Dairy Sci. Technol.
© INRA, EDP Sciences, 2010
DOI: 10.1051/dst/2010036

Available online at:
www.dairy-journal.org

Note

Waste valorization: Recovery of lactose from partially deproteinated whey by using acetone as anti-solvent

Sanjay R. Patel, Z. V. Panchakshari Murthy*

Chemical Engineering Department, Sardar Vallabhbhai National Institute of Technology, Surat 395007, Gujarat, India

Received 15 December 2009 – Revised 31 May 2010 – Accepted 8 June 2010
Published online 2 August 2010

Abstract – The treatment of dairy wastewater which conforms to environmental regulations is a crucial problem due to its high biological oxygen demand (BOD). The main cause of the BOD in dairy wastewater is due to residual whey which consists mainly of lactose. Recovery of lactose from the whey would solve the problems of whey utilization as well as pollution reduction as lactose recovery itself can reduce the BOD of whey by > 80%. In the present study, the recovery of lactose from partially deproteinated whey by the use of an anti-solvent (acetone) was investigated. Process parameters, such as acetone concentration (65–85% v/v), time (1–3 h), stirring speed (500–1000 rpm), seeding (1–3% w/w) and pH, were varied. The results suggest that > 90% of lactose was recovered from whey after 1 h of stirring at an acetone concentration of 85% v/v. The crystal size distribution of the lactose recovered from whey was estimated by image analysis and found to be affected by crystallization time as well as seeding.

whey / acetone / lactose recovery / crystallization

摘要 – 丙酮反溶剂法从部分脱蛋白乳清中回收乳糖。乳制品生产废水的生物需氧量 (BOD) 较高、废水必须经过处理以达到环保排放标准。 奶废水中生物需氧量 (BOD) 较高的主要原因是由于残留的乳清含有大量的乳糖。从乳清中回收乳糖既能够解决乳清再利用的问题、乳糖回收能够将乳清中的生物需氧量减少 80% 以上、同时也减少了乳糖造成的环境污染问题。本文通过利用丙酮作为反溶剂、从部分脱蛋白乳清中回收乳糖。实验参数为丙酮浓度 (65–85% v/v) 、时间 (1–3 h)、搅拌速度 (500–1000 rpm)、添加的晶种量 (1–3% w/w) 和 pH。实验结果表明、在丙酮浓度为 85% (v/v) 的情况下搅拌 1 h 后、乳糖回收率可以达到 90% 以上。从乳清中回收的乳糖的晶体粒径分布 (CSD) 通过影像分析仪发现、乳糖的晶体粒径分布 (CSD) 受结晶时间和添加的晶种量影响。

乳清 / 丙酮 / 乳糖回收 / 结晶化

1. INTRODUCTION

Lactose is produced from whey, a waste stream from the manufacture of *paneer*/cheese in dairy industries. Approximately 47% of the 115 million tons of whey produced worldwide every year are disposed into rivers, lakes or water bodies [16]. Whey is a serious pollutant as it imposes a high biological oxygen demand (BOD) of

*Corresponding author (通讯作者): zvpm2000@yahoo.com, zvpm@ched.svnit.ac.in

This article should be cited as: Patel S.R. and Murthy Z.V.P., Waste valorization: Recovery of lactose from partially deproteinated whey by using acetone as anti-solvent, Dairy Science & Technology (2010), doi:10.1051/dst/2010036

35 000 mg·L^{-1}, which is mainly due to the lactose content [7]. The recent environmental regulations do not allow whey to be discharged into the water bodies due to the presence of lactose. The principal components of whey are lactose (44–52 g·L^{-1}), proteins (6–8 g·L^{-1}) and mineral salts (4.3–9.5 g·L^{-1}) [10]. The increased capacity of *paneer*/cheese plant and the high BOD of whey make it necessary for dairy companies either to process whey or to dispose it in some environmentally acceptable manner. Therefore, the dairy industry will have to decrease the lactose concentration and recover enormous amounts of lactose from whey and effluent streams before discharging the wastewater into the environment [5]. The recovered components can be supplied to the food, pharmaceutical, dairy and beverage production industries, which use them as ingredients [2]. To make lactose recovery an economical process, recovery of lactose should be a rapid process from low concentration whey and with high purity of lactose in the first step of crystallization itself. In the conventional lactose recovery process, raw lactose is re-dissolved, charcoal treated and re-crystallized to yield various grades of lactose with a maximum recovery of lactose around 50–60% [4]. Every crystallization process is influenced by factors such as solubility, supersaturation, seed loading, crystal shape factor, agitation, growth rate kinetics, nucleation rate kinetics, agglomeration kinetics, etc. [11, 14]. One of the ways to separate organic products from aqueous solutions is adding non-solvent compounds that reduce the solute solubility without creating a new liquid phase. Therefore, it is possible to imagine that crystal formation will occur differently when one of the solvents is changed, which may provide information that could improve the industrial productivity of this high value added product. Acetone can greatly reduce the solubility of lactose in water [3]. Very few reports are available for the recovery of lactose from the actual concentrated deproteinated whey in an anti-solvent acetone [3, 15]. Most of the reported studies have been done with reconstituted lactose solution [3, 15]. In an earlier study [8] with acetone to recover lactose from whey, up to 65% acetone was added to concentrated whey (18–20% lactose) which allowed recovery of 85% of lactose in 3.5 h period. The yield of lactose and rapidity of crystallization were influenced by the rate of acetone addition [8]. In another study of lactose crystallization in water-acetone solutions [3], lactose yield was found to be 92.63% from 27.02 g lactose·100 g^{-1} solvent reconstituted lactose solution during 2.9 h period, stirred at 350 rpm. Another study with ultrasound assisted crystallization of lactose in an anti-solvent acetone [15], the recovery of lactose from 16% w/v reconstituted lactose solution was found to be in the range of 80–92% (w/w) within 4 min of sonication.

In the present study, use of an anti-solvent acetone has been investigated for the rapid recovery of lactose from deproteinated whey obtained from a nearby small scale dairy industry.

2. MATERIALS AND METHODS

2.1. Materials

Whey generated during the manufacture of *paneer* was used to study the recovery of lactose in an anti-solvent crystallization. The whey had a varied concentration of lactose and protein as shown in Table I. The whey was stored at 7 ± 2 °C. The fat, observed after the storage at this temperature, was separated by centrifugation (high-speed research centrifuge, Bio-Laboratory Instruments, Mumbai, India) at 7168× *g* for 30–35 min. The heat-induced deproteination process of whey was conducted at the optimized conditions of 92 ± 2 °C and 30 min at pH 5.3 ± 0.1 followed by centrifugation for 30 min [1].

Table I. Protein and lactose content of the whey.

Protein content (% w/v)	Lactose content (% w/v)	pH
0.145	2.8	3.6
0.183	3.2	3.8

The resultant partially deproteinated whey had a protein concentration of < 0.1% w/v, which was then concentrated in a steam heated open porcelain pan to achieve lactose concentration in the range of 9.5–10% w/v. A 15 mL concentrated whey, having a known protein and lactose concentration, was taken into a 250 mL beaker kept in an ice bath. Stirring (Remi Instruments, Mumbai, India) was done by a three-blade radial type impeller agitator. The temperature was maintained at 5 ± 1 °C throughout the experiment. The effects of operating parameters; viz., acetone concentration (65–85% v/v), time (1–3 h), stirring speed (500–1000 rpm), seeding of 1% and 3% (i.e. 1% means, 0.01628 g of analytical grade commercial lactose/1.6284 g of initial lactose content in whey), which was added immediately after the addition of acetone and pH; have been investigated on the recovery of lactose from whey. The recovered lactose from whey was filtered by a vacuum filtration and dried in a vacuum oven at 60 °C for 3 h. The percentage recovery of lactose was calculated based on the initial lactose content in the concentrated whey. After recovery of lactose from the acetone-water mixture, the acetone was separated from water and non-crystallized lactose using simple distillation (55–65 °C). The recovery of acetone was found to be 94.89% (v/v). The purity of acetone was analyzed by gas chromatography (Clarus 500 FID FPD, Perkin-Elmer, California, USA) and found to be 98.99%. The coil used for the analysis was DC 5 (30 m × 0.32 mm × 0.25 μm) and the identification was done with flame ionization detector. Oven temperature was kept at 50 °C initially, which was then raised to 280 °C and kept constant for 5 min. The injector and detector temperatures were maintained at 250 and 280 °C, respectively. The flow rate of carrier gas (nitrogen) was maintained at 1 $mL \cdot min^{-1}$.

2.2. Determination of lactose and protein concentration

Lactose content was estimated with iodometric titration by the method of Willstatter and Schudel [9]. For the determination of protein concentration, modified Folin-Lowry method was used [4]. A 1 mL alkaline copper reagent was added to 0.1 mL sample and incubated at 30 °C for exactly 10 min. Then, a 0.1 mL of Folin-Ciocalteau reagent, diluted with equal part of distilled water (pH 5.9 ± 0.2, conductivity 1.0 $\mu S \cdot cm^{-1}$ produced by Millipore, Elix, Bangalore, India), was added and mixed immediately and then kept it for 30 min at 30 °C. The absorbance of the mixed sample was measured at 660 nm with an UV-VIS spectrophotometer (Hach, DR 5000, Loveland, Colorado, USA). The calibration plot of absorbance vs. protein concentration was prepared in the range of 0–0.8 $mg \cdot mL^{-1}$. The protein used for the calibration is α-lactalbumin (Sigma-Aldrich, St. Louis, MO, USA) dissolved in distilled water. Concentration of the protein was determined using the calibration plot of UV absorbance and concentration.

2.3. Crystal size analysis and distribution

The recovered lactose crystals were observed under microscope at 40× magnification (Coslab Laboratory, Ambala Cantt, Haryana, India). The images were photographed using digital camera attached with the microscope and analyzed for crystal

sizes and their distribution using Coslab Image analyzer. Approximately 80 crystals were measured in each sample and length (L), width (W), perimeter (P) and surface area (A) were recorded. Shape descriptors, like shape factor and elongation, were derived as follows: Elongation ratio: L/W; Shape factor: $(4\pi A)/P^2$. The shape factor is a two-dimensional shape descriptor used in many image analyzer software programs and combines properties of surface roughness and shape. A spherical particle with smooth surface will have a shape factor 1, while non-spherical particles or spherical particles with rough surface will have a shape factor value < 1. The more irregular the shape and/or rougher the surface, then smaller will be the shape factor.

2.4. Crystallization kinetics

The crystallization kinetics was studied for the lactose samples recovered at different time intervals of 1–3 h. The lactose samples at these processing conditions were utilized for determination of equivalent diameter, shape factor and the total number of crystals in 1 mL using Coslab Image analyzer. The data have been used to estimate the growth rate of lactose crystals using mixed suspension mixed product recovery (MSMPR) model [4]. The growth rate expression [4] is given as:

$$n = n_0 \exp\left(\frac{-L}{Gt}\right),$$

where n is the number of crystals per milliliter, n_0 is the number of embryo size crystals having diameter practically equal to zero which presents at the beginning of nucleation process, L is the average crystal diameter (μm), G is the crystal growth rate ($\mu m \cdot s^{-1}$) and t is crystallization time (s). The crystal growth rate over crystallization time was calculated by plotting ln(n) vs. L.

3. RESULTS AND DISCUSSION

3.1. Effect of acetone concentration

To study the effect of acetone concentration on the recovery of lactose from the whey, acetone at room temperature of 30 ± 3 °C was added in an appropriate quantity at once to a 15 mL concentrated whey, to achieve acetone concentration of 65–85% v/v (i.e. to achieve 85% v/v acetone concentration, 15 mL of whey considered as 15% and 85 mL acetone was added, hence the total volume of material stirred was 100 mL) in the system. Each sample was stirred at 500 rpm for 1 h by a three-blade radial type impeller agitator (Remi Instruments, Mumbai, India) and the temperature was maintained at 5 ± 1 °C. The recovered lactose was found to be 84.58%, 85.80% and 87.03% w/w with respect to acetone concentration of 65%, 75% and 85% v/v, respectively (Fig. 1). Lactose solubility is directly proportional to the solution temperature and inversely proportional to the acetone concentration and the solubility is close to zero in pure acetone [3]. Therefore, an increase in acetone concentration will decrease the lactose solubility in water, which leads to rapid supersaturation and then precipitation of lactose. The crystallization speed is proportional to the supersaturation; however, if this speed is too high then agglomeration may occur. Agglomeration increases the size of crystals and reduces the number of crystals and it depends on the crystallization system [3]. The main problem associated with crystallization of lactose is that it takes very long induction time (i.e. the time elapsed between creation of supersaturation and appearance of crystals) and extremely slow growth rate as well as the high metastable zone width (which is responsible for the generation of high supersaturation), which can be reduced by stirring the solution [15]. When

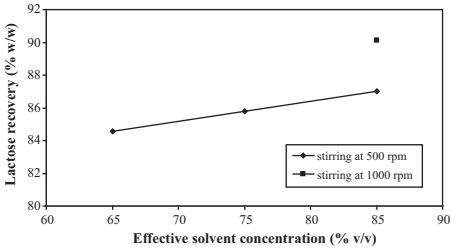

Figure 1. Effect of acetone concentration on the recovery of lactose.

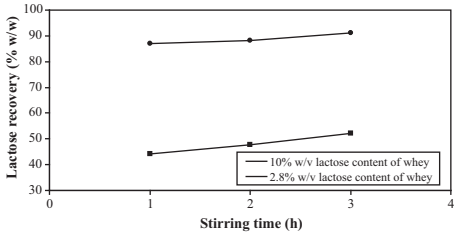

Figure 2. Effect of stirring time on the recovery of lactose.

the stirring speed in a sample was increased to 1000 rpm, recovery of lactose from concentrated whey was found to be increased to 91.14% w/w, for 1 h of crystallization time in an anti-solvent acetone concentration of 85% v/v as shown in Figure 1. It is known [6] that agitation markedly increases the rate of crystallization by increasing the surface area on which crystallization can occur. The anti-solvent technique involves addition of a second solvent (acetone), in which the solute (lactose) is insoluble, which initially generates local supersaturation at the point of addition and then eventually leads to global supersaturation [4]. The recovered mother liquor (acetone) with the purity of 98.99% obtained by simple distillation was further used for recovery of lactose from whey. Lactose recovery was found to be 85.90% w/w in 1 h of crystallization time and 85% v/v acetone concentration, which is close to lactose recovery of 87.03% w/w obtained at the same conditions in actual experiments. The residue of distillation bottoms with known lactose concentration can also be recycled for further recovery of lactose.

3.2. Effect of crystallization time

Crystallization time was varied from 1 to 3 h to study the recovery of lactose in an anti-solvent acetone (85% v/v) from concentrated whey, keeping the stirring speed at 500 rpm. The rate of crystallization influences the crystal habit and the factor that governs the rate of crystallization is the supersaturation, which increases with concentration. Therefore, experiments were carried out to study the effect of initial lactose concentration in whey on the rate of crystallization. It can be seen from Figure 2 that the recovery of lactose increases with increase in time. The recovery of lactose was found to be 87.03%, 88.25% and 91.23% w/w, with the crystallization time of 1, 2 and 3 h, respectively, for 10% w/v lactose content of concentrated whey. When the recovery of lactose was studied with the whey brought from a nearby dairy industry (initial lactose content of whey was 2.8% w/v), the lactose recovery was found to be 52.03% w/w in 3 h of crystallization time. The lactose crystal growth rates obtained using the data from the lactose crystallization from 10% w/v lactose content of concentrated whey using 85% v/v acetone concentration in 1–3 h of crystallization time are shown in Table II and Figure 3. The lactose crystal growth rates were obtained as 4.22×10^{-4} to 1.4×10^{-4} $\mu m \cdot s^{-1}$ for 1–3 h of crystallization time and the number of embryo size crystals observed was 3.24×10^{9} crystals$\cdot mL^{-1}$. It was observed that the crystal growth rate was rapid in the first 1 h of crystallization time and hence, the lactose recovery. Lactose recovery was also greatly influenced by initial lactose content of the whey.

Table II. Lactose crystal growth rates.

Crystallization time (s)	Average diameter (μm)	n (number of crystals·mL^{-1}) × 10^{-8}	1/Gt (μm·s^{-1})	n_0 (number of embryo crystals·mL^{-1}) × 10^{-9}	G (μm·s^{-1}) × 10^4
3600	3.28	3.78	0.658	3.24	4.22
7200	4.44	1.67			2.11
10 800	5.10	1.17			1.40

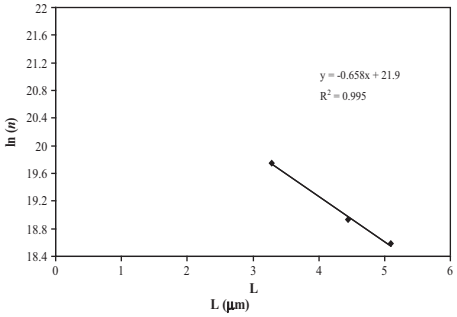

Figure 3. Calculation of embryo size crystals and growth rate from lactose crystallization data.

Higher lactose content of the parent solution results in faster supersaturation, which in turn leads to increase in the nucleation rate and hence, high lactose recovery.

3.3. Effect of seeding

The seeding of lactose with 1–3% w/w of the initial lactose content of concentrated whey was studied with 85% v/v acetone concentration and 1 h of crystallization time at 500 rpm stirring. The seeding was carried out immediately after the addition of acetone to the concentrated whey sample. Drastic increase in lactose recovery was observed with increase in the seeding. It can be seen from Figure 4 that the lactose recovery was observed to be 95.32% and 99.48% w/w with seeding of 1% and 3% w/w, respectively, when compared with that of without seeding sample, which was found to be only 87.03% w/w.

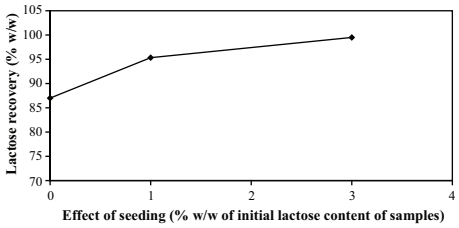

Figure 4. Effect of seeding on the recovery of lactose.

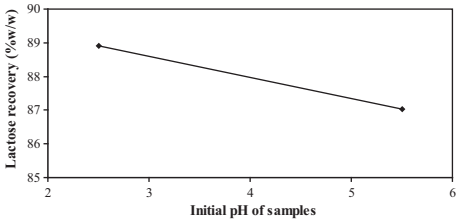

Figure 5. Effect of initial pH of samples on the recovery of lactose.

3.4. Effect of initial pH

There have been reports in the literature [12, 17] that variations in pH cause changes in both the mutarotation equilibrium and the growth rate of α-lactose crystals. This effect has been attributed to its influence on mutarotation, which is accelerated by alkali and high acid condition [13]. The mutarotation equilibrium was noted to be constant and minimum between pH 3 and 7 [15]. In the present study, the initial pH of the concentrated whey sample was 3.6 which was

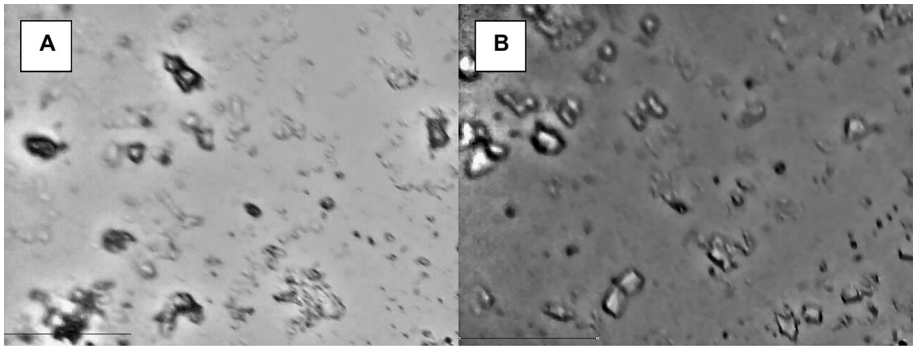

Figure 6. Photographs (50 μm scale) of stirred samples: (A) 85% (v/v) acetone concentration, 1 h stirring time and (B) 85% (v/v) acetone concentration, 3 h stirring time.

increased to 5.5 after the addition of acetone. To study the effect of initial pH on the lactose recovery, initial pH of the mixture of concentrated whey and anti-solvent acetone was kept at 2.5 and 5.5 using 1 N HCl. The samples were stirred at 500 rpm for 1 h of crystallization time in 85% v/v of acetone. It can be seen from Figure 5 that the recovery of lactose was found to be slightly increased with lowering the pH from 5.5 to 2.5. Acids like H_2SO_4 or HCl can greatly accelerate the lactose crystallization, as acids catalyze mutarotation [13]. It is also reported [17] that at low pH lactose crystallization rate increases greatly as a result of increase in mutarotation rate and hence, increases rate of orientation of lactose molecules on the lactose crystals.

3.5. Crystal size distribution analysis

Photographs of lactose crystals recovered at the end of 1 and 3 h of stirring time from concentrated whey, observed under the microscope (40× magnifications), are shown in Figures 6A and 6B, respectively. The processing conditions, viz., stirring time and seeding, caused a lot of variation in the shape characteristics and crystal size distribution (CSD) as shown in Figure 7.

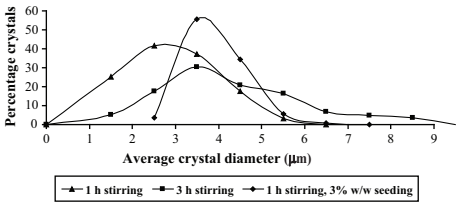

Figure 7. CSD of lactose recovered from concentrated whey.

Maximum percentage crystals indicate that the maximum numbers of crystals were corresponding to a particular average crystal diameter. The spread of CSD was found to be increasing with increase in stirring time. It was also observed that the peak of the CSD shifted from 2.5 to 3.5 μm with seeding of 3% w/w for the same crystallization time of 1 h. This might be due to the higher crystal size of the seed of analytical grade commercial lactose (14.78 μm^2 as estimated by image analysis) used for seeding. Bund and Pandit [4] studied the CSD of the recovered lactose from deproteinized *paneer* whey in an anti-solvent ethanol. They observed that the peak of the CSD shifted from average diameter of 6–4 μm with a decrease in crystallization time from 5 to 1 h and with seeding, the peak shifted

Table III. Characteristics of crystals recovered from samples at different conditions.

Sample stirring time	Average area (μm^2)	Average diameter (μm)	Standard deviation of average diameter (±)	Average roundness	Elongation ratio
1 h	8.45	3.28	0.80	0.69	1.70
3 h	15.47	4.44	1.56	0.64	1.58
1 h, 3% (w/w) seeding	12.12	3.93	0.63	0.68	1.65

back to 6 μm at the same crystallization time of 1 h. The slow growth of lactose crystals led to the need for long crystallization periods. Over long periods of time, the particles eventually reach constant size, despite remaining in supersaturation. Simultaneously, smaller particles will be developed by secondary nucleation due to attrition in the stirred slurry. Thus, long growth periods are required to give significant increases in particle sizes [15]. The crystal size analysis of various recovered lactose samples showed that the average projected area of crystals was found to be 8.45, 15.47 and 12.12 μm^2 with respect to stirring time of 1, 3 h and 1 h with 3% w/w seeding, respectively (see Tab. III). The crystal habit in this case was expressed in terms of roundness (shape factor) and the elongation ratio (i.e. ratio of the longest length to the width at the right angles to that length). Average projected area of lactose crystals increased with increase in crystallization time, which indicates better mass transfer of solute (lactose) on the surface of lactose crystals. The average projected area of lactose crystals obtained with seeding crystallization was found to be larger in comparison with that of lactose crystals obtained without seeding.

4. CONCLUSIONS

In the present work, the effects of various operating parameters in the recovery of lactose, from partially deproteinated concentrated whey using acetone as an anti-solvent, have been studied. Recovery of lactose was found to be directly proportional to the anti-solvent acetone concentration. Effects of initial pH, crystallization time, initial lactose content of the whey and seeding were also found to be influencing the lactose recovery from concentrated whey. Spread of the CSD was affected by the crystallization time as well as seeding. It was found that > 90% lactose recovery was obtained from whey in 1 h of stirring time at 85% v/v acetone concentration.

Acknowledgments: The authors acknowledge S.V. National Institute of Technology, Surat 395007, Gujarat, India, for financial support through Research and Development Project (A.P/R&D/ChED/SP/2007-08).

REFERENCES

[1] Agrawal S.G., Bund R.K., Pandit A.B., Effect of agitation on heat-induced deproteination process of buffalo milk whey, J. Food Eng. 87 (2008) 398–404.

[2] Barba D., Beolchini F., Cifoni D., Veglio F., Whey protein concentrate production in a pilot scale two-stage diafiltration process, Sep. Sci. Technol. 36 (2001) 587–603.

[3] Brito A.B.N., Giulietti M., Study of lactose crystallization in water-acetone solutions, Cryst. Res. Technol. 42 (2007) 583–588.

[4] Bund R.K., Pandit A.B., Rapid lactose recovery from buffalo whey by use of antisolvent, ethanol, J. Food Eng. 82 (2007) 333–341.

[5] Chollangi A., Hossain M.Md., Separation of proteins and lactose from dairy wastewater, Chem. Eng. Process. 46 (2007) 398–404.

[6] Haase G., Nickerson T.A., Kinetic reactions of alpha and beta lactose. II. Crystallization, J. Dairy Sci. 49 (1966) 757–761.

[7] Hobman P.G., Review of processes and products for utilization of lactose in deproteinated milk serum, J. Dairy Sci. 67 (1984) 2630–2653.

[8] Holsinger V.H., Lactose, in: Wong P.N., Jenness R., Keeney M., Marth E.H. (Eds.), Fundamentals of Dairy Chemistry, 3rd edn., Van Nostrand Reinhold, New York, USA, 1999, pp. 279–342.

[9] Judd H.M., The iodometric estimation of sugars, Biochem. J. 14 (1920) 252–262.

[10] Kargi F., Ozmyhcy S., Utilization of cheese whey powder (CWP) for ethanol fermentations: Effects of operating parameters, Enzyme Microb. Technol. 8 (2006) 711–718.

[11] Mullin J.W., Crystallization, Butterworth-Heinemann, New York, USA, 2001.

[12] Nickerson T.A., Moore E.E., Factors influencing lactose crystallization, J. Dairy Sci. 57 (1974) 1315–1319.

[13] Nonoyama N., Hanaki K., Yabuki Y., Constant supersaturation control of anti-solvent addition batch crystallization, Org. Process Res. Dev. 10 (2006) 727–732.

[14] Patel S.R., Murthy Z.V.P., Ultrasound assisted crystallization for the recovery of lactose in an anti-solvent acetone, Cryst. Res. Technol. 44 (2009) 889–896.

[15] Raghavan S.L., Ristic R.I., Sheen D.B., Sherwood J.N., The bulk crystalllization of α-lactose monohydrate from aqueous solution, J. Pharm. Sci. 90 (2001) 823–832.

[16] Saddoud S., Hassairi I., Sayadi S., Anaerobic membrane reactor with phase separation for the treatment of cheese whey, Bioresour. Technol. 98 (2007) 2102–2108.

[17] Twieg W.C., Nickerson T.A., Kinetics of lactose crystallization, J. Dairy Sci. 51 (1968) 1720–1724.