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# A straightforward logistic method for feeding a fed-batch baker's yeast culture

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ABSTRACT

The aim of this work was to find a straightforward, low-cost method for nutrient medium delivery during fed-batch cultivation of baker's yeast; a method that provides a high specific growth rate, a high biomass yield, and a high dough-leavening ability. The time profile for the inflow of the nutrient medium, $g(t)$, is a logistic model which takes the form of $g(t) = a/(1 + be^{-ct})$, with $a$, $b$ and $c$ standing for its parameters. The initial values of $a$, $b$ and $c$ were calculated considering the results of a baker's yeast process where dissolved oxygen tension was used as a control parameter of medium inflow. A method was developed for modifying the parameters of the initial model so as to obtain a formula that would cause the inflow of the nutrient medium to maximize the criterion $K(K = Y\mu)$, where $Y$ stands for yeast biomass yield and $\mu$ denotes specific growth rate. The parameters of the modified model depended on initial biomass concentration. The relation was described by a logarithmic function and incorporated into the control algorithm. This algorithm produced a biomass yield exceeding 0.55 g g$^{-1}$ and a high dough-leavening ability. The method proposed is recommendable for small and medium-sized firms producing baker's yeast.



## 1. Introduction

A major goal in baker's yeast manufacture is to achieve the highest possible specific growth rate and yield of the biomass, as well as a high dough-leavening ability of the yeast produced. In fed-batch processes each of these goals can be achieved with the same method, *i.e.* by the control of nutrient medium inflow. Therefore, the strategy of control must be varied according to the goal that is to be reached. On the one hand, this is what undoubtedly complicates the optimization of nutrient medium delivery in fed-batch baker's yeast cultivation. On the other hand, however, these complications have spurred extensive studies into the problem of how to optimize the baker's yeast process, which has resulted in the development of a variety of methods. Some of the methods involve mathematical models, which serve as a basis for the control of nutrient medium inflow. Applicability tests were performed not only for simple models, such as the parabolic or the exponential one [1,2], but also for those more advanced [3–5]. In recent times much attention has been given to artificial intelligence techniques, which were found to be equally useful for yeast growth control. Among these, knowledge-based systems [6,7], neural networks [8,9], fuzzy logic [10–12] and evolutionary algorithms have found the widest acceptance [13,14].

It is essential to note, however, that the use of advanced methods in baker's yeast production is linked with the necessity of installing complicated control equipment and implementing sophisticated control algorithms. This is a serious obstacle in the way to modernization of small and medium-sized companies, as the majority of them cannot afford such costly investments.

The aim of this work was to develop a simple, low-cost method of nutrient medium supply during fed-batch baker's yeast cultivation, which makes it possible to achieve a high specific growth rate and yield of the biomass, as well as a sufficiently high leavening ability of the dough. In the method proposed, the supply of the nutrient medium is controlled using a logistic model of the formulation $g(t) = a/(1 + be^{-ct})$. A previous study [16] has demonstrated that the logistic model very well describes the time profile for glucose medium supply for the fed-batch baker's yeast process, with DOT as the control parameter.

## 2. Material and methods

### 2.1. Nutrient media

Two nutrient media were used. One of these was composed of glucose (360 g l$^{-1}$), $(NH_4)_2SO_4$ (104 g l$^{-1}$), $KH_2PO_4$ (60 g l$^{-1}$), $MgSO_4 \cdot 7H_2O$ (5 g l$^{-1}$), $CaCl_2 \cdot 6H_2O$ (5 g l$^{-1}$), yeast extract (2 g l$^{-1}$), calcium panthotenate (0.36 g l$^{-1}$), mesoinositol (0.72 g l$^{-1}$), thiamine (0.072 g l$^{-1}$), pyridoxine (0.018 g l$^{-1}$) and biotin (0.36 mg l$^{-1}$). The glucose medium was sterilized using a

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**Nomenclature**

| | |
|---|---|
| $A$, $B$, $C$ | polynomial coefficients |
| $a$ | parameter of the logistic model ($g$) |
| $b$ | parameter of the logistic model |
| $c$ | parameter of the logistic model ($h^{-1}$) |
| $CO_2$ | off-gas content of $CO_2$ (%v $v^{-1}$) |
| DOT | dissolved oxygen tension (% of saturation) |
| $f$ | auxiliary variable |
| $g(t)$ | logistic glucose medium supply term as a function of time |
| $G$ | total amount of glucose (g) |
| $G_c$ | glucose concentration in the nutrient medium ($g\,l^{-1}$) |
| $G_m$ | glucose concentration in the culture medium ($g\,l^{-1}$) |
| $K$ | criterion: $K = Y\mu$ ($g\,g^{-1}\,h^{-1}$) |
| $m$ | modifier (g) |
| $m_{opt}$ | optimal value of modifier (g) |
| $n$, $p$ | parameters of logarithmic function |
| $O_2$ | off-gas content of $O_2$ (%v$v^{-1}$) |
| $R^2$ | coefficient of determination |
| $RQ$ | respiratory quotient |
| $t$ | time of the process (h) |
| $t_{on}$ | delivery time for nutrient medium (h) |
| $\Delta t$ | time increment (0.083 h) |
| $W$ | rate of delivery of nutrient-dosing peristaltic pump ($0.96\,l\,h^{-1}$) |
| $X$ | biomass concentration in the culture medium ($g\,l^{-1}$) |
| $Y$ | biomass yield ($g\,g^{-1}$) |
| $\mu$ | specific growth rate ($h^{-1}$) |
| subscripts: $i$, $m$ | $i$ = initial, $m$ = modified |

cellulose acetate membrane of a 0.2 μm pore diameter (Sartorius AG) and was used for the construction of the new control algorithm.

The other nutrient medium (250 $g\,l^{-1}$ of reducing substances expressed as glucose) consisted of molasses wort from Lesaffre Bio-Corporation S.A., Wolczyn, Poland (800 $ml\,l^{-1}$), $(NH_4)_2SO_4$ (90 $g\,l^{-1}$), $MgSO_4{\cdot}7H_2O$ (3.75 $g\,l^{-1}$ g), $CaCl_2{\cdot}6H_2O$ (1.415 $g\,l^{-1}$), calcium panthotenate (0.25 $g\,l^{-1}$), mesoinositol (0.5 $g\,l^{-1}$), thiamine (0.05 $g\,l^{-1}$), pyridoxine (0.0125 $g\,l^{-1}$) and biotin (0.36 $mg\,l^{-1}$). The medium was pasteurized twice in the Koch apparatus at 96 °C for 45 min. The molasses-based medium was primarily used to verify the functioning of the control algorithm when use is made of a nutrient medium commonly applied in yeast factories.

### 2.2. Inoculum

The inoculum was a pure culture of the baker's yeast *Saccharomyces cerevisiae* B1 (obtained from a yeast producing plant Śląska Fabryka Drożdży, Wołczyn, Poland), suspended in sterile distilled water. The quantity of the yeast biomass in the suspension was derived from the plan of the experiments.

### 2.3. Experimental set-up

The experiments (each with a 12-h duration) were performed in a bioreactor of an overall volume of 7 l, using the same laboratory set-up as in our previous study [16]. The reactor was filled with such a quantity of sterile distilled water that enabled the total volume of both distilled water and the inoculum added to be kept at 3.5 l. Temperature was set to 30 °C and the value of DOT to the level of 100% of saturation; thereafter the inoculum (suspension of *S. cerevisiae* culture in distilled water) was added and the cultivation process commenced. Control was carried out using a PC with an Axiom AX 5412 data acquisition card. Temperature was maintained automatically at 30 ± 0.5 °C. The pH (kept within the range of 4.75–5.0) was controlled with 25% ammonia water, which was supplied with a peristaltic pump (Ecoline VC-280, made by Ismatec). Continuous measurements were conducted for determining DOT (Mikro Sauerstoffsensor 301, made by UMS GmbH), the volume percent of off-gas $CO_2$ (Guardian II Infrared $CO_2$ Monitor, made by Edinburgh Sensors Ltd.) and the volume percent of off-gas $O_2$ (1100 Oxygen Analyser, made by Servomex). Computed nutrient portions were added into the bioreactor with a peristaltic pump (Ecoline VC-280, made by Ismatec). Sterile air was supplied at a constant rate of 0.3 vvm, the initial stirrer speed being 300 rpm. When there was an indication that DOT might decrease below a level limiting biomass growth, stirrer speed was increased manually. Such corrections were made twice to four times during each cultivation (Fig. 4). Every hour, samples were taken for analysis to determine the concentration of biomass, glucose and ethanol.

**Table 1**
Parameters of the initial logistic model, $X(0) = 2.1\,g\,l^{-1}$, $G(0) = 0.36\,g$.

| Parameter | Number of cultivation | | | Parameters of the initial logistic model (arithmetic means) |
|---|---|---|---|---|
| | 1 | 2 | 3 | |
| $a$ (g) | 70 | 63 | 62 | 65 |
| $b$ | 24 | 17 | 19 | 20 |
| $c$ ($h^{-1}$) | 0.35 | 0.28 | 0.26 | 0.30 |

### 2.4. Feeding method

#### 2.4.1. Computation of the parameters for the initial logistic function

In the cultures used for computing the parameters of the initial logistic function the inflow of the glucose medium was controlled by the DOT value. The medium was introduced in pulses when DOT exceeded 45% of saturation. In this way consecutive portions were added after the yeast had consumed the previous doses. All portions were of the same size (1 ml and 0.36 g of glucose). The results obtained with these cultures are summarized in Table 1.

#### 2.4.2. Other experiments

Nutrient media for the other cultures were dosed at 5-min intervals (a dosing frequency established in a previous work [16]) according to the logistic function described by the general Eq. (1):

$$g(t) = \frac{a}{1 + be^{-ct}} \tag{1}$$

The size of consecutive nutrient medium portions was expressed as the time of their delivery ($t_{on}$) and calculated every 5 min in terms of the relation of (2) (the calculating procedure being visualized in Fig. 1):

$$t_{on} = \frac{G(t + \Delta t) - G(t)}{G_c W} \tag{2}$$

### 2.5. Computation of the parameters for the modified logistic model

It seemed advisable to change the parameter values for the initial model in order to find such a formula that would optimize the inflow of the nutrient media in terms of a defined criterion, *e.g.* maximization of $K$ ($K = Y\mu$).

It has been assumed that all of the logistic models used in this work for the description of nutrient medium supply take the same form, *i.e.* the one defined by the relation of (1), and that they