Journal of Industrial Microbiology, 6 (1990) 85–90
Elsevier

SIM 00252

Original Papers

Fermentation of *Candida utilis* for uricase production

Vladimír Adámek[a], Martina Suchová[a], Kateřina Demnerová[a], Blanka Králová[a], Ivan Fořt[b] and Petr Morava[2]

[a]*Department of Biochemistry and Microbiology, Institute of Chemical Technology, Prague, and* [b]*Department of Chemical and Food Process Equipment Design, Czech Technical University, Prague, Czechoslovakia*

(Received 21 January 1988; revised 5 October 1989; accepted 8 October 1989)

Key words: Uricase production; *Candida utilis*; Inducer of uricase production; Fermentation kinetics

SUMMARY

Conditions for the production of microbial uricase by *Candida utilis* were studied. For the selected strain, hypoxanthine proved to be the most effective inducer of uricase formation. The highest values of biomass as well as uricase activity in the mechanically agitated fermentor were obtained under the following conditions: 50 h, rotation impeller speed 7 s^{-1}, air flow rate 1.25×10^{-5} $m^3 s^{-1}$, concentration of inducer 0.1%.

INTRODUCTION

Uricase (urate: O_2 oxidoreductase, EC 1.7.3.3), an enzyme playing an important role in nitrogen metabolism, is inducible. In medicine and in clinical biochemistry, it is used as a diagnostic reagent. Commercial preparations are either of mammalian or microbial origin. A number of microorganisms have been described as uricase producers, mainly bacteria and molds. Of the yeasts, strains of *Candida* were studied most often [2,3,7].

Microbial uricase is synthesized in cells only in the presence of a suitable inducer [1]. Since the substrate of a catalyzed reaction often operates as an inducer, the natural inducer of uricase should be uric acid. However, due to its low solubility in water, uric acid is far from ideal as an inducer to be added to the culture medium. Watanabe and Ohe [8] found that in the presence of xanthine, hypoxanthine and 6,8-dihydroxypurine, *Streptomyces* sp. was able to synthesize uricase even better than in the presence of uric acid. The above-mentioned inducers are more soluble than uric acid and are also cheaper.

Correspondence: V. Adámek, Department of Biochemistry and Microbiology, Institute of Chemical Technology, Prague, Czechoslovakia

The aim of this paper was: (a) to find an inducer with suitable properties for uricase formation in *Candida utilis*; and (b) to scale up the fermentation process for uricase production by *Candida utilis* in a mechanically agitated fermentor.

MATERIALS AND METHODS

Strain. Candida utilis was obtained from the Collection of the Department of Biochemistry and Microbiology, Institute of Chemical Technology, Prague.

Medium. C. utilis was cultivated in a liquid medium with 0.5% yeast extract, 2.0% glucose, 0.1% ammonium sulphate and 0.1% inducer at 28°C.

Extraction. After cultivation, the medium was centrifuged and the cells were harvested. After storage in the deep freeze, the cells were disrupted with an X-press 3 times [4] and suspended in 0.05 M phosphate buffer, pH 8.5 (4 ml of buffer per g biomass). The disrupted cells were centrifuged, washed twice with buffer, and the supernatant fluid was measured for protein content [5] and uricase activity [6].

Uricase assay. The enzyme activity was calculated from the rate of decrease of uric acid in the presence of uricase as follows. To 2 ml of the solution containing

TABLE 1

Basic geometric parameters of laboratory and pilot plant fermentors

Type of fermentor	Quantity dimension (mm)							
	Ød	ØD	H_2	H	h	L	b	c
Laboratory	70	158	143	42	12	16	30	78
Pilot plant	120	328	348	120	24	30	44	440

10 μg uric acid in borate buffer pH 8.5, 0.8 ml of water and 0.1 ml of enzyme were added and the reaction mixture was incubated at 25 °C. After 5 min, 0.2 ml of 0.1 M KCN solution was added to stop the enzyme reaction. In the reference sample, KCN was added to the mixture before the addition of enzyme. The absorbance of both samples was measured at 293 nm. The difference between the absorbance of the sample and reference sample is proportional to the decrease of uric acid during the enzyme reaction.

Cultivation. Experiments with inducers were carried out for 66 h on a rotary shaker in 500 ml flasks with 150 ml of medium. Substances tested as possible inducers: uric acid; dithiouric acid; 8-hydroxyguanine; hypoxanthine; thioxanthine, 6,8-dihydroxypurine; 4-aminouracil; 4,5-diamino-6-hydroxypyrimidine (free base); 4(5) formamido-imidazole-5(4)-carboxyamide hydrochloride. Tested substances were added to the culture medium in two different concentrations (0.1 and 0.01%).

Fermentation. Fermentations were carried out in laboratory and pilot plant jacketed, mechanically agitated cylindrical fermentors made from stainless steel with a standard turbine impeller and baffles. The main dimensions of the fermentors are listed in Table 1.

Dry cell weight. During fermentation, the samples for determination of dry cell weight were taken at specific

TABLE 2

Conditions of fermentation of *Candida utilis* in laboratory fermentor

Parameters	Fermentation conditions					
	1	2	3	4	5	6
Air flow rates $\dot{V}_g \times 10^{-5}$ ($m^3 s^{-1}$)	5.0	2.5	1.25	1.25	1.25	1.25
Rotational speed of impeller N (s^{-1})	10.0	10.0	10.0	5.0	7.7	10.0

Volume of batch, 0.028 m^3; temperature, 28 °C; pH, 5.0; time of cultivation, 72 h.

TABLE 3

Conditions of fermentation of *Candida utilis* in the pilot plant fermentor

	Fermentation conditions	
	1	2
Temperature T (°C)	28	28
Air flow rates $\dot{V}_g$ ($m^3 s^{-1}$)	5.4×10^{-5}	5.4×10^{-5}
pH	5.0	5.0
Rotational speed of impeller N (s^{-1})	5.83	7.00
Time of cultivation T (h)	70.0	70.0

Volume of the batch $V = 0.0325$ m^3.

intervals. Dry cell weight was estimated gravimetrically.

Scaling up. Scaling up experiments were done to find suitable conditions for uricase production by *Candida utilis*. Tables 2 and 3 summarize the independent variables examined in both types of fermentors. The fermentors were equipped for automatic monitoring and control of batch temperature, rotational speed of the impeller, pH and air flow rate. Scaling up at the turbulent regime of flow of the agitated batch can be carried out according to the following criteria:

(1) geometric similarity of agitated fermentors (d/D, H/D, H_2/d), type of impeller, type and size of intervals (e.g. baffles b/D).

(2) constant tip speed of impeller:

$$Nxd = \text{const.} \tag{1}$$

(3) constant gas (air) superficial velocity:

$$V_g/D^2 = \text{const.} \tag{2}$$

(4) impeller Reynolds number:

$$Re_m = Nd^2\,\varrho/\eta \geq 1 \times 10^4 \tag{3}$$

(5) the impeller sufficiently distributes gas (air) in an agitated batch when:

$$(Fr_M/Kp_g)/(d/D)^{3.40} \geq 0.03 \times 10^{-2} \tag{4}$$

i.e. impeller is not flooded, Eqn. 4 contains the impeller Froude number:

$$Fr_M = N^2 d/g \tag{5a}$$

and the impeller flow rate number

$$Kp_g = V_g/Nd^3 \tag{5b}$$

Because the density of the cells practically equalled the density of the liquid phase (water) in the batch, the quantity was considered to be the density of water at temperature of fermentation *T*. Viscosity of the batch <*eta*< (suspension of microbial culture) was calculated according to the Einstein formula

$$\eta = \eta_1 (1 + 2.5\,\Phi_r) \tag{6}$$

where η_1 is the viscosity of the pure liquid and Φ_r is the volumetric fraction of dry matter of cells in a microbial suspension.

RESULTS AND DISCUSSION

Induction of uricase activity

Candida utilis was grown in the culture medium with different substances as potential inducers of uricase. Among the tested substances, only those summarized in

TABLE 4

Effect of analogs and precursors of uric acid on the formation of uricase activity

Inducer	Conc. of inducer (%)	Uricase activity	
		volumetric (nkat/ml)	specific (nkat/mg protein)
Uric acid	0.1	1.53	0.20
	0.01	0.51	0.06
Hypoxanthine	0.1	1.93	0.27
	0.01	0	0
4(5)-Formamido-imidazole-5(4)-carboxamide HCl	0.1	0.42	0.04
	0.01	0	0
8-Hydroxyguanine	0.1	0.42	0.04
	0.01	0	0
6,8-Dihydroxypurine	0.1	0.72	0.06
	0.01	0	0
4,5-diamino-6-hydroxypyrimidine	0.1	0.51	0.05
	0.01	0	0

TABLE 5

Uricase activity formed by *Candida utilis* in laboratory fermentor under different air flow rates

V_g (s^{-1})	Uricase activity after 50 h of fermentation		
	nkat/mg protein	nkat/kg medium	nkat/g dry matter
5.0×10^{-3}	0.04	7.9	2.2
2.5×10^{-3}	0.04	7.8	2.1
1.25×10^{-3}	0.06	13.5	3.9

Rotational speed of impeller $N = 10.0\ s^{-1}$.

TABLE 6

Uricase activity formed by *Candida utilis* in laboratory fermentor under different rotational speed of the impeller

N (s^{-1})	Uricase activity after 50 h of fermentation		
	nkat/mg protein	nkat/kg medium	nkat/g dry matter
5.0	0.24	36.20	11.44
7.67	0.25	35.82	11.41
10.0	0.06	13.50	3.96

Air flow rate $\dot{V}_g = 1.25 \times 10^{-3}\ m^3 s^{-1}$.

Table 4 showed a positive effect on uricase formation. No thioderivatives of purine had an induction effect. In addition to uric acid (Table 4), five substances gave positive results as inducers. Hypoxanthine proved to be the most effective inducer at a concentration of 0.1%, even better than uric acid. During cultivation, hypoxanthine is probably gradually transformed into uric acid by the cells. As hypoxanthine is more soluble in water than is uric acid, the actual concentration of intracellular uric acid yielded by hypoxanthine is probably higher than from uric acid transported into the cells from the medium. When a lower concentration of hypoxanthine was used, the intracellular concentration of uric acid was probably not high enough to induce uricase. The same holds for the other substances tested except for uric acid which need not be transformed, only transported.

In subsequent studies with other strains of *Candida utilis*, we found that each strain responds differently to the presence of inducers. This is probably a function of the different ability of these different microorganisms to convert these analogs to uric acid. Hypoxanthine as an inducer was employed in subsequent experiments because it was best for the strain of *C. utilis* chosen for scale up.

Scale up of the fermentation

Several fermentations of *Candida utilis* were carried out in laboratory and pilot plant fermentors under the different conditions shown in Tables 2 and 3.

Laboratory fermentor. From the results summarized in Tables 5 and 6 the best combination of air flow rate ($1.25 \times 10^{-5}\ m^3 s^{-1}$) and rotational speed of the impeller ($5.0\ s^{-1}$) was chosen for further work.

Pilot plant fermentor. From the results of experiments in the laboratory fermentor, two rotational impeller speeds and a constant value of air flow rate were chosen.

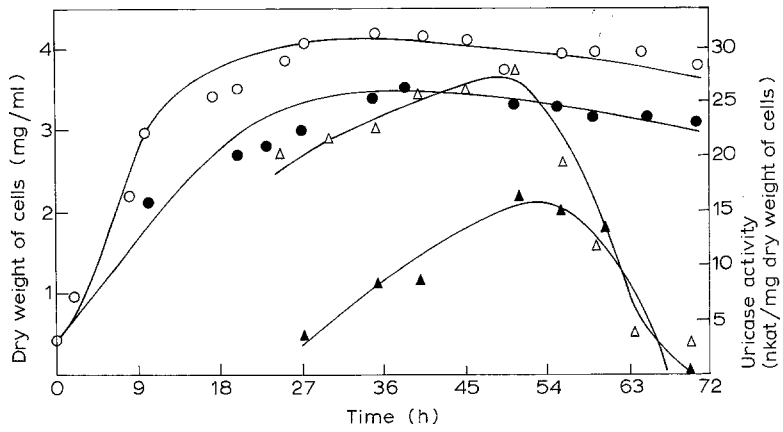

Fig. 1. Course of biomass production and uricase formation in the pilot plant fermentor. ●——● ▲——▲ $N = 5.83\ s^{-1}$; ○——○ △——△, $N = 7.0\ s^{-1}$; ●——● ○——○, biomass production; ▲——▲ △——△, uricase activity.

TABLE 7

Values of dimensionless criteria of mixing for the pilot plant fermentor

N (s^{-1})	$V_g \times 10^{-5}$ ($m^3 s^{-1}$)	$\upsilon \times 10^{-3}$ (Pa s)	$Re_m \times 10^{-4}$	Fr_m	Kp_g
5.83	1.25	0.843	9.97	0.416	0.0063
7.00	1.25	0.844	11.90	0.599	0.0050

Note: in both cases the impeller was not flooded.

Scaling up criteria (1) and (2) were taken into consideration. The results of *Candida utilis* cultivation in the pilot plant fermentor are summarized in Fig. 1 and Table 7. Table 7 shows the dimensionless criteria of the mixing process defined for the examined process. From the results (see Fig. 1), it can be concluded that the optimum time of fermentation for uricase production in the pilot plant fermentor is 50 h and optimum rotational impeller speed is 7.0 s^{-1} when the air flow rate is 1.25×10^{-5} m^3 s^{-1}. Under these conditions, the highest values of uricase activity as well as biomass were obtained.

From the description of the mixing process (see dimensionless criteria in Table 7), it follows that the regime of flow of the agitated batch was fully turbulent (see Eqn. 3) and further, that the impeller was not flooded. Both of these factors proved to be suitable for the optimum cultivation mode. It should be pointed out that the air flow rate necessary for efficient fermentation is relatively low. This can lead to the sparing of energy for a gas pump or compressor in the technological size of the equipment.

ACKNOWLEDGEMENTS

The authors would like to express their gratitude to Dr. J. Gree and Mr J. Sustr from the Research Institute of Food Industry in Prague.

LIST OF SYMBOLS

b	width of baffle, m
c	length of baffle, m
D	diameter of cylindrical fermentor, m
d	diameter of impeller, m
d_1	diameter of impeller disc, m
Fr_m	impeller Froud number
g	gravitional acceleration, ms^{-2}
H	height of batch surface above bottom, m
H_2	height of impeller disc above bottom, m
h	height of impeller blade, m
Kp_g	flow rate number
L	length of impeller blade, m
N	rotational speed of impeller, s^{-1}
Re_m	impeller Reynolds number
T	time, h
V	volume of batch, m^3
V_g	air (gas) flow rate, $m^3 s^{-1}$
x	mass fraction of the dry matter of cells
x_0	initial value of the mass fraction of the dry matter of cells
Φ_r	volume fraction of the dry matter of cells
$<eta<_1$	viscosity of pure liquid, Pa s
η	viscosity of batch (suspension of microbial suspension), Pa s
ϱ	density of batch, $kg\ m^{-3}$

REFERENCES

1 Bongaerts, G.P.A., J. Uitzetter, R. Brouns and G.D. Vogels. 1978. Uricase of *Bacillus fastidiosus* properties and regulation of synthesis. Biochim. Biophys. Acta 527: 348–358.

2 Itaya, K., T. Yamamoto and J. Fukumoto. 1967. Studies on yeast uricase. Agric. Biol. Chem. 31: 1256–1264.

3 Itaya, K., T. Yamamoto and J. Fukumoto. 1971. Studies on urate oxidase of *Candida utilis*. Agric. Biol. Chem. 35: 813–821.

4 Králová, B., R. Lehejčková, K. Demnerová and T. Dobránský. 1986. Extraction of uricase from *Candida utilis*. Biotechnol. Lett. 8: 99–10.

5 Lowry, O.H., N.J. Roseborough, A.L. Farr and R.J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193: 265–275.

6 Mahler, J.L., 1970. A new bacterial uricase for uric acid determination. Anal. Biochem. 38: 65–68.

7 Nishimura, M., K. Yoshida, Y. Yokota, A. Matsushida and Y. Mada. 1982. Physiochemical properties and states of sulphydryl groups of uricase from *Candida utilis*. J. Biochem. 91: 41–48.