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Enhanced in situ dynamic method for measuring K_La in fermentation media

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ABSTRACT

The overall oxygen mass transfer coefficient (K_La) is often used as scale-up factor of fermentation systems. In fermenter scale-up, it is desired to achieve the same K_La values at the larger scale than the one that was obtained at a smaller scale during the development stage. It is therefore important to be able to measure K_La in situ during fermentation and to also determine the action to be taken to maintain its value at its design set point. These objectives can be obtained by measuring K_La using the dynamic method and enhancing the K_La information by immediately conducting a series of changes in agitation speed and/or aeration rate to determine the influence of these variables on K_La. This enhanced dynamic method is demonstrated with two filamentous microorganisms: *Trichoderma reesei* for the production of cellulase and *Aspergillus niger* for the production of citric acid. Two different types of bioreactor were used: a reciprocating plate bioreactor and a stirred (Rushton) bioreactor. It is shown that the proposed method can provide a simple way to measure the local variation of K_La and to adjust its value to its set point during the course of fermentation.

1. Introduction

The main challenge in scale-up is to determine the operating conditions and mass transfer characteristics that should prevail in a fermenter of a different size in order to achieve the same process yield. Scale-up is the reproduction in a production-scale fermenter of results obtained from a successful fermentation carried out in laboratory or pilot-plant equipment. An important goal in most aerobic fermentations is therefore to maintain the operating conditions at the optimal level that were usually derived at a smaller scale. Many methods for scale-up have been proposed to ensure the same productivity when the scale of production is increased. Some of these methods, which have been applied successfully, are: (1) fixing K_La such that it is identical at both scales [1–3]; (2) keeping constant the power per unit volume (P_G/V_L) required for mixing the fermentation broth [1]; (3) keeping constant the speed at the tip of the agitator; (4) maintaining a constant dissolved oxygen concentration, and (5) keeping equal mixing times in fermenters of different scales [4]. Margaritis and Zajic [5] surveyed the first four scale-up methods and reported that they were used industrially in 30%, 30%, 20%, and 20% of the time, respectively.

For the scaling-up of aerobic fermentation, the effect of gas–liquid mass transport was found to be the most significant factor [6]. The oxygen mass transfer coefficient (K_La) often serves to compare the efficiency of bioreactors and mixing devices and it is also considered as an important scale-up factor because it is desired to achieve a given oxygen mass transfer capability that can meet with the oxygen demand of the culture. Often, aeration and agitation are selected to achieve the desired oxygen mass transfer coefficient, since this is the controlling parameter in most fermentations [7].

The objective of this paper is to demonstrate how the in situ dynamic method to determine K_La during the course of fermentation can be easily extended to obtain information on the variation of K_La with agitation and aeration and therefore offer a way to use the information from these additional measurements to manipulate the speed of agitation and/or the inlet gas flow rate to achieve the desired K_La value that is dictated in the scale-up process. It is important to realize that for filamentous microorganisms, the drastic increase in viscosity, associated with the growth and morphology of microorganisms, will affect K_La throughout the fermentation. This is the case of *Aspergillus niger* which forms mycelia and/or pellets [8] and of *Trichoderma reesei* which can adopt mainly four types of morphological states [9]. The oxygen mass transfer coefficient is significantly reduced as the concentration of the microorganism increases with time, this reduction being more important for some morphological states.

2. Materials and methods

2.1. Bioreactors

The two bioreactors, a reciprocating plate bioreactor (RPB) and a stirred tank bioreactor (STB) with three Rushton turbines (Fig. 1),

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Nomenclature

C_L	dissolved oxygen concentration (mol/m^3)
C_L^0	pseudo-steady-state dissolved oxygen concentration recorded at the initiation of the dynamic method (mol/m^3)
C_L^*	dissolved oxygen concentration in equilibrium with mean gaseous oxygen concentration (mol/m^3)
DO	dissolved oxygen
J	data reconciliation objective function (Eq. (6))
K_La	overall oxygen mass transfer coefficient (s^{-1})
P	pressure (Pa)
P_G	average gassed power input (W)
Q_G	gas flow rate (m^3/s)
Q_{O_2}	respiration rate (mol/s (g biomass))
R	gas constant (8.306 Pa m^3/(mol K))
RPB	reciprocating plate bioreactor
RPM	revolution per minute
RQ	respiratory quotient
STB	stirred tank bioreactor
SLPM	standard litre per minute
t	time (s)
T	temperature (K)
U_G	gas superficial velocity (m/s)
V_L	liquid volume in the fermenter (m^3)
X	biomass concentration (g/L)
y	gaseous mole fraction

Greek letters

α	weighting factor in Eq. (6) (inverse units of each term in Eq. (6))
α	parameter in Eq. (7)
β	parameter in Eq. (7)
γ	parameter in Eq. (7)
τ	time constant (s)

Subscripts

inlet	stream
outlet	stream
CO_2	carbon dioxide
G	gas
O_2	oxygen
P	dissolved oxygen probe

were used in these experiments [10]. These bioreactors, built in our laboratories, are identical except for the mixing mechanism. The two bioreactors have a total volume of 22 L. The bioreactors are made of stainless steel and have an inner diameter of 228 mm and a column height of 550 mm. The outer tube has an internal diameter of 236 mm that leaves an annular gap of 3.5 mm to form a jacket where water, at an appropriate temperature, is continuously circulated to maintain the temperature of the fermentation broth constant. The top of the bioreactor has ports for sampling, feeding, and to hold a dissolved oxygen probe, pH probe and a thermocouple. Compressed air is fed at the bottom of the bioreactor after passing through a flow meter, a mass flow meter and a sterile gas filter. The gas sparger at the bottom of the bioreactor is a thin plate perforated with one hundred uniformly distributed holes, 1 mm in diameter. The gas flow rate is controlled by a mass flow controller and a dissolved oxygen probe measures the dissolved oxygen (DO) at a point located 82 mm from the centre of the column and 255 mm from the bottom of the reactor for *A. niger* fermentation and 130 mm from the bottom for *T. reesei* fermentation. Positions of the probes are different due to different volumes of the fermentation broth. The

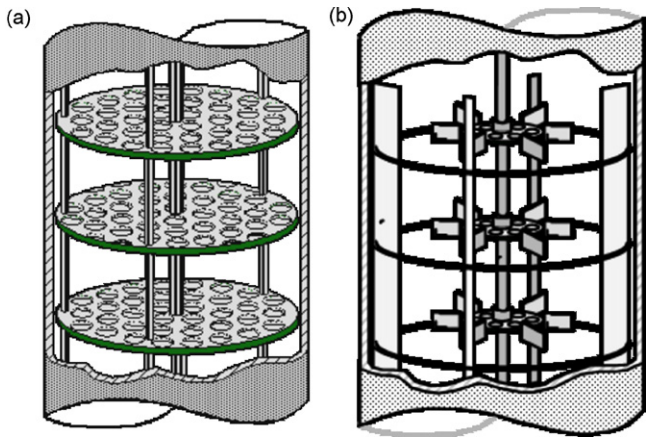

Fig. 1. Schematic diagram of the RPB (a) and STB (b).

exit and inlet gas streams were dehumidified before analyzing their composition.

2.1.1. Reciprocating plate bioreactor

The plate stack, reciprocated axially, consisted of 6 perforated stainless steel plates, 221 mm in diameter and 1.25 mm thick. Each plate was spaced 50 mm apart from one another. The perforations have a diameter of 19 mm and holes are distributed on an equilateral triangular pitch. The plate fractional free area, including the 3.5 mm annular space between the plate edge and bioreactor wall, is 0.36. The driving unit consists of a connecting rod, which imparts the reciprocating motion, a tenfold reducing speed transmission and a variable speed motor controlled by a microcomputer. An aluminium disc, containing 100 uniformly distributed perforations and mounted on the output shaft of the reducing transmission, is used in conjunction with an infrared optical switch (HOA-2001, Honeywell) to measure and control, with a microcomputer, the frequency of the reciprocation by manipulating the power to the motor.

2.1.2. Stirred tank bioreactor

The mixing mechanism of the stirred tank bioreactor consisted of three identical Rushton turbines mounted on the central rotating shaft. The location of the impellers, measured from the bottom of the column, is 56, 199 and 327 mm for *A. niger* fermentation and 54, 132 and 210 mm for *T. reesei* fermentation. As mentioned earlier, due to different volumes of the fermentation broth, the impeller positions were also adjusted so that all the impellers were immersed in the fermentation broth. Each turbine has 6 blades mounted on the periphery of a 50 mm diameter disk. Each blade is 25 mm long, 15 mm high and 1.5 mm thick. Four baffles were placed inside the mixing vessel.

2.2. Experimental procedure

The microorganisms used for the fermentation experiments were *A. niger* (ATCC 1015) and *T. reesei* RUT C-30 (ATCC 56765). For *A. niger*, the freeze-dried culture was rehydrated and grown on Petri dishes and subsequently transferred to an agar slant (SIGMA, Potato Dextrose Agar, P-2182). It was then used to inoculate 50 mL of culture medium. The composition of the culture medium [11] was identical for both bioreactors and composed of the following constituents: Sucrose, 140 g/L; NH_4NO_3, 2.5 g/L; KH_2PO_4, 2.5 g/L and $MgSO_4{\cdot}7H_2O$, 0.25 g/L. Three days before the start of an experiment, the microorganisms were transferred into two 730-mL Erlenmeyer flasks of culture medium. The bioreactors and their contents were autoclaved for a period of 20 min at 121 °C. At the start of each experiment, the content of one Erlenmeyer was transferred into