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Study of whey fermentation by kefir immobilized on low cost supports using ^{14}C-labelled lactose

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HIGHLIGHTS

- ^{14}C-labelled lactose uptake rate by kefir immobilized on BSG and MSR was studied.
- Lactose uptake rate by immobilized cells was correlated to fermentation rate in whey.
- Biocatalysts with low cost BSG, MSR, GP or DCM increase the whey fermentation rate.
- BSG cellulosic biocatalyst can in 8 h ferment the lactose of whey and produce alcohol.
- Hydrophilic cellulose protects immobilized cells and increase biocatalytic activity.

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ABSTRACT

Brewer's Spent Grains (BSG) and Malt Spent Rootlets (MSR) were used as supports for kefir cells immobilization and the role of lactose uptake rate by kefir in the positive activity of produced biocatalysts during whey fermentation was investigated. Lactose uptake rate by the immobilized cells was recorded using ^{14}C-labelled lactose and the effect of various conditions (pH, temperature and kind of support) on it and consequently on fermentation time and ethanol production was examined. The results showed that lactose uptake rate was correlated to fermentation rate and increased as temperature was increased up to 30 °C at pH 5.5. The same results have been recently noticed by using biocatalysts with Delignified Cellulosic Materials (DCM) and Gluten Pellets (GP), but fermentation time of about 7 h by kefir immobilized on DCM and BSG resulted to two fold lower than that on GP and MSR. The highest alcohol concentration was observed by MSR.

1. Introduction

It is well known that microorganisms cells take up carbohydrates through their cell wall and it is obvious that this diffusion of carbohydrate molecules could play an important role on the fermentation rate. Therefore, research to examine cell carbohydrate uptake rate will visualize this phenomenon and will create the practical and theoretical background in supported productivity in bioprocesses. As recently has been reported, ^{14}C-labeled lactose was used for monitoring lactose uptake rate by free kefir cells and by immobilized ones in an attempt to confirm directly the positive effect of immobilized kefir on fermentation rate (Golfinopoulos et al., 2009, 2011, 2012). So, it was found that lactose uptake rate is strongly correlated to fermentation rate and increased during whey fermentation by kefir cells immobilized on Delignified Cellulosic Materials (DCM) and Gluten Pellets, (GP), in comparison with fermentation by free cells (FC).

Whey is the main liquid by-product of the dairy activities, in which a large quantity of the lactose of the milk is removed (~4.2–5%). But, it is usually discarded as a waste in the environment, representing a grave pollutant. So, except of protein and whey lactose recovery processes, the improvement of whey lactose fermentation was necessary for the production of various bioproducts, through biotechnological means. In that way the streams of whey could be used as an abundant and renewable potential raw material for microbial fermentations (Panesar et al., 2007). Over the last two decades, the natural mixed culture kefir was chosen for whey exploitation, due to its ability to ferment the lactose of whey mainly to ethanol and lactic acid (Athanasiadis et al., 2002; Panesar et al., 2010).

In the present work two new biocatalysts prepared by immobilization of kefir on Brewer's Spent Grains (BSG) or Malt Spent Rootlets (MSR) were used for whey fermentation. The BSG

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and MSR are important solid by-products of the brewing industry and have been used in animal feed production (Bekatorou et al., 2007) and as ingredients in the food industry due to their high nutritional properties (Loetterle et al., 2011). They are available at low or no cost throughout the year and are produced in high quantities not only by large, but also by small breweries.

Cells immobilized on BSG have been applied successfully as biocatalysts in various brewing processes (Kopsahelis et al., 2007) but their use as supports for kefir immobilization to develop biocatalysts for use in whey fermentation has not been reported before. The production of potable alcohol or whey alcoholic beverages using kefir immobilized on BSG and MSR is a very attractive perspective, because they are natural, easily available, abundant and have food grade purity and many dietary fibers.

In this study the lactose uptake rate by kefir cells immobilized on BSG and MSR during whey fermentation was examined and the results were compared with the previously reported ones (Golfinopoulos et al., 2009, 2011, 2012). For this reason the optimum physicochemical conditions like pH, temperature, and kind of support for lactose uptake rate by the new biocatalysts were investigated for fast whey fermentation. The results were compared with results by DCM and GP biocatalysts as well as by FC in an attempt to propose the best system for fast and easy removal of lactose from whey.

2. Methods

2.1. Microorganism and cell growth

The kefir yeast commercial product bought from the local market used in Caucasus for homemade kefir drink was employed in the present work. As the manufacturer and researchers report the kefir grains are mainly consisting of (% w/w) 5.0 ± 0.6 protein, 8.2 ± 1.6 sugars και 80.7 ± 1.3 H_2O (Rimada and Abraham, 2001). Cell growth and production of biomass were done according to previous work (Golfinopoulos et al., 2009).

2.2. Materials and media

Liquid cheese whey used for all fermentation experiments was obtained from the regional dairy industry "Agricultural Cooperative Union of Kalavryta" (Kalavryta, Greece). It remained after the production of feta cheese and after removal of whey proteins lactalbumins and lactoglobulins and contained about (% w/w) 5 of lactose, 0.8 of proteins, while its pH value was 6.5. The BSG and MSR were obtained from the Athenian Brewery S.A. and used as cells supports. The BSG contained about (% w/w on dry matter) 21.6 of crude protein, 6.8 of fats, 17 of cellulose, 22 of lignin, 28 of non-cellulosic polysaccharides and 4.7 of ash. The MSR consist of about (% w/w on dry matter) 36.2 protein contents, 12.1 cellulose, 18.4 fibers, 13.3 carbohydrates, 3.7 glucose, 1.0 polyphenols, 0.6 fats and 3.0 ash. The compositions of BSG, MSR and whey provided by the manufacturers.

2.3. Preparation of supports and cell immobilization

For cell immobilization fresh BSG were utilized, while the MSR were sieved and the particles with diameter less than 0.36 mm were selected and remained in water for two days before use. All media were sterilized by autoclaving at 120 °C for about 15 min prior to their use. Then, kefir immobilization on BSG or MSR was performed according to previous investigations, where DCM and GP were also used as supports for kefir cells immobilization (Kopsahelis et al., 2007; Golfinopoulos et al., 2012). Finally, the produced immobilized biocatalysts were washed twice with 12% lactose culture medium for removal of free cells and used for whey fermentation.

2.4. Effect of pH and temperature on lactose fermentation rate and lactose uptake rate by kefir immobilized on BSG or MSR during whey fermentation

In order to study the effect of pH value and temperature on whey fermentation rate and lactose uptake rate by kefir cells immobilized on BSG or MSR, whey fermentations experiments were carried out in 500-mL Erlemeyer flasks without agitation or air supply, as immobilized biocatalysts were distributed in all reacting volume and the lactose fermentation is an anaerobic process. Specifically, an amount of about 122.00 g BSG biocatalyst or 120.00 g of wet MSR biocatalyst was added in 250 mL of pasteurized whey and fermentations were carried out (i) at various pH values 4, 5, 5.5, 6 and 6.5 at 30 °C and (ii) at various temperatures of 10, 20, 25 and 30 °C under 5.5 pH value.

In all cases initial trial pH value was achieved by the addition of tartaric acid (7% w/v). During of each whey fermentation the pH value was monitored by pH meter (Hanna 9024C) at time intervals of about two hours and maintained stable to the selected trial pH by the addition of 6 M NaOH solution.

At the beginning of each run a small quantity of ^{14}C-labelled lactose [[D-glucose-1-^{14}C], (ARC 0466 lactose 0.1 mCi/mL)] was added, in order to determine the lactose uptake rate by kefir during the processes by liquid scintillation.

Samples of the fermented liquids were collected on fixed time intervals and stored at −20 °C until further analysis. Also, whey samples at the beginning and at the end of each fermentation were used for determination of Ca^{2+} ions concentration.

Fermentation kinetics was monitored by total sugar measurements by HPLC and ethanol concentration by GC. For statistical reasons all fermentations were carried out in triplicate and the recorded results were the mean value of the three repetitions.

2.5. ^{14}C-labelled lactose determinations

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

2.6. Determination of residual sugar, ethanol and Ca^{2+} ions concentration

Residual sugars in the fermented whey samples were determined in a Shimadzu LC-9A HPLC system comprising a Shim-pack SCR-101 N, an LC-9A, an RID-6A refractive index detector, a CTO-10A column oven, and a DGU-2A degassing unit. Ultra pure water obtained by a Milli-Q water purifier system, (resistivity 18.2 MΩ cm^{-1}), 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 the injection volume was 40 μL.

Ethanol was determined according to a recent investigation (Golfinopoulos et al., 2012).

Finally, the Ca^{2+} ions concentration was measured by Flame Atomic Absorption Spectrometry (FAAS) using a Shimadzu (AA-6500) spectrophotometer equipped with SR hollow cathode lamps for good background correction and a corrosion resistant nebulizer. Analytical precision was better than 10% on the basis of replicate analyses.