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Development of a New Bioprocess for Production of 1,3-propanediol I.: Modeling of Glycerol Bioconversion to 1,3-propanediol with *Klebsiella pneumoniae* Enzymes

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Abstract Glycerol is a renewable resource for it is formed as a byproduct during biodiesel production. Because of its large volume production, it seems to be a good idea to develop a technology that converts this waste into products of high value, for example, to 1,3-propanediol (1,3-PD). We suggested an enzymatic bioconversion in a membrane reactor in which the NAD coenzyme can be regenerated, and three key enzymes are retained by a 10-kDa ultrafilter membrane. Unfortunately, some byproducts also formed during successful glycerol to 1,3-PD bioconversion runs, as we used crude enzyme solution of *Klebsiella pneumoniae*. To study the possibilities to avoid this byproduct formation, we built a mathematical description of this system. The model was also used for simulation bioconversions of high glycerol concentration with and without elimination of byproduct formation and of continuous operation.

Keywords 1,3-Propanediol · *Klebsiella pneumoniae* · Enzymatic bioconversion · Modelling

Abbreviations

1,3-PD	1,3-propanediol
DHA	1,3-dihydroxyacetone
GDH	glycerol dehydrogenase enzyme
GDHt	glycerol dehydratase enzyme
PDOR	1,3-propanediol oxydoreductase
DHAK	dihydroxyacetone kinase
TPI	triose-phosphate isomerase
GAPD	glyceraldehyde-3-phosphate dehydrogenase
PGK	phosphoglycerate kinase
PGM	phosphoglycerate mutase
PPH	phosphopyruvate hydratase
PK	pyruvate kinase
PS	pyruvate synthase
PAT	phosphate acetyltransferase

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PTA phospholipid-translocating ATPase
LDH lactate-dehydrogenase
LMO lactate 2-monooxygenase
BB "black-box"
Clha chloro-3-hydroxyacetone

Introduction

1,3-propanediol(1,3-PD) has become one of the most interesting raw materials for chemical industries in the last 15 years because it can be widely used in different areas from inks to medical applications (e.g., one of its derivatives can be used to prevent rejection of a new organ after transplantation [1]). In addition, 1,3-PD was most extensively used in the polymer industry since 1995, when Shell Chemical launched a new efficient synthetic technology for its production as a monomer for the new plastic called Corterra. This new kind of plastic proved to be most advantageous for producing fibers and textiles for use in the clothing industry. Nowadays, 1,3-PD production of the world is over 100,000 t yearly. Although bioproduction of 1,3-PD would be environmentally more friendly than the chemical synthesis, Degussa started only in 2006 the first bioprocess based on recombinant *Escherichia coli* fermentation. This technology utilizes glucose as a raw material, which is first converted to glycerol by the gene products of *Saccharomyces cerevisiae* genes, and the glycerol is then converted to 1,3-PD by the gene products of the dha regulon of *Klebsiella pneumoniae*. This process needs the addition of the rather expensive coenzyme B_{12} that is absolutely necessary for the dehydration of glycerol by glycerol-dehydratase (GDHt, EC 4.2.1.30), and *E. coli* is unable to produce this cofactor. An additional disadvantage of the de novo fermentation processes is that they usually have lower product yields as a consequence of the necessary cell growth and maintenance carbon and energy requirements and the occasional formation of byproducts.

The primary aim of our research was to develop a novel biological technology utilizing a better defined enzymatic process for the 1,3-PD production. This methodology is to apply three key enzymes: glycerol-dehydratase, (GDHt, EC 4.2.1.30), 1,3-propanediol-oxydoreductase (PDOR, EC 1.1.1.202), and glycerol-dehydrogenase (GDH, EC 1.1.1.6) in a batch stirred membrane reactor, where the $NAD^+/NADH_2$ coenzyme is regenerated, and B_{12} can be retained by a proper immobilization technique. Theoretical sketch of this bioprocess is shown on Fig. 1.

In this paper, we describe our experimental results obtained with our initial bioconversion system operated with a crude enzyme mixture solution taken from sonicated *K. pneumoniae* cells and applying 4–8 g/l glycerol as the initial substrate concentration and containing added B_{12} and NAD^+. The mathematical model we constructed on the basis of the measured data with this simplified system and the simulations realized with this model helped a thorough study of several features of the system; whereas various ideas arose on how to avoid the side reactions.

Materials and Methods

K. pneumoniae was obtained from the DSMZ (no. 2026). Strain maintenance, culture media and fermentation parameters, and the method of enzyme solution preparation were published earlier [2]. For resuspending the harvested cells, HEPES buffer pH=7.4 containing 50 mM N-(2-Hydroxyethyl)-piperazine-N′-(2-ethanesulfonic acid) and 0.1 mM $MnCl_2$, and 2 mM dithiothreitol was used.

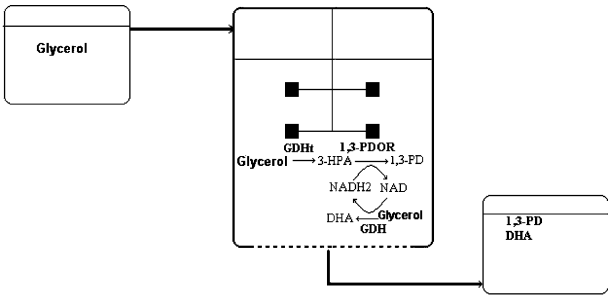


Fig. 1 The sketch of enzymatic 1,3-PD production process

Glycerol, NAD, and $MnCl_2$ were obtained from Reanal (Hungary); the other chemicals were from Sigma.

The PDOR enzyme activity was measured with the modified Lin's method [3]. The assay mixture contained 100 mM NAD^+, 100 mM 1,3-PD, and 100 mM pH=9.0 bicarbonate buffer with 30 mM $(NH_4)_2SO_4$ in 1 cm^3 final volume. The change of absorbance (caused by the formation of reduced NADH) was followed photometrically at 340 nm and 25 °C. Because of the use of crude enzyme solution containing a lot of different other compounds, we monitored the changes in the absorbance 8 min long before starting the reaction, and when the absorbance change caused by the background reactions has stopped, we gave the substrate 1,3-PD to the assay mixture and continued monitoring the absorbance changes for further 7 min.

The GDH activity was determined similarly replacing 1,3-PD with 1 M of glycerol solution as substrate. GDHt activity was measured with Toraya's MBTH method [4].

The bioconversion was carried out in a Solvent Resistant Stirred Cell (Millipore) equipped with a magnetic stirrer and a 10-kDa ultrafilter membrane for retaining the enzymes during sampling.

The composition of the samples taken from fermentation and enzyme reaction broths were analyzed with a Waters Breeze high-performance liquid chromatography (HPLC) system equipped with RI detector and autosampler with BioRad Aminex HPX-87H 300×7.8 mm column at 65 °C and 0.5 ml/min flowrate in ultrapure water containing 5 mM H_2SO_4.

As glycerol and dihydroxyacetone cannot be measured at the same time from the same sample with this chromatographic method, glycerol was determined photometrically at 37 °C and 500 nm using an enzyme reagent kit (GPO; Reanal, Hungary; http://www.reanal.hu/magyar/humagy/triglicerid-m-a.pdf).

1,3-Dihydroxyacetone (DHA) was measured with *o*-toluidine reagent kit (Sigma T-1199).

Berkeley Madonna 8.01 software was used for mathematical model simulations.

Results and Discussion

Successful enzymatic bioconversions were performed with crude enzyme solution of *K. pneumoniae*. The starting reaction mixture consisted of 4 g/l glycerol, 0.28 g/l NAD^+, and 0.25 g/l B_{12} in 20 ml of reaction volume. The enzyme concentrations were 0.015 U/ml PDOR, 1.365 U/ml GDH, and 0.085 U/ml GDHt. After 24 h, the conversion was 88%, and the reaction mixture contained 46% 1,3-PD, 10% DHA, and 15% acetic acid (AcOH). The appearance of the byproduct acetic acid means that there have to be a series of side reactions present after the phosphorylation of DHA as it can be followed in Fig 2. To create the possibility to avoid these unwanted side reactions, we have set up a mathematical description of this system.

Model Building

To formulate mathematical model equations describing the glycerol bioconversion and the further metabolic reactions according to the map in Fig. 2, we used the simple Michaelis–Menten equation (e.g., Eq. 1) of one-substrate reactions for reactions no. 1, 5, 8, 9, 11, and 12; whereas the random bi-bi mechanism of two-substrate reactions was assumed to operate in the case of the coenzyme-linked reactions no. 2–4, 6, 7, 10, and 13 (as an example, see Eq. 2).

$$v_1 = v_{1,\max} \cdot \frac{[\text{Glycerol}]}{K_{11} + [\text{Glycerol}]} \tag{1}$$

$$v_2 = v_{2,\max} \cdot \frac{\frac{\text{HPA}\cdot\text{NADH}_2}{a\cdot\text{HPA}\cdot\text{NADH}_2}}{1 + \frac{\text{HPA}}{K_{21}} + \frac{\text{NADH}_2}{K_{22}} + \frac{\text{HPA}\cdot\text{NADH}_2}{a\cdot\text{HPA}\cdot\text{NADH}_2}} \tag{2}$$

Reactions no. 1–13 were taken into account for the utilization of the nonseparated enzyme "soup" in our first mathematical model. This mathematical description consists of the rate equations of the 13 reactions in Fig. 1 and 21 mass balance expressions for the 18 various compounds and the three key enzymes (GDHt, PDOR, and GDH) of the pathway. Because samples were taken from the reaction system through an ultrafilter membrane, the enzymes were retained in the system. At the same time during the 24-h bioconversion, the total volume decreased; however, this volume change was taken into account in the mass balance equations.

Unfortunately not all necessary kinetic constants for *K. pneumoniae* enzymes can be found in literature sources; thus, we used the *Brenda Enzyme Data Bank* to determine lower and higher possible limits for the unknown parameters on the basis of other micro-

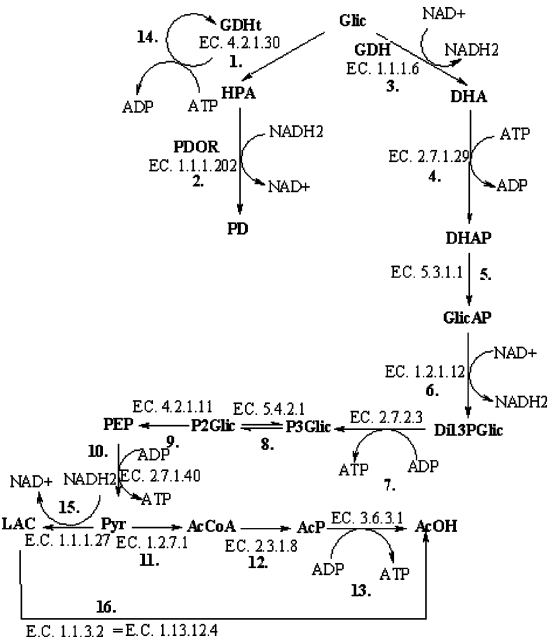


Fig. 2 Biochemical reaction pathways of *Klebsiella pneumoniae*

organisms. During the parameter estimation process, the model fitting software was allowed to move between these restrains to avoid the more than one "best-fitted" parameter combinations that usually result when one has a multiparameter model and limited number of measured data.

According to reactions no. 4, 7, 10, and 13 of Fig. 2, the byproduct acetate formation needs adenosine triphosphate/adenosine diphosphate (ATP/ADP) that is present in the enzyme "soup" at an unknown concentration. Thus, this concentration was also a parameter to be determined. The parameters of the first model describing 1,3-PD as a main product and determined by the model fitting are collected into Table 1.

With these combination of model parameters, the predicted behavior of the reaction system follows the continuous lines in Fig. 3 along with the measured points.

On the basis of the relatively good correspondence between the model curves and the measured data, we tried to predict the behavior of another bioconversion experiment where the enzyme solution came from another fermentation run. However, the mathematical model with parameters of Table 1 was not able to properly describe the behavior of those experiments in which instead of 1,3-PD and DHA formation only, AcOH arose with 83% yield and 96% conversion (PDOR, 0.003 U/ml; GDH, 0.258; GDHt, 0.025 U/ml; V=0.06 l, without an ultrafilter membrane, i.e., with enzyme effluent due sampling). To understand how the former byproduct acetate became the main product, we tried to improve our model.

The bottleneck of our first model was that when too little enzyme activity was present in the 1,3-PD path, $NADH_2$ generated from the Glycerol-DHA-AcOH pathway could not be regenerated effectively. Because, according to the model, acetic acid formation cannot reoxidize $NADH_2$ to NAD^+, which is to be reused in the acetic acid pathway, and the model cannot predict such a high acetic acid yield, concentration, and conversion as the measured value. Biebl et al. [5] described the pyruvate utilization in *Enterobactericae*. They claimed that from pyruvate, lactic acid can arise using $NADH_2$ along the anaerobic glycerol utilization pathway. Albeit we were able to detect the appearance of lactate, but its concentration was so small that it could not be measured quantitatively either in the fermentation broth or after the bioconversion. Studying the *Brenda Enzyme Database*, we found that lactate 2-monooxygenase (EC 1.13.12.4) is able to convert lactic acid to acetic acid in one step. Nevertheless, this reaction is still not enough for the necessary $NADH_2$ regeneration; moreover, to map the whole pathway was not our primary goal. We built the 17th reaction into the model (following Michaelis–Menten kinetics) as a "black-box" simplification, with which $NADH_2$ can be regenerated to NAD^+.

On the other hand, many literature sources refer to the fact that GDHt enzyme undergoes suicide inactivation with its coenzyme (coenzyme-B_{12}) after converting one molecule of glycerol (decay constant of GDHt $k_{d,1}$=0,1489 [1/h]) [6]. In the living cells, there is a reactivating protein complex, which can separate and reactivate the coenzyme and also the enzyme.

Meanwhile, we learned [7] that GDH's mechanism is known, and it follows an ordered bi-bi multisubstrate kinetics.

We completed Model 1 with these features, getting the new Model 2 and fitting it to the experimental data, which resulted in 1,3-PD as the main product (Fig. 4; Table 1, second column).

As a second step, we fitted Model 2 to those experimental data, where acetic acid was the main product formed. During this fitting process, all the $r_{i,max}$ values were newly determined because the enzyme solution came from a different fermentation run, so the enzyme content of the cells differed from the former one. Initial ATP and ADP content of the reaction mixture (originally present in the crude enzyme solution) was also newly fitted (Fig. 5; Table 1, Model 2 for AcOH).

Table 1 Summary of the kinetic constants used in the simulations.

		1. Model for description of 1,3-PD as main product formation					2. Model for description of 1,3-PD as main product formation					2. Model for description of AcOH as main product formation				
Reaction	Enzyme mechanisms	Michaelis constants Km, i, j (g/l)	Maximal reaction rate rmax, i [g/(l× h)]	Turnover number kcat, i (1/h)	Random bi-bi constants a-g	Inhibition constants kI, i	Michaelis constants Km, i, j (g/l)	Maximal reaction rate rmax, i [g/(l× h)]	Turnover number kcat, i (1/h)	Random bi-bi constants a-h	Inhibition constants kI, i	Michaelis constants Km, i, j (g/l)	Maximal reaction rate rmax, i [g/(l× h)]	Turnover number kcat, i (1/h)	Random bi-bi constants a-h	Inhibition constants kI, i
1	GDHt	0.1380 [9]	=E1× kcat1	88.4024		0.0129	0.1380 [9]	=E1× kcat1	86.8629		0.0006	0.1380 [9]	=E1× kcat1	86.8629		0.0006
2	PDOR	0.0104	=E2× kcat2	160.4070	0.7562		0.0018	=E2× kcat2	81.7837	0.5595		0.0018	=E2× kcat2	81.7837	0.5595	
	Random bi-bi	0.0200					20.0095					0.0095				
3	GDH	3.5880	=E3× kcat3	11.5163	0.1872		0.1904 [7]	=E3× kcat3	6.9526	Ordered bi-bi		0.19044 [7]	=E3× kcat3	6.9526	Ordered bi-bi	
	Random bi-bi	0.0995					0.2719 [7]					0.272 [7]				
4	DHAK	0.0004 [10]	1.7290		0.0705	0.04133 [8]	0.0004 [10]	0.0787	53.0041	0.0025	0.04133 [8]	0.0004 [10]	1.6016	53.0041	0.0025	0.04133 [8]
	Random bi-bi	0.1263 [10]					0.0166 [10]					0.016 [10]				
5	TPI	0.0440	0.0704				0.0470	0.0940				0.0470	0.1985			
6	GAPD	0.0138	0.6380		0.3165		0.0397	0.0879		0.0012		0.0397	0.8786		0.0012	
	Random bi-bi	0.7190					0.0226					0.0226				
7	PGK	0.3290	0.4175		0.0092		0.0085	0.0215		0.0006		0.0085	0.0514		0.0006	
	Random bi-bi	0.0664					0.0290					0.0290				
8	PGM	0.0344	0.0718				0.1071	0.0855				0.1071	0.1966			
9	PPH	0.1503	0.2681				0.0915	0.0853				0.0915	0.4966			
10	PK	0.0187	0.8335		1.0409		0.2333	0.4109		0.0643		0.2333	3.8659		0.0643	
	Random bi-bi	0.0130					0.1453					0.1453				
11	PS	0.0072	0.0412				0.0063	0.0510				0.0063	0.9713			
12	PAT	0.0074	0.0836				0.0071	0.0650				0.0071	0.5756			
13	PTA	0.1477	0.1771		0.0134		0.1466	0.0738		0.000002		0.1466	0.3259		0.000002	
	Random bi-bi	0.0005					0.00007					0.00007				
14	Reg (GDHt)						0.0018	0.0111				0.0018	0.4003			
15	LDH						0.0025	0.0010		0.0970		0.0025	0.0370		0.0970	
	Random bi-bi						0.0086					0.0086				
16	LMO						0.0024	0.0015				0.0024	0.0454			
17	BB						0.0024	0.0491				0.0024	0.0556			
Initial ATP concentration determined with simulation (g/l):0.01174							0.02610					0.41592				
Initial ADP concentration determined with simulation (g/l):0.00349							0.00050					0.00269				

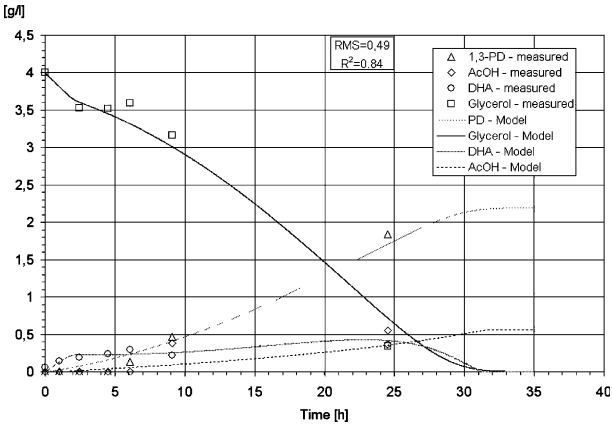


Fig. 3 First model fitted to the measured data (1,3-PD as main product)

This two-step model development and fitting procedure finally resulted in a parameter value set, equally suitable to fairly well described either 1,3-PD or AcOH formation.

Inhibition Study with Model Simulations

It seemed to be a good idea to prevent the further conversion of DHA to DHAP via phosphorylation in the reaction step no. 4 (Fig. 2) by inhibiting the activity of dihydroxyacetone-kinase enzyme (EC 2.7.1.29).

Simulation experiments were carried out with the first experimental run (where the main product was 1,3-PD), applying the model to examine the possible ways to reduce the byproduct formation with DHA structure analogons as potential competitive or irreversible inhibitors. Figure 6 shows the effects of different types of DHAK inhibition according to the model simulations.

In this inhibition study, we had to turn off the "black-box" reaction (no. 17), as it continuously reoxidized $NADH_2$ independently on DHAK inhibition, although it certainly must be placed after the DHA phosphorylation.

It is known [8] that Chloro-3-hydroxyacetone (Clha) is a competitive inhibitor for DHAK. Figure 6a illustrates the simulation results with this competitive inhibitor, Clha. Addition of this competitive inhibitor has no effect on the final concentration of the products. It is not surprising because Bowden [11] states that competitive inhibitions (in living cells) are irrelevant, and uncompetitive inhibitions play much more regulation role. Figure 6b shows a simulation with adding a fictive noncompetitive inhibitor with a K_i that is supposed to be the same as of Clha. This way, it can reach an effective inhibitor concentration, where the products are only 1,3-PD and DHA with yields ca. 47 and 53%. However, theoretically, it would be more advantageous to use an irreversible inhibitor (Fig. 6c), which would completely eliminate the unwanted enzyme (DHAK) and its reaction. This way, it was simulated with a hypothetical first order decay of the DHAK (we supposed a very high $k_{d,4}$= 0.0405 [1/h]). Nevertheless, using such an inhibitor can result only 48% 1,3-PD and 48% DHA beside of 4% AcOH if the inhibitor was applied in 0.0002 g/l of concentration. Higher concentration of irreversible inhibitor is disadvantageous because if the total DHAK activity is eliminated, ATP/ADP could not be regenerated; although ATP is needed by the GDHt regeneration (Fig. 2, reaction no. 14). Without ATP regeneration, the GDHt regeneration will also stop and, certainly, the product formation, too. In this case, just a slight product formation

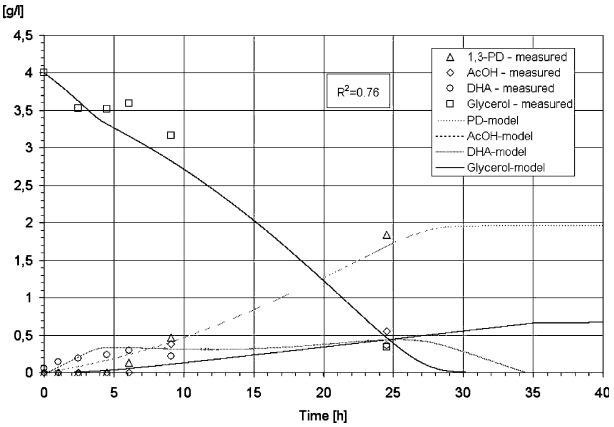


Fig. 4 Second model fitted to the measured data (1,3-PD as main product)

occurs because until the initial ATP diminishes from the system, the GDHt will continuously be regenerated, and when ATP is totally exhausted, GDHt slowly runs out.

As a conclusion of this inhibition studies, we can state that without inhibition, 1,3-PD and AcOH as main products will be formed; however, adding a noncompetitive inhibitor, the main products will be DHA and 1,3-PD beside a little AcOH. Moreover, applying irreversible inhibitor in lower concentrations than DHAK, the situation is the same as in the noncompetitive case; whereas higher irreversible inhibitor concentration is disadvantageous. Acetic acid formation can only be eliminated with applying noncompetitive inhibitor; however, in that case, a very high concentration of inhibitor is needed, and more DHA than 1,3-PD will be formed (1,3-PD is more valuable).

Simulation of the Effect of Enzymes and Glycerol Concentration

To enhance bioreactor performance, the simplest way is to increase the concentrations of the bioconverting enzymes and the initial substrates. To convert higher amounts of glycerol to 1,3-PD, we must have higher enzyme activity in the reactor. To simulate this experiment, we used a concentration factor for the enzymes, which can be practically achieved, e.g., by concentrating the enzyme solution with ultrafiltration. It can be seen on Fig. 7a that a 20-fold concentrated enzyme solution converts 100 g/l of glycerol into 45 g/l 1,3-PD and 19 g/l AcOH

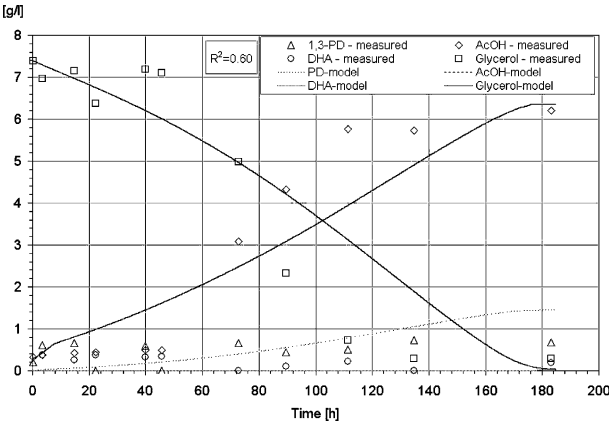
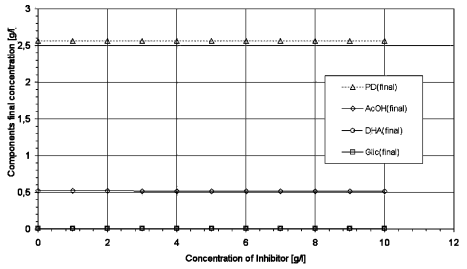
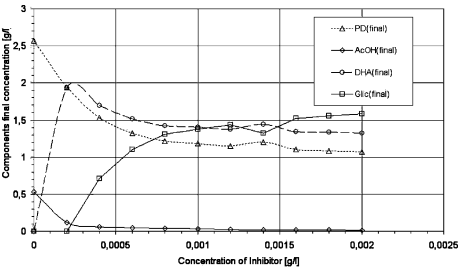


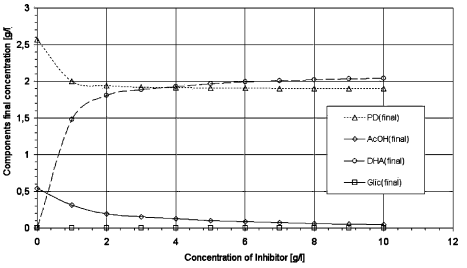
Fig. 5 Second model describing formation of AcOH as main product



a competitive inhibition

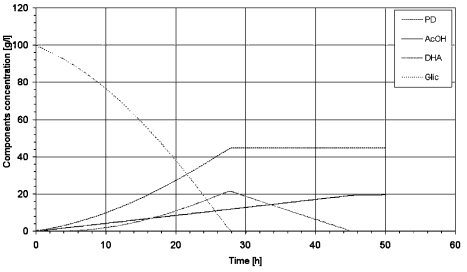


c irreversible inhibition

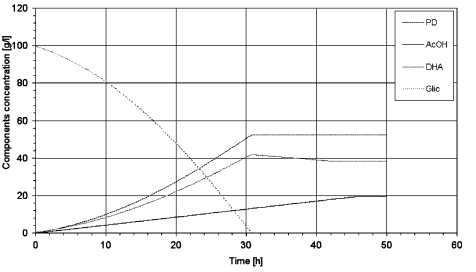


b noncompetitive inhibition

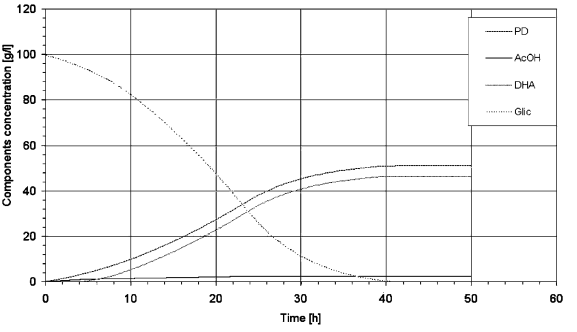
Fig. 6 Different inhibitions on the second model describing 1,3-PD as main product



a



b +1g/l non-competitive inhibitor



c +0.0031 g/l irreversible inhibitor

Fig. 7 Simulation of higher enzyme activity and initial glycerol concentration

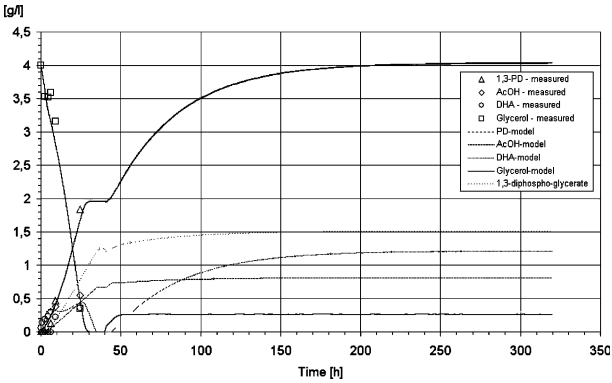


Fig. 8 Continuous operation with batch startup

without an inhibitor (the accumulated intermediers run out very slowly [c.a. 560 h] and finally form 56 g/l AcOH). Applying 1 g/l of noncompetitive inhibitor (Fig. 7b) reaches a conversion to 53 g/l 1,3-PD and 38 g/l DHA beside of 5 g/l AcOH in batch operation quite rapidly. Using 0.0031 g/l irreversible inhibitor results in 51 g/l 1,3-PD and 46.4 g/l DHA beside of 2.5 g/l AcOH (Fig. 7c).

Modelling the Mode of Operations

The mathematical description is also suitable to simulate the system working in continuous operational mode. We supposed that the coenzymes are retained by the reactor membrane, as they can really be immobilized experimentally [12–14]. In Fig. 8, an example without inhibition is shown where two different parts of the bioconversion process has to be distinguished: (1) batch start and (2) continuous operation at constant feed rate. A steady state occurs where 8 g/l glycerol with F=0.0001 l/h feed rate results in a reaction mixture containing 0.81 g/l AcOH, 1.21 g/l DHA, 4 g/l 1,3-PD, and 0.25 g/l unconverted glycerol. According to the simulation, 1,3-diphospho-glycerate equalizes the mass balance, its steady state concentration is 1.56 g/l.

As it was suggested regarding the inhibition study, applying an irreversible inhibitor in a concentration lower than DHAK, AcOH always remains as a byproduct (0.17 g/l). However, if the inhibitor concentration is higher than the DHAK concentration, acetic acid formation stops, but at the same time, the ATP regenerating steps will be eliminated, which consequently breaks the GDHt regeneration and product formation, too.

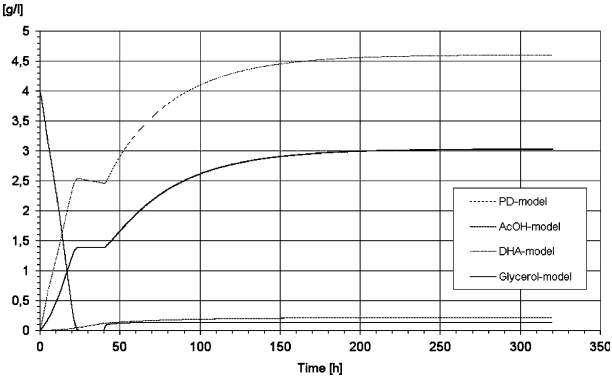


Fig. 9 Continuous operation with batch startup using a 3-g/l noncompetitive inhibitor

Noncompetitive inhibition (3 g/l inhibitor concentration) leads to 4.6 g/l DHA and 3 g/l 1,3-PD, and 0.13 g/l remaining glycerol (Fig. 9). Acetic acid is quite well eliminated (0.21 g/l).

Our simulations showed that the original goal of our work—namely, simultaneous DHA and 1,3-PD production on glycerol substrate—cannot be realized with raw enzyme mixture of *K. pneumoniae* because either AcOH is formed as a byproduct, or if its production is under various inhibition, then the ATP-producing pathway is also eliminated. Thus, the ATP-dependent GDHt regeneration cannot reactivate the suicide inactivated B_{12}-GDHt-complex.

The only possibility to overcome this problem is to use the enzymes of such a microorganism in which GDHt does not need ATP-dependent regeneration. The only bacterium with such a GDHt is the *Clostridium butyricum* VPI1718 [15] because its GDHt use *S*-adenosyl-methionine as coenzyme. This enzyme and coenzyme do not form any inactive complex, so there is no need for regeneration. We suggest that using these *Clostridium* enzymes can solve byproduct formation problem, for DHAK activity can be eliminated anyhow (for example, removing ATP-dependent enzymes or ATP or applying inhibitors).

Conclusion

It would be very advantageous to develop a coenzyme-regenerating enzymatic bioconversion in a membrane reactor for the production of 1,3-PD. With the crude key enzymes (more correctly that enzyme "soup") of *K. pneumoniae*, we made several successful glycerol transformation to 1,3-PD and set up a complex mathematical model to describe the whole process. These simulations clearly showed that it is not possible to produce DHA and 1,3-PD as sole main products with this mixture of enzymes because AcOH is always produced as an unfavored byproduct. The simulations also showed that this byproduct formation cannot be eliminated any way because it serves not only the unwanted byproduct but also the ATP, which is essential for the regeneration of the first key enzyme (GDHt). On the basis of our in silico experiments, it is of high probability that it is impossible to carry out this glycerol biotransformation to 1,3-PD and DHA simultaneously with this crude unpurified enzyme source from *K. pneumoniae*. The existence of another possible enzyme source, i.e., *Clostridium butyricum*VPI1718 may solve the problems hitherto discussed as its GDHt enzyme does not need ATP-dependent regeneration. The successful glycerol biotransformations to 1,3-PD with such an enzyme mixture will be presented in our next report.

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