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The Oxygen Transfer and Oxygen Uptake in Antibiotic Fermentation using *Streptomyces kanamyceticus*

Kye Joon Lee and Moo Bae
Applied Microbiology Laboratory
The Korea Advanced Institute of Science and Technology
P.O. Box 131 Dong Dae Mun, Seoul, Korea
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抗生物質醱酵에서의 酸素傳達 및 吸収速度에 關한 研究

李啓準 裵 武
韓國科学技術院・應用微生物研究室
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Abstract

The aim of the present study was to assess the oxygen transfer rate and oxygen uptake rate in antibiotic fermentation. As a model study, cultures of *Streptomyces kanamyceticus* in a complex medium were analyzed to evaluate the oxygen transfer and uptake rates using oxygen balance technique. Quantitative evidence for the effect of oxygen transfer rate on the volumetric antibiotic production was clearly demonstrated. The oxygen uptake rates and the specific oxygen requirements were significantly changed with culture time. Those phenomena were indicative of biological turnover in the antibiotic fermentation.

Introduction

A primary importance has being given to the phenomenon of gas transfer, especially of aerobic cultures, in a fermentation broth. The transfer of oxygen from air to the respiring microor-ganisms in the water has been a major problem because of oxygen's low solubility. Recent attempts to construct complex rich media and to design fermentation system have resulted in industrial cultures which have high microbial concentrations, thereby requiring more rapid rates of oxygen transfer to maintain dissolved oxygen above critical levels. Inadequate control of oxygen levels results in suboptimum fermentations. In batch culture studies, the physical and chemical properties of the fermentation broth vary with time. Therefore it would be very useful to assess the antibiotic fermentation in terms of oxygen transfer and oxygen uptake as well as the changes of biomass, substrate and antibiotic concentrations produced in the culture broth. For this reason *Streptomyces kanamyceticus* which produces kanamycin was chosen as the subject for this study.

Materials and Methods

Microorganisms and preservation

Streptomyces kanamyceticus strain no. KIAM 307 was used in these studies. This strain was selected from a strain ATCC 12853 after sequential mutagenesis for in-

creasing the kanamycin production. It was preserved as spores in a soil culture, and stored at 10°C. *Bacillus subtilis* strain no. ATCC 6633 was used for the determination of antibiotic activity in the fermentation broth. It was preserved by the being transferred to fresh nutrient agar slant each month and stored at 4°C.

Media compositions

For the stock culture and pre-seed culture of *St. kanamyceticus,* the following media compositions were used: glucose 1%, meat extract 0.5%, peptone 1%, NaCl 0.3%. For fermentation studies the medium compositions were as follows: soluble starch 2%, soy bean meal 1%, $NaNO_3$ 0.2%, KCl 0.05%, $MgSO_4$ $7H_2$ 0.05%. The pH of each medium was adjusted to 7.0 before steam sterilization. Sterilization of the media and fermentor was carried out by steam flow for 15 minutes under 15 Psig.

Procedure for batch cultures

A single colony of *St. kanamyceticus* was transferred from the stock culture plate to 20 ml of pre-seed culture medium and incubated for 2 days with the shaking incubator at 30°C. The pre-seed culture was inoculated into 100 ml of the fermentation medium for seed culture, then the exponentially growing cell was transferred to 5l of the fermentation medium in a 10l fermentor. A 2% (V/V) inoculum size was used for all batch experiments. Temperature was maintained at 30°C, the pH was adjusted at the start to 7.0, then left with no further control. The supply of air for the growth of *St. kanamyceticus* was requlated using a manostat and a rotameter. Agitation of the culture broth was achieved by means of a variable transformer.

Analytical Procedures and methods

The first 10 ml of culture broth contained in the sampling line was discarded, then about 10 ml of culture broth was taken for further analysis. The supernatant of the culture broth after centrifugation at 3,000 x g for 10 minutes was used for determining the kanamycin and substrates concentrations. Cell mass was measured by the dried cell weight (DCW) after 18 hours at 105°C. The concentration of kanamycin was analyzed by means of bioassay using *Bacillus subtilis* and plate cup methods. The residual concentrations of starch were detected by using dinitrosalicylic acid after acid hydrolysis. In this analyzing procedure the concentrations of total reducing sugars before acid hydrolysis were also analyzed by the same methods in order to calculate the concentrations of starch remaining in the samples.

Measurement of oxygen concentrations and oxygen uptake rates

The concentrations of dissolved oxygen in the culture broth were measured by a steam sterilizable membrane type oxygen probe (Fuji Electric Co.,) and the concentrations of oxygen in the inlet and exhausted air were detected by an oxygen analyzer (Beckmann Company). The measurement of oxygen concentrations in the culture broth and air phases were carried out throughout the fermentation runs, and the values were recorded simultaneously with recorders.

In a well agitated fermentor, it is assumed that the dissolved oxygen concentration is uniform throughout the bulk liquid. The overall oxygen balance in a fermentation broth is:

Change of O_2 concentration in the culture	=	Oxygen transfer rate by aeration and agitation (O.T.R)	–	Oxygen uptake rate by cell respiration (O.U.R)

Thus

$$\frac{d\bar{C}}{dt} = K_{L}a\,(C^{*}-\bar{C}) - Q_{02}X \quad \text{..... Equation 1}$$

where $K_{L}a$ = the volumetric oxygen transfer coefficient (h^{-1})

K^{*} = the concentration of dissolved O_2 which is in equilibrium with partial pressure in the bulk gas phase (Mol of O_2/liter)

$\bar{C}$ = the actual concentration of oxygen in bulk liquid (Mol of O_2/liter)

Q_{02} = the specific oxygen uptake rate by cell respiration (Mol of O_2/g of cell·hr)

X = the concentration of respiring organisms (g/liter)

Many methods have been proposed to determine the $K_{L}a$ or to further the oxygen transfer rate.

One of the proposed methods is the "static gassing out" method which measures the oxidation rates of sodium sulfite solution in a presense of suitable catalyzing ion (viz. Co^{-2}, Cu^{-2})[2] While a simple and reproducible procedure, the static-gassing out method has the disadvantage that the sodium sulfite solutions do not ade-

quately simulate the real fermentation broth[2].

Another method that has been extensively used is the "dynamic gassing out" technique which uses an oxygen sensor to detect the dissolved oxygen concentration in the culture.[3, 4] It's applicability, however, to viscous fermentation culture broths such as the *Sttreptomyces* culture seems to be limited by the fact that air bubbles are held on the mycelia masses.[5]

Another method that could be applied to those viscous culture broths is the "steady-state oxygen balance" method using both on oxygen analyzer and a dissolved oxygen measuring electrode.[6] In the fermentation system where a steady-state is maintained, $\frac{d\bar{c}}{dt}$ becomes zero. Equation 1 can also be rearranged as follows:

$$Q_{o2}X = K_{L}a(C^* - \bar{C}) \quad \text{..... Equation 2}$$

The oxygen uptake rate is equivalent to the oxygen transfer rate which is controlled by the given fermentor system and operating conditions. This method was chosen in the presented studies for evaluating the oxygen transfer phenomenon in a fermentation broth because no assumptions must be adopted and no manipulation of fermentation variables such as aeration agitation is required.

Results and Discussion

The oxygen transfer rates of the fermentor system varying with the air flow rate and agitation speed were determined by the application of the steady state oxygen balance method using a 0.8N $NaSO_3$ and a $1x10^{-3}$ Mol $CoSO_4 7H_2O$ solution. Agitation speed was increased from 200 RPM to 600 RPM at a fixed air flow rate and the air flow rate was varied from 0.4 to 0.1 volume of air to total liquid volume per minute (VVM) at the corresponding agitation speed. Data for the changes of oxygen transfer rates are shown in Fig. 1. It is clear that the oxygen transfer rates are a function of agitation speed and air flow rate in a given fermentor. The sodium sulfite solution containing Co^{++} ions as a catalyst is considered as an inexact simulation of the real fermentation broth. However the data may indirectly provide some information to estimate the oxygen transfer rates in the real fermentation broth. It was found that this technique was very simple and the steady state was reached very rapidly, therefore, the assessment of the oxygen transfer rate could effectively be carried out.

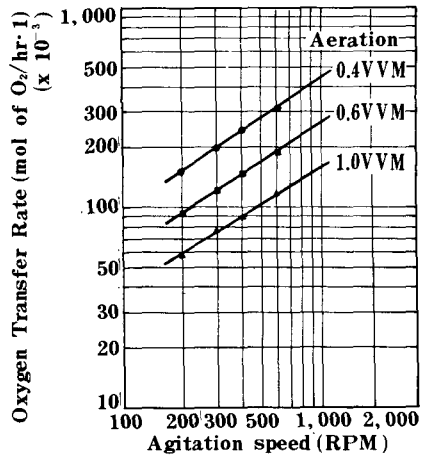

Figure 1. Effect of Agitation and Aeration on the Oxygen Transfer Rate measured by the Sulfite Oxidation Method.

Oxygen transfer rates and kanamycin production

Batch culture experiments were conducted under 15 different sets of conditions to evaluate the effect of agitation and air flow rate on kanamycin production. As shown in Fig. 2, it is apparent that kanamycin production is stimulated by the appropriate control of agitation and aeration. The air flow rate and agitation speed should be optimized in order to avoid contamination risks, and to maximize the kanamycin productivity. It is evident that the maximum production of kanamycin was achieved with a 300 RPM of agitation speed and a 1 VVM of aeration rate.

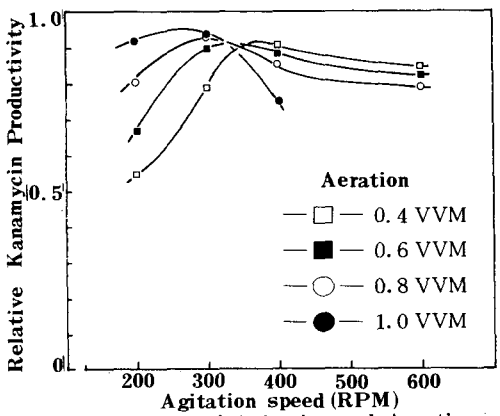

Figure 2. Effect of Agitation and Aeration on Kanamycin Production for *St. kanamyceticus*

Oxygen uptake rate and specific oxygen requirements

Data for the growth of *St. kanamyceticus* and production of kanamycin in the fermentation medium are shown in Fig. 3 (A). Changes in pH and the dissolved oxygen were also plotted. The oxygen uptake rates were measured by the oxygen balance method and the specific oxygen requirements were obtained by Equation 2. These values are given in Fig. 3 (B). The rapid disappearance of starch and a concomitant increase of reducing sugars was the distinct characteristic in the trophophase. In the subsequent stage of idiophase, the starch was not detected. The accumulation of reducing sugars in the culture broth of trophophase were indicative of the presence of starch hydrolyzing enzymes and of that the reducing sugars could be used for kanamycin production. Other interesting points were the significant changes in the concentrations of dissolved oxygen, volumetric oxygen uptake rates and the specific oxygen requirements of the used strain. The maximum values of the specific oxygen requirement was observed with the growing cell in the middle of an exponential growth phase. It is clear that the volumetric oxygen uptake rates were a function of the specific oxygen requirement and total respiring cell mass. The concave curve of the volumetric oxygen uptake graph revealed that the initial stage of kanamycin production had evidence of biological turnover, for example, the derepression of key enzymes in the kanamycin formation. The concentration of dissolved oxygen decreased as the volumetric oxygen uptake rate increased, however, this value was not below the critical level. These results indicate that the dissolved oxygen is not a limiting factor in kanamycin fermentation. It is therefore desirable to increase the biomass for greater kanamycin productivity. The presented study was carried out initially to evaluate the effects of aeration and agitation on antibiotic fermentation. From these studies, it is clear that the oxygen balance technique offers a rapid assessment of oxygen control in aerobic fermentation.

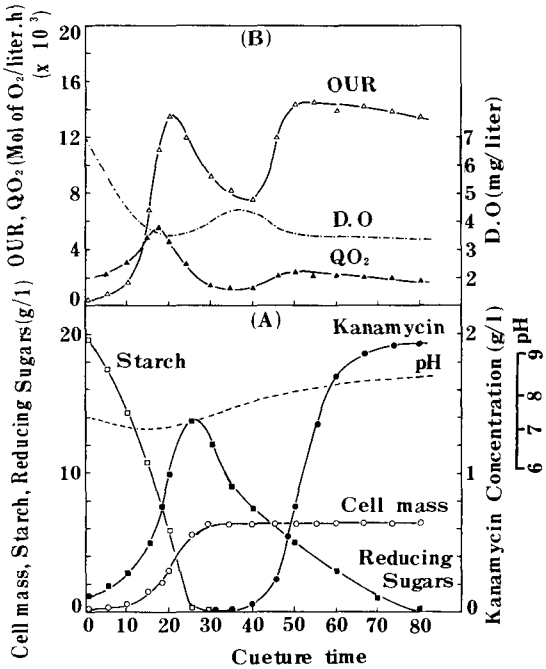

Figure 3. Batch Culture Data for the increase of Cell mass, Uptake of Substrate, Kanamycin Production. pH, Dissolved Oxygen (D.O), Oxygen Uptake Rate (OUR) and Specific Oxygen Requirement (QO_2).

要　　約

通気醱酵에서 중요한 通気 및 振盪효과를 측정함에 있어 공기로부터 물속으로 溶存해 가는 산소의 전달속도와, 溶存된 산소가 미생물에 의해 吸収되는 속도를 算出하였다. 抗生物質醱酵에서 通気效果는 현저하였으며, 배양시간에 따른 산소흡수 속도와 比酸素吸収 速度가 変化하는 것을 알았다. 이러한 変化는 대사이차 산물인 抗生物質生合成의 生化学的인 전환에 기인하는 것으로 판단된다.

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