduate School of Engineering, Tottori University, Japan
4) Da Nang Environ. Tech. Center, Institute of Environ. Tech., VAST, Vietnam

Introduction

- What is waste milk?
  - Milk from antibiotic-treated cows and mastitic milk
  - Colostrum and transitional milk

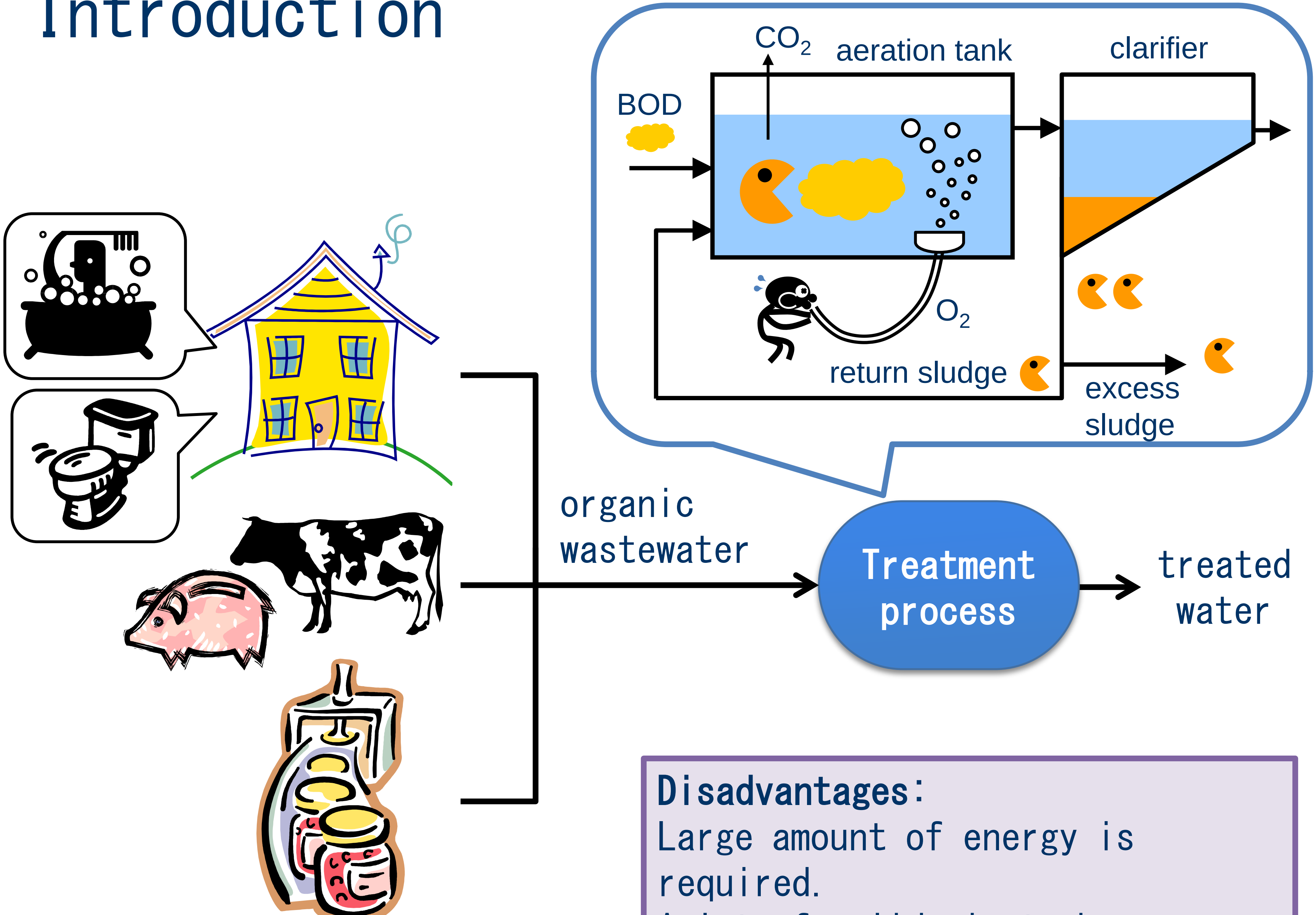
    = the early milk produced by cows during the first 5 days after the calf's birth
- How is it treated now in Japan?
  - Land spread
  - Treated in wastewater treatment facilities in the farm
    - However, treatment capacity is very small because the BOD of waste milk is extremely high. Discharging such large quantities into the facility will cause the system to fail.

Introduction

- Impact of discharging waste milk to the environment
  - about 10% of milk is wasted
  - shipment of milk in 2011 = ca. 7,500,000 t in Japan
    → 750,000 t of milk could have been wasted in the year.
  - BOD in milk = 120,000 mg/L
    c.f. BOD in typical domestic wastewater = 150–250 mg/L
  - BOD load of waste milk i
    = 120,000 mg/L x 750,0 t/yr

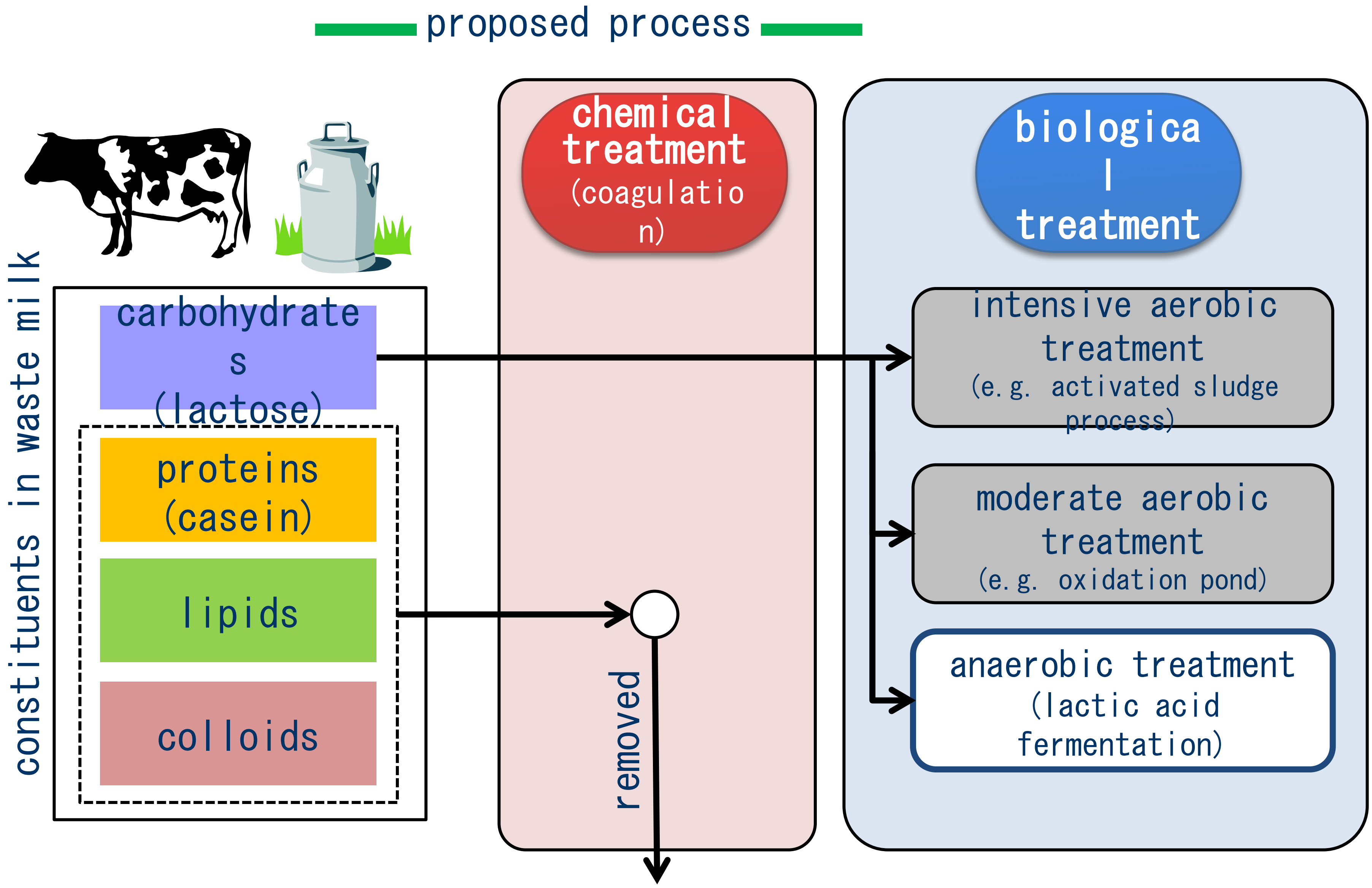
as large as the BOD load from 4.3 million people!

Introduction



Disadvantages:
Large amount of energy is required.
A lot of solids is to be

Introduction



Introduction: coagulation

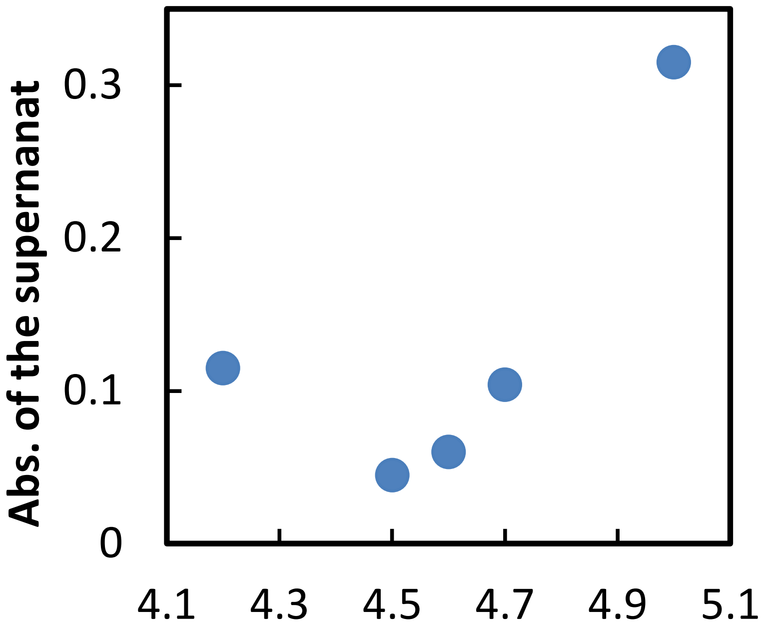


Fig.　Turbidity of the supernatant

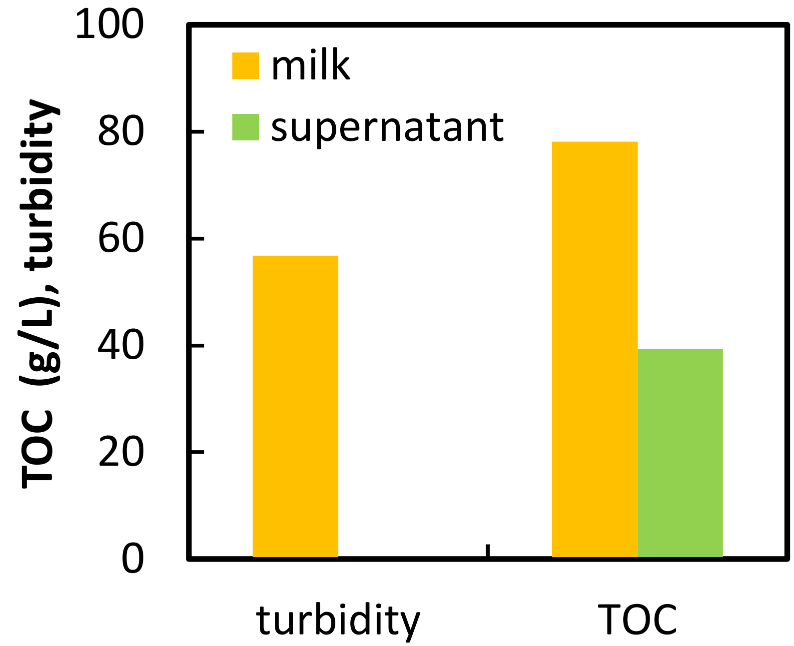
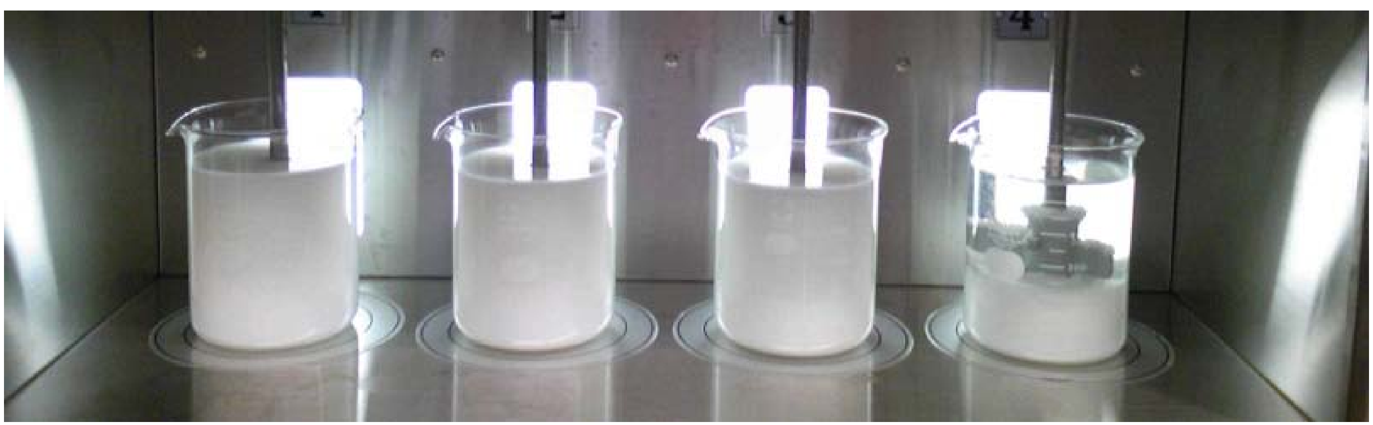


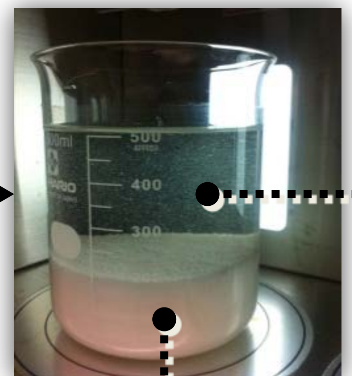
Fig.　Removal of turbidity & TOC



Introduction: coagulation → fermentation

Bacillus coagulans
JCM 2258

coagulation →

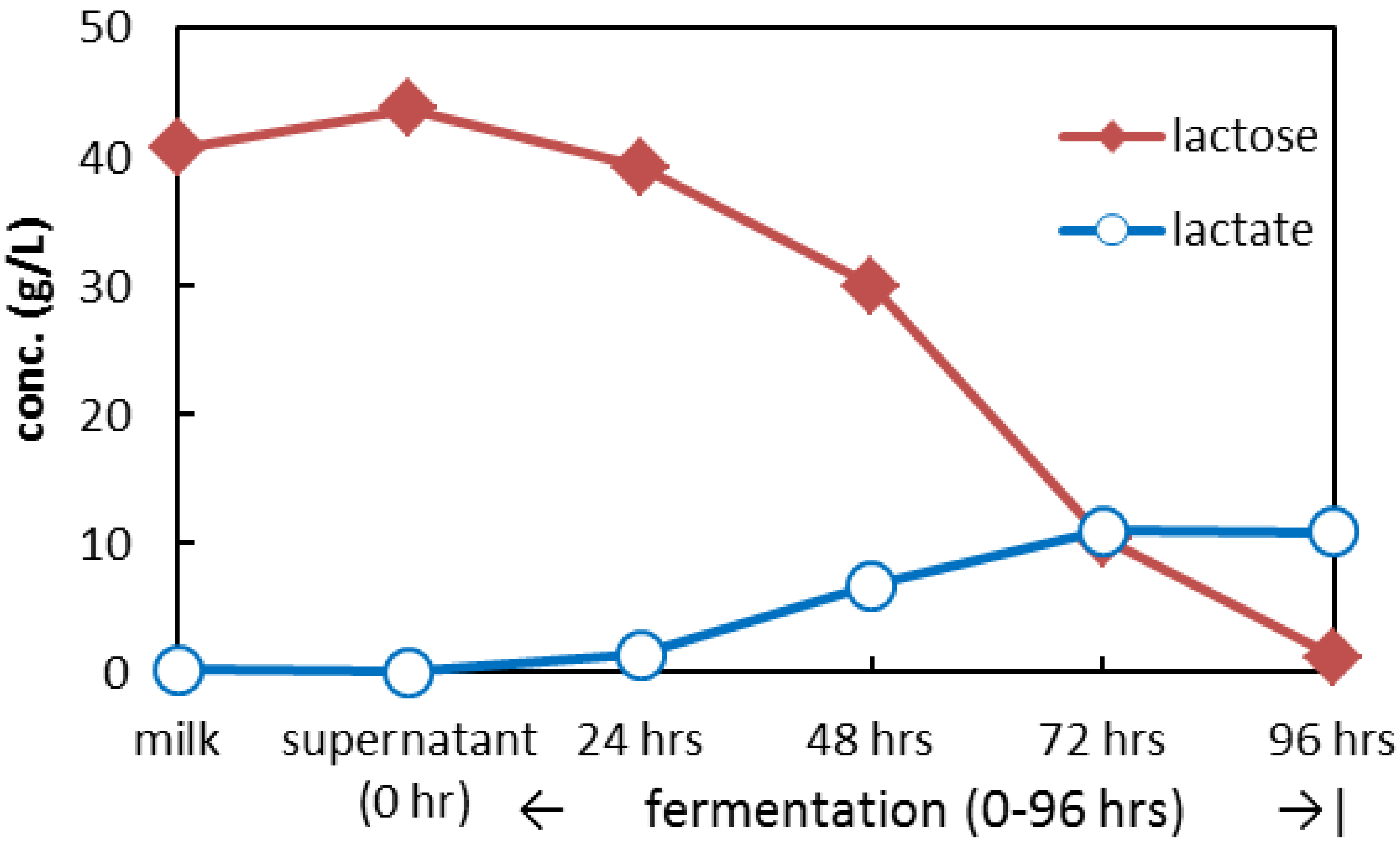


→ biological treatment

Casein floc, which can be reused as materials for biodegradable casein plastic production.

This time, lactic acid fermentation was applied to recover lactate.

Introduction: lactic acid fermentation



Objectives

- Investigate the reaction in fermentation process w. *Bacillus coagulans* JCM2258 to improve lactate yield.

- Why *Bacillus coagulans* JCM2258?
  - high L-lactic acid yield
  - dominating in a specified condition (pH5.5, 55℃)

Methodology

- L-lactic acid fermentation
  - Commercial milk was coagulated with HCl.
  - A 100 mL of the supernatant was supplied to L-lactic acid fermentation (LAF).
  - After adding 2 g of east extract as the nitrogen source and 1 mL of *Bacillus coagulans* JCM2258, the supernatant in a grass reactor was incubated at pH5.5 and 55 degree for four to five days (Maeda et al., 2011).
- Analysis of contents
  - Contents of the fermentation broth was analyzed by various methods.

Results: fermentation

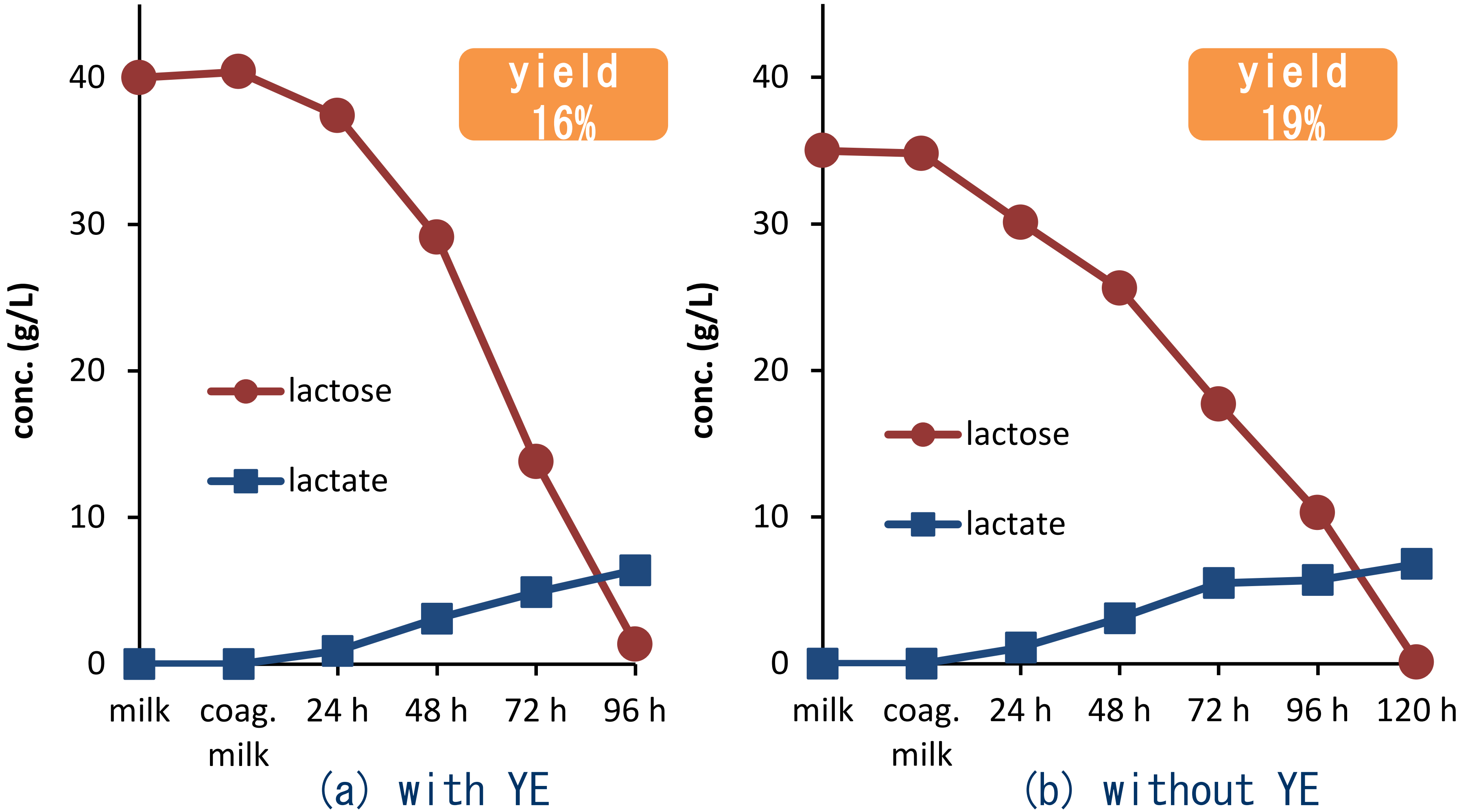


Fig. 1 Concentration changes of lactose and lactate during LAFs. Two cases of fermentation were studied: with and without yeast extract (YE) as nitrogen source.

Results: DOC balance

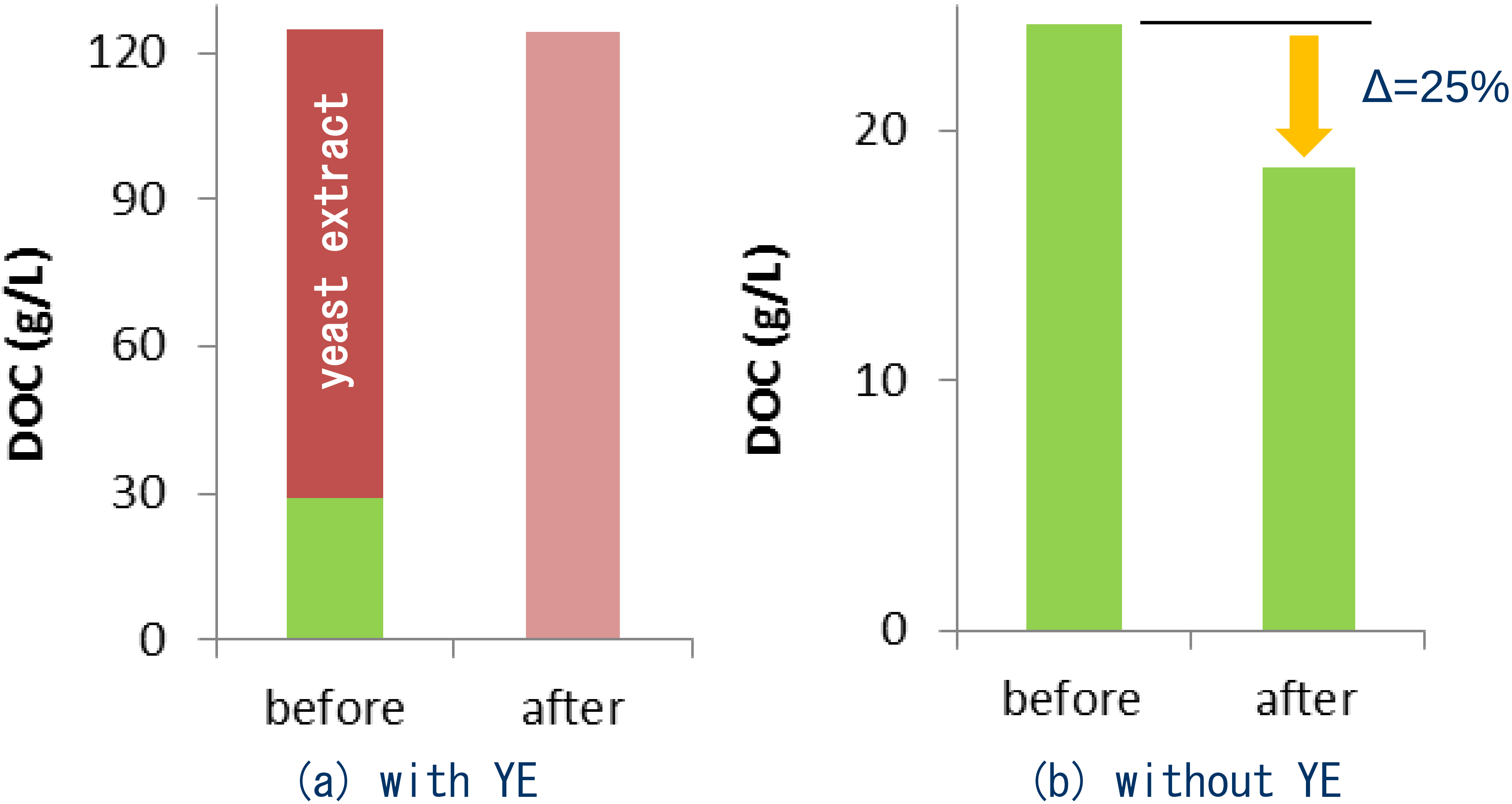


Fig. 2 Changes in DOC during LAFs.

Results: changes in possible CHs conc.

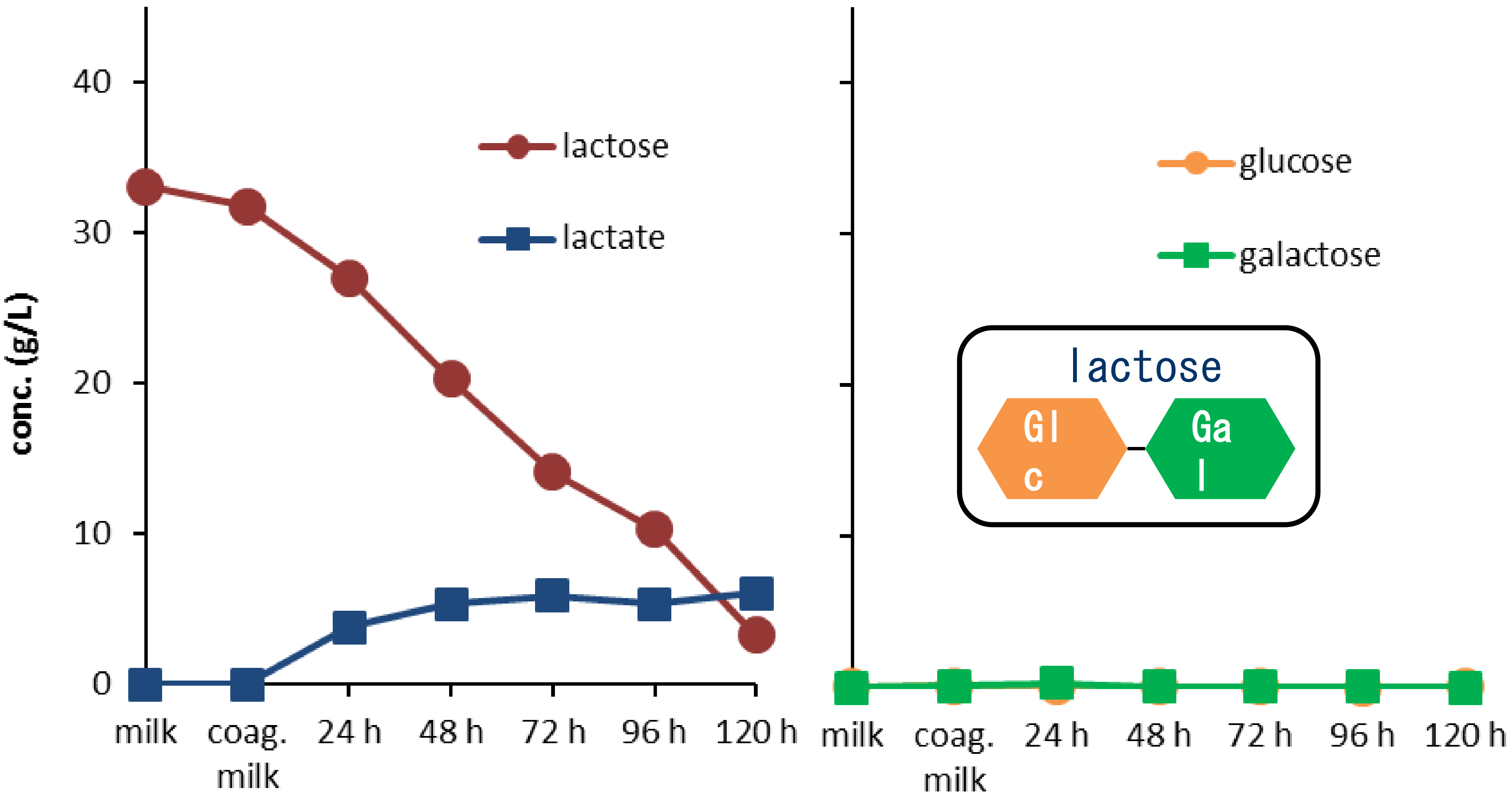


Fig. 3 Concentration changes of carbohydrates during LAFs.

Results: CE analysis

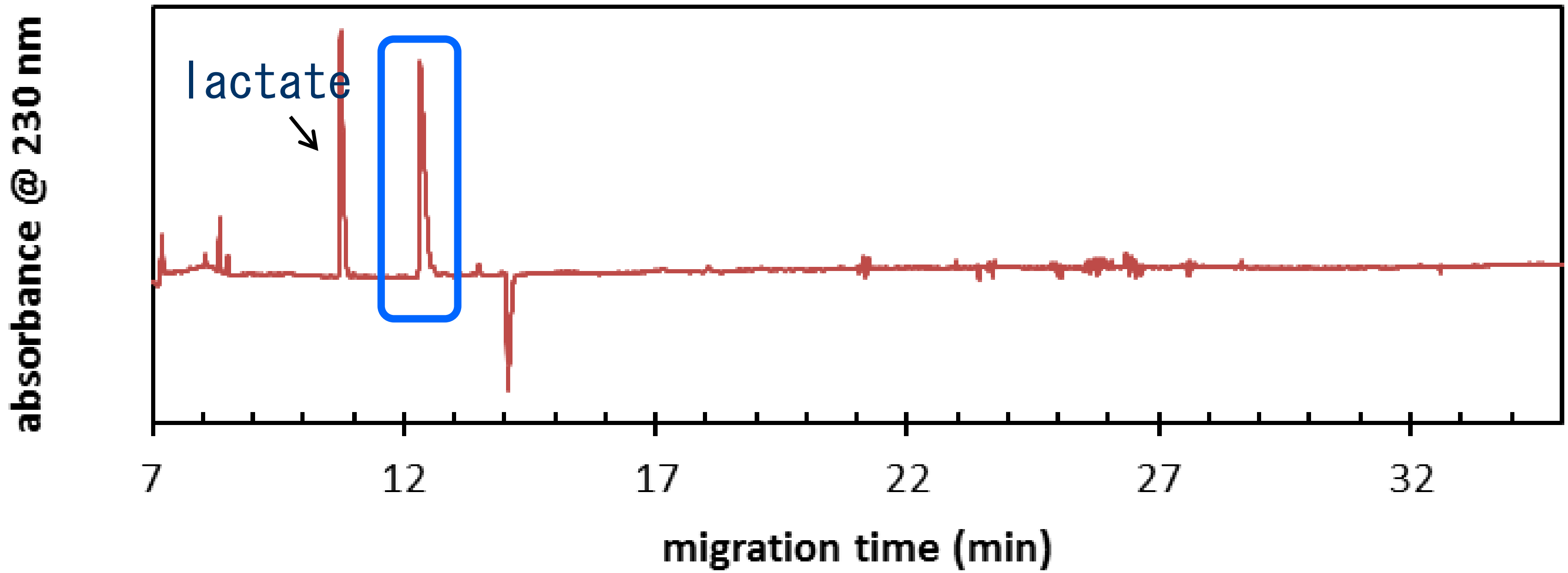


Fig. 4 Electropherograms of coagulate milk and filtered fermentation broth.

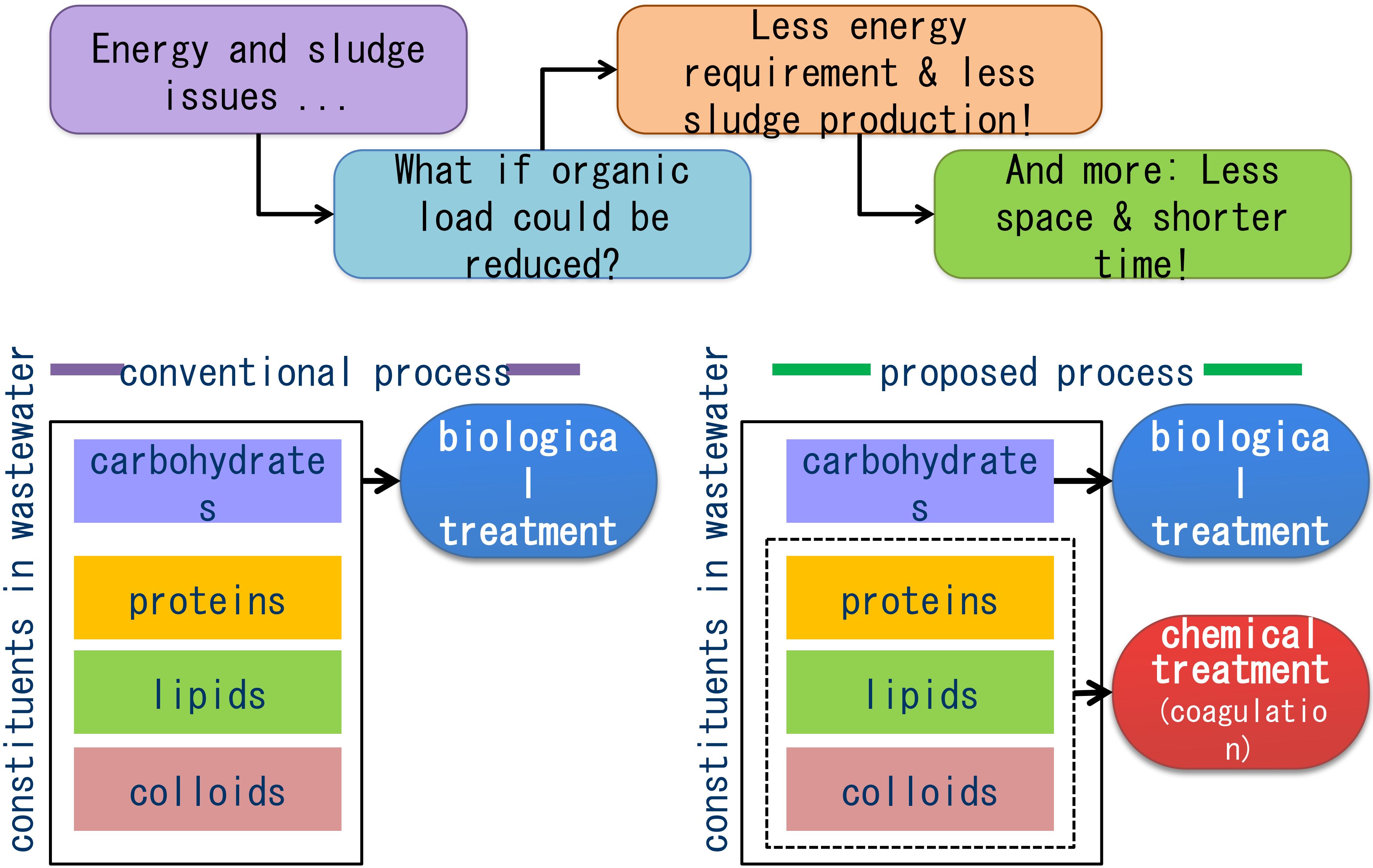
Conclusions

- In this research, lactic acid fermentation from wasted milk was studied for improvement of lactate yield.
- The reason for the low yield was not specified.
- Possible fermentation products such as amino sugars and monosaccharides were found.
  - ➡ further investigation

Summary

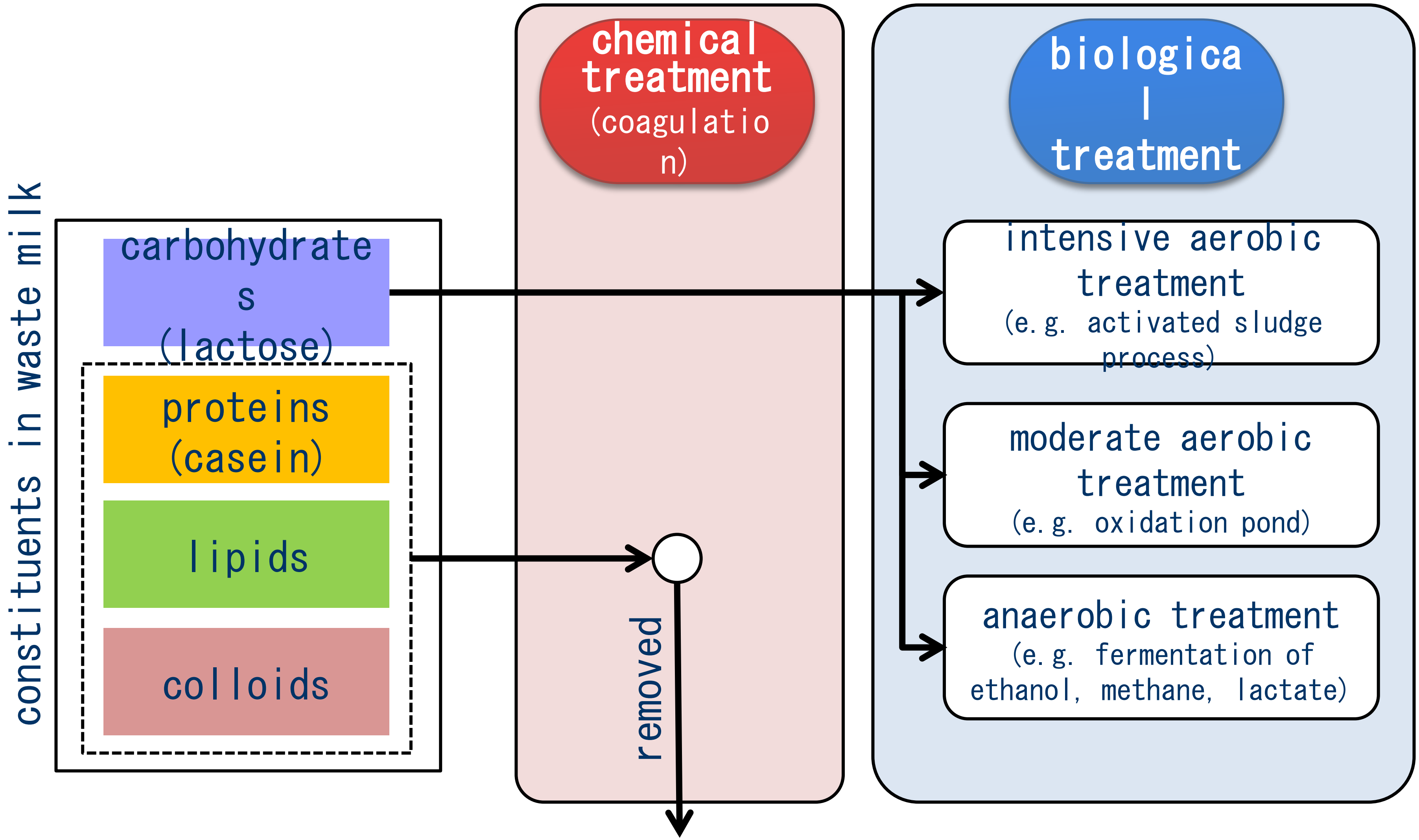
- Wastewater treatment process with chemical treatment (coagulation) is proposed to overcome the disadvantages of traditional process such as activated sludge process.
- As a case study, waste milk treatment with coagulation and lactic acid fermentation was conducted.
    - Clear supernatant was obtained after coagulation.
    - Lactose in the supernatant was assimilated during lactic acid fermentation to be converted to lactate.

New concept for wastewater treatment



New concept for wastewater treatment

proposed process



Treatment of wasted milk:

lactic acid fermentation

(w/o yeast extract)