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The effects of nutrient supplements on the production of lactic acid from cheese whey

[Peynir altı suyundan laktik asit üretimine besin ilavelerinin etkileri]

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ABSTRACT

Purpose: To investigate the effects of microorganism strain, substrate concentration and yeast extract (w/v), $MnSO_4.H_2O$, $MgSO_4.7H_2O$ addition to cheese whey solution on the lactic acid production and enantiomeric excess of produced lactic acid.

Material and Methods: Fermentations were performed as batch fermentations with 100 mL liquid medium in 250 mL erlenmeyer flasks. The different species of lactic acid bacteria (seven bacteria species), concentrations of yeast extract (0-1% w/v), manganese sulfate (0.003-0.02 % w/v) and magnesium sulfate (0.005-0.01% w/v) were investigated on the lactic acid production. The medium was inoculated with about 0.04 mg/mL inoculum of bacteria. Lactic acid and lactose was determined by HPLC, with a Phen. Rezex. RHM Mon. (300x7.8 mm) column. Lactic acid enantiomers were determined also by HPLC, using a chiral column (Shodex Orpak CRX-853).

Results: Lactic acid concentration was increased about three times according to the control experiment by adding additional nutrient supplements (yeast extract, $MnSO_4$) into the cheese whey. After 48 hour of fermentation, the highest lactic acid concentration was achieved with *Lactobacillus casei* NRRL-B-1922 with the presence of 0.4% yeast extract and 0.007% $MnSO_4$. The highest lactic acid concentration and conversion were 61 g/L and 94%, respectively. When only 0.5% yeast extract (w/v) was added to the fermentation medium, approximately the same conversion (93%) was obtained at 48 h of fermentation. Lactic acid produced by *L. casei* NRRL-B-1922 was mostly L(+) lactic acid and 99% ee was obtained at all conditions.

Conclusion: It is provided that only addition of yeast extract into cheese whey should be increase the lactic acid conversion and productivity.

Key Words: Lactic acid, cheese whey, batch fermentation, *Lactobacillus casei,* enantioselectivity

Conflict of Interest: The authors do not have conflict of interest.

ÖZET

Amaç: Çalışmanın amacı, Laktik asit üretimi ve üretilen laktik asitin enantiyomerik aşırılığına, mikroorganizma türü, substrat derişimi, peynir altı suyuna maya özütü (w/v), $MnSO_4.H_2O$, $MgSO_4.7H_2O$ ilavesinin etkisinin araştırılmasıdır.

Gereç ve Yöntemler: Fermentasyon, 250 mL'lik erlenlerde 100 mL çalışma hacminde, kesikli olarak gerçekleştirilmiştir. Laktik asit üretimine farklı laktik asit bakteri türlerinin (yedi bakteri türü), maya özütü derişimi (% 0-1 w/v), mangan sülfat derişimi (%0.003-0.02 w/v) ve magnezyum sülfat derşimi (% 0.005-0.01 w/v) etkileri incelenmiştir. Laktik asit ve laktoz Phen. Rezex. RHM Mon. (300x7.8 mm) kolon ile HPLC'de belirlenmiştir. Laktik asit enantiyomerleri kiral kolon (Shodex Orpak CRX-853) kullanılarak yine HPLC'de belirlenmiştir.

Sonuç: Peynir altı suyuna ilave besin maddeleri (maya özütü, $MnSO_4$) eklendiğinde, laktik asit derişimi kontrol deneyine göre yaklaşık üç kat artmıştır. En yüksek laktik asit derişimi 48 saat süren fermentasyon sonunda, *Lactobacillus casei* NRRL-B-1922 ile % 0.4 maya özütü ve %0.007 $MnSO_4$ varlığında elde edilmiştir. En yüksek laktik asit derişimi 61 g/L ve dönüşümü % 94'dür. Fermentasyon ortamına sadece % 0.5 maya özütü eklenerek yaklaşık olarak aynı dönüşüme (% 93) 48 saat süren fermentasyon ile ulaşılmıştır. L. casei NRRL-B-1922 ile üretilen laktik asit genellikle L(+) laktik asittir ve bütün koşullarda % 99 ee elde edilmiştir.

Tartışma: Bu çalışmada, laktik asit dönüşümünü ve verililiğini artırmak için peynir altı suyuna sadece maya özütü eklenmesi gerektiği önerilmiştir.

Anahtar kelimeler: Laktik asit, peynir altı suyu, kesikli fermentasyon, *Lactobacillus casei,* enantiyo seçimlilik.

Çıkar çatışması: Yazarların çıkar çatışması bulunmamaktadır.

Introduction

Lactic acid is one of the most important organic acids produced by lactic acid bacteria (LAB), discovered by Swedish scientist C. W. Scheele in 1780. Lactic acid (2-hydroxypropionic, $CH_3CHOHCOOH$) is the most widely utilized organic acid in the food, pharmaceutical, cosmetics, leather and chemical industries [1, 2]. In recent years, there has been an interest in lactic acid production because of its potential use as a raw material in the production of polylactic acid. Polylactic acid exhibits many properties that are equivalent to or better than many petroleum-based plastics, which makes it suitable for a variety of applications [3]. As a result, lactic acid production is increasing every year, which represented 86.000 tonnes in 2001, is now expected to reach more than 500.000 tones in 2010 [4].

Lactic acid can be manufactured either by chemical synthesis or by microbial fermentations. Chemical synthesis results in rasemic DL-lactic acid whereas optically pure lactic acid can be produced by fermentation with suitable bacteria. LA produced worldwide every year about 90% are made by lactic acid bacterial fermentation and the rest is produced synthetically by the hydrolysis of lactonitrile [5]. Significant advantage over chemical synthesis is that biological production can use cheap raw materials such as whey, molasses, starch waste, beet, cane sugar, and other carbohydrates rich materials. Mild conditions and low energy consumption are the other important advantages that biotechnological production offer [6, 7]. Raw material accounts for 47-68 % of the manufacturing cost of lactic acid production by microbial fermentation [8].

Whey permeate is a by-product of the cheese industry. Approximately about 10 L of milk is used to make 1 kg of cheese. Only about 50% of the solids in milk are incorporated into cheese; the remainder (90% of the lactose, <20% of the protein, and <10% of the fat) are present in the whey [9, 10]. The disposal of excess whey and whey permeate presents a pollution problem. Approximately 47% of the 115 million tons of whey produced worldwide every year are disposed into rivers, lakes or water bodies [11]. To overcome this problem, a better alternative is using whey and its protein concentrate as ingredients in the production of numerous value added product, e.g. edible films, animal food, sports foods, food additives, leather and textile industries, clinical diets, and even pharmaceuticals [12, 14]. Another alternative is the production of lactic acid from whey permeates by fermentation with lactic acid bacteria [15]. Lactase (β-galactosidase, β-D-galactoside, galactohydrolase, EC 3.2.1.23) an enzyme occurs in lactic acid bacteria that hydrolyzes lactose to glucose and galactose [16, 17].

Nutrient supplements such as yeast extract, corn steep liquor and lactamine AA can improve the nutritional quality of the medium, because they contain, all essential amino acids growth promoting compounds, in addition to organic nitrogen, and carbonaceous compounds. However, it is very expensive to use these nutrient supplements in large quantities and can reach as high as 32% of the total lactic acid production cost [18, 19].

It has been reported by Senthuran et al [20] and Fitzpatrick et al [21] that the supplementation of whey hydrolysate with yeast extract (YE) and manganese ions was necessary to improve lactic acid production and lactose utilisation. Gao et al [22] studied the effect of YE concentration on lactic acid production for *L. rhamnosus*. Productivity of lactic acid increased with YE concentration increasing as expected. However, YE concentration above 15 g/L did not come to higher productivity of lactic acid.

Lactic acid bacteria have complex nutrient requirements due to their limited ability to biosynthesize B-vitamins and amino acids. These comprise many of the known vitamins, amino acids and even small peptides [23, 24]. By the way, supplementation of nutrients introduces additional cost to production of lactic acid beside their positive effects. Therefore the least amount of nutrient must be used. When a pure sugar is fermented the purest lactic acid would be obtained with lower purification costs. However this is also not an economical process, because pure sugars are expensive. Another important thing is to produce the purest optical isomer, because it affects the properties of polylactic acid.

The aim of this work is to produce lactic acid using waste of cheese industry, whey. In this study effects of microorganism strain, substrate (cheese whey containing 5-6% lactose) concentration and nutrient supplementation on the lactic acid production, enantiomeric excess of lactic acid and lactose utilization during fermentation under batch conditions were investigated.

Experimental

Microorganism and culture conditions

The organisms used in the experiments, their growth mediums and optimum growth temperatures are presented in Table 1. In Table 1 first six microorganisms were supplied by the US Department of Agriculture, National Center for Agricultural Utilization Research (NRRL) and the last microorganism obtained from DİSTAM (Dipartimento di Scienze e Tecnologie Alimentari e Microbiologiche) Laboratories. The inoculum was obtained from cultures in the logarithmic phase of growth cultivated in 100 mL medium. All chemicals were purchased from Merck. The pH of the broth was adjusted to 6 with NaOH and H_2SO_4 solutions.

Treatment of whey

Cheese whey powder was purchased from SÜTAŞ (TURKEY). It contains 60-62% lactose. Whey powder was diluted with distilled water. Protein precipitation was induced by autoclaving the whey at 121 °C, 20 min. Precipitated proteins were removed by filtration. Whey was supplemented with different nutrients. The pH of

Table 1. Microorganisms used in this study

Species	Origin	Growth Medium	Opt. Growth Temp.
Lactobacillus casei	NRRL-1922	MRS	28 °C
Lactobacillus casei	NRRL-441	MRS	28 °C
Lactococcus lactis	NRRL-23802	MRS	37 °C
Lactobacillus jensenii	NRRL-4550	Lıver Infusion (LI)	28 °C
L. delbr. subsp. *bulg.*	NRRL-548	Lıver Infusion (LI)	37 °C
L. delbr. subsp. *delbr.*	NRRL-763	Lıver Infusion (LI)	37 °C
L. delbr. subsp. *lactis*	DİSTAM	MRS	37 °C

the solution was adjusted to 6 with NaOH and H_2SO_4 solutions prior to sterilization at 121 °C for 20 min.

Fermentation conditions

Fermentations were performed as batch fermentations with 100 mL liquid medium in 250 mL erlenmeyer flasks. To control the growth pH during the fermentation, neutralizing agents need to be added into fermentation medium. Among these agents, calcium carbonate has been widely used in the shake flask investigations and bioreactor processes (25). We have used also $CaCO_3$ (60% (w:w) of the initial lactose concentration) as a neutralizing agent. The medium was inoculated with about 0.04 mg/mL (10^8 cfu/mL) inoculum of bacteria. Fermentations were carried out in a temperature controlled orbital shaker at 150 rpm stirring rate and fermentation temperatures were the optimum growth temperatures which are given in Table 1. All the experiments were conducted in two replicates.

Analysis

For estimation of the bacterial density in the MRS/LI medium, the optical density was measured using a UV-Spectrophotometer (UV-160A, Shimadzu Co., Tokyo, Japan), at a wavelength of 660 nm. Cell weight was calculated from the optical density using calibration curve for each LAB strain.

Since the HPLC is very sensitive to contaminants, before the analysis of lactic acid and residual lactose in the fermentation medium, 1.5 mL fermentation medium was centrifuged at 13000 rpm for 15 min and the supernatant was ultrafiltrated (0.22 µm pore size, Millex) to a HPLC vial.

Lactic acid and lactose was determined by HPLC, with Phen. Rezex. RHM Mon. (300x7.8 mm) column and a refractive index detector. The operating conditions were the following: H_2SO_4 solution (0.005 N) was used as eluent at a flow rate of 0.6 mL/min and column temperature of 45 °C. Lactic acid enantiomers were determined also by HPLC, using a chiral column (Shodex Orpak CRX-853 - 8.0 x 50 mm). For this analysis the eluent was 2 mM $CuSO_4$/1% acetonitrile with a flow rate of 1 mL/min and the temperature was 25 °C. The enantiomeric excess was calculated as follows;

$$\%ee = 100x(L(+)-D(-))/(L(+)+D(-)) \quad (1)$$

Where, L(+) and D(-) are the concentrations of L-isomers and D-isomers, respectively.

Results and Discussion

Effect of microorganism on lactic acid production

At first we investigated the effect of microorganism on lactic acid production and compared seven different lactic acid bacteria (*L. casei*-1922, *L. casei*-441, *Lc. lactis*, *L. jensenii*, *L. delbr.* subsp. *bulgaricus*, *L. delbr.* subsp. *delbrueckii*, *L. delbr.* subsp. *lactis*) with regard to the lactic acid produced from deproteinized cheese whey. 0.4% YE were added to the fermentation medium.

The production of lactic acid and lactic acid conversion by different LAB strains given above are shown in Figs. 1-7. The effectiveness of a process can be measured as the concentration of lactic acid produced, as the conversion of lactic acid based on substrate. The conversions (X_{LA}) were calculated as g produced lactic acid per g initial lactose. As expected the concentration of residual lactose decreased and lactic acid concentration increased during the fermentation.

Minimum lactic acid production was obtained using *L. jensenii*, *L. delbr.* subsp. *bulgaricus*, *L. delbr.* subsp. d*elbrueckii* as 15 g/L, 11 g/L and 11 g/L, respectively (Figs. 4, 5, 6).

L. delbr. subsp. *bulgaricus* and *L. delbr.* subsp. *lactis* are present in milk, they are able to ferment lactose but *L. delbr.* subsp. *delbrueckii* colonizes vegetable sources and it is unable to ferment lactose. *L. delbrueckii* subsp. *bulgaricus* is known to catabolize a much lower number of carbohydrates than *L. delbrueckii* subsp. *lactis*. Germond et al [26] showed that the *L. delbrueckii* subsp. *bulgaricus* strains metabolize only glucose, fructose, mannose, and the disaccharide lactose, whereas *L. delbrueckii* subsp. *lactis* strains can metabolize, in addition, galactose and different modified carbohydrates.

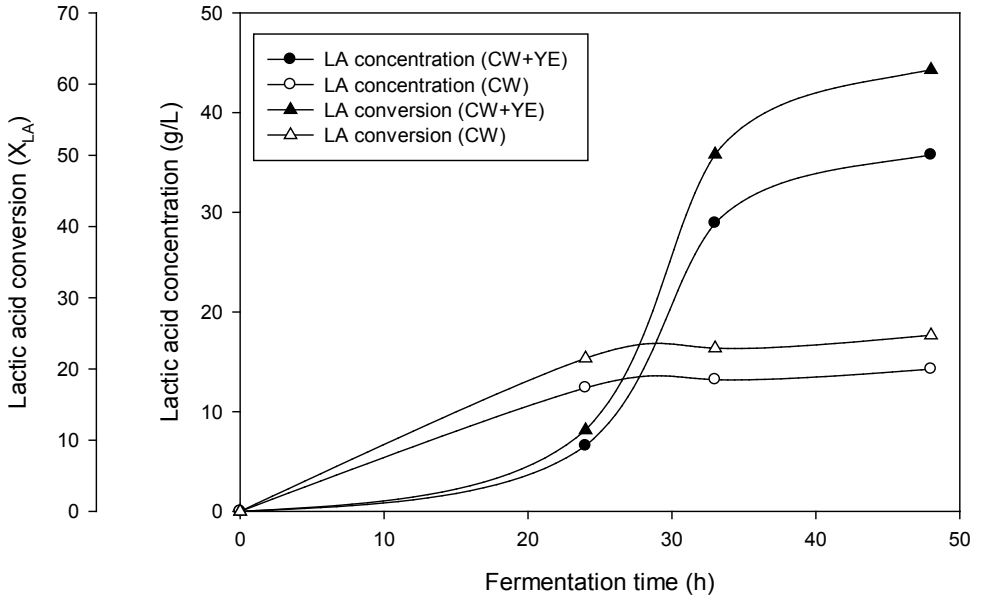

Figure 1. Lactic acid fermentation by *Lactobacillus casei* NRRL-B-1922 (YE: 0.4% (w/v), Initial Lactose Concentration (ILC): 57.6 g/L, T= 28°C, CW: Only cheese whey without any supplement)

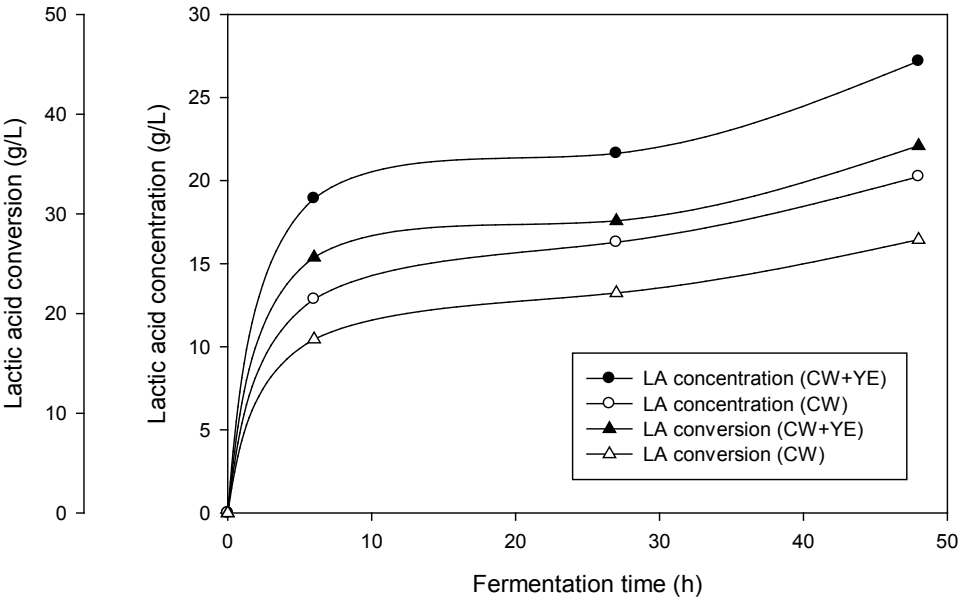

Figure 2. Lactic acid fermentation by *Lactobacillus casei* NRRL-B-441 (YE: 0.4% (w/v), ILC: 71.8 g/L, T= 28°C)

By *L. delbr.* subsp. *lactis* almost two times higher lactic acid production was observed because of the galactose metabolism in this microorganism (Fig. 7).

Enantiomeric purities (ee%), lactic acid concentrations and lactic acid conversions are given in Table 2. The results are for the end of 48 h of fermentation.

Enantiomeric excess (for L(+) lactic acid) of lactic acid was obtained more than 99% with *Lactobacillus casei* NRRL-B-1922 and *Lactobacillus lactis.* When *Lactobacillus jensenii* was used almost racemic lactic a cid (61% ee) was obtained. The lactic acid having different ee value was produced with other lactic acid bacteria (Fig 1, Table 2).

After 48 h of fermentation the highest lactic acid was achieved with *Lactobacillus casei* NRRL-B-1922 with the present of 0.4% (w/v) YE. The highest lactic acid concentration and conversion was 36 g/L and 62%, respectively (Table 2).

Many parameters influence the efficiency of a fermentation process. From our study discussed above, the effect

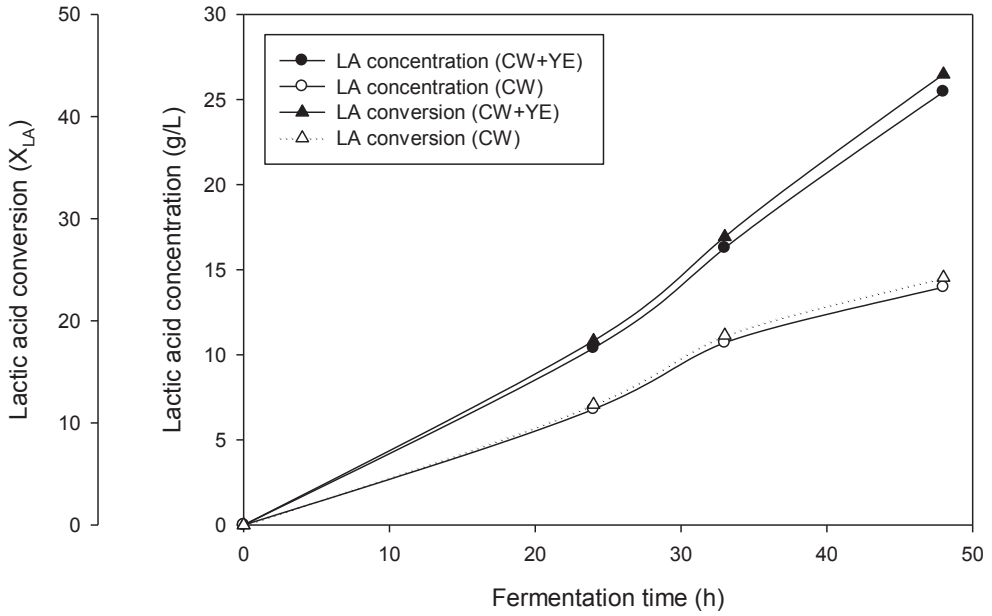

Figure 3. Lactic acid fermentation by *Lc. lactis* NRRL-B-23802 (YE : 0.4% (w/v), ILC: 57.6 g/L, T= 37°C)

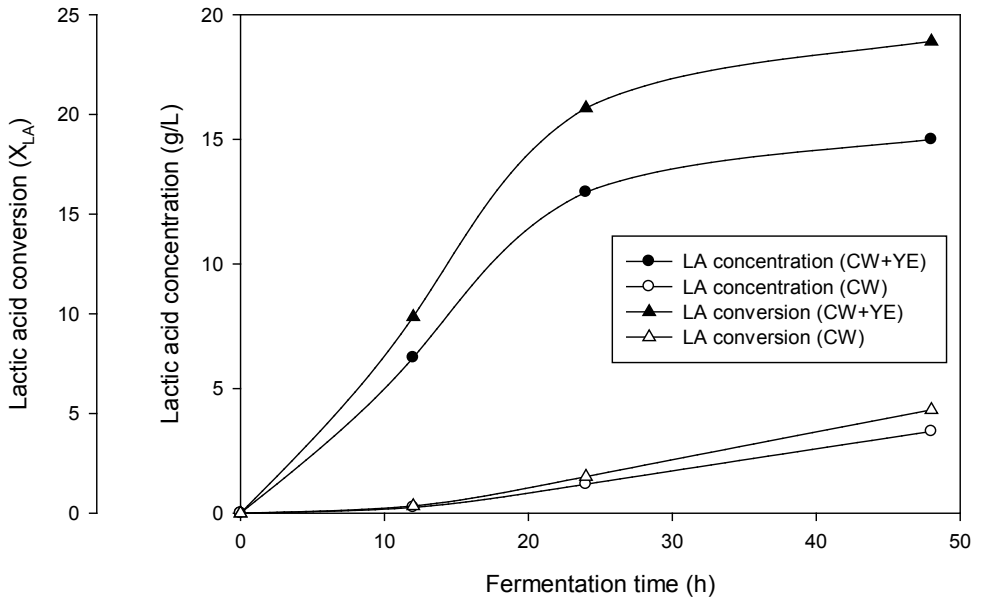

Figure 4. Lactic acid fermentation by *Lactobacillus jensenii* NRRL-B-4550 (YE: 0.4% (w/v), ILC: 63.4 g/L, T= 28°C)

of initial substrate concentration and effect of nutrient supplement on lactic acid production were investigated by using *L. casei* NRRL-1922. Due to the oxygen tolerance of *L. casei* all fermentations were performed without aeration control.

Effect of initial substrate concentration on lactic acid production

The effect of initial substrate concentration was investigated. We have prepared the fermentation medium from cheese whey powder by diluting with distillated water to obtain different lactose concentration. Initial lactose concentrations were the following; 53.2 g/L, 60.1 g/L, 77.5 g/L, 82.6 g/L, 92.1 g/L. The results of lactic acid conversion and lactose utilization are given in Fig. 8. Lactose consumption curves are parallel and maximum conversion 80 % was obtained for 50 g/L of lactose concentration at the end of 48 hours. The time needed for the *L. casei* to utilize higher lactose concentration is longer.

In Figure 8, we see the results of remaining lactose amounts after 48 h. The amount of lactose remained was dif-

Table 2. Comparison of different strains for lactic acid production

Species	LA (g/L)	LA conversion (X_{LA})	% ee (L-LA)	Initial lactose concentration(g/L)
Lactobacillus casei-1922	36	62	>> 99	57.6
Lactobacillus casei-441	27	37	82	71.8
Lactococcus lactis	25	44	>> 99	57.6
Lactobacillus jensenii	15	24	61	63.4
L. delbr. subsp. *bulgaricus*	11	17	84	69.1
L. delbr. subsp. *delbr.*	11	17	79	63.4
L. delbr. subsp. *lactis*	21	29	87	71.3

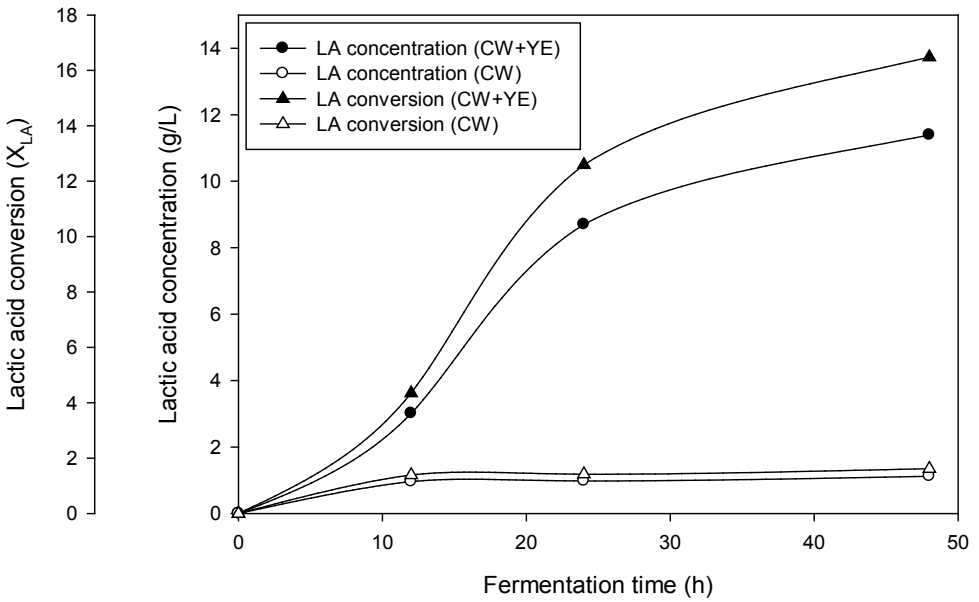

Figure 5. Lactic acid fermentation by *L. delbr.* subsp. *bulgaricus* NRRL-B-548 (YE: 0.4% (w/v), ILC: 69.1 g/L, T= 37°C)

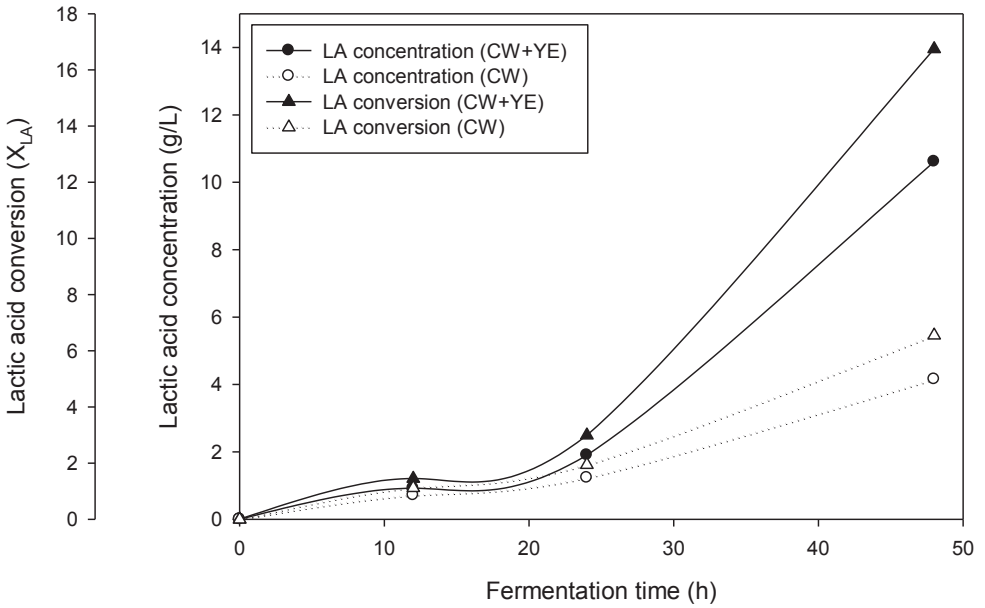

Figure 6. Lactic acid fermentation by *L. delbr.* subsp. *delbrueckii* NRRL-B-763 (YE: 0.4% (w/v), ILC: 63.4 g/L, T= 37°C)

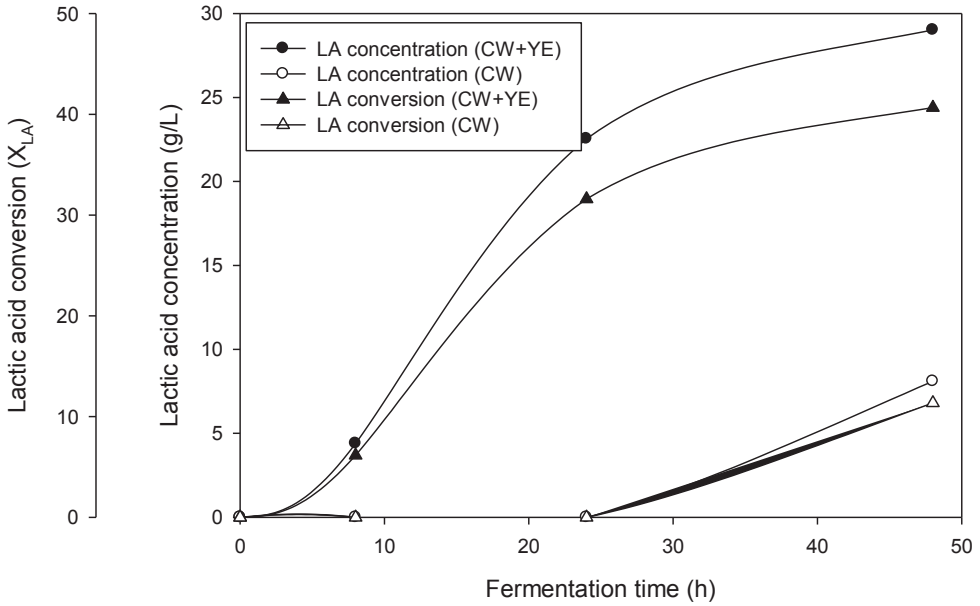

Figure 7. Lactic acid fermentation by *L. delbr.* subsp. *lactis* DİSTAM (YE: 0.4% (w/v), ILC: 71.3 g/L, T= 37°C)

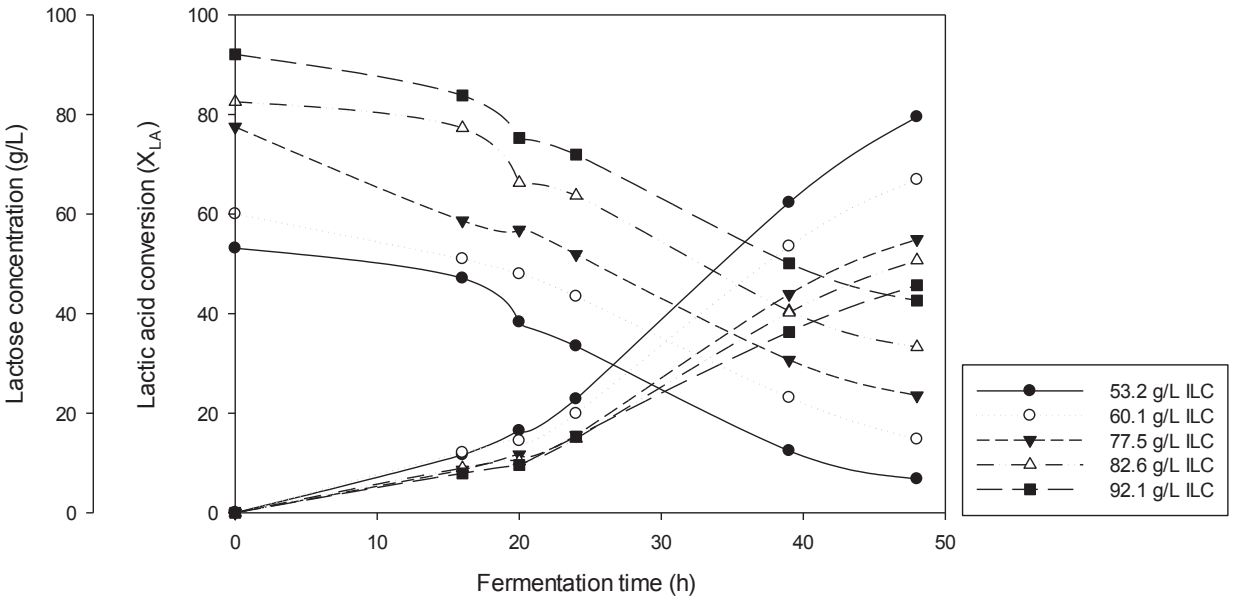

Figure 8. Effect of substrate (LACTOSE) concentration on lactic acid conversion and lactose consumption by *Lactobacillus casei* NRRL-B-1922 (YE: 0.4%; ILC: 53.2 g/L, 60.1 g/L, 77.5 g/L, 82.6 g/L, 92.1 g/L, T= 28°C)

ferent depending on the initial substrate concentration. With the increasing of the substrate concentration remaining lactose amounts increases at the end of the fermentation. After 48 h similar lactic acid concentrations (40-42 g/L) were achieved with all substrate concentrations and lactic acid conversions varies 46-80% due to the initial substrate amount. Most probably *Lactobacillus casei* could utilize the lactose completely after more then 48 h and lactic acid production would be higher for all initial lactose concentrations. If we would have waited for that time we would have see obviously different lactic acid concentrations but similar lactic acid conversions. For the 48 h lactic acid productivities are varies 0.83-0.88 g/Lh. We can say that the initial lactose amount has not any effect on productivity and concentration of lactic acid.

Effect of nutrient supplement on lactic acid production

Supplementation of yeast extract (0.4% w/v), $MnSO_4.H_2O$ (0.005% w/v), $MgSO_4.7H_2O$ (0.01% w/v) addition to fermentation medium were investigated. Because yeast

extract was proven to be most effective nitrogen source, we used yeast extract as nitrogen source for lactic acid production.

Manganese is an essential growth factor for *L. casei*, because of its role as a constituent of lactate dehydrogenase [27]. Also magnesium is a critical cation and cofactor in numerous intracellular processes. Mg^{++} has an efficient role in the 1., 3., 7., 9. and 10. steps in EMP pathway [28]. We supplemented these nutrients in the forms of $MnSO_4 \cdot H_2O$ and $MgSO_4.7H_2O$ with 0.4% w/v YE to the fermentation medium. The results of lactic acid conversions are given in Fig. 9. Resultantly conversions of lactic acid were close to another. However we can easily indicate from Fig. 9, the microorganism requires a nutrient supplement for complete conversion of lactose to lactic acid. Beside this, manganese sulphate and magnesium sulphate addition has not a more significant beneficial effect on the production of lactic acid from cheese whey by *L. casei* NRRL-B-1922.

The lactic acid conversion was 60% with only YE addition. This conversion was increased only 63% when additional Mn and Mg nutrient were added to the medium.

In this step of our study we wanted to see the effects of nutrient amounts one by one.

Effect of yeast extract

The effect of YE concentration on lactic acid production by *L. casei* NRRL-1922 was investigated by varying YE concentrations from 0% (w/v) to 1% (w/v). Fermentations were performed without manganese and magnesium addition, for 96 hours. Fig. 10 shows the lactic acid conversion and lactose utilization. The lactic acid conversion increased with adding YE.

In all the cases (with YE), lactose was consumed after 96 h and the final concentrations of lactic acid, conversions of lactic acid were close to one another. Beside this after 48 h of fermentation, with 0.5-1% YE addition, lactose was completely consumed and 90-99% lactic acid conversions were achieved. These results were achieved with lower YE concentrations (0.2% and 0.4%) only after 96 h of fermentation. We can easily mention that increasing the yeast extract supplementation from 0.2% to 1%, reduced the fermentation time from 96 h to 48 h to obtain maximum conversion. This is because increasing cell growth with the addition of nitrogen source. When there is no yeast extract in the fermentation medium time for lactose consumption gets longer. Without any YE supplement the lactic acid conversion is 57% at the end of fermentation. It is obviously shown in Fig. 10. YE is an important nutrient for an efficient lactic acid production.

However, increasing the nutrient addition represents an additional source of impurities as well as being costly. So the important thing is to use optimum yeast extract for lactic acid production as well as being cost effective.

Effect of manganese sulfate

The effect of manganese sulfate concentration on lactic acid production by *L. casei* NNRL-1922 was investigated. 0.003%, 0.005%, 0.07%, 0.01% and 0.02% (w/v) $MnSO_4.H_2O$ was supplemented to the fermentation medium presence of 0.4% (w/v) YE. For observing the effect of nutrient supplement a control sample was studied that contains only CW. The results of lactic acid conversion and lactose utilization are given in Fig. 11.

For cheese whey which contains different concentrations of $MnSO_4.H_2O$, despite the way of fermentation process is different after 96 h production of lactic acid is similar with each other.

The average lactic acid production is about 61 g/L. It is shown Fig. 11 that the lactic acid conversion is 94% with 0.007% manganese sulfate at the end of 72 h fermentation time. When there is only 0.4% yeast extract and no $MnSO_4.H_2O$ in the fermentation medium lactic acid conversion is 88% at 72 h. However, from the results given above we realized that $MnSO_4.H_2O$ concentration has not a significant effect on lactic acid production.

As seen in Fig. 11 with 0.003% (w/v) $MnSO_4.H_2O$ addition, lactose consumption is slower than other manganese sulfate concentrations. With all manganese sulfate concentrations, before 96 h of fermentation residual lactose amounts are close to zero as expected.

Effect of magnesium sulfate

Different concentrations of $MgSO_4.7H_2O$ (0.005%-0.01% w/v) were used for the investigation of magnesium effect on lactic acid production.

Likewise magnesium sulfate, when the different concentrations of $MgSO_4.7H_2O$ were added to the fermentation medium, lactic acid concentration did not change during the fermentation. The results of lactic acid conversion and lactose utilization are given in Fig. 12.

When there is not $MgSO_4.7H_2O$ in the fermentation medium 90% lactic acid conversion was occurred. Except 0.03% $MgSO_4.7H_2O$ concentration, lactic acid conversions are similar (91-93%). The highest value (97%) for lactic acid conversion was achieved after 96 h fermentation with 0.03% $MgSO_4.7H_2O$ addition to the fermentation medium.

After several experiment, it is mentioned that, when there was only 0.5% yeast extract in the fermentation medium only 93% lactic acid conversion was observed after 48 h. But when 0.007% (w/v) $MnSO_4.H_2O$ with 0.4% yeast extract were added to the fermentation medium, only 94% lactic acid conversion was occurred. Therefore, it is not necessary to use $MnSO_4.H_2O$ in lactic acid processes.

$MgSO_4.7H_2O$ concentration has not a beneficial effect on lactic acid production. It would be more cost effective if do not use the magnesium sulfate.

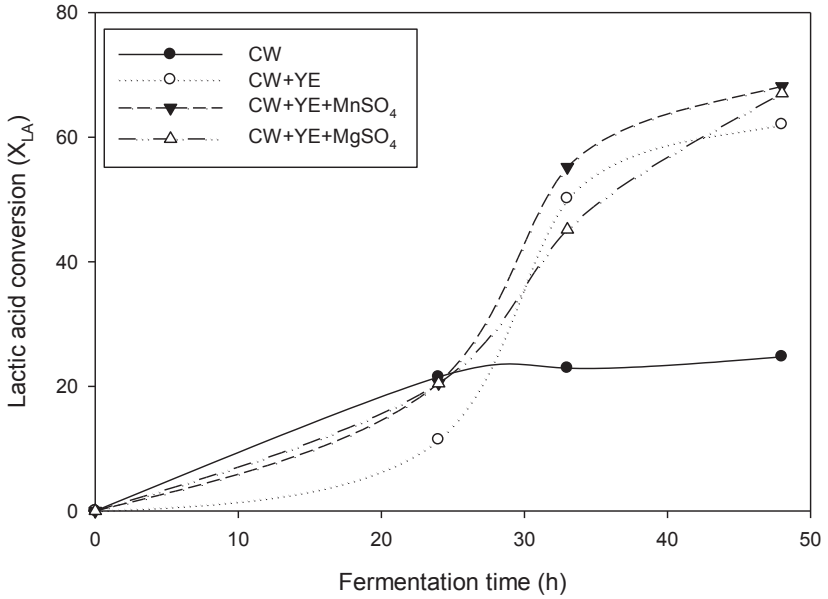

Figure 9. Effect of nutrient supplement on lactic acid conversion by *L. casei* NRRL-B-1922 (YE: 0.4%, ILC: 57.7 g/L, T= 28°C)

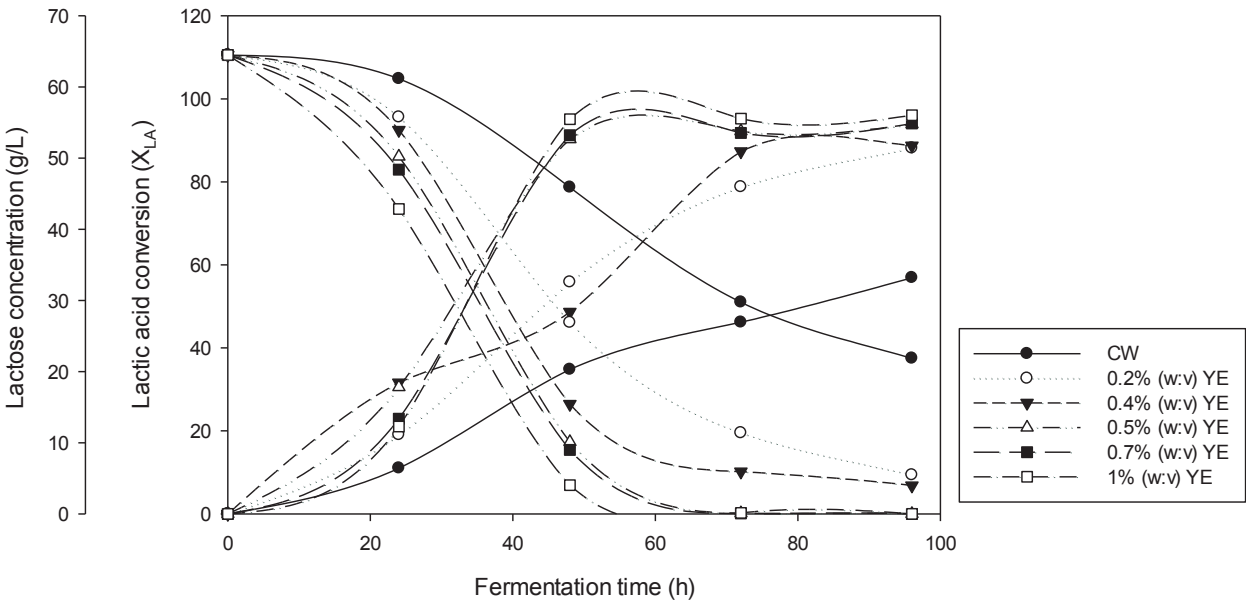

Figure 10. Effect of yeast extract amount on lactic acid conversion and lactose consumption by *Lactobacillus casei* NRRL-B-1922 (ILC: 64.5 g/L, T= 28°C

Conclusions

Lactic acid has an extensive interest for recent years since its potential in wide industrial applications and production of biodegradable plastics. Use of low-cost materials could lead to the reduction of fermentation cost.

The above results have demonstrated that efficient lactic acid production with high optical purity (>>99%) from cheese whey by *L. casei* is possible.

Both lactic acid concentration and lactic acid conversion were very low when cheese whey was used without nutrient supplement. Generally, the addition of nutrients and higher nutrient concentrations has a positive effect on the lactic acid production. The highest lactic acid concentration (61 g/L) and conversion (93%) for 48 h of fermentation was achieved from whey when supplemented with 0.5% YE (w/v) by using *L. casei* NRRL-1922. Therefore, it is proposed that cheese whey should inclu-

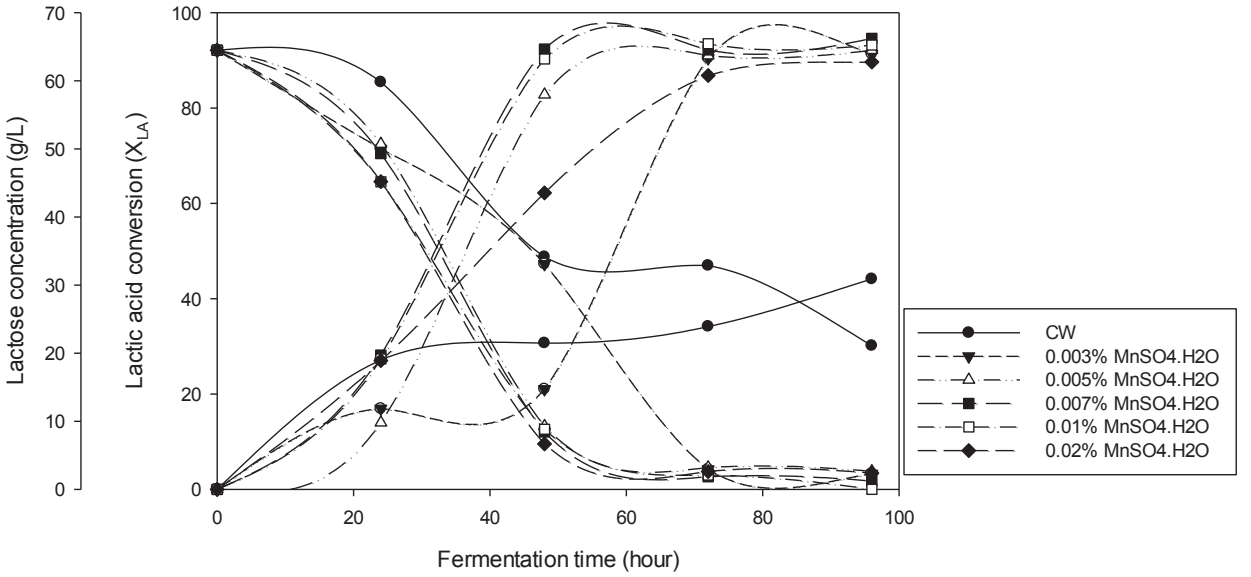

Figure 11. Effect of $MnSO_4.H_2O$ amount on lactic acid conversion and lactose consumption by *Lactobacillus casei* NRRL-B-1922 (YE: 0.4% (w/v), ILC: 64.5 g/L, T= 28°C

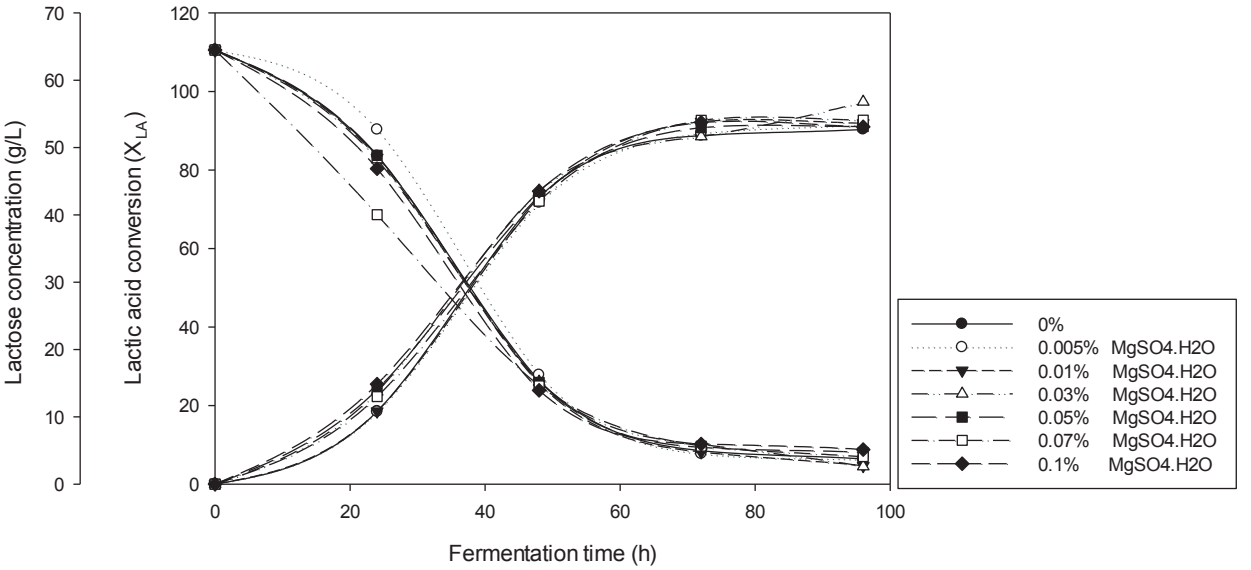

Figure 12. Effect of $MgSO_4.7H_2O$ amount on lactic acid conversion and lactose consumption by *Lactobacillus casei* NRRL-B-1922 (YE: 0.4% (w/v), ILC: 64.5 g/L, T= 28°C)

de only yeast extract without other nutrient supplements mentioned in literature to increase lactic acid conversion and productivity.

We highlight that the main advantage of this study is using an environmental pollutant as a substrate for economical production of lactic acid with high conversion. And it is sufficient only YE adding to the fermentation medium.

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