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Effect of aeration on xylanase production by *Bacillus sp*. I-1018

P.L. Pham, P. Strehaiano, P. Taillandier

Abstract The effect of aeration on xylanase production by *Bacillus sp*. I-1018 grown in batch cultures on xylan was investigated. Efficiency of aeration and agitation was evaluated through the *Kla* coefficient, and the consumption of oxygen by bacteria was evaluated by measuring batchwise the dissolved oxygen pressure (pO_2).

During the exponential growth phase pO_2 was not zero only for the highest value of *Kla*. Growth and enzyme production were faster when *Kla* was increased (productivity in xylanase was up more than 50%).

List of symbols

Kla	volumetric mass transfer coefficient, h^{-1}
pO_2	dissolved oxygen pressure, % of saturation
V.V.M.	volume of air by volume of liquid in the fermentor by minute, L/L/min
P_{xyl}	productivity in xylanase = maximal activity/time of the maximum, nkat/mL/h

1 Introduction

Endo-1,4-β xylanases are of great interest in the field of pulp paper bleaching [1]. Among the organisms able to produce cellulase-free extra-cellular xylanases, *Bacillus* are potent producers [2] and are more interesting than fungi because of their higher growth rates. Despite the fact that most of *Bacillus* are aerobic organisms [3], no informations are available in literature about the effect of aeration on enzyme production.

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In a previous work [4] we have chosen a strain producing high levels of xylanase with no contaminant activities in aerobic batch cultures on xylan. Then, we have optimized the culture medium composition for concentrations of carbon and nitrogen sources.

In this work, we are investigating the effect of various aeration and agitation conditions on growth and xylanase production in order to improve the latest.

2 Materials and methods

2.1 Organism

Bacillus sp. I-1018 from CNCM (Collection Nationale de Cultures de Microorganismes, Institut Pasteur, France) was used in this study. Cellular suspensions of the strain grown for 18 hours in culture medium described in the following are stored at −18 °C with adjunction of 10% (v/v) glycerol.

2.2 Culture medium

The same medium was used for conservation, inoculum growth and batch cultures. Its composition was the following (g/l): oat spelt xylan (Sigma) 3.16, casein hydrolysate 1.94, NH_4Cl 0.8, $MgSO_4 \cdot 7H2O$ 0.25, K_2HPO_4 2.3, NaH_2PO_4 0.6 and 10 ml/l of each trace element and vitamin solution; the trace element solution contained (in g/l): nitrilotriacetic acid (NTA) 12.8, $FeCl_3 \cdot 6H_2O$ 1.35, $MnCl_2 \cdot 4H_2O$ 0.1, $CoCl_2 \cdot 6H_2O$ 0.024, $CaCl_2 \cdot 2H_2O$ 0.1, $ZnCl_2$ 0.1, $CuCl_2 \cdot 2H_2O$ 0.025, H_3BO_3 0.01, $Na_2MoO_4 \cdot 2H_2O$ 0.024, NaCl 1.0, $NiCl_2 \cdot 6H_2O$ 0.12, $Na_2SeO_3 \cdot 5H_2O$ 0.026; the vitamin solution contained (in mg/l): biotin 2.0, folic acid 2.0, pyridoxine-HCl 10.0, thiamine-HCl 5.0, riboflavin 5.0, nicotinic acid 5.0, DL-calcium panthotenate 5.0, vitamin B12 0.1, p-aminobenzoic acid 5.0, lipoic acid 5.0.

The pH of the medium was adjusted to 7.3 with 1N NaOH prior to autoclaving (121 °C, 15 min).

2.3 Batch cultures

For preparation of inocula, 50 ml of culture medium placed in 250 ml conical flasks were seeded with 5 ml of a conservation suspension brought to ambient temperature. Inocula were grown for 18 h at 50 °C, 250 rev/min.

Batch cultures was carried out in a 2 l Setric fermentor (1.6 l working volume). The fermentors were equipped for measurements of agitation, temperature and dissolved

oxygen concentration (pO_2). The cultivation temperature was 50 °C; aeration and agitation rates were controlled as indicated in the text. The dissolved oxygen was measured with a membrane-covered polarographic oxygen electrode (Ingold) which was heat-sterilizable. The "zero" was realized by sparging nitrogen into the sterilized medium. The full scale was established by sparging air so that a dissolved oxygen concentration of "100%" corresponded to a medium saturated in air. The fermentors were seeded with inocula to an initial concentration of about $3 \cdot 10^6$ bacteria/ml.

2.4
Measurement of growth

Growth was monitored by cells numeration. A "Petit-Salumbéni" hematocymeter was used for direct cell counting [5]. The number of cells per grid square was counted using a microscope (40 × 10 magnification).

After numeration, the cultures were centrifuged at 14,000 rev/min for 15 min at 17 °C and the supernatant fluids were stored at −18 °C until assayed.

2.5
Enzymes assays

In this study, "xylanase" refers to the total enzymatic activity present in the culture supernatant that contributes to the release of reducing sugars from purified birchwood xylan. Xylanase was assayed as described by the method of Bailey et al. [6] using 1% birchwood xylan (Sigma, lot 113H0900) as substrate. The enzyme activities were determined at 60 °C and pH 5.8 using Britton-Robinson universal buffer. Xylanase was expressed as nkat (1 nkat is the amount of enzyme that can catalyse the release of 1 nmol of product in 1 second under the specified conditions).

2.6
Determination of *Kla*

For determination of the initial value of *Kla* the indirect method (without microorganisms) was used [7]. The principle of the method is to measure the oxygen absorption in the culture medium after degassing it with nitrogen. Then, aeration and agitation are started at the desired values and the pO_2 is recorded as a function of time. The value of *Kla* is the opposite of the slope of semilogarithmic plot of $(1 - pO_2)$ versus time according to the equation:

$$\ln\,(1 - pO_2) = -Kla \cdot t + \text{constant}$$
$$\text{where t} = \text{times in hours}\ .$$

3
Results and discussion

3.1
Determination of *Kla*

Oxygen supply to the microorganisms grown in the fermentors depends on the *Kla*. The *Kla* values were determined for four different combinations of two agitation and aeration rates (Table 1). Although the highest value of *Kla* obtained was not very great we could hardly use better conditions for air feeding, especially for aeration rates.

Table 1. Determination of *Kla* values for various aeration and agitation rates

Aeration (*V.V.M.*)	Agitation (rev/min)	*Kla* (h^{-1})
0.5	250	21.2
0.5	350	36.7
1.5	250	46.4
1.5	350	73.8

3.2
Cellular growth and oxygen consumption

Figure 1 shows the growth kinetics followed by cells counting for each condition of fermentation. Oxygen consumption was evaluated by measuring the pO_2 batchwise (Fig. 2).

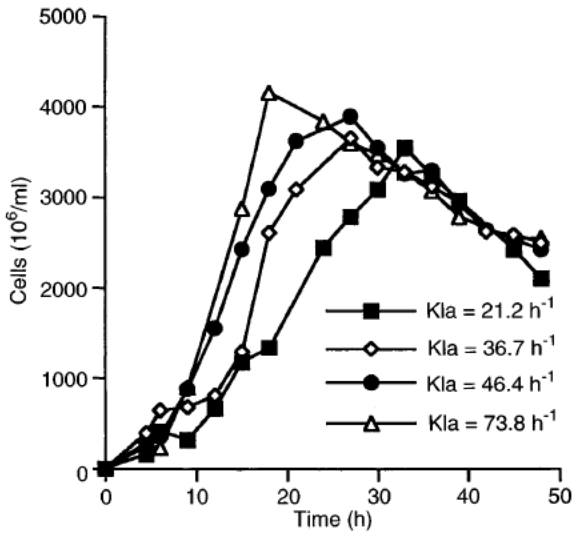

Fig. 1. Growth kinetics for various aeration conditions

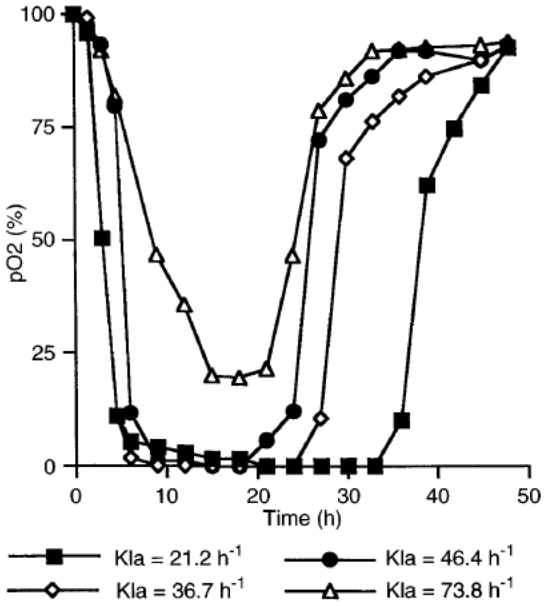

Fig. 2. Evolution of pO_2 during batch cultures

For all fermentations excepted the one with the highest value of *Kla*, pO_2 was zero during the exponential growth phase, indicating that the oxygene supply was not sufficient. In these cases, the time of duration of the limitation was longer when the value of the *Kla* was smaller. Nevertheless, the values of the maximal biomass concentration was affected only for $Kla = 21.2\ h^{-1}$.

For the 4 cultures growth rates were increased with *Kla*.

3.3 Xylanase production

The xylanase production as a function of time is shown in Fig. 3 for all cultures. The production started when the growth stopped. The values of the maximal reached enzyme activity were slightly different. Like for growth, the effect of aeration was obvious only on xylanase production rates.

The productivity P_{xyl} could be calculated for each fermentation (Table 2). It was up 1.57 times when the *Kla* was increased from 21.2 to 73.8 h^{-1}. So, the rise was not linear.

Since no oxygen limitation was detected for the highest value of *Kla*, P_{xyl} can not be improved. The agitation and aeration were optimized. Compared to *Bacillus polymyxa* [8], the oxygen supply necessary for *Bacillus sp.* I-018 was higher.

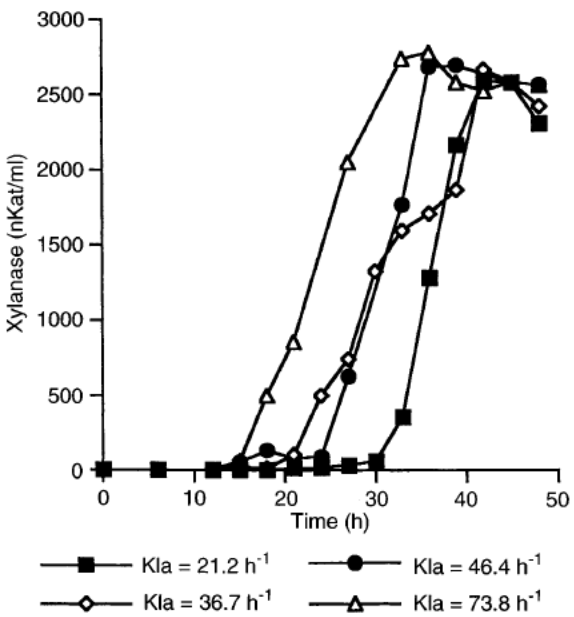

Fig. 3. Kinetics of xylanase production for various aeration conditions

Table 2. Productivity in xylanase as a function of *Kla*

Kla (h^{-1})	P_{xyl} (nkat/ml/h)
21.2	59.7
36.7	61.9
46.4	75.4
73.8	94

3.4 Conclusion

The effect of aeration was shown to be important on metabolic rates (growth and enzyme production) but not on maximal values of produced biomass xylanase. The oxygen limitation could be avoided for the highest value of *Kla* corresponding to 1.5 *V.V.M.* and 350 rev/min. More intensive aeration and agitation conditions are not necessary.

The phase of oxygen limitation ($pO_2 = 0$) always occurred during the exponential growth phase. Although the enzyme production was observed during the stationary growth phase when pO_2 was not zero, the effect on xylanase production was the same than on growth: the increase of aeration enhances the production rate but not the final concentration of enzyme. This could indicate that the xylanase synthesis starts when a substrate is exhausted [9]. We are now investigating the xylan consumption. Oat spelt xylan which is partially insoluble needs a special procedure of analysis. The production of xylanase obtained for the better conditions of aeration was very high compared to literature [10].

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