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# Lactose uptake rate measurements by $^{14}$C-labelled lactose reveals promotional activity of porous cellulose in whey fermentation by kefir yeast

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ABSTRACT

Lactose uptake rate by kefir yeast, immobilized on tubular cellulose and gluten pellets during fermentation of lactose and whey, was monitored using $^{14}$C-labelled lactose. Results illustrated that, in all cases, lactose uptake rate was strongly correlated with fermentation rate and the fermentation's kinetic parameters were improved by kefir yeast entrapped in tubular cellulose. As a result, twofold faster fermentations were achieved in comparison with kefir yeast immobilized on gluten. This is probably due to cluster and hydrogen bonds formation between cellulose and inhibitors, such as $Ca^{++}$ and generated lactic acid, by which they leave the liquid medium. The findings, regarding the promotional effect of cellulose, seem promising for application in industrial whey fermentations.



## 1. Introduction

Whey is the liquid by-product of cheese processing units, which constitutes an important environmental problem due to its bulk and its high organic load of 40–70 g/l BOD and 60–80 g/l COD (Athanasiadis, Paraskevopoulou, Blekas, & Kiosseoglou, 2004; Guimarães, Teixeira, & Domingues, 2010). Additionally, whey contains significant amounts of nutrients and is therefore a source of useful and high added-value products (Kosseva, Panesar, Kaur, & Kennedy, 2009). So bioconversion of whey to ethanol and lactic acid represents an alternative use of this co-product. Due to its ability to ferment lactose, kefir yeast was thought to be ideal for exploitation of whey (Athanasiadis, Boskou, Kanellaki, & Koutinas, 2001; Djuric, Caric, Milanovic, Tekic, & Panic, 2004; Dragone, Mussatto, Oliveira, & Teixeira, 2009; Kourkoutas et al., 2002; Koutinas et al., 2009; Panesar, Kennedy, Gandhi, & Bunko, 2007). However, whey fermentation time using free cells of kefir yeast was lengthy. In addition residual lactose was high (Athanasiadis, 2003; Athanasiadis et al., 2001). Cell immobilization on delignified cellulosic materials showed acceleration of lactose and whey fermentation (Athanasiadis et al., 2001). The most acceptable explanation for this promotion of fermentation was the enhancement of catalytic action of some enzymes involved in the process. Recent studies of lactose uptake rate by kefir cells in synthetic media containing lactose (Golfinopoulos, Papaioannou, Soupioni, & Koutinas, 2009) and whey (Golfinopoulos, Kopsahelis, Tsaousi, Koutinas, & Soupioni, 2011) explained kinetic aspects in relation to lactose uptake rate. These investigations showed that $Ca^{++}$ of whey may cause the inhibition, as was also reported for glucose uptake in previous studies (Akrida-Demertzi, Drainas, & Koutinas, 1990; Akrida-Demertzi & Koutinas, 1992). However, due to the fact that fermentation rate depends on lactose uptake rate by microorganisms and whey fermentation is promoted by immobilized cells, research efforts have been directed to study fermentation ability of immobilized kefir yeast. So, in the present work, kefir yeast cells, immobilized on delignified cellulosic material (porous cellulose) or gluten pellets, were used to ferment initially synthetic lactose media and then whey.

In a previous study, $^{14}$C-labelled lactose was used to record glucose uptake rate by *Saccharomyces cerevisiae* (Soupioni, Polichroniadou, Tokatlidou, Kanellaki, & Koutinas, 1998). In this work, lactose uptake rate by immobilized kefir yeast, during fermentation, was recorded by using $^{14}$C-labelled lactose. The aim of this investigation was to examine the effect of various conditions on lactose uptake rate during whey fermentation by immobilized kefir yeast cells, in order to reveal the role of uptake rate in the promotional activity of immobilized cells.

## 2. Material and methods

### 2.1. Microorganism and cell growth

The kefir yeast commercial product, which is used in the Caucasus for a homemade kefir drink, isolated and available at the

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Department of Chemistry of the University of Patras, was employed in the present work (Athanasiadis, Boskou, Kanellaki, & Koutinas, 1999; Athanasiadis et al., 2001; Koutinas, Athanasiadis, Bekatorou, Iconomopoulou, & Blekas, 2005). Cell growth and production of biomass were done according to previous work (Golfinopoulos et al., 2009).

### 2.2. Preparation of delignified cellulosic material and cell immobilization

Delignified cellulosic (DC) material (porous cellulose) was prepared from sawdust treated with 1% w/v NaOH for 3 h at the boiling point, in order for the lignin to be removed. After filtration and washing with water, DC was used for immobilization. In order to carry out cell immobilization, 170 g of delignified cellulosic material were added to 800 ml of culture medium containing 12% w/v lactose and 16 g wet weight of kefir culture and the whole were allowed to ferment for approximately 12 h. Then, the immobilized biocatalyst was filtered and used for fermentations.

### 2.3. Preparation of gluten pellets and cell immobilization

Wheat flour was mixed with tap water, kneaded, and the dough was washed exhaustively with water in order to remove starch. The prepared wet gluten was shaped manually into pellets of 1.5–2 cm diameter, which were dried at 105 °C for 5 h. These pellets were cooled to room temperature and used for cell immobilization. 170 g of gluten pellets were added to 800 ml of culture medium containing 12% w/v lactose and 16 g wet weight of kefir yeast culture and were allowed to ferment for about 12 h. Then, pellets were taken (after decanting of the fermentation broth) and used as biocatalyst in fermentations.

### 2.4. $^{14}$C-labelled lactose determination

The determination of $^{14}$C-labelled lactose and liquid scintillation measurements were done according to a recent investigation (Golfinopoulos et al., 2011).

### 2.5. Determination of residual sugars and ethanol

Residual sugars in the whey samples were determined on a Shimadzu LC-9A HPLC system consisting of a Shim-pack SCR-101N column, an LC-9A pump, an RID-6A refractive index detector, a CTO-10A column oven, and a DGU-2A degassing unit. Three times distilled water was used as the mobile phase (0.8 ml/min), and 1-butanol (0.1% v/v) was used as an internal standard. Column temperature was 60 °C. Sample dilution was 1% v/v, and injection volume was 40 μl (Tsaousi, Dimitrellou, & Koutinas, 2008). Residual sugar concentrations were calculated using standard curves prepared by 8 standard solutions of lactose, galactose and glucose (containing 0.1, 0.5, 1, 2, 3, 4, 5 and 6 g/100 ml of each sugar), by correlating the ratio of the residual sugar divided by the 1-butanol peak areas with residual sugar concentrations.

Ethanol was determined by a Shimadzu GC-8A gas chromatograph system, using a Porapac S column. Nitrogen was used as carrier gas at 40 ml/min. The column temperature was programmed at 120–170 °C at a rate 10 °C/min. The temperatures of the injector and FID detector were 210 and 220 °C, respectively. For the ethanol determination, a total volume of 2 μl (for each sample) was injected directly into the column and the concentration of ethanol was determined using standard curves prepared by eight standard solutions of ethanol (0.1%, 0.5%, 1%, 1.5%, 2%, 2.5%, 3% and 4%v/v). 1-Butanol was used as internal standard, at a concentration of 0.5% (v/v) (Kopsahelis, Nisiotou, Kourkoutas, Panas, Nychas, & Kanellaki, 2009).

### 2.6. Effect of lactose concentration, on lactose uptake rate during lactose synthetic medium fermentation by kefir yeast immobilized on gluten pellets and on delignified cellulosic materials

In order to study the effect of lactose concentration on lactose uptake rate, it was necessary (as whey contains always about 5% of lactose) to perform fermentations using synthetic media containing lactose. Based on previous results of lactose synthetic medium fermentation by free natural kefir culture (5 g) (Golfinopoulos et al., 2009), a series of sterilized synthetic lactose medium fermentations were carried out using 120 g of wet, gluten pellets-based, immobilized biocatalyst in 250 ml of synthetic lactose medium of different concentrations (2%, 4%, 6%, 10% and 15% w/v) at 30 °C and 5.5 as the initial pH value. In order to be comparable with the case of gluten pellets, the same conditions were used in a series of sterilized synthetic lactose medium fermentations, carried out by 102.24 g of wet, delignified cellulosic materials-based, immobilized biocatalyst in 250 ml of synthetic lactose medium. The above-mentioned fermentations were all conducted by using 5 g of wet weight kefir yeast cells in 250 ml of synthetic lactose medium, as in a previous study (Golfinopoulos et al., 2009). Calculations were also based on previous studies, where it was estimated that, on 100 g of gluten pellets, the amount of the immobilized kefir yeast cells was 4.2 g (Bardi, Bakogianis, Koutinas, & Kanellaki, 1996) whereas, on 100 g of delignified cellulosic materials, this amount was 4.9 g. (Bardi & Koutinas, 1994). Throughout the fermentations, Baume density (°Be) was measured and recorded in order to study the kinetics of the fermentation process. Likewise, samples of 2 ml were obtained and filtrated at various time intervals and the amount of the consumed $^{14}$C-labelled lactose by the cells was determined and expressed as cpm of lactose per gramme of kefir biomass per hour. For statistical reasons the recorded results were the mean values of three repeats. All fermentations were carried out under anaerobic conditions.

### 2.7. Effect of pH value, temperature and biocatalyst quantity on lactose uptake rate in whey fermentation by kefir yeast cells immobilized on gluten pellets and delignified cellulosic materials

Two series of whey fermentations were carried out using, for the first one, 142.8 g of kefir yeast immobilized on gluten pellets and, for the second, 122.7 g of kefir yeast immobilized on DC materials. The quantities were chosen, as mentioned above, based on previous studies, in order to use (in both cases) 6 g of wet weight kefir yeast cells in 250 ml of whey (Bardi et al., 1996; Golfinopoulos et al., 2009; Bardi & Koutinas, 1994). Likewise, in both cases, 250 ml of whey (rennet whey) were used and fermentations were carried out at 30 °C and at different initial pH values, e.g. 4, 5, 5.5, 6 and 6.5. Trial pH value was achieved by the addition of tartaric acid (7% solution), as the original whey initial pH value was 6.5. During whey fermentation the pH value was maintained stable to the selected trial pH, by the addition of 6 M NaOH solution. The optimum pH value, in each case, was used in order to study the effect of temperature on lactose uptake rate by immobilized kefir yeast. Thus, fermentations were carried out at various temperatures (10, 20, 25 and 30 °C) at pH values 5.5 or 5, in the case of kefir yeast immobilized on gluten pellets or delignified cellulosic materials, respectively.

Furthermore, the effect of biocatalyst amount was studied by two series of fermentations, using, for the first one, 100, 142.8 and 200 g of biocatalyst (kefir yeast cells immobilized on gluten pellets) and, for the second, 122.7, 140 and 180 g of biocatalyst (kefir yeast cells immobilized on delignified cellulosic materials). All fermentations were carried out under anaerobic conditions at 30 °C and pH values were kept constant at 5.5 or 5, respectively (which were the optimum conditions of the previous experiments).

### 2.8. Data analysis

All fermentations were performed in duplicate, while all measurements, analyses and results presented are the mean values of three repetitions. Calculations, figures, mean values and standard deviations were obtained using Microsoft Office Excel 2007.

## 3. Results and discussion

### 3.1. Effect of lactose concentration, on lactose uptake rate by kefir yeast immobilized on gluten pellets and DC materials during synthetic media fermentation

Figs. 1 and 2(a) and (b) illustrate fermentation kinetics and lactose uptake rate by kefir yeast immobilized on gluten pellets and porous cellulose respectively, during fermentation of synthetic media containing lactose at various concentrations. It is shown, that the lactose uptake rate by kefir yeast cells immobilized on gluten pellets was high and only with small differences up to 15% lactose. In the case of porous cellulose-supported kefir yeast cells, lactose uptake rate was higher than that of gluten. In both supports, the lactose uptake rate increased as the rate of fermentation increased. However, the increase of lactose uptake rate in the case of porous cellulose, in comparison with gluten pellets, did not result in a substantial increase in the fermentation rate. This phenomenon can be attributed to the different activities of intracellular enzymes, which was also observed in a previous study, when free cells of kefir were used to ferment lactose media of similar concentrations (Golfinopoulos et al., 2009). However, lactose uptake rate and fermentation rate were much higher in the case of cells immobilized on DC and gluten. Lactose uptake rate was higher at low concentrations, due to cells experiencing lower osmotic stress (Guimarães et al., 2010). High sugar concentrations inhibit cell growth of lactic acid bacteria which are present in kefir microflora (Athanasiadis et al., 2001). Kinetic parameters were improved for fermentation by kefir yeast immobilized on DC materials in comparison to fermentation by kefir yeast immobilized on gluten pellets (Figs. 1 and 2). It is also obvious that the fermentation activation energy, $E_a$, is strongly reduced in the case of cells immobilized on DC and gluten, as reported for other materials (Converti, Bargagliotti, Cavanna, Nicolella, & Del Borghi, 1996; Kandylis & Koutinas, 2008), suggesting that immobilization on DC and gluten acts as a promoter of the catalytic activity of the enzymes involved in the fermentation process (Bardi et al., 1996).

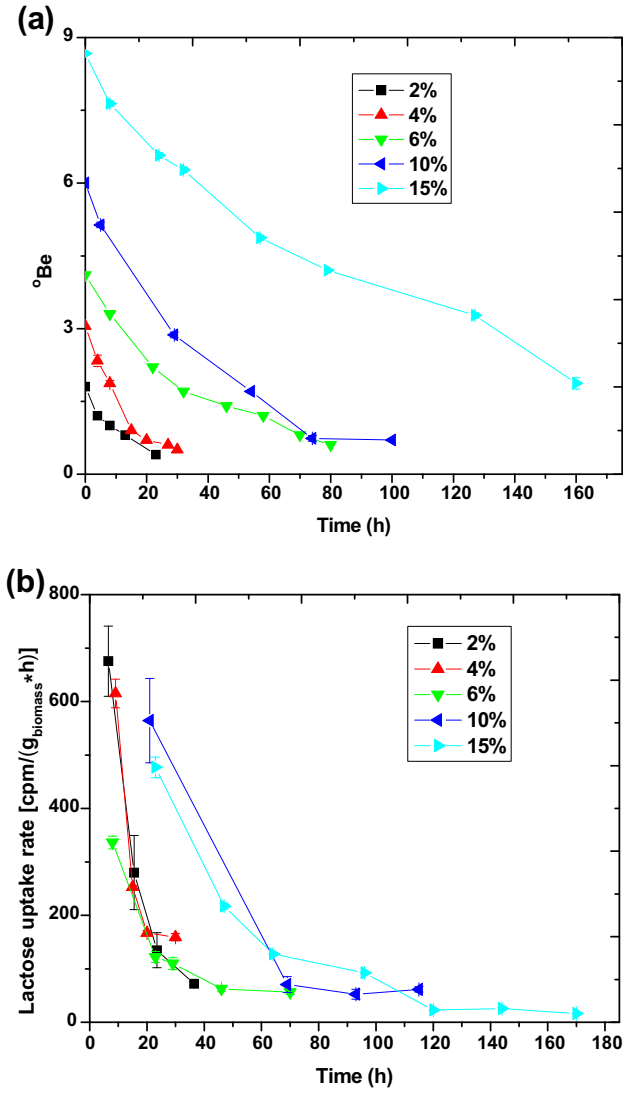


**Fig. 1.** Effect of lactose concentration on (a) Fermentation kinetics of lactose in synthetic medium by kefir yeast immobilized on gluten pellets, (b) in relation to lactose uptake rate.

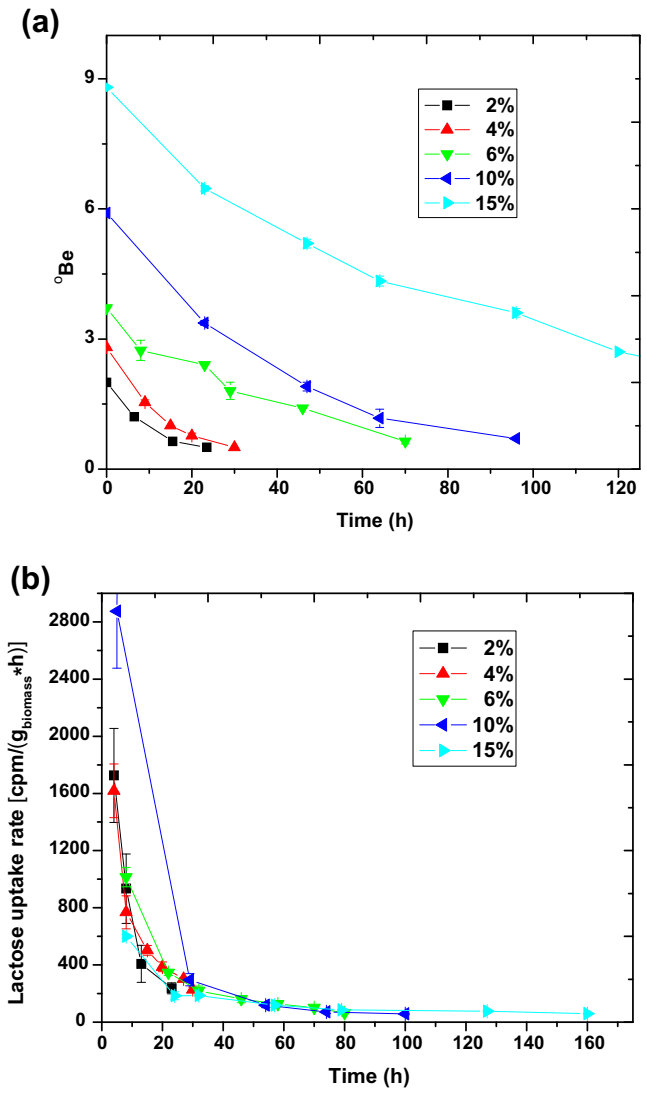


**Fig. 2.** (a) Fermentation kinetics of lactose in synthetic medium by kefir yeast immobilized on delignified cellulosic material, (b) in relation to lactose uptake rate, observed at various lactose concentrations.

*3.2. Effect of pH on lactose uptake rate by kefir yeast immobilized on gluten pellets and DC materials, during whey fermentation*

Figs. 3 and 4(a) and (b) show pH effect on fermentation kinetics and lactose uptake rate by kefir yeast immobilized separately on gluten pellets and DC material, respectively, during whey fermentation at various pH values. The best pH value for gluten seems to be 5.5. This result agrees with results from previous studies where synthetic lactose medium (Golfinopoulos et al., 2009) and whey were fermented by kefir free cells (Golfinopoulos et al.,

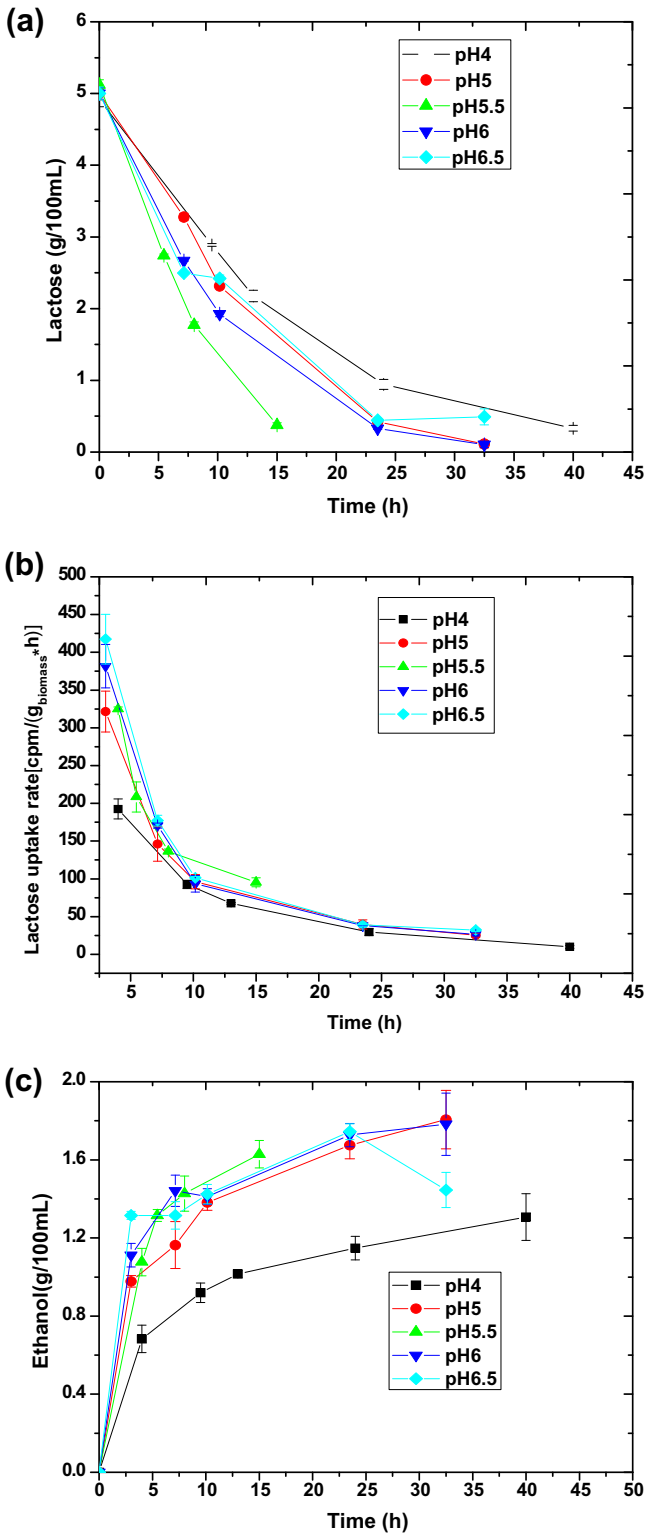


**Fig. 3.** (a) Fermentation kinetics of lactose by kefir yeast immobilized on gluten pellets, (b) in relation to lactose uptake rate and (c) ethanol concentration during whey fermentation, observed at various initial pH values.

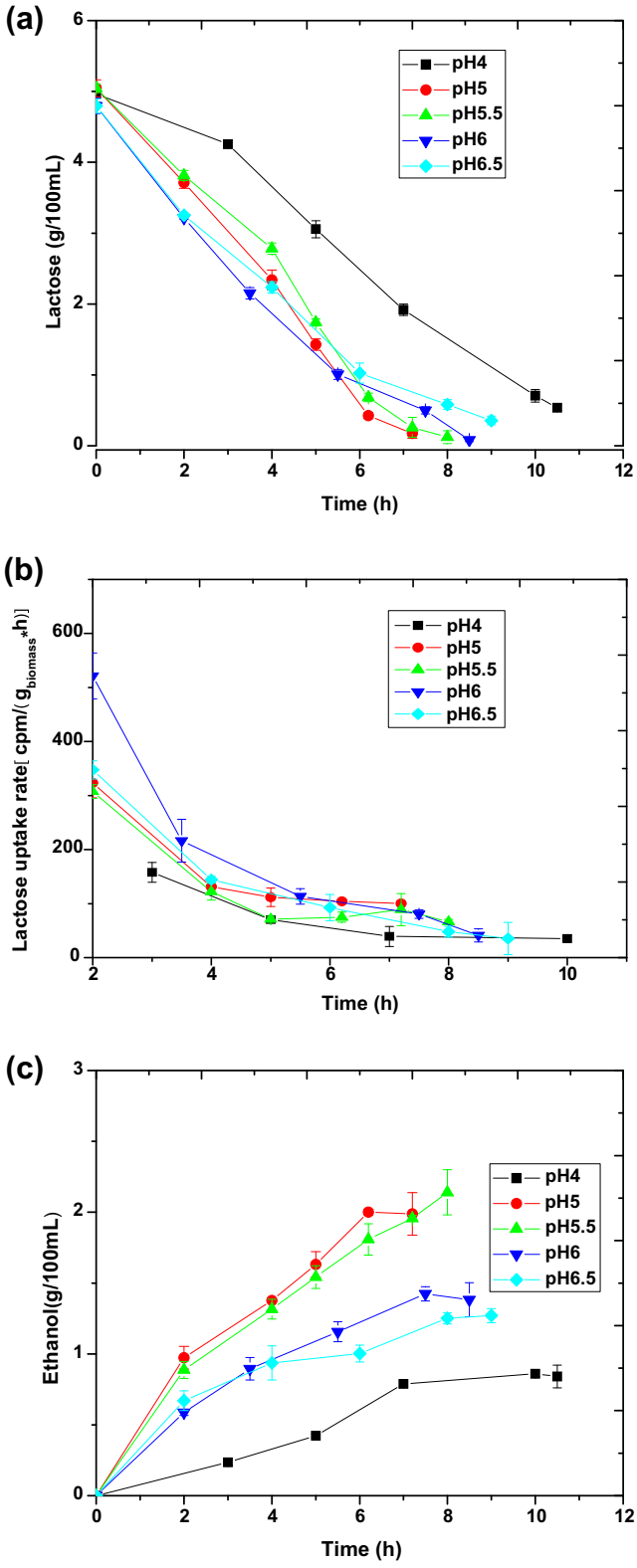


**Fig. 4.** (a) Fermentation kinetics of lactose by kefir yeast immobilized on delignified cellulosic material, (b) in relation to lactose uptake rate and (c) ethanol concentration during whey fermentation, observed at various initial pH values.

2011). At the 5.5 pH value, the fermentation lasted 15 h and was about 7-fold faster than was the fermentation by free cells. In the case of porous cellulose, the rate of fermentation was also higher at pH 5.5. However this does not give the highest lactose uptake rate, for both supports. Maybe this can be attributed to lower enzyme activity at that pH value than at 6–6.5. Furthermore, porous cellulose gave faster fermentations than did gluten. Specifically, at pH 5 the fermentation lasted only 7.2 h and was 2-fold faster than was fermentation by kefir yeast immobilized on gluten pellets (Tables 1 and 2). Fig. 3(c) shows that the highest ethanol production was at pH 5, but this was also high at pH 6 and 5.5. At the same pH values ethanol concentration, during whey fermentation, reached almost 1.80 g/100 ml (2.29% v/v) (Table 1).

During kefir yeast natural co-culture fermentation, the pH value affects lactose hydrolysis through differentiation of lactase activity. Therefore, the optimum 5.5 pH value results in the optimum activity of lactase. That explains why, even though there was higher lactose uptake rate at some pH values, this was not accompanied by an increase in the fermentation rate. Differences in lactose uptake rate at various pH values, in the range 4–6.5, may be attributed to the effect of pH on the cell wall charges, which affects cell uptake of metal ions contained in the fermentation broth. Possible increase of ion uptake by the cell wall reduces lactose uptake rate, due to ions preventing its diffusion into the cell. That might reflect upon the rate of fermentation. Correlation of glucose uptake rate with copper uptake by the cell wall was previously reported (Akrida-Demertzi et al., 1990). Ethanol concentration was at the same level or higher than that in previous studies (Magalhães et al., 2011; Papapostolou, Bosnea, Koutinas, & Kanellaki, 2008). Likewise, Fig. 4(c) shows that higher ethanol production was observed at pH 5.5, yielding an ethanol concentration of 2.14 g/100 ml (2.70% v/v) (Table 2) in the case of DC biocatalyst and 1.80 g/100 ml (2.29% v/v) (Table 1) in the case of gluten pellets.

### 3.3. Effect of temperature on fermentation kinetics and lactose uptake rate by kefir yeast cells immobilized on gluten pellets and DC materials during whey fermentation

Fig. 5(a) and (b), illustrate the effect of temperature on fermentation kinetics of lactose, in relation to lactose uptake rate by kefir yeast immobilized on gluten pellets and DC materials, respectively, during whey fermentation. It is clear that, as temperature increased, fermentation rate and lactose uptake rate increased too, in accordance with a previous study (Golfinopoulos et al., 2009). In this case, increase of fermentation rate is identified with lactose uptake rate. So the increase of fermentation rate is attributed to lactose uptake increase by kefir. Generally, fermentation rate was higher when DC materials were used as biocatalyst at all temperatures compared to gluten pellets. Fig. 5(c) shows ethanol production under the above mentioned conditions. The highest ethanol production was recorded at 30 °C when DC materials were used as biocatalyst support, paralleling the fermentation rate (Table 2). At constant pH value, the increase of temperature resulted in increase of fermentation rate that was identified with the increase of lactose uptake rate. That was attributed to the increase of the enzyme activity for lactose hydrolysis by the increase of temperature at constant pH value (Whitaker, 1996).

### 3.4. Effect of biocatalyst concentration on fermentation kinetics and lactose uptake rate by kefir yeast cells immobilized on gluten pellets and DC materials during whey fermentation

Figs. 6 and 7(a) and (b) show fermentation kinetics of lactose, in relation to lactose uptake rate by kefir yeast immobilized separately on gluten pellets and DC materials, respectively, with various amounts of biocatalyst. DC materials, as support, were more effective than were gluten pellets. So, fermentation time with immobilized cells on DC materials was two times shorter in comparison with immobilized cells on gluten pellets (Tables 1 and 2). The increase of biocatalyst concentration, up to 140 g, also caused an increase of fermentation rate (Table 2). Higher concentrations did not have any significant effect on fermentation rate. Lactose uptake rate was increased as the amount of biocatalyst was increased and went in parallel with the increase of fermentation rate in both supports. The use of 140 g of DC material biocatalyst, resulted in a 50% increase of lactose uptake rate compared with the gluten biocatalyst. Figs. 6(c) and 7(c) show higher ethanol production in the fermentation with 142.8 g of gluten pellets and 122.7 g of DC materials.

### 3.5. Lactose uptake rate study as a whole

The results based on lactose uptake rate of (i) synthetic medium containing lactose fermentation, using free cells of kefir (Golfinopoulos et al., 2009), (ii) whey fermentation using free cells of kefir yeast (Golfinopoulos et al., 2011) and (iii) whey fermentation by kefir yeast immobilized on biopolymers (gluten and DC) are discussed in this paragraph. The three investigations showed that the increase of fermentation rate is accompanied by increase of lactose uptake rate. Lactose uptake rate is optimum at pH 5.5. Fermentation rate and lactose uptake rate using free cells were higher in synthetic media containing lactose than in whey fermentation. Similar results were also observed in a previous study (Vallet, Masud, & Martin, 1998) and have been lately attributed to $Ca^{++}$ contained in whey (Golfinopoulos et al., 2011). However, DC material and gluten resulted in a sharp reduction of the whey fermentation time. This means that the inhibition caused on free cells was lowered or eliminated. Comparing DC and gluten, the cellulosic support resulted in much higher ethanol productivity as compared with the gluten support (Tables 1 and 2). After the aforementioned description of the overall research that has been done in the frame of whey fermentation, it may be concluded that fermentations by DC material-supported cells of kefir yeast leads to higher lactose uptake and fermentation rate and higher ethanol concentration.

### 3.6. Promotional effect of lactose fermentation by biopolymers

The results of this investigation indicate that the use of kefir yeast immobilized on gluten and delignified cellulose sharply reduces whey fermentation time and increases lactose uptake rate. Porous cellulose resulted in increased lactose fermentation rate and increased lactose uptake rate, in general. Lactose uptake rate for porous cellulose was 5-fold higher in the case of synthetic medium containing lactose than for whey fermentation, even though this was not identified with the fermentation rate (Figs. 2 and 4). Fermentation rate, using biopolymer, was much higher in the case of whey than with lactose synthetic medium. The inhibition in whey fermentation using free cells (Golfinopoulos et al., 2011) by $Ca^{++}$ was lowered or eliminated, maybe due to cluster formation of $Ca^{++}$ with cellulose which leaves the liquid medium. It is also probable that the formed lactic acid, which usually acts as a yeast inhibitor, generates hydrogen bonds with the hydroxyl groups of cellulose and also leaves the liquid medium (Rhee & Tanaka, 2000). This explains why whey is fermented in a reduced fermentation time by porous cellulose-supported kefir yeast cells, as compared with free cells (Golfinopoulos et al., 2011). Delignified cellulose was much more effective regarding fermentation rate than was gluten (Figs. 3–7). Specifically, Figs. 6 and 7 and Tables 1 and 2 show that the fermentation time for porous cellulose dropped 30% in comparison with gluten. This substantial increase of the fermentation rate cannot be explained by the limited increase of lactose uptake rate. The increase of the promotional

**Table 1**
Effect of temperature, pH and amount of biocatalyst on kinetic parameters of whey fermentation by kefir cells immobilized on gluten pellets (G.P.).

| Temp. (°C) | Amount of biocatalyst (g/250 ml) | pH | Initial sugar (g/100 ml) | Fermentation time (h) | Ethanol concentration (%v/v) | Daily ethanol productivity (g/l) | Ethanol yield (g/g) | Residual sugar (g/100 ml) | Conversion (%) |
|---|---|---|---|---|---|---|---|---|---|
| 30 | 142.80 | 4 | 4.92 ± 0.10 | 40 | 1.66 ± 0.12 | 7.86 ± 0.57 | 0.29 ± 0.03 | 0.33 ± 0.04 | 93.3 ± 0.95 |
| 30 | 142.80 | 5 | 5.00 ± 0.09 | 32.5 | 2.29 ± 0.15 | 13.4 ± 0.88 | 0.37 ± 0.03 | 0.11 ± 0.05 | 97.8 ± 0.98 |
| 30 | 142.80 | 5.5 | 5.12 ± 0.10 | 15 | 2.06 ± 0.07 | 26.1 ± 0.82 | 0.34 ± 0.01 | 0.38 ± 0.03 | 92.6 ± 0.61 |
| 30 | 142.80 | 6 | 5.00 ± 0.08 | 32.5 | 2.26 ± 0.16 | 13.2 ± 0.92 | 0.36 ± 0.03 | 0.10 ± 0.04 | 98.0 ± 0.83 |
| 30 | 142.80 | 6.5 | 5.00 ± 0.09 | 32.5 | 1.84 ± 0.09 | 10.7 ± 0.51 | 0.32 ± 0.02 | 0.49 ± 0.11 | 90.2 ± 2.37 |
| 10 | 142.80 | 5.5 | 5.06 ± 0.14 | 104 | 1.99 ± 0.10 | 3.63 ± 0.18 | 0.38 ± 0.01 | 0.95 ± 0.09 | 81.2 ± 1.60 |
| 20 | 142.80 | 5.5 | 5.10 ± 0.14 | 38 | 2.09 ± 0.12 | 10.4 ± 0.62 | 0.37 ± 0.03 | 0.68 ± 0.14 | 86.7 ± 2.65 |
| 25 | 142.80 | 5.5 | 5.19 ± 0.08 | 28 | 2.05 ± 0.09 | 13.9 ± 0.58 | 0.35 ± 0.01 | 0.61 ± 0.05 | 88.2 ± 1.11 |
| 30 | 142.80 | 5.5 | 5.12 ± 0.10 | 15 | 2.06 ± 0.07 | 26.1 ± 0.82 | 0.34 ± 0.01 | 0.38 ± 0.11 | 92.6 ± 0.61 |
| 30 | 100.00 | 5.5 | 5.01 ± 0.09 | 27.5 | 1.71 ± 0.17 | 11.8 ± 1.17 | 0.28 ± 0.03 | 0.16 ± 0.11 | 96.8 ± 2.30 |
| 30 | 142.80 | 5.5 | 5.12 ± 0.10 | 15 | 2.06 ± 0.07 | 26.1 ± 0.82 | 0.34 ± 0.01 | 0.38 ± 0.03 | 92.6 ± 0.61 |
| 30 | 200.00 | 5.5 | 5.12 ± 0.10 | 15 | 1.93 ± 0.14 | 24.4 ± 1.78 | 0.32 ± 0.02 | 0.31 ± 0.06 | 94.0 ± 1.02 |

**Table 2**
Effects of temperature, pH and amount of biocatalyst on kinetic parameters of whey fermentation by kefir cells immobilized on delignified cellulosic materials (D.C.).

| Temp. (°C) | Amount of biocatalyst (g/250 ml) | pH | Initial sugar (g/100 ml) | Fermentation time (h) | Ethanol concentration (%v/v) | Daily ethanol productivity (g/l) | Ethanol yield (g/g) | Residual sugar (g/100 ml) | Conversion (%) |
|---|---|---|---|---|---|---|---|---|---|
| 30 | 122.70 | 4 | 4.96 ± 0.12 | 10.5 | 1.06 ± 0.08 | 19.1 ± 1.43 | 0.19 ± 0.01 | 0.54 ± 0.14 | 89.1 ± 3.01 |
| 30 | 122.70 | 5 | 5.04 ± 0.12 | 7.2 | 2.51 ± 0.15 | 66.1 ± 4.05 | 0.41 ± 0.03 | 0.17 ± 0.07 | 96.6 ± 1.48 |
| 30 | 122.70 | 5.5 | 5.04 ± 0.06 | 8 | 2.70 ± 0.16 | 64.0 ± 3.91 | 0.43 ± 0.02 | 0.12 ± 0.09 | 97.6 ± 1.76 |
| 30 | 122.70 | 6 | 4.80 ± 0.11 | 8.5 | 1.75 ± 0.12 | 39.0 ± 2.71 | 0.29 ± 0.02 | 0.08 ± 0.02 | 98.3 ± 0.40 |
| 30 | 122.70 | 6.5 | 4.80 ± 0.10 | 9 | 1.61 ± 0.05 | 33.9 ± 1.11 | 0.29 ± 0.01 | 0.36 ± 0.06 | 92.5 ± 1.14 |
| 10 | 122.70 | 5 | 5.11 ± 0.20 | 73 | 1.72 ± 0.10 | 4.47 ± 0.25 | 0.31 ± 0.03 | 0.68 ± 0.14 | 86.7 ± 2.79 |
| 20 | 122.70 | 5 | 5.02 ± 0.08 | 24 | 1.66 ± 0.09 | 13.1 ± 0.67 | 0.29 ± 0.01 | 0.50 ± 0.10 | 90.1 ± 1.90 |
| 25 | 122.70 | 5 | 5.03 ± 0.09 | 14 | 1.80 ± 0.13 | 24.4 ± 1.70 | 0.29 ± 0.03 | 0.17 ± 0.08 | 96.6 ± 1.56 |
| 30 | 122.70 | 5 | 5.04 ± 0.12 | 7.2 | 2.51 ± 0.15 | 66.1 ± 4.05 | 0.41 ± 0.03 | 0.17 ± 0.07 | 96.6 ± 1.48 |
| 30 | 122.70 | 5 | 5.04 ± 0.12 | 7.2 | 2.51 ± 0.15 | 66.1 ± 4.05 | 0.41 ± 0.03 | 0.17 ± 0.07 | 96.6 ± 1.48 |
| 30 | 140.00 | 5 | 4.86 ± 0.12 | 5 | 1.17 ± 0.10 | 44.4 ± 3.85 | 0.20 ± 0.02 | 0.22 ± 0.09 | 95.5 ± 1.77 |
| 30 | 180.00 | 5 | 4.84 ± 0.08 | 4.45 | 1.83 ± 0.14 | 78.0 ± 6.01 | 0.31 ± 0.02 | 0.20 ± 0.04 | 95.9 ± 0.76 |

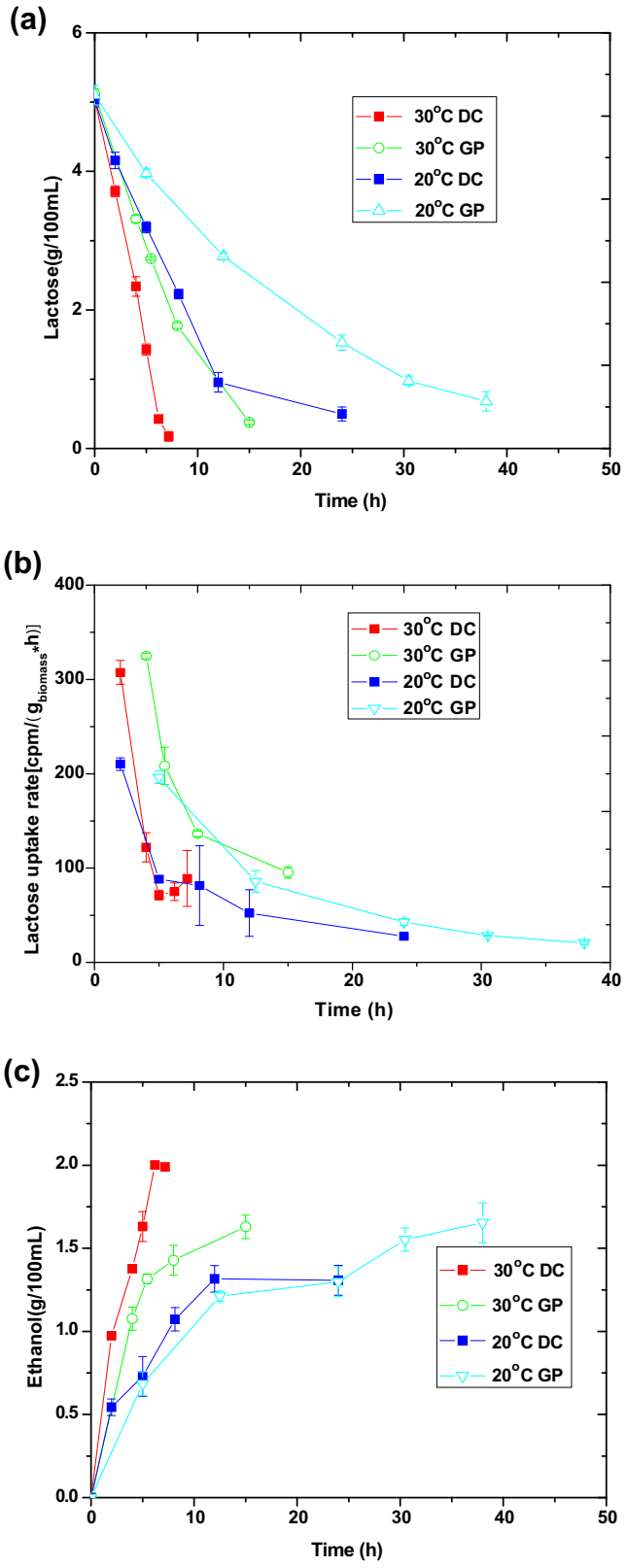


**Fig. 5.** (a) Fermentation kinetics of lactose by kefir yeast immobilized on gluten pellets (GP) and delignified cellulosic (DC) material, (b) in relation to lactose uptake rate and (c) ethanol concentration, during whey fermentation at 20 and 30 °C.

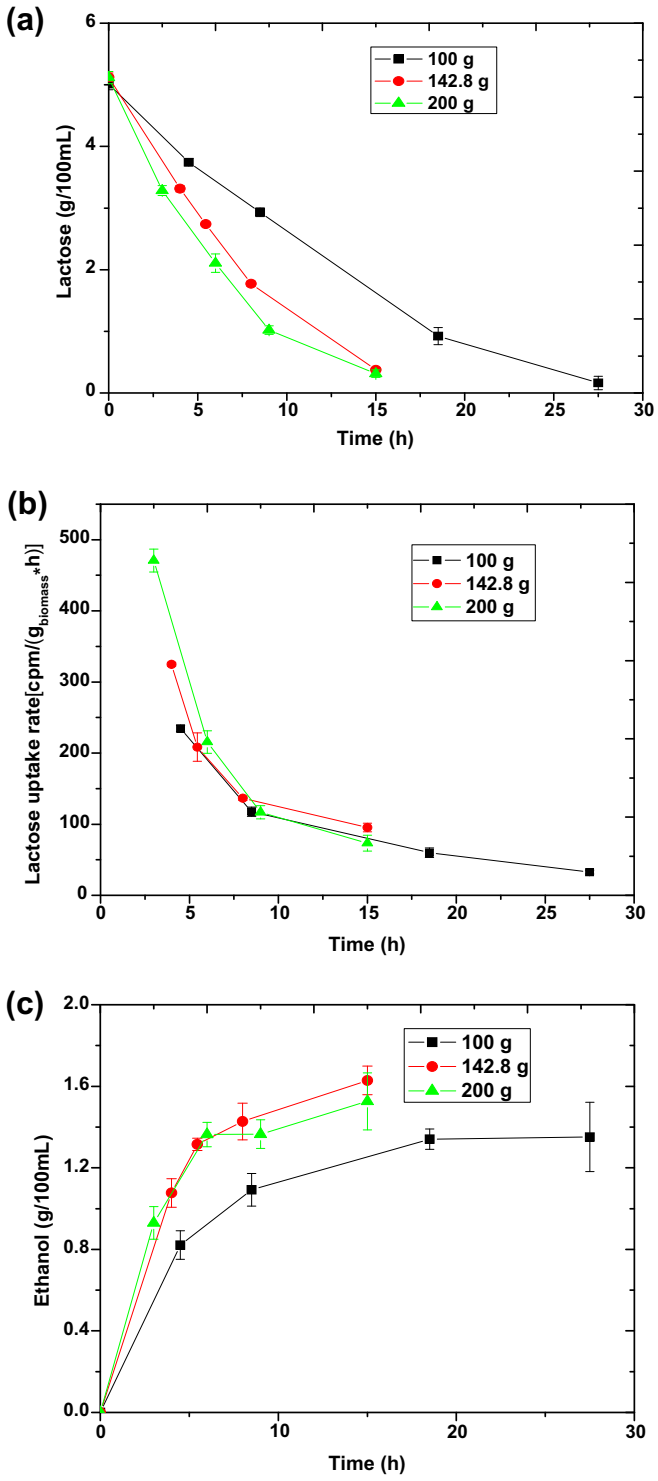


**Fig. 6.** (a) Fermentation kinetics of lactose by kefir yeast immobilized on gluten pellets, (b) in relation to lactose uptake rate and (c) ethanol concentration during whey fermentation, observed with various amounts of biocatalyst.

activity of delignified and porous cellulose versus protein gluten can be attributed (i) to the increase of lactose concentration on the surface of DC due to hydrogen bonds between hydroxyl groups of lactose and cellulose or (ii) to the promotion of the lactase enzyme activity, causing increase of lactose hydrolysis rate. In order to certify this possibility research is now in progress.

### 3.7. Impacts of the investigation on research and development

The substantial increase (by porous cellulose) of the fermentation rate, except for practical application in industry, will have to

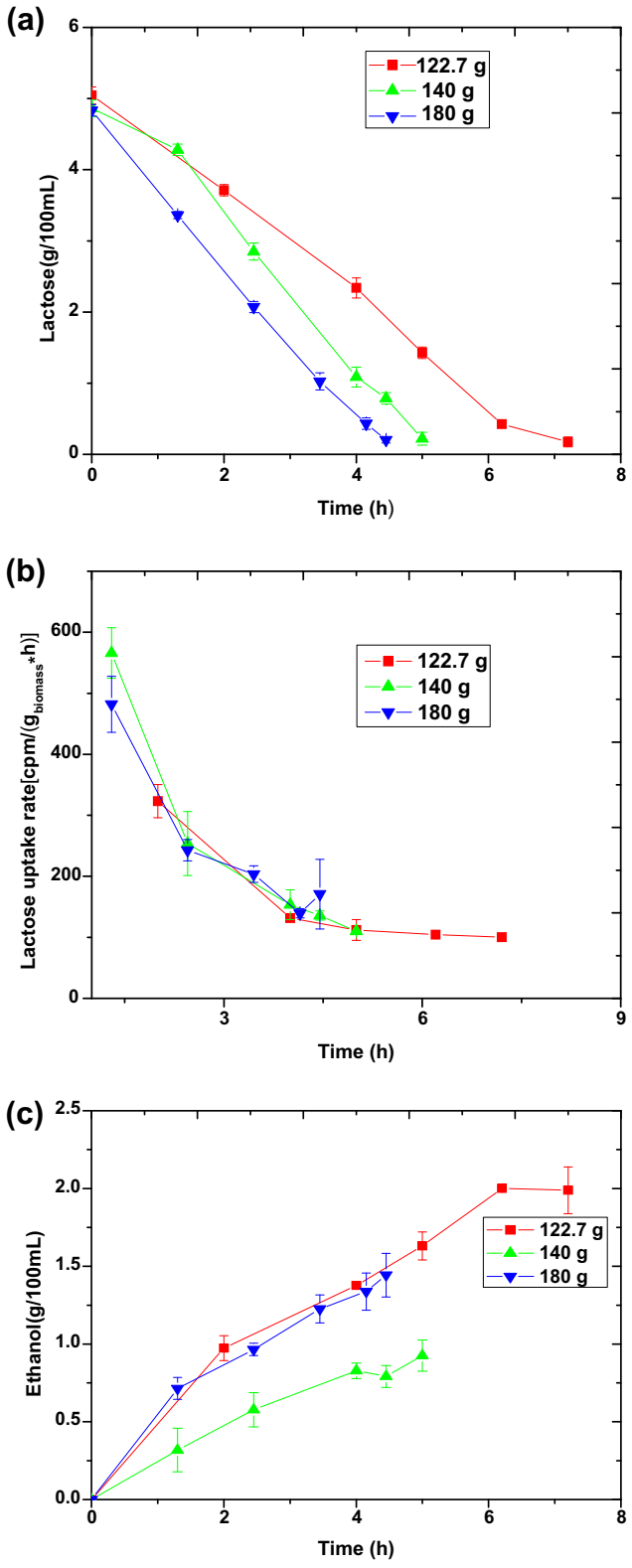


**Fig. 7.** (a) Fermentation kinetics of lactose by kefir yeast immobilized on delignified cellulosic material, (b) in relation to lactose uptake rate and (c) ethanol concentration during whey fermentation, observed with various amounts of biocatalyst.

be examined also in a series of bioconversion and biochemical processes. The latter can be examined in parallel with sugar uptake rate in each biochemical reaction. In the frame of this research, the mechanism of the promotional effect of porous cellulose can be examined, proving (i) the increase of sugar concentration on its surface and (ii) the effect of porous cellulose on hydrolytic enzyme activity. Another topic could be the study of porous cellulose and other biopolymers as materials that can be used for the production of biocatalysts and in the production of new materials. Therefore, it is necessary to develop methods to produce nano- and micro-porous biopolymers. The production of these nano- and micro-porous biopolymers will lead to nano- and micro-composites that could be used for research in the area of sustainable and intelligent food packaging materials.

## 4. Conclusions

Lactose uptake rate was strongly correlated with fermentation rate and it increased during whey fermentation by kefir yeast cells, as temperature increased up to 30 °C. Moreover the highest lactose uptake rate was recorded at pH 5.5 for gluten pellets and 5 for DC materials. The increase of the amount of the biocatalyst did not seem to have a high impact on lactose uptake rate by kefir.

Biopolymers, such as gluten pellets and DC materials, can be used as promoters during the whey fermentation process. The biocatalytic activity of porous cellulose can be attributed to (i) hydrogen bonding between hydroxyl groups of cellulose, with inhibitors and/or lactose and (ii) attachment of cells to the internal porous cellulose surface.

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