PRODUCTION OF L-RIBULOSE BY DEHYDROGENATION OF RIBITOL WITH *GLUCONOBACTER OXYDANS*

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INTRODUCTION

The *Acetobacteraceae* are known for their incomplete oxidation of a wide range of carbohydrates and alcohols. In most cases, the products are formed in the production medium by dehydrogenases connected to the respiratory chain in the perisplasmic space (Deppenmeier *et al.* 2002). We studied these dehydrogenation reactions by using the bioconversion of ribitol as a model. Since ribitol is not a good carbon source for the growth of these bacteria, we chose to separate the growth from the production phase. The microbial conversion of ribitol to L-ribulose was first noticed by Reichstein (Reichstein 1934), and has later been reported by several other authors (Moses *et al.* 1962; Bhuiyan *et al.* 1998; Adachi *et al.* 2001; Kylmä *et al.* 2004). L-Ribulose is an interesting lead molecule for the synthesis of valuable sugars, such as L-ribose that is used in the production of (retroviral) drugs (Kylmä *et al.* 2004).

MATERIALS AND METHODS

Bacterial strains – *Gluconobacter oxydans* LMG 1673 and *Acetobacter aceti* LMG 1512 were obtained from the Laboratory of Microbiology Ghent Collection (LMG), whereas *Gluconobacter oxydans* MC14 is a mutant strain selected at our laboratory.

Culture conditions – The bacterial strains were cultured in Erlenmeyer flasks or on fermentor scale at 30°C in a liquid medium containing yeast extract, peptone, phosphate and glucose or mannitol as a carbon source. To control the acidity of the medium, in the shake flask cultures bicarbonate was added as a buffer whereas in the fermentor cultures, a 5N NaOH solution was gradually added. Under both culturing conditions, efficient aeration could be reached by agitation and/or aeration with sterile air (1 vvm).

General bioconversion method – After reaching the stationary phase, the cells were harvested by centrifugation, washed twice with physiological solution (0.85% NaCl) and resuspended in a ribitol solution in distilled water for bioconversion. The bioconversion proceeded at 30°C with thorough aeration.

Carbohydrate analysis – The carbohydrate levels in the growth medium and in the bioconversion solution were analysed with high performance liquid chromatography using, respectively, an Aminex HPX-87C and HPX-87K column from Bio-Rad Laboratories.

RESULTS AND DISCUSSION

Strain selection

Resting cells of *Acetobacter aceti* LMG 1512, *Gluconobacter oxydans* LMG 1673 and *Gluconobacter oxydans* MC14, were incubated with ribitol and evaluated as to their oxidizing capacities. From Figure 1 can be seen that *G. oxydans* MC14 shows a slightly higher activity then *G. oxydans* LMG 1673 and that both perform much better than *A. aceti* LMG 1512.

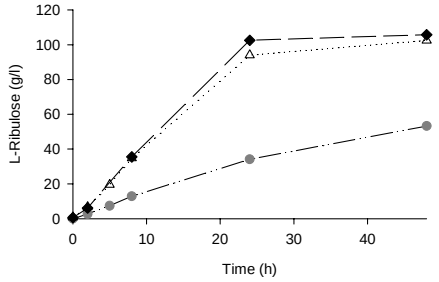

Figure 1. Bioconversion profile of *A. aceti* LMG 1512 (●), *G. oxydans* LMG 1673 (Δ) and *G. oxydans* MC14 (♦)

On the basis of these profiles, we derived important bioconversion parameters in order to be able to compare our results with literature data. As seen from Table 1, our mutant strain, *G. oxydans* MC14 has a fairly good activity. It displays an average initial productivity but the total efficiency of its bioconversion is very high. With further optimisation both characteristics could be improved. Therefore, not only the bioconversion process, but also the biomass production step, was optimised in order to obtain a high-yielding economically viable process.

Table 1. Bioconversion parameters for different strains, compared with literature data; *(Bhuiyan *et al.*, 1998); ‡(Adachi *et al.*, 2001)

	Max. conversion rate (g/l*h)	Initial specific productivity (mg/g CDW*h)	Bioconversion efficiency (%)
A. aceti LMG 1512	1.62	78	39
G. oxydans LMG 1673	4.29	211	88
G. oxydans MC14	4.48	280	91
IFO 3281 (= LMG 1512)		268*	
IFO 3172 (= LMG 1673)		104*	
IFO 3264		292*	
IFO 12528 (= LMG 1673)		1200‡	70‡

Biomass production

In order to optimise the preculture needed to inoculate the fermentation, we first optimised the biomass production step on Erlenmeyer scale. Parameters such as initial pH, carbon source (concentration), buffer, phosphate concentration and nitrogen source (concentration) were evaluated. Some of the results are shown in Figure 2 and 3. It is clear that mannitol, glycerol and sorbitol were the highest yielding carbon sources and that the bacteria grew best in a medium set at a pH value around 6.0. However, glucose also proved to be a good carbon source as long as the medium is very well buffered. The production of (keto)gluconic acids, causing a strong acidification of the culture broth, is probably the reason why glucose was an inferior carbon source in the experiment shown in Figure 2.

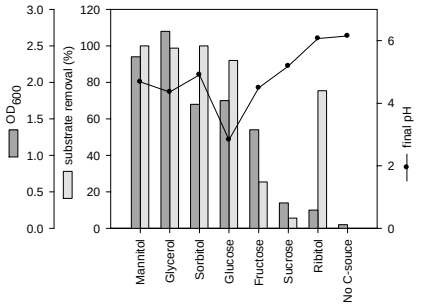

Figure 2. Effect of carbon source on the growth of *G. oxydans* MC14

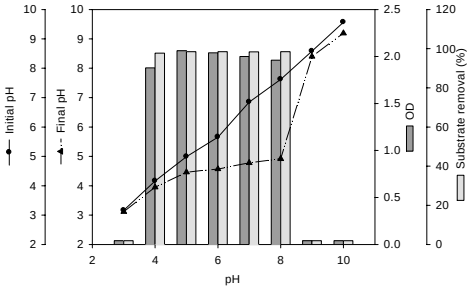

Figure 3. Effect of initial pH on the growth of *G. oxydans* MC14

On fermentor scale, the biomass yield could be doubled to an average production of 3.5 g CDW L^{-1}. This means an average production of 0.3 g CDW per hour. A typical fermentation profile is represented in Figure 4

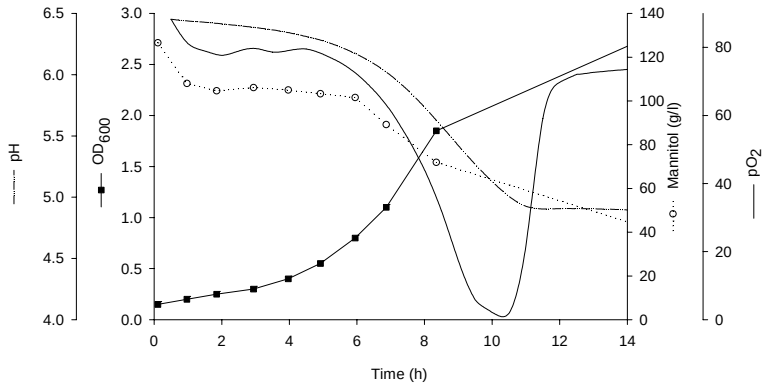

Figure 4. Fermentation profile of *G. oxydans* MC14 on mannitol medium

Bioconversion characteristics

The most important parameter for the bioconversion process proved to be the level of aeration. An excellent aeration is of crucial importance to create enough dissolved O_2 for the dehydrogenation reaction to occur. Hence, performing the bioconversion in a still Eppendorf tube gives a totally different and poor profile as compared to an Erlenmeyer shake flask reaction (Fig. 5).

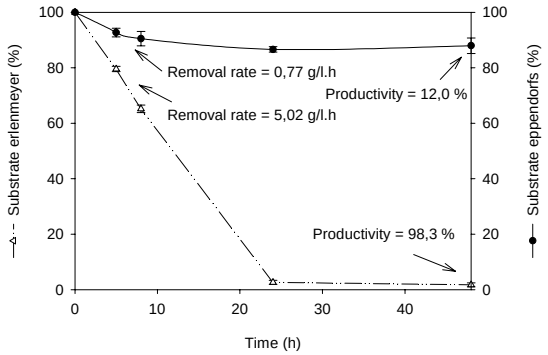

Figure 5. Bioconversion in Eppendorf (•) and in Erlenmeyer (Δ)

Furthermore, we have evaluated the effects of the initial ribitol concentration, the cell concentration, the solution acidity, the influence of the substrate for cell growth and the time of cells harvest from the growth medium. In the end, a quantitative conversion into L-ribulose could be reached with up to 300 g L^{-1} ribitol solutions.

ACKNOWLEDGEMENT

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