ENVIRONMENTAL SOUND MANAGEMENT OF LACTOSE MOTHER LIQUOR THROUGH BACTERIAL FERMENTATION

Priyanka *

ABSTRACT

Lactose mother liquor is very high strength waste water generated by the lactose manufacturing milk plants. It is the residual liquor left behind after recovery of lactose from concentrated whey permeate. Due to its high strength, it is not easily amenable for treatment to meet the prescribed effluent standards. Every 10 liters of raw milk processed in the milk plant to produce lactose, generates 1 liter of lactose mother liquor. LML has high residual lactose (up to 15% or more), whey proteins (up to 8% or more) and milk minerals and the salts (as high as 7%) hence it can't be viewed as waste water. Instead it should be used as a resource or efforts should be made to recover byproducts and resources from it, and appropriate techniques should be developed for enhancing its recycling and reuse potential. Due to high lactose content (15% or more), LML was used as a base culture medium for the production of value added products using biochemical conversion process. The utilization of mother liquor as fermentation feed stock generates usable and valuable products while reducing waste disposal problem. The produced lactic acid can be used in the milk plant for curding the skimmed milk in place of HCl.

Keywords: *Lactic acid, Lactose Mother Liquor, Whey, Lactose, Fermentation.*

* Indian Institute of Technology, Roorkee.

INTRODUCTION

Lactose mother liquor is very high strength waste water generated by the lactose manufacturing milk plants. It is the residual liquor left behind after recovery of lactose from concentrated whey permeate [1, 2]. Due to its high strength, it is not easily amenable for treatment to meet the prescribed effluent standards. Every 10 liters of raw milk processed in the milk plant to produce lactose, generates 1 liter of lactose mother liquor [3].

Liquid portion left after separation of casein from curded skimmed milk is known as whey. The whey is ultra-filtered for concentrating the native whey proteins and obtaining whey protein concentrate with varying protein content i.e. 35 to 90% proteins. The whey permeate generated from the ultra-filtration is concentrated through vacuum evaporation into supersaturate lactose concentrate. From this concentrate, through crystallization and separation of the resultant crystals, lactose is recovered. Residual liquor left behind after crystallization and recovery of lactose is discarded as LML [5, 6]. Strength of lactose mother liquor (LML) is very high and variable because of the presence of residual lactose, whey proteins that slipped into whey permeate during ultra-filtration of whey, milk minerals and the salts from the milk being processed and from the water being used in the processing[7,8].

LML has high residual lactose (up to 15% or more), whey proteins (up to 8% or more) and milk minerals and the salts (as high as 7%) hence it can't be viewed as waste water [9,10]. Instead it should be used as a resource or efforts should be made to recover byproducts and resources from it, and appropriate techniques should be developed for enhancing its recycling and reuse potential [11].

Due to high lactose content (15% or more), LML may be used as a base culture medium for the production of value added products using biochemical conversion process. One of the promising ways to use the lactose as low carbon source for the production of organic acids by fermentation [12, 13, 14]. The utilization of mother liquor as fermentation feed stock generates usable and valuable products while reducing waste disposal problem. Lactic acid is one such a product which is under increasing demand in Food, Pharmaceutical and Chemical Industries and for production of Polylactic acid polymers and possesses excellent biomedical applications [15].

CHARACTERISTICS OF LACTOSE MOTHER LIQUOR (LML)

- The total solid content of LML is 25-30%. Lactose is the major constituent of all the solids, i.e. 50% of the total solids.

- Temperature of LML is relatively low. On storage LML becomes acidic and its pH drops, may be due to fermentation. For preserving the LML different concentration of various preservatives are used. Formaldehyde (0.01%), H2O2 (0.02%) are most economical preservatives which maintain pH value above 6.0 even at high temperature [16]
- Drying LML into powder is difficult. High salts content and lactose make the LML highly hygroscopic.

LML has a potential to serve as a renewable carbon and energy resource for the microbial production of liquid fuel and chemical feedstock either through chemical or microbial route via fermentative mode [17, 18].

Due to high strength of LML, it presents a major disposal problem to the dairy industry. As LML is rich in lactose, whey protein and milk minerals and other salts, hence it is not treated as wastewater. LML can be managed either through minimizing its generation (strength and volume) within the industrial plant (dairy unit) or through recovery of residual lactose and proteins and by recovery of useful byproducts and resources from it and by increasing its recycling and reuse potential.

Present study included management of LML through fermentation. Literature indicated that proteins can be inhibitory to fermentation and high salt content may make the LML unsuitable for use as a base culture medium. Hence, proteins and salts removal from the LML was also tried prior to use as substrate in the fermentation. The study comprises of desalting, deproteinization of mother liquor and fermentation of lactic acid through shake flask method.

MATERIALS AND METHODS

Inoculum Preparation

Bacterial strain was obtained from NDRI, Karnal. The bacterial culture was revived and maintained in De-Man-Rogosa-Sharpe (MRS). For maintaining, the cultures were subcultured every 15 days. For subculturing incubation period was 24 hours at 37ºC.

All the cultures were stored in a refrigrator at 4ºC till they were used.

LML Preparation

LML was deproteinized by heat denaturation and salts were removed by precipitation methods.

Fermentation of LML

The liquor after making the necessary amendments and adjusting pH to the desired level was sterilized and inoculated with active culture for fermentation. For fermentation, the

inoculated liquor flasks were incubated at desired temperature for desired period. The fermented liquor was centrifuged and the supernatant liquor was checked for lactose and lactic acid level.

RESULTS & DISCUSSIONS

LML Desalting

For this, pH of the LML was adjusted to a value in the range of 7-10, by sodium hydroxide, heated to about 70°C and then the contents were allowed to stand and cool for the settling of the precipitated salts. Precipitated salts were then removed from the liquor by centrifugation. TDS level in the supernatant was estimated.

Results obtained from the salts removal studies are given in **table 1**

Table 1: Residual TDS level after salt removal from LML at different pH values

TDS (initial) in the liquor = 326 gm/l

Ph	TDS in the LML(gm/l)
3	294.8 (90%)*
6	151.6 (46.5%)*
7	145.4 (44.6%)*
8	129.7 (39.7%)*
9	101.2 (31%*)

* Values given in the paratheses are percentage salts left in the liquor after the treatment.

Salts removed were observed to increase with the increasing pH. At 9 pH 69% removal efficiency was observed.

LML Deproteinization

For protein removal, pH of the liquor was adjusted to different values in the range of 6-9 by adding sodium hydroxide, and then it is heated to 121°C through autoclaving. Then pH of the liquor was brought down to 4.6-4.7 by adding HCl. As a result, denaturation and precipitation of proteins occurred. Then the liquor was centrifuged to remove the suspended material and the supernatant was tested for protein, lactose and lactic acid.

Results obtained from the thermal denaturation studies are given in **table 2.**

Table 2: Effect thermal denaturation on the Protein, lactose and lactic acid concentrations in LML

pH	Protein (g/l)	% Lactose	Lactic Acid (g/l)
3	13	16.5	0.12
6	7	--	0.12
7	0	10	0
8	0	6.7	0
9	2.8	6.5	0

Initial concentration of protein, lactose and lactic acid in LML were 50gm/L, 17% and 0.12 g/L respectively.

Thermal denaturation of LML, after adjusting its pH to 7 or 8, resulted complete removal of proteins. Thermal denaturation, without prior pH adjustment, gave only 74% removal.

LML Fermentation

After desalting and deproteinization, LML was fermented with *Lactobacillus bulgaricus* at pH 5.5, incubation temperature 42°C and at 150 rpm. Lactic acid production achieved with *Lactobacillus bulgaricus* was 8.0 g/L

CONCLUSIONS

The high lactose content of LML may make it suitable as a fermentation feedstock. As proteins can be inhibitory to fermentation and high salt content may make the LML unsuitable for use as a base culture medium, proteins and salts were removed from LML. At pH 7 and 8, complete removal of proteins was observed by thermal denaturation. At pH 9, with sodium hydroxide, 69% removal of salts was achieved through precipitation. After desalting and deproteinization, LML was fermented to produce lactic acid. The fermentation was carried out with bacterial strain *Lactobacillus bulgaricus*, at **pH 5.5, incubation temperature 42**°C and at 150 rpm. Lactic acid production achieved with *Lactobacillus bulgaricus* was 8.0 g/L.

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