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APPROACHES TO $K_L a$ MEASUREMENTS IN SOLID STATE FERMENTATION

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Summary : A convenient method for measuring $K_L a$ in a solid state medium is proposed. Due to the particular nature of the substrate used in solid state fermentation, different modifications of the sulfite oxidation method have been necessary. This first approach allows to study the influence of air inflow rate and dry matter percentage of the medium on the oxygen volumetric mass transfer coefficient.

INTRODUCTION

The solid-state fermentation (SSF) can be defined as a method of culturing microorganisms on and within the particles of solid matrix (solid substrate), where the liquid content, bound with them, is at the level corresponding to the water activity assuring correct growth and metabolism of cells, but not exceeding the maximum water holding capacity of the solid matrix. (Durand et al, 1987). This system comprises three phases :

- a solid phase which is generally a vegetable substrate containing the microorganism nutrients
- a liquid phase for the different mass transfers
- a gaseous phase

The last phase is very important because it plays three parts in SSF
- to regulate substrate temperature,

- to regulate moisture level of the medium during the course of the fermentation,
- to maintain aerobic conditions

So, to know the oxygen availability in the liquid phase for the microorganism needs, it is very important to measure the oxygen volumetric mass transfer coefficient, $K_L a$. Furthermore, when the air goes through a 0.5 m or 1.0 m thick layer, the aerobic conditions can change from the bottom to the top of the layer (Durand and Chereau, 1987).
Many measuring methods of $K_L a$ have been proposed in the literature (Tsao and Kempe, 1960 ; Cooper et al, 1944 ; Wise, 1951 ; Matsumura et al, 1979 ; Taguchi et al, 1968 ; Imai et al, 1986 ; Fukuda et al, 1968 ; Reit, 1979). But most of them do not fit to our purpose or even are impossible to use, taking into account the particular nature of the solid-state medium and mainly the thin layer liquid phase.
This paper describes an adaptation of the sulfite oxidation method (Cooper et al, 1944). Results are presented on the influence of the air inflow rate and dry matter percentage of the medium on the oxygen volumetric mass transfer coefficient.

MATERIALS AND METHODS

Experimental device
Figure 1 shows the reactor used in our experiments. This glass column includes at the bottom a fine wire-mesh helping to distribute gas equally. Two valves allow the introduction of air or nitrogen. With a flowmeter, the air inflow rate can be adjust at different values from 2.0 to 15.0 l/min. The column, is filled up with the medium up to 25 cm height. The quantity involved is about 2.0kg of sugar beet pulp at 25% dry-matter

Medium preparation

The solid-state substrate we used is leached sugar beet pulp. The required quantity of dried pulp is placed in a hermetically sealed bag under nitrogen flow to eliminate air. By a small hole a sulfite solution, without oxygen is added to moisten the pulp at the required moisture level.
(Na_2SO_3, 0.5 mol/l with a catalyser $CoCl_2$, 0.19 g/l.).
Always under nitrogen flow, a gentle manual agitation allows

the homogeneous moistening of the substrate. The time necessary to have a good moistening is two hours.
Just before and during the filling of the column, the nitrogen valve is open. At time t = 0, the nitrogen valve is turned off and the air valve is turned on. Each minute a sample of about 10 g is taken just under the pulp surface and immediately put in a small flask containing 50 ml of a iodine solution (0.1 mol/l). The weight of each sample was measured, so the sulfite quantity could be calculated.

Measuring procedure

Each sample of pulp was in contact with the iodine solution for exactly 20 minutes. Ten milliliters were taken for the titration. The oxidation rate of sulfite was determined by the usual iodometric procedure of back titration with a sodium thiosulfate solution ($Na_2S_2O_3$, $5H_2O$, 0.2 mol/l).
Figure 2 shows an example of the $K_La C^*$ measurement. The slope of the straight line represents the sulfite decrease during the time ($d(SO_3^{2-})/dt$), which is proportional to dC_L/dt :
$dC_L/dt = (d(SO_3^{2-})/dt).\ (60/2) = K_La\ C^*$ (in mol/l.h)
where C_L is the dissolved oxygen concentration at time t (mn) and C^* is the maximum dissolved oxygen concentration determined by "the Winkler method" (1888) in our experimental conditions.

RESULTS AND DISCUSSION

For the substrate preparation, we found that the duration of the pulp moistening is very important and can affect the K_La measurements. It is absolutely necessary that liquid gets time to enter the capillary system of the substrate (about 2 hours). If not,the sulfite solution is mainly at the pulp surface and the K_La measurements do not represent the reality of a raw material. Another problem has been encountered for the titration of the residual sulfite. The iodine solution must also enter the capillary system.By testing different contact durations, we observed that 20 minutes are necessary to titrate about 70 % of the remaining sulfite. These two remarks can be very attractive to appreciate the oxygen availability in the different parts of the substrate in relation to the microorganism growth. Also, it would be possible, in the future, to estimate the gas-liquid interfacial area and its evolution during a solid state fermentation.
By the Winkler method, we established a relation between

maximum dissolved oxygen concentration, C^*, and sulfate concentration. According to Hitchman (1978), we obtained :

$$C^* = 2.95\ 10^{-4} - 2.08\ 10^{-4}\ (SO_4^{2-})$$

with a correlation coefficient of 0.998.
The influence of temperature is well known. During the experiments, an increase of the temperature occures up to about 35°C and it is necessary to correct C^*.

Influence of the cobaltous chloride concentration

The Table 1 shows the evolution of the sulfite oxidation rate in relation to Co^{2+} concentration. The experimental conditions were :pulp medium at 25 % dry-matter ; air-flow rate at 5 l/mn.

Table 1 : Influence of cobaltous chloride concentration on $K_L a$ measurements

Cobaltous chloride Concentration (g/l)	$K_L a$ (h^{-1})
0.064	389 ± 26
0.128	803 ± 25
0.190	1774 ± 36
0.250	2511 ± 20
0.400	4197 ± 19
0.640	4626 ± 31
1.00	5045 ± 25

Between 0.128 g/l and 0.40 g/l, we have a linear evolution of the $K_L a$ values. Above 0.40 g/l the catalyst concentration has less influence. We have choosen for all our experiments a concentration of 0.19 g/l essentially for two reasons :
- to have a lower rate of oxydation allowing sampling and accurate measurements,
- to avoid a quick increase of temperature.

Influence of the air inflow rate

The increase of the air inflow rate allows an increase of turbulence and a faster renew of the gaseous phase composition. Table 2 gives the results obtained for different air inflow rates.

Table 2 : Influence of the air inflow rate on the $K_L a$ (Dry-matter percentage : 25 %)

Air inflow rate (l/min)	$K_L a$ (h^{-1})
2	1140 ± 22
5	1775 ± 36
10	2650 ± 30
15	3050 ± 35

We can see that even at a low air inflow rate (2 l/min. so about 4 l/min. for 1 kg of dry-matter), the $K_L a$ value is high, compared with classical values obtained in stirred liquid reactor. In static SSF the air inflow rate is much higher (about 50-100 l/min. x kg dry-matter) to remove heat energy produced by microbial metabolism. So the oxygen availability is never a limiting factor.

Influence of the moisture content in the medium

As the liquid phase plays a great part in the mass transfers, we studied the influence of the moisture content of the pulp in the range 25 % - 35 % of dry-matter (Table 3). We can observe an increase of the $K_L a$ when the moisture content of the medium is decreasing. Two hypothesis can explain these results : an increase of K_L by a decrease of the film liquid thickness, an evolution of the layer porosity, of the interparticle volume so an increase of the gas liquid interfacial area.

Table 3 : Influence of the dry matter percentage on the $K_L a$ for an air inflow of 5 l/min..

Dry-matter percentage (%)	$K_L a$ (h^{-1})
25	1775 ± 36
30	1880 ± 17
32.5	2280 ± 17
35	2350 ± 14

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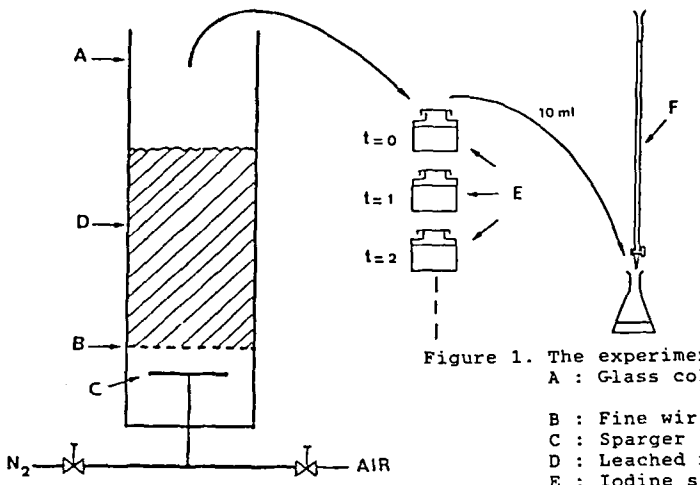

Figure 1. The experimental device
A : Glass column Diameter : 16 cm
Height : 50 cm
B : Fine wire mesh
C : Sparger
D : Leached moistened sugar beet pulp
E : Iodine solution flasks
F : Sodium thiosulfate solution

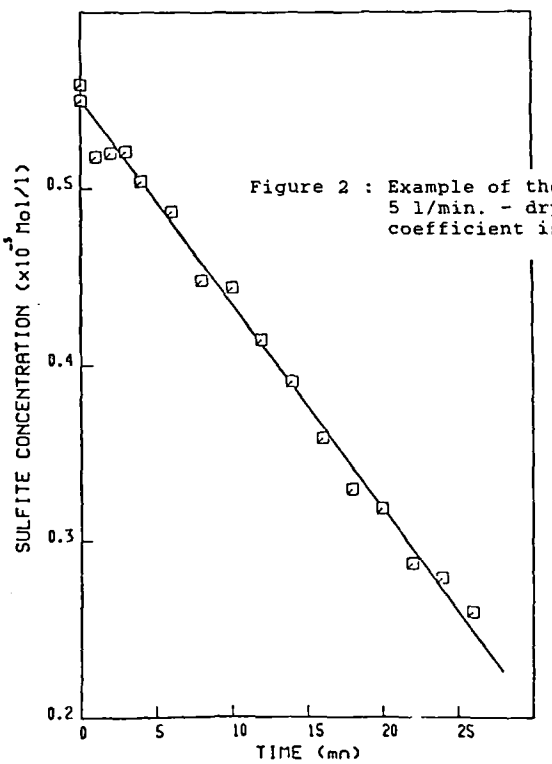

Figure 2 : Example of the $K_L aC^*$ measurement (Air flow rate 5 l/min. - dry-matter : 32.5%). The regression coefficient is 0.996.