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Lactose uptake rate by kefir yeast using ^{14}C-labelled lactose to explain kinetic aspects in its fermentation

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ABSTRACT

The present work shows the relation between kefir fermentation ability and carbohydrate uptake rate. This was examined in a model system containing kefir co-culture and lactose in order to study fermentation induced by yeasts and bacteria at the same time. Lactose uptake rate was recorded by using ^{14}C-labelled lactose. The effect of lactose, cell concentration and pH on lactose fermentation was examined. Results have shown increase of lactose uptake rate at lower cell concentrations and specifically the maximum values of lactose uptake rate were obtained at 30 °C, 5.5 pH value and initial lactose and cell concentration 10% w/v and 16 g/L, respectively. Likewise, lighten that the increase of the fermentation rate by immobilized cells can be attributed also, in addition to other factors, to lower cell concentration on the surface of the support or of the promoter. Besides, it is shown that the effect of pH value on the biochemical reactions, carried out by intracellular enzymes can be attributed, except to the effect of pH on enzyme ability, in addition to the effect of pH on carbohydrate uptake rate.

1. Introduction

Kefir is a mixed culture of various species of yeasts and bacteria (Pintado et al., 1996; Garrote et al., 1997). Due to its ability to ferment lactose, kefir was thought to be ideal for the exploitation of whey lactose. Its microbiological composition ensures simultaneous alcoholic and lactic acid fermentations.

Research efforts have been undertaken over the last years to study kefir yeasts at low temperature fermentations by immobilized cells on suitable supports (Athanasiadis et al., 1999). Furthermore, kefir has been already used for the production of bread with improved quality and shelf life (Plessas et al., 2005) and for the production of novel whey beverage with good organoleptic properties (Athanasiadis et al., 2004). Likewise, in the production of whey cheese with longer shelf life and enhanced quality when it is used as a starter culture (Dimitrellou et al., 2007). Cheese whey represents an important environmental problem due to its bulk capacity and its high organic load of a 60,000–80,000 ppm COD (Athanasiadis et al., 2004). In order to convert this waste to value added products, firstly the kinetic parameters of lactose fermentation need to be examined thoroughly. Thus, the effect of lactose concentration, pH value, temperature and kefir biomass concentration, have been previously studied (Athanasiadis et al., 2005). Also, under the above conditions a study for lactose uptake rate by kefir is necessary, in order to contribute on the clarification of the kefir cells behaviour during the fermentation of a raw material as the whey. It is more appropriate to study both alcoholic and lactic acid fermentations. In order to do so, the model system of kefir-lactose was preferred for examination. Research efforts of the last years showed that both fermentations are affected by various conditions (Parmjit et al., 2007; Papavasiliou et al., 2008). This is a challenge to study the role of cell physiology in those effects and may the results be the precursor of a more complete theoretical background and approach of intracellular enzyme activity and in approach of the increase of the fermentation rate by cell immobilization. Previous study of lactose fermentation using kefir yeast showed that the fermentation leads to increased alcohol yield as compared with lactic acid formation. Likewise, by-products are formed as in the alcoholic fermentation of glucose using yeasts of the genus Saccharomyces. In the frame of these works by-products quantity was formed in lactose fermentation using kefir yeast is low and in the level of traditional glucose fermentation (Athanasiadis et al., 1999, 2001; Kourkoutas et al., 2002).

Bioprocesses using yeast and bacteria show multiple behaviour in productivities affect production cost. The theoretical background of that is supported on enzyme catalytic activity. However, it is well known that cells take up carbohydrates through the cell wall and it is obvious that the diffusion of carbohydrate molecule could play a role in the fermentation rate. Therefore, research to examine cell carbohydrate uptake rate will visualise this phenomenon and will create the practical and theoretical background is supported the productivity in bioprocesses. The industrial carbohydrates employed are glucose, fructose, sucrose and lactose. To

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study cell carbohydrate uptake rate in relation to fermentation, the natural co-culture kefir was finally preferred because it contains several yeast genera and lactic acid bacteria species most of which convert lactose. Therefore, the model system preferred was kefir co-culture as active organism and lactose as carbohydrate, because in practice raw materials containing lactose are treated with kefir yeast (Athanasiadis et al., 2001, 2002; Harta et al., 2004). To organise this investigation, fermentations were performed using synthetic medium containing lactose and kefir biomass as the active organism. The effects of cell and lactose concentrations as well as pH and temperature on fermentation of lactose and lactose uptake rate were examined. This model of kefir co-culture and lactose was selected due to the simultaneous alcoholic and lactic acid fermentation performed.

Therefore, due to the fact that cheese whey is a very complex medium of high capacity, in this investigation lactose synthetic medium was used to study kefir fermentation ability through lactose uptake rate, using ^{14}C-labelled lactose, which is the experimental aim of this work, with theoretical aim the challenges that have been aforementioned.

2. Methods

2.1. Microorganism and cell growth

Kefir, a commercial product usually used to produce kefir drink, was employed in the present study. Kefir grains were preserved into 1 L fresh pasteurised full cream milk, which was renewed every week, at approximately 4 °C. Cell growth of kefir biomass took place in a sterilized synthetic medium (2% lactose, 0.4% yeast extract, 0.1% $(NH_4)_2SO_4$, 0.1% KH_2PO_4 and 0.5% $MgSO_4 \cdot 7H_2O$) at 30 °C under anaerobic conditions. Pressed wet-weight cells were prepared and used directly in anaerobic fermentation. All treatments were carried out in triplicate and the mean values are presented.

2.2. ^{14}C-labelled lactose determination

At the beginning of each run 1 mL of labelled lactose [D-glucose-1–14C], (ARC 0466 lactose, and 0.1 mCi/mL) was added, as we did in previous investigations (Soupioni et al., 1998). The labelled lactose was fermented in the same way as the non active one. At various time intervals samples of 2 mL were filtered using cellulose membrane filters (0.45 μm) and ^{14}C within the cells was determined by liquid scintillation. The amount of the labelled lactose consumed by a specific amount of kefir biomass during fermentation was determined and expressed as cpm of lactose per gram of kefir biomass per hour.

2.3. Liquid scintillation measurements

All cellulose filters with cells contained ^{14}C were put one by one in appropriate vials and 5 mL of liquid scintillation cocktail Opti Fluor (Perkin Elmer) was added. The measurements were performed on a PACARD-3255 liquid scintillation counter, interfaced to an APPLE-2 personal computer for data evaluation.

2.4. Effect of lactose concentration, pH value, temperature and cell concentration on lactose uptake rate

Sterilized synthetic lactose medium (250 mL) of different concentration (2%, 4%, 6%, 10% and 15% w/v) having 5.5 pH initial value was introduced into an Erlemeyer flask. A fixed amount of wet kefir biomass to provide 20 g/L cell concentration was added along with ^{14}C-labelled lactose and allowed to ferment at 30 °C under anaerobic conditions. To study the effect of pH value, the above medium of 6% lactose was fermented at different pH values of 3.5, 4.0, 5.0, 5.5, 6.0 and 6.5.

The effect of the temperature on lactose uptake rate by kefir biomass was studied at various temperatures of 10, 15, 20, 25 and 30 °C. The pH value during fermentation was kept constant at 5.5 by the addition of NaOH solution (6 M).

Furthermore, the effect of kefir biomass concentration was studied by a series of fermentations conducted using 12, 16, 20, 24 and 28 g/L kefir biomass, carried out at 30 °C in a pH value of the fermented medium kept constant at 5.5 by the addition of NaOH solution (6 M).

Measurements of the Baume density (°Be) and filtration of samples (2 mL) were followed in order to study the kinetics of fermentation. The recorded results were the mean value of three repeats.

3. Results and discussion

3.1. Effect of lactose and cell concentration, on kefir-lactose uptake rate

In Fig. 1a and b fermentation kinetics and lactose uptake rate by kefir free cells, that was observed for each synthetic medium respectively, are illustrated. The best lactose uptake rate was observed when the lactose concentration of the fermented medium was 10% w/v and in general we can say that lactose uptake rate was higher at 2% than 4% and 6% may be due to osmotic pressure

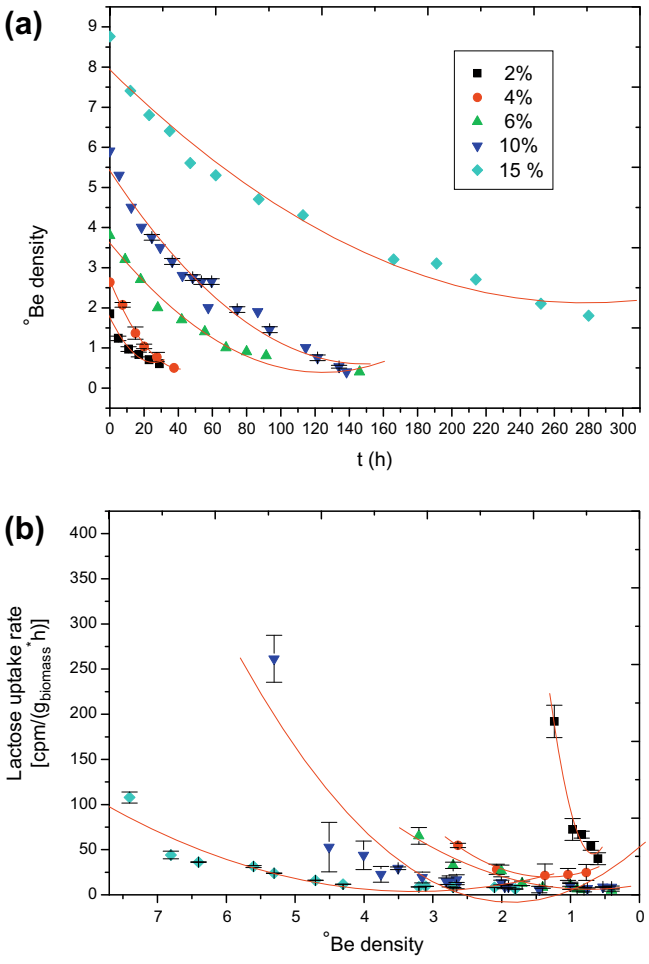

Fig. 1. (a) Fermentation kinetics of lactose, (b) in relation with lactose uptake rate, observed at various lactose concentrations.

balance of cell reduced stress of them. At 10% even though the osmotic pressure is higher the uptake increased after an adaptation period, which is higher than that of 2%, 4% and 6%. The increase of the adaptation period can be attributed to increased stress of cells. May be increasing stress more contributed to a reduction of lactose diffusion through of the cell wall. However, increasing further lactose concentration lactose uptake rate came back to reduction due to cell stress increment (Athanasiadis et al., 2001).

Also, Fig. 2a and b show the effect of kefir biomass concentration on fermentation kinetics and lactose uptake rate of kefir free cells, respectively. The higher biomass concentration resulted to higher fermentation rate and lower lactose uptake rate. Due to the higher dispersion of lactose substrate on larger amount of kefir cells, the lactose uptake rate of an individual kefir cell is reduced. Thus, keeping the substrate concentration constant and increasing the biomass concentration resulted in lower lactose uptake rate. Therefore, at low cell concentrations cells looked to be more active having the ability to consume more lactose per hour. This ability competed the increment of fermentation rate was obtained by the increased cells concentrations and consequently by the increased concentration of enzymes. The more active cells at low concentrations led to perspective to find ways for the increment of lactose uptake rate by yeast and bacteria in bioprocesses. May be this reduction could be obtained by the minimisation of cell stress.

3.2. Effect of pH and temperature on kefir-lactose uptake rate

Furthermore, Fig. 3a and b show the effect of pH on fermentation kinetics and lactose uptake rate by kefir free cells respectively. On 5.5, pH value showed the higher lactose uptake rate. Lactose uptake rate was increased up to 5.5 pH value. At 6 pH value we had a reduction of the uptake rate and then it increased again. That was identified by the changes of the rate of fermentation where up to 5.5 was increased and then was reduced. Therefore, the high effect of pH value in the case of kefir yeast natural co-culture fermentation can be attributed either to lactose hydrolysis was affected by pH, through of the pH effect on lactase enzyme activity, or to the low resistance at lower pH value of various lactic acid bacteria were contained in kefir microflora. The high differences in lactose uptake rate at various pH values in the range 3.5–6.5 may be attributed, to the effect of pH on the cell wall charges, affect the cell uptake of metal ions were contained in the fermentation broth. The ion uptake increment by the cell wall reduced lactose uptake rate due to ions prevents its diffusion into the cell. That reflected to the rate of fermentation. The correlation of glucose uptake rate with copper uptake by the cell wall was reported in the past (Akrida-Demertzi and Koutinas, 1992; Akrida-Demertzi et al., 1990). May be this ion uptake possibility on the fermentation rate effect was a second parameter, with the first to be the effect of pH on lactase hydrolytic enzyme activity.

Moreover, in Fig. 4a and b the fermentation kinetics and lactose uptake rates by free kefir cells at various temperatures are illustrated. The higher lactose uptake rate was observed when the fermentation was carried at temperatures higher of 25 °C. Lactose

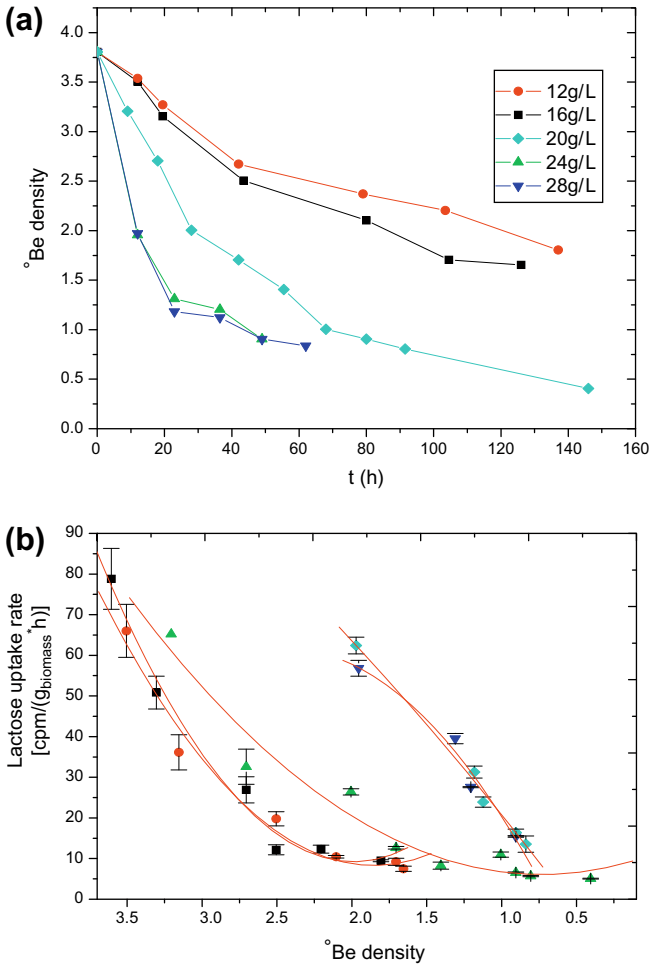

Fig. 2. (a) Fermentation kinetics of lactose, (b) in relation with lactose uptake rate observed at various kefir biomass concentrations.

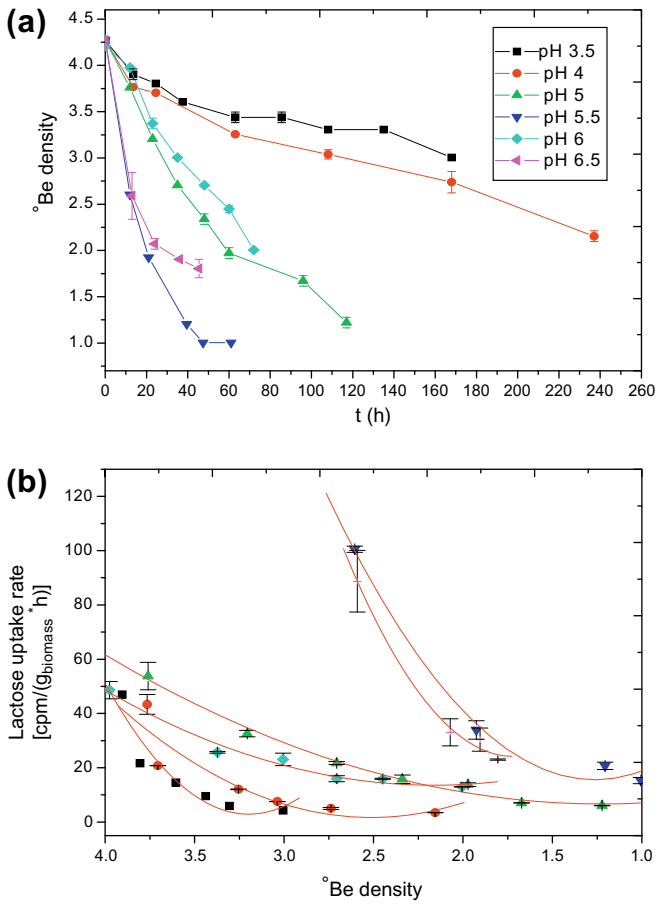

Fig. 3. (a) Fermentation kinetics of lactose, (b) in relation with lactose uptake rate observed at various initial pH values.

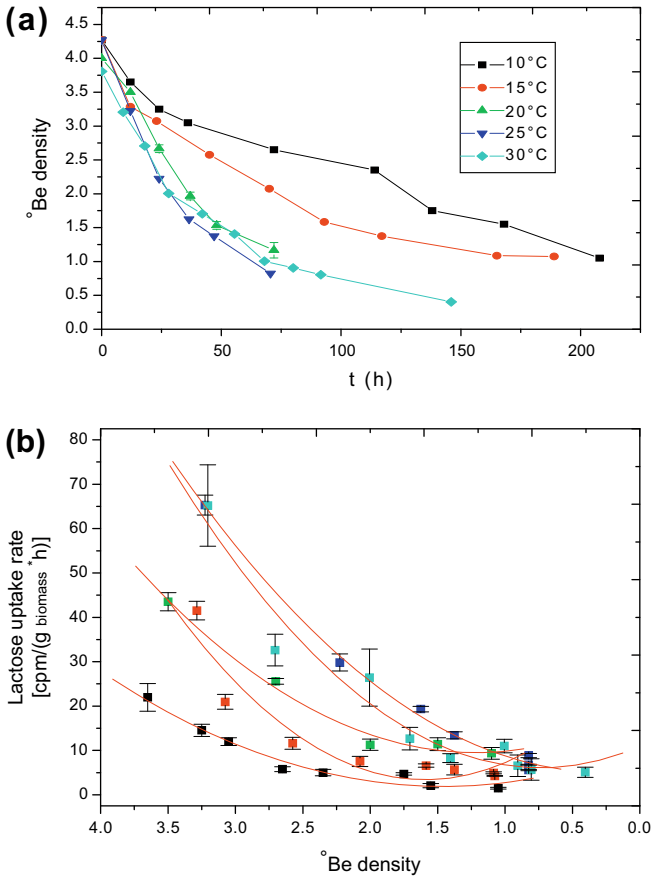

Fig. 4. (a) Fermentation kinetics of lactose, (b) in relation with lactose uptake rate observed for various temperatures.

uptake rate as well as fermentation rate was increased when the temperature increased too. This can be explained considering the fact that approximately 83%–90% of the microbial count of kefir grains was consisted by lactic acid bacteria species, the majority of them being mesophilic (Simova et al., 2002). These predominated mesophilic species – like *S. lactis*-demonstrated optimum growth at 30 °C. Yeast species also contained in kefir microflora were able to ferment at these temperatures. The increment of fermentation rate as the temperature increased was usually attributed to enzyme activity increment. However, this uptake rate increment, as the temperature increased, was related also with the increment of the fermentation rate as it has been found in this investigation. This also indicated that the fermentation rate increment depended on the lactose uptake rate increment, went in parallel with the fermentation rate increment caused by the enzymatic activity. The lactose uptake rate increment as the temperature increased was attributed to the stress of cells at low temperatures (Garbutt, 1997).

3.3. Perspectives of the investigation

The sharp increment of lactose uptake rate obtained at lower cell concentrations was identified with the increased fermentation rates were obtained by immobilized cells having low cell concentrations (Bardi and Koutinas, 1994). However, immobilized cells having low cell concentration gave increased fermentating rates as compared with free cells of the same concentrations. This opens the way to study carbohydrate uptake rate by immobilized cells. Likewise, is useful to find ways for increasing carbohydrate uptake rate leading to higher productivities in bioprocesses. Finally, to study if this carbohydrate uptake rate increment is related with the bioconversion rate increment, can be correlated with any reduction of the activation energies of biochemical transformations.

4. Conclusions

Lactose uptake rate was related to fermentation rate. The uptake rate by kefir was reduced at high lactose concentrations and as the temperature was also reduced due to the stress of cells. Furthermore, lactose uptake rate had the best result at 5.5 pH value, whilst the lower cell concentrations led to higher lactose uptake rate as compared with that of higher cell concentrations. Lactose uptake rate was correlated with fermentation kinetics aspects and opened a way for further research.

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