Biochemical Engineering Journal 42 (2008) 153–158

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Kefir grains production—An approach for volume optimization of two-stage bioreactor system

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ARTICLE INFO

Article history:
Received 17 January 2008
Received in revised form 9 June 2008
Accepted 15 June 2008

Keywords:
Kefir grains production
Bioreactor design
Modeling
Optimization
Continuous operation mode

ABSTRACT

The main objective of the present study was to design a continuous two-stage bioreactor system for kefir grains production. Based on the experimental data of time-depended kefir grain mass increase and average pH profile during kefir grains batch propagation, parameters of predictive growth and exponential pH models were initially estimated. Afterwards, the non-linear programming (NLP) optimization problem for estimating the optimal volumes of two unequal in series connected continuously stirred tank bioreactors (CSTB) has been developed. The NLP problem is based on criterion of minimal total holding time (MTHT), kefir grain mass balances in CSTB and parameters of the growth and pH models. The results showed that novel kefir grains production plant with two-stage continuous operation, at capacity, $q_{m,\mathrm{KG,pr}} = 3\,\mathrm{kg\,h^{-1}}$ and conversion, $X_{\mathrm{o},2} = 0.980$, would primarily require investment in two CSTB with $V_1 = 5.93\,\mathrm{m^3}$ and $V_2 = 5.62\,\mathrm{m^3}$, respectively. Technological and economical authorization for a two-stage continuous bioreactor system was mainly confirmed with a more than five times lower total bioreactor volume compared to one-stage plant.

1. Introduction

Kefir grains are natural mixed culture starter, containing diverse spectrum of bacteria and yeasts [1]. Mentionable, the variegated microbial population found in kefir grains represents a pattern of symbiotic community [2]. Traditionally kefir grains have been used for centuries in many countries, especially in Eastern Europe, as the natural starter in the production of the unique self-carbonated dairy beverage known as kefir [3]. However, many recent studies indicate also their potential novel applications in bread production as baker's yeast [4], polysaccharide production [5] and novel fermented drink production from mixture of whey and raisin extract [6–8]. Nevertheless, if kefir grains would be commercially used in above-quoted novel applications, then their production (based on traditional propagation of kefir grains in milk with relatively low daily biomass increase fraction) has to be improved.

Some studies exist reporting considerable improvements in kefir grains increase during their production in laboratory level [9,10]. However, efficient and rapid transfer of process improvements from laboratory, via pilot, to production scale requires knowledge of predictive kefir grain growth model. Therefore, in our previous study different well-known predictive non-linear sigmoidal models were analyzed [11]. We established, that the growth of classically activated kefir grain biomass during batch propagation in milk at optimal process conditions can be statistically most successfully described by the Gompertz predictive model. Unfortunately, the reported values of growth parameters are specific for used initial kefir grains culture and cannot be considered as general one for all batch propagations.

Furthermore, we also investigated the influence of inoculum activity on pH profiles of fermentation medium during kefir grains propagation in milk [12]. The results show that frequent repeatability of fermentation medium concentration profiles (followed by dynamic pH measurements) can be ensured only by the grains previously activated for at least 11 successive days.

Increased demand on kefir grains in global market requires development of new economically efficient grains production processes with high capacity. Traditionally, kefir grains propagations operate in batch mode. However, there are several well-known major advantages for using continuous operation instead of discontinuous one [13,14]. Irrespective of the promising idea of incorporation the continuous mode operation in classic kefir grains production, there is still a lack of published information concerning design of this type of bioreactor system.

The main objective of the present study was to design a continuous two-stage bioreactor system for kefir grains production. In order to minimize total volume of bioreactors in two-stage system

Abbreviations: CSTB, continuously stirred tank bioreactor; GAMS, general algebraic modeling system; MTHT, minimal total holding time; NLP, non-linear programming.

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doi:10.1016/j.bej.2008.06.009

Nomenclature

a	asymptotic value (1)
CV	coefficient of the variation (%)
f_m	rotational frequency of the stirrer (min^{-1})
k, h	auxiliary function parameters (1)
n	parameter of the exponential pH model (1)
p	auxiliary function parameters (1)
q_m	mass flow rate (mass capacity) (kg h^{-1})
q_V	fermentation medium volumetric flow rate (L h^{-1})
R^2_{adj}	adjusted coefficient of the determination (1)
s	parameter of the exponential pH model (1)
$S.E.$	standard error
t	time (h)
v	designation number (1)
V_T	total volume of bioreactors (L)
V_1, V_2	volume of CSTB-1 and CSTB-2 (L)
X	conversion with regard to decreasing of fermentation medium pH value (1)
Y	yield (kefir grains increase regarding changing the pH value) (g L^{-1})
z	parameter of the exponential pH model (h^{-1})

Greek symbols

γ	mass concentration (g L^{-1})
ϑ	temperature (°C)
μ	specific kefir grains growth rate (h^{-1})

Subscripts

a, b	designation of the location (e.g. decreasing pH value from pH_a to pH_b)
avr	average
G	glucose
i	inlet
j	designator
KG	kefir grains
lag	lag phase
max	maximum
mod	model (predicted)
o	outlet
pr	production
0	initial (concentration in batch reactor)
1	first bioreactor in cascade (CSTB-1)
2	second bioreactor in cascade (CSTB-2)

for desired kefir grains production capacity a simple NLP problem has been formulated. It is based on MTHT criterion of two in series connected CSTB, on kefir grain mass balances in CSTB and on experimentally evaluated predictive Gompertz growth and exponential pH models. For this purpose some experiments with optimally activated inoculum (kefir grains were previously activated for at least 11 days) were initially performed in the RC1 batch reactor (Mettler Toledo). On the basis of experimental data of time-depended kefir grain mass increase, growth parameters of predictive Gompertz model were determined. Simultaneously, during individual experiments at different batch propagation times the pH values of fermentation medium were monitored. pH profiles were mathematically analyzed and the values of shape parameters of the supposed exponential pH model, which describes average pH profile during batch propagation of kefir grains, were determined. In the second part of the study, we estimated the optimal volumes of two in series connected CSTB for desired kefir grains production capacity by implementing optimization NLP problem using high-level modeling system GAMS [15]. At the same time we also compared the total bioreactor volume of two-stage system with potential volume if only one CSTB is considered.

2. Materials and methods

2.1. Kinetic model of two-stage bioreactor system

The process scheme (Fig. 1) of continuous two-stage bioreactor system for kefir grains production can be described by a cascade of two CSTB [16]. If we assume equal inlet and outlet fermentation medium volumetric flow rates ($q_{V,\text{i},1} = q_{V,\text{o},1} = q_{V,\text{o},2} = q_V$) then the mass balance of kefir grains using the CSTB model under steady state conditions, can be described separately for first (Eq. (1)) and second bioreactor in two-stage system (Eq. (2)), by equations as follows:

$$\frac{q_V}{V_1}\gamma_{\text{KG,o,1}} = \frac{q_V}{V_1}\gamma_{\text{KG,i,1}} + \mu_{\text{o,1}}\gamma_{\text{KG,o,1}} \tag{1}$$

$$\frac{q_V}{V_2}\gamma_{\text{KG,o,2}} = \frac{q_V}{V_2}\gamma_{\text{KG,o,1}} + \mu_{\text{o,2}}\gamma_{\text{KG,o,2}} \tag{2}$$

where $\gamma_{\text{KG,i,1}}$, $\gamma_{\text{KG,o,1}}$, inlet and outlet kefir grain mass concentrations in CSTB-1; $\gamma_{\text{KG,o,2}}$, outlet kefir grain mass concentration in CSTB-2; V_1 and V_2, volumes of CSTB-1 and CSTB-2; $\mu_{\text{o,1}}$ and $\mu_{\text{o,2}}$, specific kefir grains growth rate in CSTB-1 and CSTB-2.

The pH value of fermentation medium is one of the most important dynamic-measured bioprocess variable of traditional kefir grains batch propagation. Let us suppose that during this process at optimal bioprocess conditions, it can be efficiently mathematically described by the three parametric (n, s and z) exponential function (exponential pH model):

$$\text{pH} = \text{pH}(t) = n + s\exp(-zt) \tag{3}$$

where t means batch propagation time. On the other side, the changing pH value of fermentation medium in batch bioreactor can be defined in a similar manner as mass balance of whichever reactant, i.e. with differential equation:

$$\frac{\text{dpH}}{\text{d}t} = -\frac{\mu\gamma_{\text{KG}}}{Y} = -\frac{\text{d}\gamma_{\text{KG}}/\text{d}t}{\text{d}\gamma_{\text{KG}}/\text{dpH}} \tag{4}$$

where Y means yield (kefir grains increase regarding changing pH value). Our previous study [11] has showed that growth curve during batch propagation of classically activated kefir grains in milk at optimal bioprocess conditions can be most successfully described by the predictive Gompertz growth model [17]:

$$\ln\left(\frac{\gamma_{\text{KG}}}{\gamma_{\text{KG},0}}\right) = a\exp\left(-\exp\left(\frac{\mu_{\max}\exp(1)}{a}(t_{\text{lag}} - t) + 1\right)\right) \tag{5}$$

where $\gamma_{\text{KG},0}$, initial kefir grain mass concentration; $\mu_{\max}$, maximum specific kefir grains growth rate; a, asymptotic value; t_{lag}, lag phase duration. Specific growth rate, μ, is defined, in accordance with predictive modeling of growth curve, as first derivative of sigmoidal function of growth model. Therefore, in case of Gompertz model (Eq. (5)), μ can be mathematically expressed as follows:

$$\mu = \mu_{\max}\exp(1)\exp\left(-\exp\left(1 - \frac{\mu_{\max}\exp(1)}{a}(t - t_{\text{lag}})\right)\right) \exp\left(1 - \frac{\mu_{\max}\exp(1)}{a}(t - t_{\text{lag}})\right) \tag{6}$$

By considering Eqs. (3) and (5), the differential Eq. (4) and expression for μ (Eq. (6)) can be rewritten to:

$$-\frac{\text{d}\gamma_{\text{KG}}}{\text{dpH}} = \frac{\mu_{\max}\gamma_{\text{KG},0}\exp(1)\exp(-k)k\exp(a\exp(-k))}{z(\text{pH} - n)} \tag{7}$$