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Modeling of Saccharide Utilization in Primary Beer Fermentation with Yeasts Immobilized in Calcium Alginate

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Abstract

Immobilized beer fermentation was studied using an industrial bottom-fermenting yeast strain *Saccharomyces cerevisiae*. The yeast cells were immobilized in 2.5% calcium alginate gel and used for brewing in a five-vessel cascade reactor. The fermentation was performed at 15°C at various flow rates. A nonstructured mathematical model was developed to simulate the performance of continuous primary fermentation of lager beer. The model was based on the following variables: maltose, maltotriose, glucose, fructose, ethanol, and cell concentration. Experimental values of these variables were determined in samples taken at regular intervals. For experimental data fitting a nonlinear regression was used. Substrate consumption was characterized by specific substrate consumption rate and saturation constant. The values of these two parameters were optimized for all four substrates. Inhibition effects of substrates and product were analyzed using various inhibition patterns. Only the inhibition effect of maltose on maltose consumption was clearly identified. A good-fitting relationship for maltose inhibition was found, and inhibition constants were calculated.

Index Entries: Beer fermentation; brewing; saccharide utilization; modeling; immobilization; calcium alginate.

Introduction

The potential of immobilized brewing technology has attracted great attention during the past few decades. The research in the application of immobilized yeasts in brewing accelerated during the 1980s, and a great potential is foreseen for immobilized yeasts in brewing technology owing

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to some advantages. The main advantage is a significant shortening of processing times owing to a high cell density. The potential of immobilized cell technology in beer production has been exhaustively reviewed recently *(1)*. Entrapment of yeast cells into a gel or their adsorption to suitable supports are now the most popular approaches to immobilization of yeasts for brewing. There are examples of experimental approaches to entrapment of yeast in alginate *(2)*, carrageenan *(3)*, and gelatin *(4)*. Yeasts can easily adsorb to the surface of sintered or porous materials (e.g., glass, ceramics, silica, and cellulose). Compared to gels, these materials offer better mechanical properties. Investigations of brewing using cells adsorbed on various materials have been recently reported. These include microporous sintered glass *(5)*, DEAE cellulose *(6)*, and cellulose sponge matrix *(7)*. Beer brewing using an immobilized yeast reactor was studied in detail by Yamauchi et al. *(8)*, including the characterization of metabolic aspects of the immobilized yeast cells *(9–11)*. Secondary fermentation of beer is also of great importance, mainly owing to a significant acceleration *(12–15)* of beer maturation.

Calcium alginate gel was mainly applied during early brewing investigations. For the first time alginate was used by White and Portno *(2)*. Later, a similar immobilization process was used in other investigations *(16–19)*. In these studies the diffusional limitations and changes in metabolism after immobilization were found and characterized. Immobilization in a gel matrix, compared to adsorption on a support, permitted a considerably higher biomass loading. However, the application of gels is considerably hampered by their diffusion properties. The most commonly employed bead size varies from 0.5 to 3.0 mm in diameter, but smaller beads are preferred because of the better diffusion properties. The type of reactor also plays an important role. Ideally, the reactor design must maximize the transport of nutrients and fermentation products, and, therefore, a packed bed or fluidized bed is usually employed.

Modeling of the fermentation process provides an aid to the engineering of biotechnologic processes. Modeling of ethanol production by yeast, by both free and immobilized yeast cells, is one of the most studied processes. Numerous models and reactor configurations, involving immobilized yeasts, have been proposed *(20–23)*. Because of the complexity of ethanol fermentation, it is preferable to use simple saccharidic substrate (glucose or sucrose) and defined media to perform these studies. The multicomponent nature and compositional variations of complex substrates such as brewer's wort makes the mathematical description difficult. Nevertheless, some approaches have been suggested recently. de Andres-Toro et al. *(24)* proposed a kinetic model taking into account biomass, sugar, ethanol, and diacetyl and ethyl acetate. Volf et al. *(25)* described and verified on an industrial scale a mathematical model of beer fermentation in a conical fermenting vessel. Virkajarvi et al. *(26)* studied the effect of aeration on flavor compounds in primary fermentation and created a mathematical model for the concentration of higher alcohols and the two acetate esters.

Previously we have looked for the optimal design of reactors and immobilization procedures for continuous primary *(27)* and secondary *(28)* beer fermentation. Our studies were focused on the quality of the beer produced by various immobilized systems *(28)*, and on the effects of cell concentration *(29)* and temperature *(30)* on the concentrations of flavor compounds. In the present study, we propose a mathematical model that can serve for brewing simulation or process control. The aim of this study was to develop a mathematical model describing the utilization of saccharidic substrates separately, distinguishing utilization of the main utilizable saccharides: maltose, maltotriose, glucose, and fructose.

Materials and Methods

Microorganism and Medium

The yeast strain used was a bottom-fermenting yeast, *Saccharomyces cerevisiae* W-96 (Collection of Research Institute of Brewing and Malting, Prague, Czech Republic). The yeast suspension was obtained from a local brewery. Yeast cells were collected by centrifugation from the wort after fermentation. Cells were washed and resuspended in sterile water. This suspension was used for the preparation of alginate beads.

Immobilization of Cells

The cells were immobilized in a 2.5% calcium alginate gel. Alginic acid sodium salt (Protanal LF 20/60) was purchased from Pronova Biopolymers (Drammen, Norway). After autoclaving the alginate solution for 20 min at 120°C, the solution was mixed with the cell suspension to obtain a cell concentration of 10^9 cells/mL of gel (corresponds to 50 g_{DW}/L). This suspension was forced through a syringe needle in an airstream and dropped into 0.1 mol/L of calcium chloride solution. The resulting beads were approx 3 mm in diameter. The beads were left for 1 h in calcium solution and then washed with distilled water.

Continuous Fermentation

Continuous fermentation was carried out in the apparatus consisting of a cascade of vessels represented in Fig. 1. Fresh wort was supplied from a local brewery. All experimental work was done using one batch of wort. The composition of wort was as follows: 59 g/L of maltose, 13.7 g/L of maltotriose, 6.9 g/L of glucose, and 3.2 g/L of fructose. The concentration of nonfermentable saccharides was in the range of 19 to 20 g/L. Each vessel contained 20 mL of alginate gel with immobilized yeasts. The volume of the liquid phase (including the volume of gel) was 200 mL. Each vessel was equipped with a magnetic stirrer. The operating temperature was maintained at 15°C. Wort was fed from a sterile flask into the cascade of vessels using a peristaltic pump. The feeding rate was set at 15, 20, and 25 mL/min and kept at each flow rate for 1 wk. Samples of liquid phase were taken at

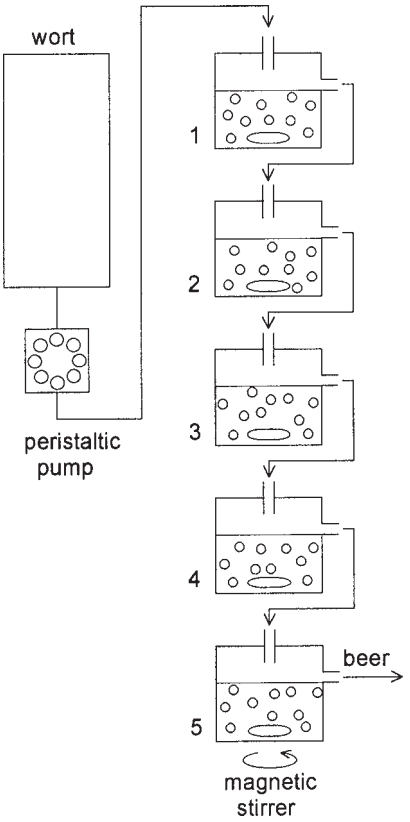

Fig. 1. Schematic diagram of the experimental apparatus for continuous beer fermentation with cells immobilized in alginate beads. Numbers 1–5 indicate vessel number in mathematical model.

24-h intervals from all vessels. Samples of gel for cell determination were taken at 3-d intervals (five beads were sampled from each vessel).

Analytical Methods

Ethanol was determined by gas chromatography using a Chrom-5 gas chromatograph (Laboratory Instruments, Prague, Czech Republic) equipped with a flame ionization detector. Analyses were run at 180°C using a column (length of 1.4 m, id of 2.5 mm) packed with Porapak Q (Sigma, St. Louis, MO).

The concentration of saccharides was determined by high-performance liquid chromatography. A high-pressure pump (Ecom Pump LCP 4000; Ecom, Czech Republic) and a refractometric detector (RID 130; Laboratorni Pristroje, Prague, Czech Republic) were used. Samples were deionized using a mixed-bed ion-exchanger Amberlite MB-150 (Sigma) and filtered through a Sep-Pak silica cartridge (Waters). Analyses were performed using a column HPX-87C (Bio-Rad) at a temperature of 85°C. Deionized water at a flow rate of 0.2 mL/min was used as the mobile phase.

The cell concentration in alginate beads was determined after dissolving alginate in citric acid. Alginate beads containing cells were put in 1% citric acid solution and left to dissolve completely (approx 5 min). The cell concentration was expressed as the concentration of dry weight matter. The dry weight matter of cells was estimated as follows: a sample was centrifuged, resuspended in a minimal volume of distilled water, and dried to constant weight at 105°C.

Mathematical Model

To construct the mathematical model, general equations describing substrate conversion and inhibition and common strategy to analyze data were used *(31)*. The mass balances of the substrates in the *n*th vessel were calculated using the following set of equations:

$$\frac{dM_n}{dt} = \frac{f}{V}(M_{n-1} - M_n) - r_{M_n} X_n \tag{1}$$

$$\frac{dMT_n}{dt} = \frac{f}{V}(MT_{n-1} - MT_n) - r_{MT_n} X_n \tag{2}$$

$$\frac{dG_n}{dt} = \frac{f}{V}(G_{n-1} - G_n) - r_{G_n} X_n \tag{3}$$

$$\frac{dF_n}{dt} = \frac{f}{V}(F_{n-1} - F_n) - r_{F_n} X_n \tag{4}$$

At a constant flow rate, the system approaches steady state and $dM/dt = 0$; this applies also for MT, G, and F. The substrate consumption rates for each vessel were calculated as follows:

$$r_{M_n} = r_{\max_M} \frac{M_n}{k_M + M_n} \left[1 - \left(\frac{M_n}{a} \right)^b \right] \tag{5}$$

$$r_{MT_n} = r_{\max_{MT}} \frac{MT_n}{k_{MT} + MT_n} \tag{6}$$

$$r_{G_n} = r_{\max_G} \frac{G_n}{k_G + G_n} \tag{7}$$

$$r_{F_n} = r_{\max_F} \frac{F_n}{k_F + F_n} \tag{8}$$

The following kinetic patterns of maltose inhibition and ethanol inhibition were also tested, although owing to insufficient correlation they were not used for the final model formulation. The inhibition of maltose consumption by maltose describes Eq. 9:

$$\frac{r}{r_{\max}} = \frac{M}{k_M + M + \frac{M^2}{k_M'}} \tag{9}$$

in which r is the substrate consumption rate, $r_{\max}$ is the maximum substrate consumption rate, and k_M' is the maltose inhibition constant. The inhibition of substrate consumption rate (tested for M, MT, G, and F) by ethanol is represented by Eq. 10:

$$\frac{r}{r_{\max}} = 1 - \left(\frac{E}{c}\right)^d \tag{10}$$

in which c and d are empirical constants. The mass balance of ethanol was calculated similarly to the calculation of substrate mass balance:

$$\frac{dE_n}{dt} = \frac{f}{V}(E_{n-1} - E_n) + r_{E_n} X_n \tag{11}$$

Ethanol production rate was calculated from substrate balance:

$$r_{E_n} = Y\left(\frac{dM_n}{dt} + \frac{dMT_n}{dt} + \frac{dG_n}{dt} + \frac{dF_n}{dt}\right) \tag{12}$$

provided that ethanol yield was constant and equal for all substrates (Y = 0.41). For the biomass concentration, the following two assumptions were adopted: (1) concentration of immobilized cells X_n were experimentally measured for each vessel, and (2) these values were used for modeling and ethanol production by free cells was neglected.

Results and Discussion

Alginate is generally not considered as an optimal material for brewing with immobilized yeasts. The possible drawbacks are diffusional limits, changes in yeast metabolism, and limited stability. On the other hand, alginate has the advantage of easy use and simple preparation of homogeneous immobilisates. For modeling studies, the ease of dissolving alginate to determine cell concentration is an advantage. The yield coefficient (Y)

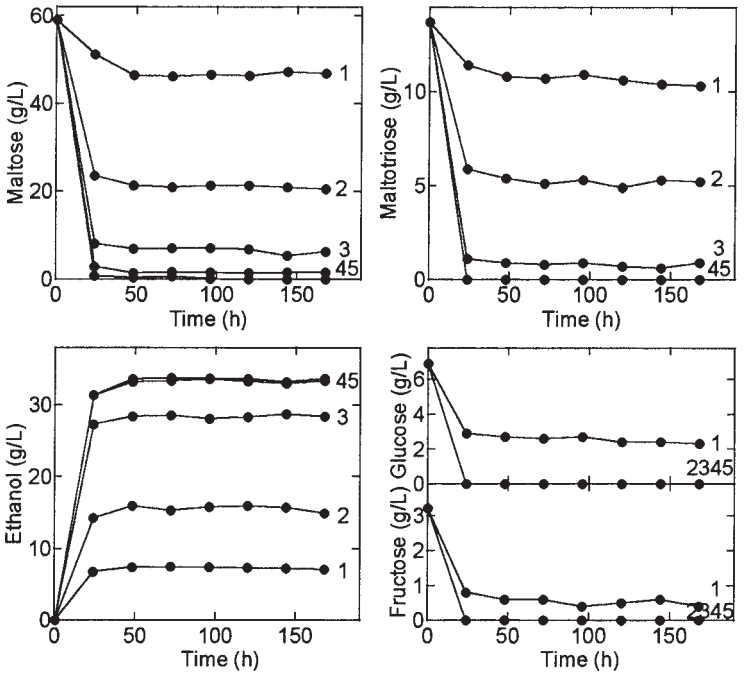

Fig. 2. Continuous beer fermentation by immobilized cells at a flow rate of 15 mL/min. Numbers 1–5 indicate the vessel as described in Fig. 1.

has been considered to be constant and equal for all substrates $Y = 0.41$. This value was obtained as a ratio of total utilizable saccharides (i.e., 82.8 g/L) (for concentration of individual saccharides see Materials and Methods) and the ethanol produced (i.e., 34 g/L). Theoretical yield is different for mono-, di-, and trisaccharides. However, the utilization of a complex mixture of substrates, such as wort, exhibits deviations from theory, and also we had considerable data dispersion. These were the reasons to use the simplified approach using one yield coefficient for all saccharides.

Continuous Beer Fermentation

Continuous beer fermentation was studied during a 3-wk experiment. The flow rate was kept at constant values of 15, 20, and finally 25 mL/min for 1-wk periods. Figure 2 shows the beginning of the experiment at a constant flow rate of 15 mL/min and reaching steady state. The steady state was reached in 3 d. Later, the experimentally determined concentrations exhibited only a small variability. For modeling purposes, the steady-state data were used. The average values of the last 5 d of operation at steady state were used for further work (the points from 72 to 168 h in Fig. 2). Small amounts of glucose and fructose present in wort were completely utilized in the first vessel, and maltose and maltotriose were simultaneously utilized in all vessels. The concentrations of other sugars that may be present in wort (e.g., maltotetraose) were below 0.1 g/L and were neglected. To

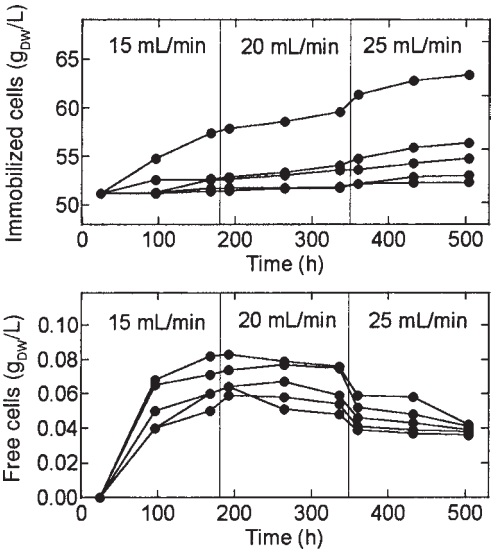

Fig. 3. Cell growth and cell release from alginate beads at various flow rates during the entire experimental period. The lines correspond to vessels 1 (upper line) to 5 (bottom line) in both top and bottom picture.

formulate the mathematical model, the utilization of the four considered substrates was considered to be simultaneous and could be characterized by its constants separately.

The behavior of yeast cells during a 3-wk experimental period is documented in Fig. 3. Cell growth was most intensive in the first vessel inside the gel and the release of cells from the gel beads was observed. Cell growth inside the gel was not possible to fit using Monod-type kinetics. The intensive cell growth in the first vessel is probably connected to the high concentration of oxygen present in fresh wort. The concentration of free cells was very low compared with the concentration of immobilized cells. The experimental complexity involving cell growth, which may proceed differently in the various vessels, and cell release makes cell growth very difficult to analyze. For the purposes of further model optimization, these two conclusions were adopted: (1) contribution of free cells to the brewing was neglected, and (2) experimentally determined values of cell concentration in the gel were used for model optimization. The cell concentration was considered to be constant during the period of constant flow rate (15, 20, or 25 mL/min) and equal to the average of three values determined during this period.

Optimization of Parameters

Because possible inhibition affects substrate consumption, inhibition by ethanol and by each substrate itself was investigated. However, no inhibition effect of ethanol on substrate consumption was ascertained after data analysis. This was probably because of the small inhibition effects that

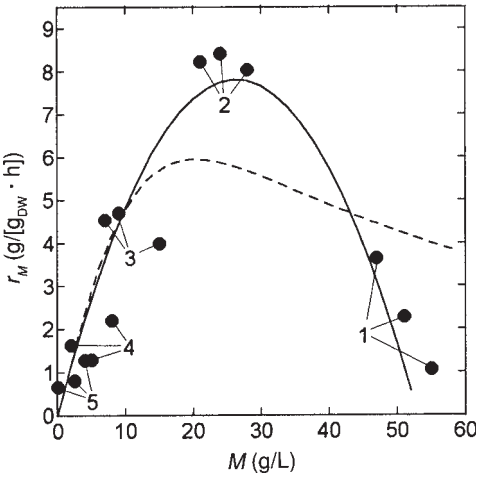

Fig. 4. Inhibition effect of maltose on specific maltose consumption rate. The solid line indicates the kinetic pattern calculated using Eq. 5, which gives values $r_{max_M} = 1.04$ (g/[g_{DW}·h]), $k_M = 54.3$ (g/L), $a = 55$ (g/L), and $b = 2.3$. The dashed line was calculated using Eq. 9, and the calculated values were $r_{maxM} = 2.29$ (g/[g_{DW}·h), $k_M = 99.0$ (g/L), and $k_M' = 4.2$ (g/L). Numbers 1–5 indicate the vessel as described in Fig. 1.

cannot be distinguished using nonlinear regression owing to data dispersion. The inhibition effect of ethanol on *Saccharomyces carlsbergensis* was previously published, and the inhibition constant of 149 g/L was reported *(20)*. The ethanol concentrations in the present system were up to 34 g/L, which suggests that little inhibition effect could be expected. Substrate inhibition was investigated for all substrates, and only the inhibition by maltose was observed. Inhibition by maltotriose, glucose, and fructose was not identified owing to their low concentrations in wort (e.g., the maximum concentration of maltose was 13.7 g/L). The inhibition of maltose consumption by maltose was clearly distinguished and is presented in Fig. 4. The maltose consumption rates were calculated using Eq. 1, for each vessel at three flow rates. The inhibition effects are obvious in the first vessel, as can be seen in Fig. 4. The steady-state maltose concentrations in the first vessel at flow rates of 15, 20, and 25 mL/min were 47, 51, and 55 g/L, respectively. The data presented in Fig. 4 were fitted using Eqs. 5 and 9. As observed in the plots (Fig. 4), a nonlinear regression using Eq. 5 gives very good correlation with experimental data. The optimized values of parameters, given in the legend to Fig. 4, were used as initial values for further model optimization. An optimization algorithm was written to calculate differences between model and experimental values and to minimize the sum of square roots. Finally, all the optimized values of the parameters of the model were obtained and are listed Table 1.

Figure 5 shows the comparison of calculated values with the experimentally measured values for maltose, maltotriose, and ethanol. Differences are mainly in vessel 1, and the data of vessels 2–5 agree quite well. The differences in vessel 1 may be connected to oxygen content, which may

Table 1
Calculated Values of Model Parameters

Substrate	Maximum specific production rate ($g/[g_{DW} \cdot h]$)	Saturation constant (g/L)
Maltose	$r_{max_M} = 1.09$	$k_M = 50.3$
Maltotriose[a]	$r_{max_{MT}} = 0.058$	$k_{MT} = 0.6$
Glucose	$r_{max_G} = 0.35$	$k_G = 19.4$
Fructose	$r_{max_F} = 0.17$	$k_F = 9.7$

[a]Calculated values of a, b for inhibition of maltose consumption by maltose: a = 53 (g/L); b = 2.0 (dimensionless).

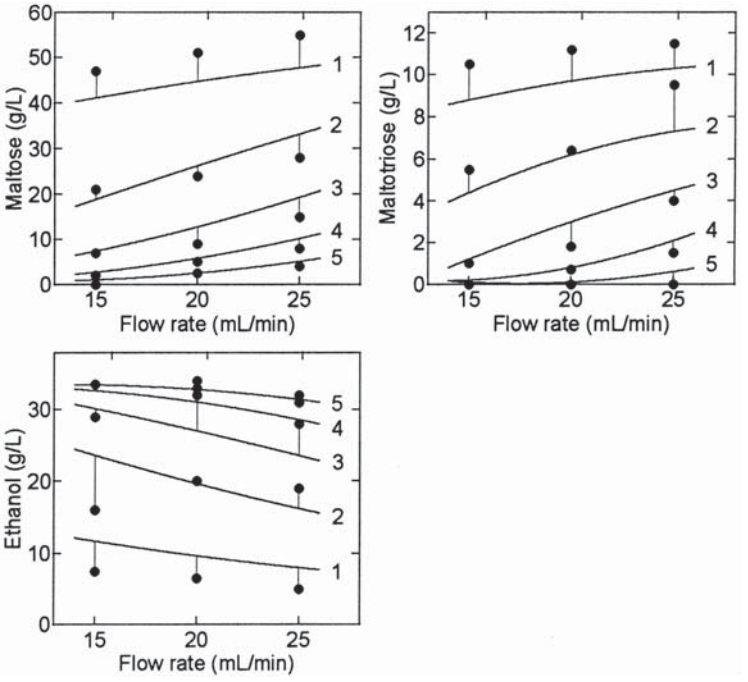

Fig. 5. Correlation of proposed mathematical model with experimental data. Solid circles represent experimental data, and lines represent values calculated using the optimized parameters listed in Table 1. Numbers 1–5 indicate the vessel of the fermentation apparatus, and experimental data are connected to the corresponding vessel using a hairline.

result in metabolic changes and biomass growth, as mentioned previously. We assume that the differences are not a consequence of diffusional limitations. The diffusional limits, if any, could appear in vessel 2, in which brewing is most intensive; however, the correlation of data of vessel 2 is good.

Conclusion

Beer brewing is a complex process; too many substrates and effects make modeling or any analysis difficult. This may be perhaps one of the reasons that the sum of sugars was used as a parameter in the previously published studies to simplify mathematical description. Our approach to beer-brewing modeling is based on the analysis of sugar consumption separately. It allows the characterization of the consumption of each sugar by two parameters: specific substrate consumption rate and saturation constant. In conclusion, the proposed model satisfactorily describes primary beer fermentation. It seems, therefore, that the differences in data for vessel 1 indicate some effect that has not been identified. The proposed model also should be applicable to other designs of brewing using immobilized systems. Future investigations will extend the modeling approach to provide a better description of biomass behavior during beer production.

Nomenclature

a,b = constants characterizing maltose consumption inhibition by maltose (g/L) (dimensionless)
E = ethanol concentration (g/L)
F = fructose concentration (g/L)
f = volumetric flow rate (L/h)
G = glucose concentration (g/L)
k = saturation constant, subscripts M, MT, G, or F indicating substrate (g/L)
M = maltose concentration (g/L)
MT = maltotriose concentration (g/L)
r = specific rate of substrate consumption or ethanol production (indicated by subscript) (g/[g_{DW}·h])
t = time (h)
V = working volume of vessel including gel volume (L)
X = biomass concentration expressed per working volume (g_{DW}/L)
Y = yield coefficient

Subscripts

DW = dry weight
max = maximum rate
n, $n-1$ = vessel (1–5)

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