Respiratory Activity of *Aspergillus niger* W78B during Gluconic and Citric Acid Biosynthesis*

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Based on experiments carried out on synthetic media with saccharose and glucose, the activity of *Aspergillus niger* W78B strain with respect to gluconic and citric acid biosynthesis was correlated with oxygen consumption and carbon dioxide evolution by the microorganisms (gases exchange). The strain revealed a high efficiency of substrate conversion to gluconic and citric acid, giving a yield factor of product on substrate, $Y_{P/S}$, close to 1 and 0.8, respectively. The product formation indicated 5 times higher rate of gluconate than citrate synthesis. It was caused by almost 1.5 times higher values of specific oxygen uptake and 3 times lower demand of microorganism cells on oxygen in the case of gluconic acid synthesis.

Gluconic (GA) and citric (CA) acids are biologically produced using fungus of *Aspergillus* genus. GA is easier to achieve by direct oxidation of glucose, but the use of this product in food processing causes reasonable reluctances [1]. In a commercial production of citric acid, the most widely used organic acid, hardly any other microorganism besides *Aspergillus niger* can be found. However, in GA production also *Penicillium* and bacteria like *Gluconobacter*, *Acetobacter*, and even *Pseudomonas* were successfully applied [2—6]. Most of the studies dedicated to various aspects of GA production kinetics and gas transfer concerned mainly processes involving bacteria [7]. Investigations of GA production by fungi scarcely appear in the literature. Anyhow, a comparison of gas exchange and respiratory activity during different product formation by the same strain of *Aspergillus* has not been published yet.

The objective of this study was to examine the possibility of gluconate biosynthesis by a highly efficient citric acid-producing strain [8], which is used also for CA production in an industrial scale. This work concentrates on different mold's respiratory activity in production of these acids and its reflection in the acid yield and product formation rate.

EXPERIMENTAL

Aspergillus niger W78B (strain collection of Food Biotechnology Department, Wroclaw University of Economics, Wroclaw, Poland) used in an industrial production of citric acid on synthetic and natural media was the production strain used in this study. A synthetic medium was used for CA and GA biosynthesis. As preferable carbon source saccharose for CA and D-glucose for GA was used. Medium for GA production contained: 200 g D-glucose, 0.4 g $(NH_4)_2HPO_4$, 0.1 g $(NH_2)_2CO$ (urea), 0.2 g KH_2PO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, tap water to 1 dm^3, and HCl solution for adjustment of pH to 5.5. CA was produced in a solution composed of: 126 g saccharose, 2 g NH_4NO_3, 0.2 g KH_2PO_4, 0.2 g $MgSO_4 \cdot 7H_2O$, tap water to 1 dm^3, and HCl solution for adjustment of pH to 2.7. During gluconic acid biosynthesis, the initial pH value was maintained by addition of 5 mol dm^{-3} NaOH solution.

Fermentations were performed in stirred tank reactor (STR) MicroFerm Fermenter MF114 (New Brunswick Scientific Co., New Brunswick, New Jersey, USA) with 14 dm^3 capacity. Citric and gluconic acid concentrations were determined by isotachophoresis [9]. Oxygen concentration in the gas leaving the fermentor was determined using paramagnetic oxygen analyzer Servomex 1100A (Servomex International, Crowborough, Great Britain). The carbon dioxide content was measured by infrared Guardian II Carbon Dioxide Monitor (Edinburgh Sensors, Edinburgh, Scotland). Airflow rate at the reactor inlet was measured by electronic rotameter ERG 2000 (Beta Erg, Warsaw, Poland). Based on continuously measured gas composition, the derivative parameters were calculated in real time, applying research

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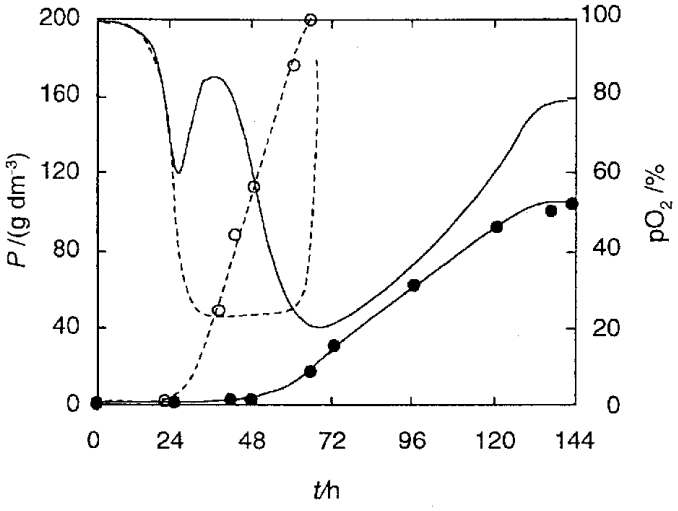

Fig. 1. Product formation (o, •) and dissolved oxygen concentration (– – –, ——) during gluconic and citric acid biosynthesis, respectively. Experimental conditions: agitation rate 600 and 700 min^{-1}, aeration rate 0.1 and 0.2 min^{-1}, temperature 303 K, pH = 5.5 and the changes from 2.7 down to 1.9, biomass growth in the range of 0.0—4.0 g dm^{-3} and 0.0—11.0 g dm^{-3} (in dry mass) during gluconic and citric acid biosynthesis, respectively.

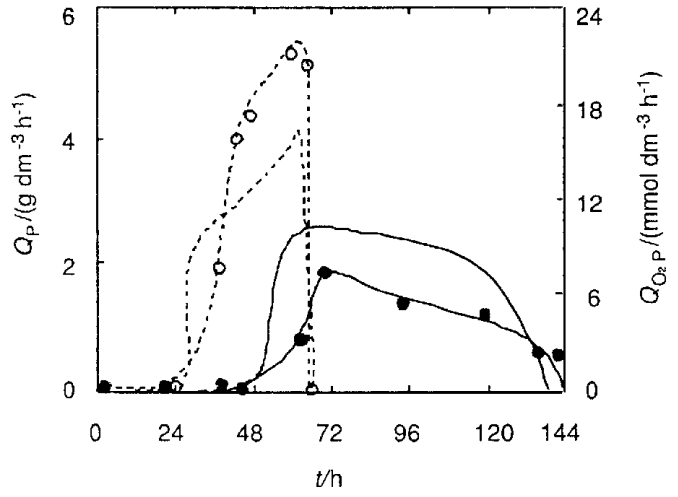

Fig. 2. Product formation rate (o, •) and oxygen uptake rate related to product formation (– – –, ——) during gluconic and citric acid biosynthesis, respectively. Experimental conditions were the same as in Fig. 1.

computer system SysLab Bio [10]. Dissolved oxygen concentration was measured by N5242 pO_2 controller with TU-4 galvanic probe (Mera Elwro, Wroclaw, Poland). Oxygen uptake (Q_{O_2}) and carbon dioxide evolution (Q_{CO_2}) rates were calculated according to *Podgorski* and *Lesniak* [11]. Corresponding specific rates were defined as the uptake (evolution) rate divided by the actual biomass concentration.

RESULTS AND DISCUSSION

The results of fermentation (Fig. 1) show that by creating suitable conditions, especially by regulation of pH at the level of 5.5, *Aspergillus niger* W78B is able to change its metabolism from citrate to gluconate formation with high homofermentative activity. The transformation efficiency of glucose into gluconic acid and saccharose to citric acid was high giving $Y_{P/S} \approx 1.00$ and 0.80, respectively. Gluconate synthesis revealed much higher production rate than the citric acid formation (Fig. 2). Fig. 2 also shows the overall oxygen uptake rates related to the product formation. The process was completed during about 70 h, in spite of high initial sugar content, 200 g dm^{-3}, in the media. During citric acid fermentation, consumption of 126 g dm^{-3} of sugar lasted two times longer (144 h). In order to identify the conditions leading to a higher GA productivity, the parameters connected with biomass respiration were taken into closer consideration. The experiments were based on the observation of the main processes occurring during fermentation, *i.e.* biomass growth and maintenance and product formation. Gluconic acid synthesis from glucose is represented by the equation

$$C_6H_{12}O_6 + 1/2O_2 \rightarrow C_6H_{12}O_7 \qquad (A)$$

Stoichiometry of citric acid formation from saccharose is described by the equation

$$1/2C_{12}H_{22}O_{11} + 3/2O_2 \rightarrow C_6H_8O_7 + 3/2H_2O \qquad (B)$$

In neither of these reactions, the product formation is accompanied by carbon dioxide evolution. The CO_2 appearance is exclusively connected with the formation of biomass precursor and with the catabolism of sucrose [12]. Taking into account these facts, the molar amount of oxygen consumed by the unit mass of *Aspergillus niger* biomass per time unit, q_{O_2}, comprises specific oxygen uptake rate related to the biomass growth and maintenance, q_{O_2X}, and specific oxygen uptake rate related to the product formation, q_{O_2P}, according to the expression

$$q_{O_2} = q_{O_2X} + q_{O_2P} \qquad (1)$$

According to [11], q_{O_2X} reflects the current biomass activity expressed as

$$q_{O_2X} = q_{CO_2}/RQ_X \qquad (2)$$

Fig. 3 shows the specific oxygen uptake rate of *Aspergillus niger* W78B during gluconate and citrate synthesis. When GA is produced, the oxygen consumption by biomass reaches 5.5 mmol g^{-1} h^{-1}, the value two times higher than the maximum oxygen uptake found during citric acid production. It is also

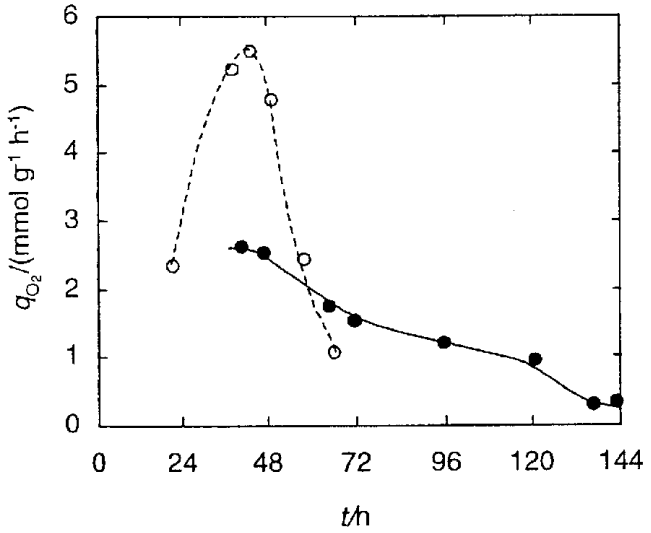

Fig. 3. Specific oxygen uptake rate during gluconic (○) and citric (●) acid biosynthesis. Experimental conditions were the same as in Fig. 1.

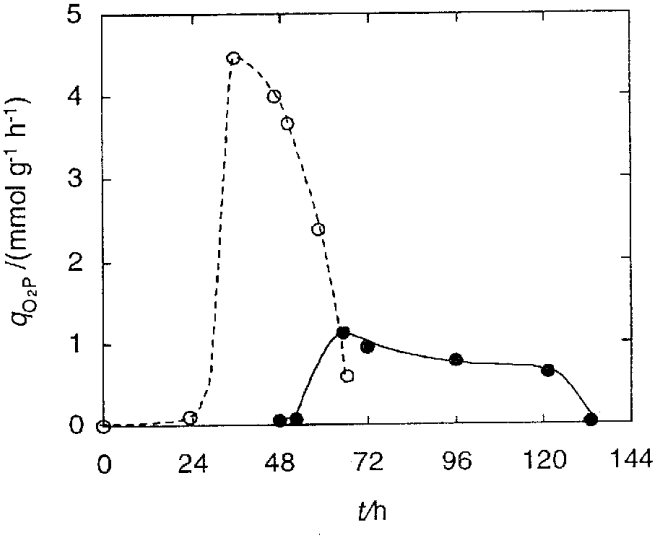

Fig. 5. Specific oxygen uptake rate related to product formation during gluconic (○) and citric (●) acid biosynthesis. Experimental conditions were the same as in Fig. 1.

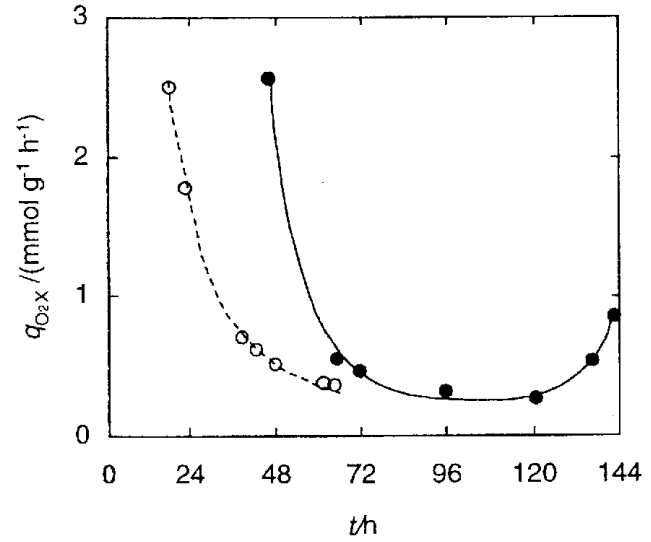

Fig. 4. Specific oxygen uptake rate related to biomass growth and maintenance during gluconic (○) and citric (●) acid biosynthesis. Experimental conditions were the same as in Fig. 1.

characteristic that the maximum of q_{O_2} is obtained in the middle of the process, *i.e.* in the intensive phase of GA formation, whilst during CA synthesis the oxygen consumption decreases constantly with the processing time.

Fig. 4 illustrates the time change of the specific oxygen uptake rate related to the biomass growth and maintenance, q_{O_2X}. Again, higher oxygen demand was observed in the case of GA synthesis, mainly as a result of increased specific maintenance requirements of oxygen, expressed by the maintenance coefficient, m_{O_2}. Probably, more energy for cells maintenance is needed in order to get high GA formation rate, in spite of the fact that the metabolism of citrate overproduction seems to be far more complex than the gluconate synthesis.

In Fig. 5 comparison of the oxygen uptake rate related to the product formation for both GA and CA production is shown. Here, differences between the oxygen uptake rates for product synthesis are clearly visible.

Based on experimental data, the average oxygen consumption rate for acid formation of 7.6 mmol dm^{-3} h^{-1} for GA and 4.7 mmol dm^{-3} h^{-1} in the case of CA synthesis was calculated. Taking into account the reaction stoichiometry (eqns (*A*) and (*B*)), about 5 (4.8) times higher theoretical (the average oxygen consumption rates were obtained experimentally) product formation rate was calculated for the case of GA biosynthesis. These preferable to GA aeration conditions allowed converting 200 g of glucose into gluconic acid during the half of time needed for that strain to convert 126 g of saccharose to citric acid. Taking into account the net time of product formation during the whole process, product formation rate (Q_P) actually agreed with theoretical statements and was about 5 times higher in favour of gluconic acid production (Q_P = 200 g GA dm^{-3}/40 h = 5 g dm^{-3} h^{-1} and Q_P = 100 g CA dm^{-3}/100 h = 1 g dm^{-3} h^{-1}).

SYMBOLS

m_{O_2}	specific maintenance requirements of oxygen	mmol g^{-1} h^{-1}
P	product concentration	g dm^{-3}
pO_2	relative concentration of dissolved oxygen	%
Q_P	product formation rate	g dm^{-3} h^{-1}
Q_{O_2P}	oxygen uptake rate related to product formation	mmol dm^{-3} h^{-1}

q_{O_2P}	specific oxygen uptake rate related to product formation	mmol g^{-1} h^{-1}
q_{O_2X}	specific oxygen uptake rate related to biomass growth and maintenance	mmol g^{-1} h^{-1}
q_{O_2}	specific oxygen uptake rate	mmol g^{-1} h^{-1}
RQ	respiratory quotient	
RQ_X	respiratory quotient related to biomass maintenance	
t	fermentation time	h
$Y_{P/S}$	yield factor of product on total sugar	

Subscripts

CA	citric acid
GA	gluconic acid
O_2	oxygen
P	product
X	biomass

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