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Propagation of Natural Starter Culture in Whey: Optimization and Kinetic Study

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The disposal of whey, the by-product of cheese or casein production, represents serious environmental problems. On the contrary, recovery of whey components or use of whey as fermentation medium may be advantageous not only for the environment but also for a sustainable economy. In this study we investigate the possibilities for whey fermentation with kefir grains. Firstly, optimal cultivation conditions were determined, afterwards construction of a mathematical model that describes the dynamic process of whey fermentation with kefir grains was developed. The attention was placed on maximum biomass increase, and not on the quality of the resulting kefir-like drink. Kinetic parameters were estimated and adjusted using a non-linear regression technique in order to fit with the experimental data. All experiments were performed in a laboratory batch bioreactor with whey from a commercial dairy plant. The gravimetric method was used to determine daily kefir grain increases under selected operating conditions (temperature, rotational frequency of the stirrer, and initial kefir grain mass concentration). Maximum specific growth rates of cells in whey and milk were compared. It was found that kefir grain increase was nearly the same in whey and in milk. From the obtained results, it is obvious that whey as dairy industry waste, could be effectively used in fermentation processes to obtain value added products. The estimated kinetic parameters could be used to bioreactor design.

1. Introduction

Whey is a dairy liquid waste of negligible cost, also called milk serum, a by-product of the manufacture of cheese or casein (Prazeres et al., 2012). This effluent of the dairy industry is produced worldwide in large amounts. It has high organic load, its treatment for disposal is very difficult, and it thus creates serious environmental pollution problems. On the other hand, liquid whey contains lactose, vitamins, proteins, minerals and small amounts of fat. All these fermentable carbohydrates give the whey nutritional value and consequently several commercial uses (Pescuma et al., 2015). Among many possible uses, it can be an alternative raw material for kefir biomass production. Development of new technologies employing whey as raw material to produce food (Jadav et al., 2015) or chemicals (Yasin et al., 2013) of added value has been undertaken over the last several years (Becerra et al., 2015).

Kefir grains have been used for centuries as the natural starter in kefir production (Beshkova et al., 2003). Real kefir can only be produced by inoculating original grains into a milk medium. Also, many reports indicate kefir grains' potential applications in polysaccharide production (Zajšek et al., 2013), different strain isolation for development of novel functional foods (Diosma et al., 2014) and range of novel fermented drinks (Abdolmaleki et al., 2015¸ Sabokbar et al., 2015). Kefir grains' commercial use as a baker's yeast is also an interesting possibility because of its relatively high resistance to spoilage and ability to produce bread of particular flavour (Mantzourani et al., 2014). Today, baker's yeast production is a large industry worldwide.

Because kefir grains are used commercially in all these applications, there is interest in improving its production capability. Some studies report considerable improvements in grain mass increase during its production in milk in laboratory conditions. Most of these studies (Schoevers and Britz, 2003; Goršek and Tramšek, 2007) are based on investigation of the influence of different process parameters and determination of their optimal values. Efficient and rapid transfer of process improvements from the laboratory, via pilot, to

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industrial production scale requires full knowledge of predictive kefir grain growth models. Same models can be used to describe product formation and substrate utilization in natural ecosystems and under research conditions (Goršek and Zajšek, 2010). In a previous study, we analysed well-known predictive non-linear sigmoidal models (Tramšek and Goršek, 2008). We established that the growth of classically activated kefir grain mass during batch propagation in milk at optimal process conditions can be statistically most successfully described using the Gompertz predictive model.

Because of all the possible applications of whey, as well as kefir grains, for our research we selected this waste of the dairy industry as a selective growth media for kefir grain mass growth. As propagation conditions influencing grain increase in milk are known from our previous study, in this study we discuss a comparison of the results obtained with fermentation of whey. The influence of key process parameters on biomass increase, as well as grain growth kinetics were studied.

2. Experimental

2.1 Materials and methods

Daily kefir grain mass increase in whey was studied using fresh whey after cheese production (by Ljubljanske mlekarne d.d.) as a culture medium. Its chemical composition is 0.44% proteins, 3.8% carbohydrates, 1.2 % fat and 5.7 % dry matter. The kefir grains originated from the Caucasus Mountains and were acquired from a local dairy (Kele & Kele d.o.o., Laze, Slovenia).

Kefir grain wet weight was determined by a gravimetric procedure taken from literature (Garrote, 1997) with a slight adjustment. The kefir grains were separated from the fermented culture medium with a household sieve, washed with cold water, dried on a paper towel and weighed using a Mettler-Toledo analytical balance.

Kefir grain biomass activation was performed in a glass lab beaker. The collected inactive kefir grains (γ= 40 g/L were inoculated in 0.5 L of whey. After incubation at room temperature (ϑ = 22 °C) for 24 h, the grains were separated from the whey based kefir beverage using a household sieve. After washing, they were inoculated again into fresh whey in order to maintain their viability. The same procedure was repeated over five subsequent days. After this procedure the kefir grains were considered active.

2.2 Kinetic model

The increase in microbial population in a closed bioreaction system can be described using a growth curve (Perni et al. 2005) that is generally sigmoid on a semi-logarithmic plot. Several growth models can be found in the literature for describing such a sigmoidal-shaped curve (Lopez et al. 2004). Most are based on mathematical equations, such as Logistic, Gompertz and Richards models (Zwietering et al., 1990). The original expressions of sigmoidal curve equations contain some mathematical parameters without biological meaning. Therefore, these equations should be reparametrized, with kinetic parameters such as maximum specific growth rate, $\mu_{\max}$, lag time duration, t_L, and asymptotic value a substituted for mathematical ones. Finally, in the predictive microbiology field, sigmoidal shaped growth models have been used to forecast cell growth rates. For a mathematical description of growth curve during kefir grain mass production using batch cultivation in milk, the Gompertz model was found to be most statistically appropriate model (Tramšek and Goršek, 2008). In the case of whey, the same model was used to fit the experimental data. A literature review shows that there is a lack of published studies concerning the mathematical description of growth curves during kefir grain mass production in whey. The original form of the Gompertz equation with parameters a, b and c, is:

$$y = a \exp\left(- \exp(b - cx) \right) \tag{1}$$

Equation (1) was modified into the form with biological meaning:

$$\ln \frac{\gamma}{\gamma_0} = a \exp\left(- \exp\left(\frac{\mu_{\max} \exp(1)}{a} \left(t_L - t \right) + 1 \right) \right) \tag{2}$$

where γ_0 is initial kefir grain mass, γ is kefir grain mass at a defined time, $\mu_{\max}$ is maximum specific growth rate, t_L is lag phase duration, t is propagation time and a is an asymptotic value.

2.3 Equipment and experimental procedure

All experiments were performed at atmospheric pressure in a batch reactor immersed in a temperature controlled water bath. The reaction mixture was agitated using an overhead glass stirrer. The pH was measured with a Mettler Toledo pH-meter.

Initially, the reactor was filled with 0.5 L of fresh whey which was then heated to the desired temperature. After reaching the steady state temperature, the reactor was inoculated with previously activated kefir grains (γ = 40 g/L). The pH of whey was measured before and immediately after the fermentation. During propagation (t = 24 h), the fermentation broth was agitated by the fixed rotational frequency of the stirrer. Once the experiment was completed, the final kefir grain mass was gravimetrically determined, thus is was possible to calculate daily kefir grain mass increase, Δm.

Fermentations were performed at different temperatures, ϑ = 24, 28 and 32 °C, and rotational frequencies of the stirrer, f_m = 100, 150 and 200 min^{-1}. Repeatability of experiments was not checked, because we were working with living microorganisms, which are very sensitive to operating conditions. Therefore our main goal was to perform all fermentations at different sets of conditions with the same batch of kefir grains. Nevertheless, according to our previous experiences, kefir grain increase of repeated fermentations normally differs for approximately (5 – 7) %.

3. Results and discussion

3.1 Temperature influence on kefir grain mass increase

Figure 1 shows the temperature effect on kefir grain mass increase during daily whey fermentations, at fixed rotational frequency of the stirrer, f_m = 100 min^{-1}. The whey solutions were not enriched with any additives.

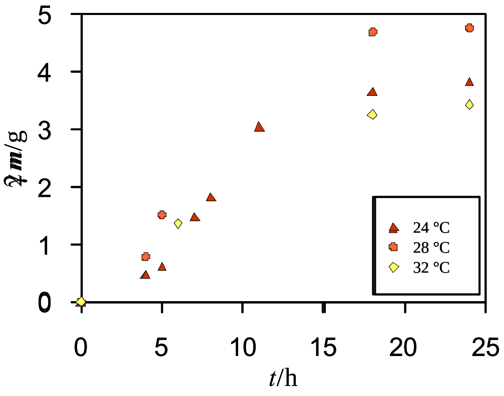

Figure 1: Kefir grain increase versus time at different temperatures

Although temperature differences between each fermentation run were small, different kefir grain mass increases were obtained. At ϑ = 32 °C, the lowest grain mass increase was noticed, this is probably the consequence of kefir grain denaturation. The highest kefir grain increase, Δm = 4.75 g (about 24 %), during the batch propagation of grains in whey was observed at ϑ = 28 °C after a 24 h. Mass production decreased again at ϑ = 24 °C. Therefore, we can establish that at temperature ϑ = 28 °C, kefir grains reached their maximum production, and consequently it represents the optimum for fermentation of whey with kefir grains.

3.2 Stirrer speed influence on kefir grain mass increase

Figure 2 reports the effect of the rotational frequency of the stirrer on the kefir grain mass increase for different temperatures. Obtained results confirm our previous claim regarding optimum temperature. The highest mass increase was achieved at all three rotational frequencies of the stirrer, f_m = (100, 150 and 200) min^{-1} at a temperature of 28 °C. This result also confirm the well-known theory about mixing, which defines the environment during cultivation in a bioreactor. Efficient fermentation is the result of many factors, including mass transfer and mixing intensity. Biomass growth in selected culturing conditions is accelerated when the rotational frequency of the stirrer is increased. At 200 min^{-1} and 28 °C the highest biomass production was achieved.

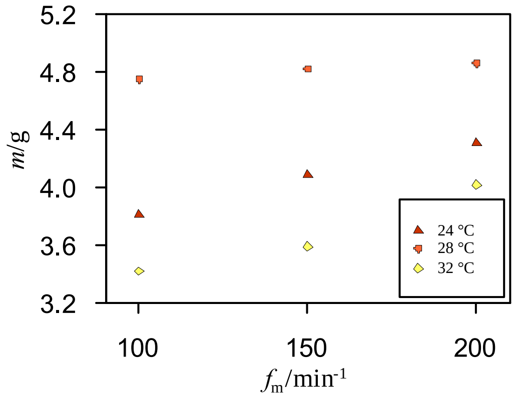

Figure 2: Kefir grain increase versus rotational frequency of the stirrer at different temperatures

3.3 Temperature-dependent pH profile during whey fermentation

A graphic comparison of experimentally measured pH profiles during fermentation at three different temperatures is presented in Figure 3. The initial pH of fresh whey was the same for all experiments (pH = 4.3). After adding the kefir grains, fermentation started, where among others, lactic, acetic and other organic acids were synthesized. Therefore pH started to decrease. The formation of acids is not the only reason for pH drop. There is also some pH gradient between the grains and the fermentation medium. Approximately 17 hours after the inoculation, the whey median pH value dropped to 3.1. The differences between pH profiles are rather small, but we can see that at a temperature of 32 °C the decrease in pH values was most pronounced.

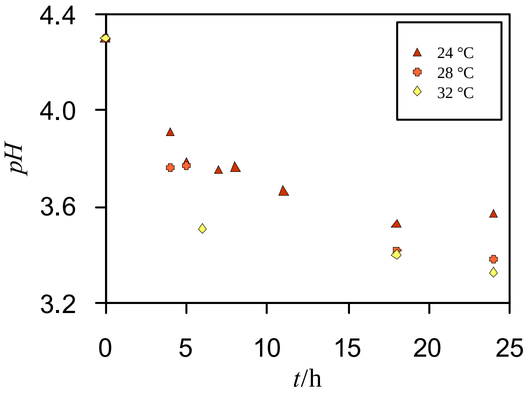

Figure 3: pH profiles during the whey fermentation at different temperatures

3.4 Whey fermentation kinetics

With the kefir grain growth model, we are able to describe mass increases over time until reaching maximum value. The growth curve has a characteristic elongated S-shape, starting out with a low slope which increases to an inflection point, and then levels off as it approaches the maximum value. Experimental data representing the growth curve of kefir grains inoculated in whey at three different temperatures of fermentation medium were fitted with the Gompertz model. Experimental and model based values for whey fermentation with kefir grains at ϑ = 28 °C are shown in Figure 4.

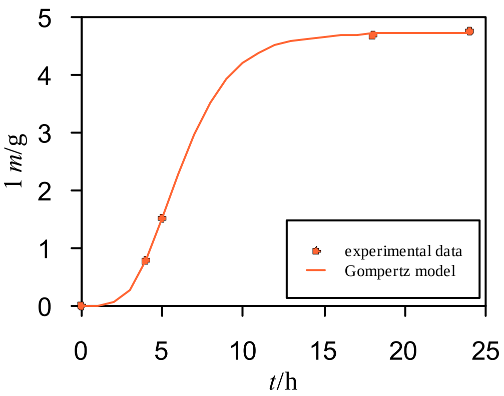

Figure 4: Kefir grain growth curve in whey medium at ϑ = 28 °C

The statistic indicator R_{adj}^2 was applied as a criterion for growth model ranking. It was equal to 0.9999, confirming the selection of the Gompertz model for representing the kefir grain increase during whey fermentation process. The key kinetic parameters for three fermentation temperatures are presented in Table 1.

Table 1: Main kinetic parameters for kefir grain mass increase in whey

ϑ/°C	a/1	μ_{max}/min^{-1}	t_L/min	R_{adj}^2
24	0.1753	0.0201	204	0.9968
28	0.2120	0.0365	181	0.9999
32	0.1572	0.0160	112	0.9995

The results of this study were compared with the results of our previous work (Goršek and Tramšek, 2008), in which HTP whole fat cows' milk was used as the fermentation medium for kefir grain propagation under selected operating conditions (ϑ = 24°C, f_m = 90 min^{-1}, $\gamma_{KG,0}$ = 75 g/L and $\gamma_{Glucose}$ = 20 g/L). Although the kefir grains were taken from the same dairy, direct comparison is not possible, as the grains did not originate from the same batch. Furthermore, the milk was enriched with glucose, while the whey was used without any additives. The optimum temperature and rotational frequency of the stirrer differed slightly from the selected process conditions of the present study. However, the results are of the same order of magnitude, indicating similar daily mass increase, Δm = 24 % (Δm = 24.85 % in milk) and similar maximum specific growth rate of kefir grains, μ_{max} = 0.0201 min^{-1} (μ_{max} = 0.0167 min^{-1} in milk). The highest deviation shows lag phase duration. Upon inoculation of kefir grains into the milk medium, 4.2 h passed before they started to reproduce, while in whey, the growth process started after 3 h.

4. Conclusion

In this study, whey was used as an effective low cost substrate for kefir grain production. Laboratory experiments were based on experiences of our previous research work where the kefir grain propagation was investigated in milk medium. Kefir grains have many commercial applications, thus their production is very important. Our results confirmed the efficient use of whey as a fermentation medium for kefir grain propagation. With the aim of developing a cost efficient kefir grain production process, fermentations in whey were performed without any enrichment of the medium with additives. Their maximum daily mass increase of about 24 % was achieved at the fermentation temperature of 28 °C and rotational frequency of the stirrer of 200 min^{-1}. We established the Gompertz mathematical growth model together with main kinetic parameters (μ_{max}, t_L, a) which are required to study batch kefir grain mass growth under selected operating conditions. They have a specific role in modelling the growth curve and consequently in the design of industrial production plants of kefir grains.

The results of the study are specific for the starter culture used, because its microbial composition varies with time and bioprocess conditions. Nevertheless, the methodology presented can be successfully used also for other starter cultures under different experimental and propagation conditions.

References

Abdolmaleki F. Assadi M.M., Akbarirad H., 2015, Assessment of beverages made from milk, soya milk and whey using Iranian kefir starter culture, International Journal of Dairy Technology, 68 (3), 441-447

Becerra M., Cerdan M.E., Gonzalez-Siso M.I., 2015, Biobutanol from cheese whey, Microbial cell factories,14 (1), 200-209.

Beshkova D.M., Simova E.D., Frengova G.I., Simov Z.I., Dimitrov Z.P., 2003, Production of volatile aroma compounds by kefir starter cultures. Int. Dairy Journal, 13, 529–535.

Diosma G., Romanin D.E., Rey-Burusco M.F., Londero A., Garrote LR., 2014, Yeasts from kefir grains: isolation, identification, and probiotic characterization, World Journal of Microbiology & Biotechnology, 30 (1), 43-53.

Garrote G.L., Abraham A.G., De Antoni G.L., 1997, Preservation of kefir grains, a comparative study, Food Science and Technology, 30, 77–84.

Goršek A., Tramšek M., 2007, Quantitative examination of process parameters during kefir grain biomass production, International Journal of Chemical Reactor Engineering, 5, S8.

Gorsek A., Zajsek, K., 2010, Influence of temperature variations on ethanol production by kefir grains - mathematical model development, Chemical Engineering Transactions, 20, 181-186.

Lopez S., Prieto M., Dijkstra J., Dhanoa M.S., France J., 2004, Statistical evaluation of mathematical models for microbial growth, International Journal of Food Microbiology, 96: 289–300.

Mantzourani I., Plessas S., Saxami G., Alexopoulos A., Galanis A. Bezirtzoglou E., 2014, Study of kefir grains application in sourdough bread regarding rope spoilage caused by Bacillus spp., Food Chemistry, 143, 17-21.

Perni S., Andrew P.W., Shama G., 2005, Estimating the maximum growth rate from microbial growth curves: definition is everything, Food Microbiology, 22, 491-495.

Prazeres A.R., Carvalho F., Rivas J., 2012, Cheese whey management: A review, Journal of Environmental Management, 110, 48-68.

Pescuma M., Font de Valdez G., Mozzi F., 2015, Whey-derived valuable products obtained by microbial fermentation, Applied Microbiology and biotechnology, 99 (15), 6183-6196.

Sabokbar N., Khodaiyan F., 2015, Characterization of pomegranate juice and whey based novel beverage fermented by kefir grains Journal of Food Science and Technology-mysore, 52 (6), 3711-3718.

Schoevers A., Britz T.J., 2003, Influence of different culturing conditions on kefir grain increase, International Journal of Dairy Technology, 56, 183-187.

Tramšek M., Goršek A., 2008, Analysis of growth models for batch kefir grain biomass production in RC1 reaction system, Journal of Food Process Engineering, 31 (6), 754-767

Yadav J.S.S., Yan S., Pilli S., Kumar L., Tyagi R.D., Surampalli R.Y., 2015, Cheese whey: A potential resource to transform into bioprotein, functional/nutritional proteins and bioactive peptides, Biotechnology Advances, 33(6), 756-774.

Yasin N.H.M., Mumtaz T., Hassan M.A., Rahman N.A., 2013, Food waste and food processing waste for biohydrogen production: A review, Journal of Environmental Management, 130, 375-385.

Zajšek K., Goršek A., Kolar M., 2013, Cultivating conditions effects on kefiran production by the mixed culture of lactic acid bacteria imbedded within kefir grains, Food Chemistry, 139 (1-4), 970-977.

Zwietering M.H., Jongenburger I., Rombouts F.M., Van't Riet K., 1990, Modelling of the Bacterial growth curve, Applied and Environmental Microbiology, 56, 1875-1881.