Application of Chemical Engineering Methodology in Process Development: A Case Study of MenD-catalyzed Synthesis of 6-Cyano-4-oxohexanoic Acid

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To speed up evaluation, development and upscaling of new processes, the use of engineering methodology can have a great impact. Here we show the application of an engineering approach to find the reaction conditions allowing the best process metrics. An experimentally validated mathematical model for the MenD-catalyzed synthesis of a commercially unavailable product, 6-cyano-4-oxohexanoic acid, with a potential industrial use as a building block, was used for process optimization. Using the optimized conditions, 62.4 g dm^{-3} of product, volume productivity of 87.1 g dm^{-3} d^{-1}, product yield of 96 %, and biocatalyst productivity of 25.8 kg_P kg^{-1}_{MenD} can be achieved. Based on the optimized production procedure, economic analysis was performed to determine minimal product price required for project to be profitable in 8 years economic lifetime. In addition, Monte Carlo analysis (MCA) was used to assess the influence of uncertainties in estimation of input variables on overall economic performance.

Keywords:

mathematical modeling, process economics, Monte Carlo analysis, C-C coupling, enzyme catalysis, process engineering

Introduction

Biocatalysis holds great potential for the development of safe, green, and selective processes[1,2]. Thus, its industrial application for the production of pharma and fine chemicals has increased significantly over the years[3] and there are several hundred biocatalytic industrial processes[4,5]. Easier access to enzymes and the ability to engineer the enzymes to meet the demands of industrial processes has certainly helped developments in that direction[6]. It is nowadays possible to carry out reactions catalyzed by enzymes with unnatural substrates, and by doing so, to gain access to new molecules that cannot be found in nature. One of such enzymes is MenD[7] as it provides biocatalytic access to new types of products not related to the products currently accessible by thiamine diphosphate (ThDP) dependent enzyme catalysis[8,9]. For further growth and development of biocatalysis, biochemical-engineering approaches can be very beneficial. They include the use of modeling techniques[10,11], process optimization tools[12–14], as well as the use of early-stage economic assessment tool[1,15]. A model-based methodology can be used to define the process design search-space within which the optimal solution can be found; it can save development time and money as simulations increase the process understanding[11], and it can have a favorable impact on product quality[16]. Pharmaceutical industry invests more money in product development and evaluation that fail than in successful products, which illustrates the importance of reducing cost and time for the development of industrial processes[17]. Consequently, any methodology or tool that can be used to evaluate process alternatives and speed up development can create a tremendous benefit. Whereas this approach is well established in the chemical and oil industry, its better exploitation is still expected in pharmaceutical industry[2]. Reaction engineering has long been used as an efficient and effective methodology to design and size the appropriate reactors for the synthesis of valuable industrial chemicals by chemical reactions[2]. The same can be done for biocatalytic reactions, but this approach is still underdeveloped in industrial settings.

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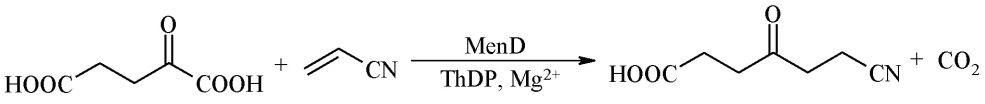

Fig. 1 – *Reaction scheme*

Even though comprehensive data are not always available, cost estimates should be made also at early stages of project development[18]. Simulation tools and a process model can help to analyze and assess suitable operating points in such complex process concepts at an early stage and reduce the experimental effort during the process[19].

In this paper, a detailed analysis and optimization of the MenD-catalyzed reaction of acrylonitrile (ACN) and α-ketoglutaric acid (KGA) to synthesize 6-cyano-4-oxohexanoic acid (COHA) (Fig. 1) was conducted to achieve optimum process metrics, such as volume productivity, product concentration, and yield. The obtained data were used for the preliminary economic evaluation of the process. This MenD-catalyzed Stetter reaction with an unnatural acceptor substrate, i.e. ACN (Fig. 1), was first introduced in 2014[8]. The relevance of this reaction is in the potential application of COHA as a building block; the molecule contains a nitrile group, which can be used to synthesize other functional groups in a straightforward manner[20]. This paper is envisaged as a case study and demonstration of the application of an engineering approach in the early stage of process development.

Methodology

Materials and apparatus

Acrylonitrile (ACN), disodium α-ketoglutarate hydrate (KGA), *p*-methoxyphenol (PMP), and ThDP were purchased from Sigma Aldrich (Germany). Magnesium chloride was purchased from Acros Organics (Belgium). MenD was kindly provided by Prozomix Ltd. (United Kingdom) as a 2.274 mg mL^{-1} pure protein suspension in 3.2 M $(NH_4)_2SO_4$ solution. The reactor set-up and the main instrumentation used for the experimental studies was the same as in Ref.[25]

Process description and the mathematical model

Proposed reactor set-up is presented in Fig. 2. Based on the preliminary model simulations, the initial reaction mixture contains the reactants ACN (120 mmol dm^{-3}) and KGA (120 mmol dm^{-3}), as well as cofactors ThDP (1 mmol dm^{-3}) and Mg^{2+} (2 mmol dm^{-3}). Reactants should be present at equimolar concentrations. The reaction is started by adding the initial amount of MenD (2.4 mg cm^{-3}) to the reactor. The feeding of all reaction components starts immediately after preparation of initial solution, and consists of two separate feeds. The addition of ACN (feed 1, q_1) was envisaged at the flowrate, which enables maintaining its concentration below 250 mmol dm^{-3} during the reaction. The addition of KGA, ThDP, Mg^{2+}, and MenD is carried out through a separate feed (feed 2, q_2). Its purpose is, besides supplying the second substrate, to maintain the concentrations of ThDP and Mg^{2+} and the enzyme activity at appropriate levels, which decrease due to volume increase. Initial volume of 10 m^3 is considered in simulations. As COHA is commercially unavailable, a moderate reaction mixture volume was chosen, since it is impossible to estimate its demand on the market.

The mathematical model[25] of the process consists of the kinetic equations (Eqs. 1 and 3) and mass balances in the fed-batch reactor (Eqs. 4–11). According to experimental results, kinetic equation 1 is modified double substrate Michaelis-Menten kinetics with included noncompetitive inhibition by PMP, which is a stabilizing additive present in commercial ACN. This equation shows that the influence of ACN on the reaction rate can be described by linear dependency. Thus, it is a substrate which controls the reaction rate.

$$r = \frac{k \cdot f \cdot \gamma_{\mathrm{MenD}} \cdot c_{\mathrm{ACN}} \cdot c_{\mathrm{KGA}}}{K_m^{\mathrm{KGA}} + c_{\mathrm{KGA}}} \cdot \frac{1}{1 + \frac{c_{\mathrm{PMP}}}{K_i^{\mathrm{PMP}}}} \tag{1}$$

$$f = \frac{c_{\mathrm{ThDP}} \cdot c_{\mathrm{Mg^{2+}}}}{\left(K_m^{\mathrm{ThDP}} + c_{\mathrm{ThDP}}\right) \cdot \left(K_m^{\mathrm{Mg^{2+}}} + c_{\mathrm{Mg^{2+}}} + \frac{c_{\mathrm{Mg^{2+}}}^2}{K_i^{\mathrm{Mg^{2+}}}}\right)} \tag{2}$$

Eq. 2 depicts the influence of cofactors ThDP and Mg^{2+} on the reaction rate. These concentrations are usually maintained at constant level during the reaction, as they are a prerequisite for enzyme activity.

$$r_1 = k_1 \cdot c_{\mathrm{ACN}} \tag{3}$$

Eq. 3 presents the rate of ACN polymerization, where $k_1 = 0.088 \cdot e^{0.0036 \cdot c_{\mathrm{ACN}}}$. Kinetic parameters used for all simulations are presented in Table 1.

Mass balances[25] for KGA, ACN, COHA, PMP, MenD, ThDP, and Mg^{2+} in the fed batch reactor are presented by Eqs. 4–10. Eq. 11 presents the change

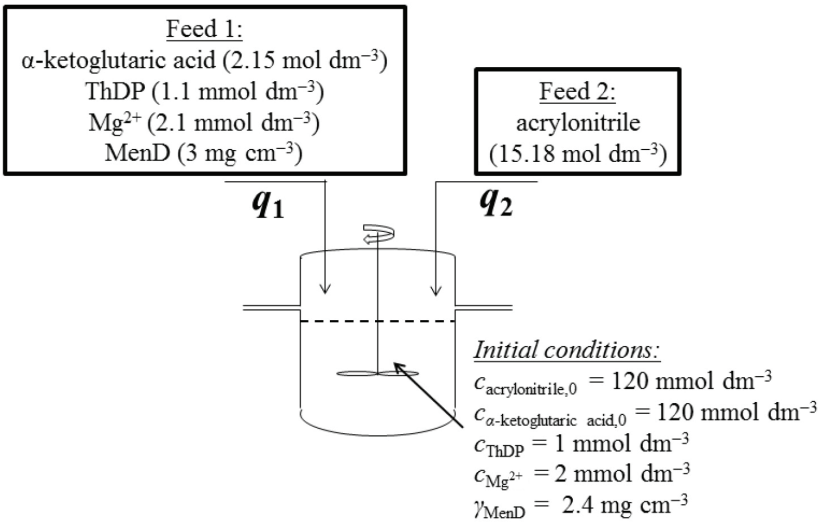

Fig. 2 – *Fed-batch reactor set-up scheme*

in reaction volume designated by the flow-rates of ACN (q_1) and KGA (q_2). As approximately constant concentrations of MenD, ThDP, and Mg^{2+} should ideally be maintained during the reaction, they are added together with KGA to the reactor.

$$\frac{dc_{KGA}}{dt} = \frac{1}{V}\cdot\left(-c_{KGA}\cdot\frac{dV}{dt} + c_{KGA,0}\cdot q_2\right) - r \quad (4)$$

$$\frac{dc_{ACN}}{dt} = \frac{1}{V}\cdot\left(-c_{ACN}\cdot\frac{dV}{dt} + c_{ACN,0}\cdot q_1\right) - r - r_1 \quad (5)$$

$$\frac{dc_{COHA}}{dt} = \frac{1}{V}\cdot\left(-c_{COHA}\cdot\frac{dV}{dt}\right) + r \quad (6)$$

$$\frac{dc_{PMP}}{dt} = \frac{1}{V}\cdot\left(-c_{PMP}\cdot\frac{dV}{dt} + c_{PMP,0}\cdot q_1\right) \quad (7)$$

$$\frac{d\gamma_{MenD}}{dt} = \frac{1}{V}\cdot\left(-\gamma_{MenD}\cdot\frac{dV}{dt} + \gamma_{MenD,0}\cdot q_2\right) \quad (8)$$

$$\frac{dc_{ThDP}}{dt} = \frac{1}{V}\cdot\left(-c_{ThDP}\cdot\frac{dV}{dt} + c_{ThDP,0}\cdot q_2\right) \quad (9)$$

$$\frac{dc_{Mg^{2+}}}{dt} = \frac{1}{V}\cdot\left(-c_{Mg^{2+}}\cdot\frac{dV}{dt} + c_{Mg^{2+},0}\cdot q_2\right) \quad (10)$$

$$\frac{dV}{dt} = q_1 + q_2 \quad (11)$$

Enzyme operational stability decay was described by the kinetics of the first order (Eq. 12), whereas the dependence of operational stability decay rate constant is directly proportional to the concentration of ACN (Eq. 13)[25]. This equation is valid up to 400 mmol dm^{-3} of ACN, which is higher than ACN concentrations in the simulations to be shown in this work.

$$\frac{d\gamma_{MenD}}{dt} = -k_d\cdot\gamma_{MenD} \quad (12)$$

$$k_d = 0.0045\cdot c_{ACN} \quad (13)$$

The influence of feed flow-rates on the reaction outcome was evaluated. Feed 1 (q_1) contained 15.15 mol dm^{-3} solution of ACN, whereas feed 2 (q_2) contained a mixture of KGA, ThDP, Mg^{2+}, and MenD at concentrations of 2.5 mol dm^{-3}, 1.1 mmol dm^{-3}, 2.1 mmol dm^{-3}, and 3 mg cm^{-3}, respectively. The simulation time in the first set of simulations was fixed at 12 hours. Process metrics, i.e. volume productivity (Q_p), product concentration and yield (Y), as well as biocatalyst productivity (BP) were calculated in these simulations. In the second set of simulations, seven flow-rates q_1 and q_2 with the best process metrics outcome were selected, and simulations were done in more detail. Reactions were run as fed-batch for 12 hours, after which feeding was stopped and the reaction was simulated as batch until one of the substrates was completely consumed for easier purification. New process metrics were calculated for each simulation.

Table 1 – *Estimated kinetic parameters in the MenD-catalyzed reaction of KGA with ACN*[25]

Parameter	Unit	Value
k	mL mg^{-1} min^{-1}	0.0097 ± 0.00009
K_m^{KGA}	μmol dm^{-3}	46.09 ± 4.32
K_m^{ThDP}	μmol dm^{-3}	9.94 ± 0.81
$K_m^{Mg^{2+}}$	μmol dm^{-3}	15.05 ± 4.97
$K_i^{Mg^{2+}}$	mmol dm^{-3}	6.44 ± 2.59
K_i^{PMP}	mol dm^{-3}	1.29 ± 0.22

Volume productivity, Q_p, was defined as the concentration of product divided by the reaction time. Product yield, *Y*, was defined as mols of product divided by the mols of ACN added to the reactor. Biocatalyst productivity, *BP*, was defined as kg of product divided by kg of enzyme added to the reactor.

Product isolation was carried out by lowering the pH of the reaction solution with 5 mol dm^{-3} HCl solution, and then the product was extracted by ethyl acetate. Organic phase was evaporated under reduced pressure to obtain COHA.

Assumptions for the economic evaluation of the process

The best result of the simulations was used as a starting point for early stage economic process analysis. Capital costs for the installation of the reactor were calculated based on the cost of 14 m^3 glass-lined carbon steel, agitated and jacketed reactor[21], multiplied by Lang factor 5.7, typical for fluid processing units[22]. The reactor volume was chosen on the basis of the final volume of the reaction mixture, which was around 12 m^3, taking into account that reactor can never be 100 % full. Annualized equipment costs were determined by multiplying total capital investment with annuity factor (*k*) calculated using Eq. 14.

$$k = \frac{i}{1-(1+i)^{-t}} \tag{14}$$

Operating costs were calculated based on raw material, waste management and utilities prices. Raw materials costs (RMC) were estimated based on market quotations of laboratory chemicals providers, divided by factor 10. Ten batches per year were assumed. It was also assumed that the quantity of liquid waste requiring treatment is equal to added volumes of distilled water, ethyl acetate, and HCl. The cost of waste treatment[3] was estimated to be 2.00 € m^{-3}. Energy cost of the process was assumed to be equal to cost of evaporation of ethyl acetate, based on 40 € t^{-1} cost of medium pressure steam[23], as reaction is conducted at 30 °C. Total operating expenses were estimated by adding 15 % for fixed costs and 10 % for maintenance to the annualized equipment costs. In this early stage of economic analysis, labor costs, cost of agitation and solvent regeneration were neglected, although these can have significant impact on economic feasibility.

To assess the influence of uncertainties in product cost estimation, Monte Carlo analysis (MCA) was performed to calculate break-even product price yielding zero net present value (*NPV*) of the project after 8 years using discounted cash flow analysis. Economic lifetime of 8 years is typical for chemical processes[3]. *NPV* was calculated[24] using Eq. 15, where C_T represents total capital investment, Φ is the product price, *m* is the production capacity per year, *t* is the economic lifetime of the project, T_N is tax percentage, O_N is the yearly operating cost, and *r* is the required internal rate of return.

$$NPV = C_T + \sum_{n=1}^{t} \frac{\phi \cdot m \cdot (1-T_N) - O_N}{(1+r)^n} \tag{15}$$

Results and discussion

Kinetic modeling and its application to process optimization

The previously developed and validated mathematical model (Eqs. 1–13) for the enzymatic synthesis of COHA (Fig. 1) was used for reaction optimization[25]. During the kinetic investigation of MenD-catalyzed 1,4-addition of KGA to ACN[25], it was found that ACN controls the reaction rate, i.e. specific activity (*S.A.*) of the enzyme (Fig. 3), as well as its operational stability, and the yield of the product. The latter is due to ACN spontaneous polymerization under the reaction conditions. With the aid of the kinetic model combined with mass balance equations in reactors, and substantiated by the experimental data[25], it was found that the fed-batch reactor is the best choice for this reaction. By using this reactor, an inactivating effect of ACN on the enzyme can be minimized and polymerization rate can be kept at low level by simply controlling ACN concentration in the reactor. As its concentration also controls the reaction rate, the initial concentration was set to 120 mmol dm^{-3} in all simulations as a compromise between these factors. The ultimate optimization goals were the values of the process metrics presented in Table 2, which show the level required for the potential industrial exploitation. The focus was put on the evaluation of the effect of different process variables on volume productivity, yield of the product, and its final concentration. Biocatalyst productivity was also calculated for the optimal operational points. Thus, the objective of this optimization was to find the process conditions at which a compromise between the goal functions can be achieved.

Model simulations were done to evaluate the effect of feed flow-rates on volume productivity, product yield, and product concentration, the results of which are presented in Figs. 4a, b, and c, respectively. The results show which flow-rates of both feeds can be combined to achieve the best process metrics. This is very important since accumulation of ACN in the reactor is unwanted. Initially, a high range of flow-rates was evaluated for both feeds. It was found that flow-rates of ACN higher than 50

cm^3 min^{-1} cause significant accumulation of ACN, thus, the upper limit of the investigated area was lowered to this value. In this area, the highest product yield limit that can be obtained is above 95 % (Fig. 4a1), which is important for simplification of product isolation. Volume productivity and product concentration lower than maximum values, i.e. Q_P of 143 g dm^{-3} d^{-1} and c_P of 442 mmol dm^{-3}, will be achieved in this area. The maximum volume productivity and product concentration that can be achieved according to the simulations (Fig. 4b1 and 4c1) are Q_P of 97.8 g dm^{-3} d^{-1} and c_P of 299.4 mmol dm^{-3}, respectively. These simulations present results after a fed-batch process; however, it is envisaged that after feeding for 12 hours, the reactor is left in batch mode for the remaining substrates to react until one of them is spent.

Since process optimization with several goal functions, as in this case, cannot have a straight forward solution, it is clearly a compromise between them. Seven operating conditions, i.e. feed flowrates, were selected for further consideration (Table 3). Processes at these conditions were simulated in detail (batch mode following the 12 hours of feeding), and the results are presented in Fig. 5. Process metrics were recalculated, and are presented in

Table 2 – *Typical process metrics goals for the potential industrial biocatalytic process carried out with free enzyme as catalyst (adapted from 1 and 15) to produce a pharma compound*

Enzyme price	1000 – 2500 € kg^{-1}
Volume productivity	48 g dm^{-3} d^{-1}
Product yield	>80 %
Product concentration	50–100 g dm^{-3}
Biocatalyst productivity	10–100 $kg_{product}$ kg^{-1}_{enzyme}
Product price	>100 € kg^{-1}

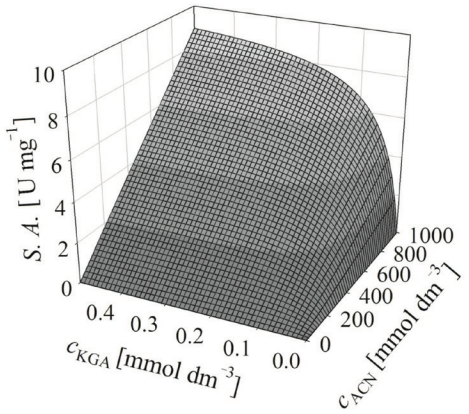

Fig. 3 – *Influence of ACN and KGA concentration on the specific enzyme activity (500 mmol dm^{-3} phosphate buffer, pH 8.0, V_r = 1 cm^3, 30 °C, 1000 rpm, γ_{MenD} = 0.23 mg cm^{-3}). One unit of enzyme activity was defined as the amount of enzyme required to produce 1 µmol of COHA per minute in 500 mmol dm^{-3} phosphate buffer pH 8.0 and at 30 °C.*

Table 3. For all seven selected operating conditions, significant values of process metrics were obtained. Nevertheless, experiments A, B, and C (Table 3, Fig. 5) were discarded from further consideration because product concentration (γ_P) was below 50 g dm^{-3}, whereas experiment F was discarded due to the obtained volume productivity (Q_P) below 48 g dm^{-3} d^{-1}. Experiment E, with the highest volume productivity (Q_P) of 87.1 g dm^{-3} d^{-1}, product concentration (γ_P) of 62.4 g dm^{-3}, and product yield (Y) of 96 % after 17 hours of reaction, was selected as the best for practical consideration and preliminary process economic evaluation. Nevertheless, experiments D and G also present viable options. Biocatalyst productivity (BP) calculated from these data (Table 3) shows that in all cases industrial requirements were met. Additionally, it is important to emphasize that all simulations were done at ACN concentrations which ensure lower rates of enzyme operational stability decay (< 250 mmol dm^{-3}), and that, for example, in experiment E, the process end-

Table 3 – *Simulated process metrics for the seven selected reaction conditions (simulation conditions: c_{ACN} = 120 mmol dm^{-3}, c_{KGA} = 120 mmol dm^{-3}, γ_{MenD} = 2.4 mg cm^{-3}, c_{ThDP} = 1.0 mmol dm^{-3}, $c_{Mg^{2+}}$ = 2 mmol dm^{-3}, V = 10 m^3, feed 1: c_{ACN} = 15.18 mol dm^{-3}, feed 2: c_{KGA} = 2.15 mol dm^{-3}, c_{ThDP} = 1.1 mmol dm^{-3}, $c_{Mg^{2+}}$ = 2.1 mmol dm^{-3}, γ_{MenD} = 3.0 mg cm^{-3})*

Exp	q_1 (dm^3 min^{-1})	q_2 (dm^3 min^{-1})	c_P (mmol dm^{-3})	γ_P (g dm^{-3})	Q_p (g dm^{-3} d^{-1})	Y (%)	BP (kg_P kg^{-1}_{MenD})
A	0.3	1.5	303.2	47.0	68.8	97	19.6
B	0.4	1.5	300.4	46.6	88.4	97	19.4
C	0.5	1.5	299.4	46.5	97.8	96	19.5
D	0.4	2.5	406.8	63.1	52.1	96	26.0
E	**0.5**	**2.5**	**402.1**	**62.4**	**87.1**	**96**	**25.8**
F	0.4	3.0	416.5	64.6	28.5	96	26.4
G	0.5	3.0	452.7	70.2	58.0	96	28.9

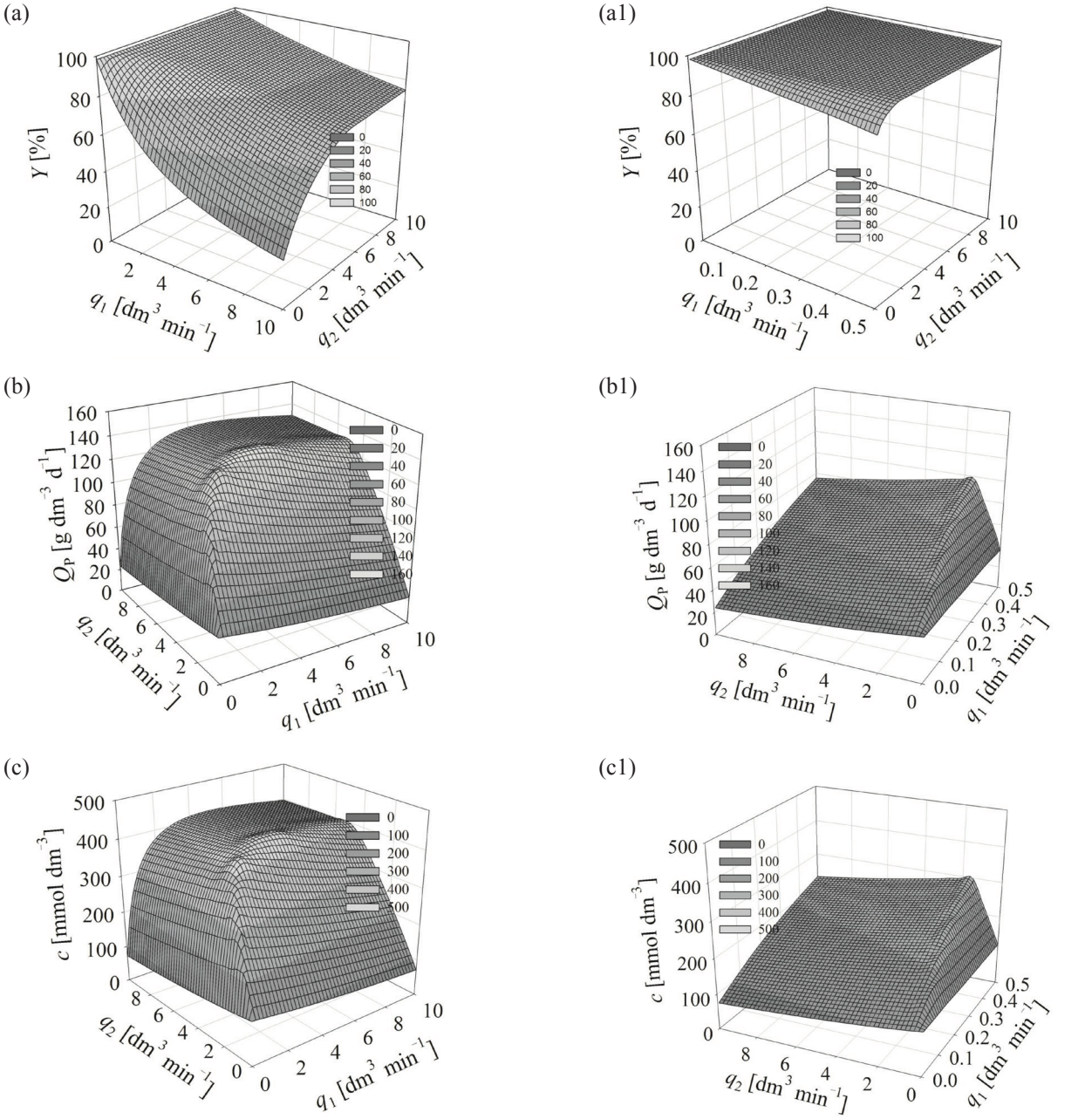

Fig. 4 – *Simulations of the COHA synthesis in the fed-batch reactor (500 mmol dm^{-3} phosphate buffer pH 8.0, 30 °C, 1000 rpm, $V_{reactor;0}$ = 10 m^3, c_{ACN} = 120 mmol dm^{-3}, c_{KGA} = 120 mmol dm^{-3}, γ_{MenD} = 2.4 mg cm^{-3}, c_{ThDP} = 1.0 mmol dm^{-3}, $c_{Mg^{2+}}$ = 2 mmol dm^{-3}, feed 1: c_{ACN} = 15.18 mol dm^{-3}, feed 2: c_{KGA} = 2.15 mol dm^{-3}, c_{ThDP} = 1.1 mmol dm^{-3}, $c_{Mg^{2+}}$ = 2.1 mmol dm^{-3}, γ_{MenD} = 3.0 mg cm^{-3}). Influence of feed flow rates on: a) product yield; b) volume productivity; c) product concentration. a1., b1., and c1. represent the more focused area where optimal conditions are searched.*

ed with enzyme activity at 89 % of initial activity. This means that enzyme can be reused and even higher values of *BP* can be reached. For the purpose of simplification, it was assumed that enzyme would be used only once. The final reaction mixture volume is 12 m^3 and 62.4 g dm^{-3} of product is obtained (Table 3, Exp. E).

Evaluation of preliminary process economics

With assumed 10 batches per year, total yearly capacity of the plant is estimated to be approximately 7,520.00 kg. Total capital investment of the plant was estimated to be 399,761.13 €. Annualized equipment cost was estimated to be 81,296.47 €, based on 6 % interest rate (*i*) and economic lifetime of 8 years (*t*), typical for chemical processes[3]. Quantities of raw materials used for synthesis in a 12 m^3 batch are presented in Table 4. Enzyme price[1] was assumed to be 2500 € kg^{-1}. Total annual operating expenses based on 10 batches per year are presented in Table 5. The estimated yearly amount of 260.4 m^3 of waste leads to the waste treatment cost of 528 € per year.

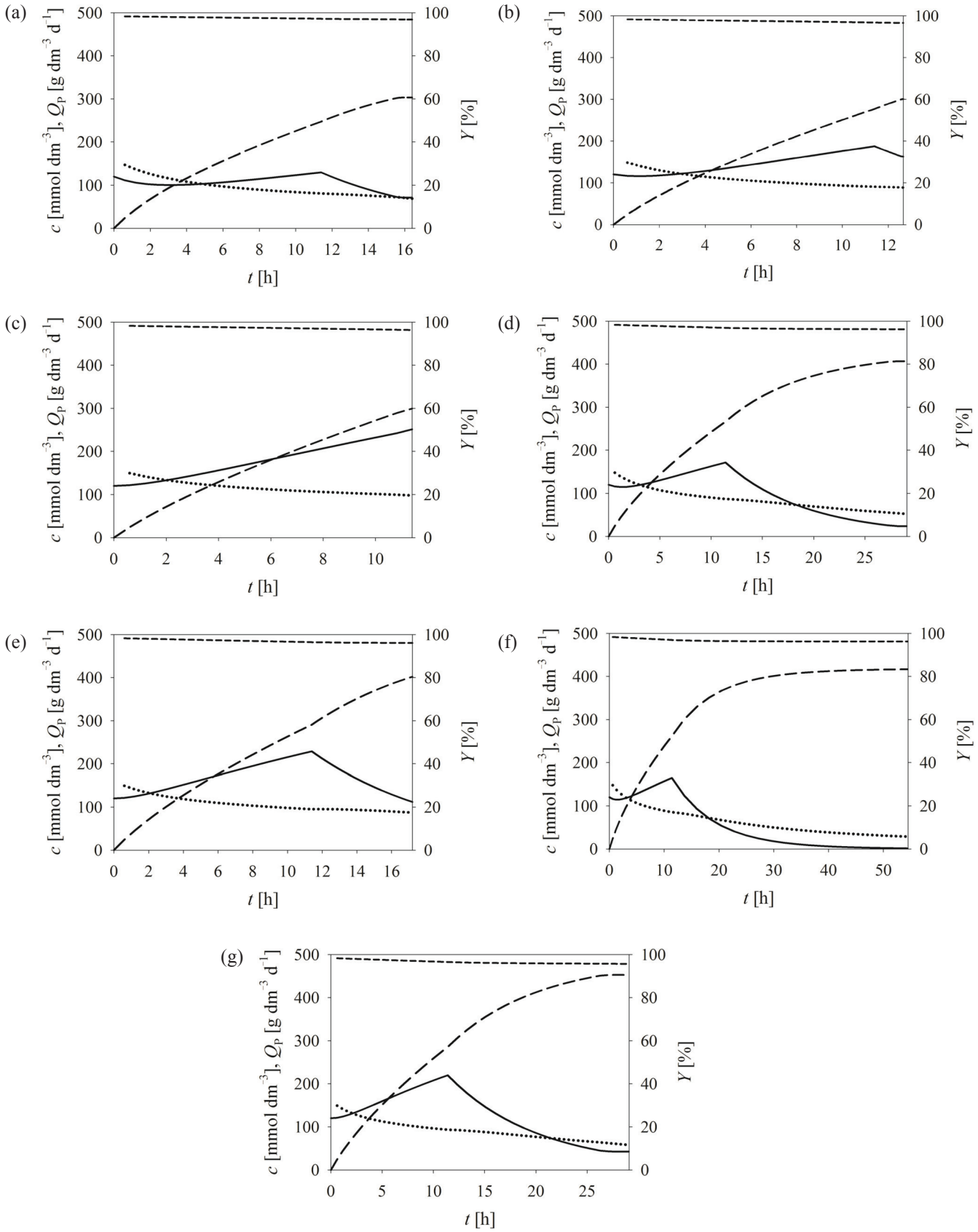

Fig. 5 – *Simulations of the fed-batch/batch processes for the selected optimal conditions presented in Table 3. Legend: line – acrylonitrile, long dash line – COHA, dotted line – volume productivity, short dash line – product yield.*

Table 4 – *Estimated raw material costs per one cycle/batch*

Chemical	Quantity	Unit price	Cost
Synthesis step			
ACN	421.07 dm^3	3.85 € dm^{-3}	1,621.12 €
KGA	975.5 kg	134.00 € kg^{-1}	130,717.00 €
MenD	29.13 kg	2,500.00 € kg^{-1}	72,825.00 €
ThDP	5.47 kg	4,925.00 € kg^{-1}	26,939.75 €
$MgCl_2$	2.29 kg	46.50 € kg^{-1}	106.49 €
Distilled water	12,000.00 dm^3	0,0005 € dm^{-3}	6.00 €
KH_2PO_4	40.80 kg	9.10 € kg^{-1}	371.28 €
K_2HPO_4	818.70 kg	19.80 € kg^{-1}	16,210.26 €
Product isolation step			
Ethyl acetate	12,000.00 dm^3	4.52 € dm^{-3}	54,240.00 €
HCl	2,400.00 dm^3	3.44 € dm^{-3}	8,256.00 €
Total RMC			311,292.90 €

Table 5 – *Annual operating costs (10 cycles)*

	Total	Per kg product
RMC	3,112,929.0 €	
Operating costs	183.2 €	
Waste treatment	528 €	
Fixed expenses	12,194.0 €	
Maintenance	8,129.6 €	
Total	3,133,963.80 €	416.8 € kg^{-1}

Table 6 – *Mode, minimum, and maximum values of independent variables used in MCA*

Variable	Minimum	Mode	Maximum
C_T	–20 %	399,761.1 €	+50 %
T_N	–30 %	42 %	+30 %
O_N	–10 %	3,112,929.0 €	+30 %
r	–30 %	10 %	+30 %

For the MCA 5000, independent variables' datasets were generated using triangular distribution according to mode, and maximum and minimum values in percentages, given in Table 6.

For each point, product price value yielding $NPV = 0$ was calculated to assess minimal product price required. Results are presented in Fig. 6. It can be seen that the minimum product cost (MPC) yielding $NPV = 0$ in 8 years with highest probability is 790 € kg^{-1}, and in 95 % of analysed scenarios, MPC is lower than 940 € kg^{-1}. Ten percent values of market quotations for similar products show much higher prices: 4-oxohexanoic acid[26] is 288 € g^{-1} and 6-nitro-4-oxohexanoic acid[27] is 12.8 € g^{-1}.

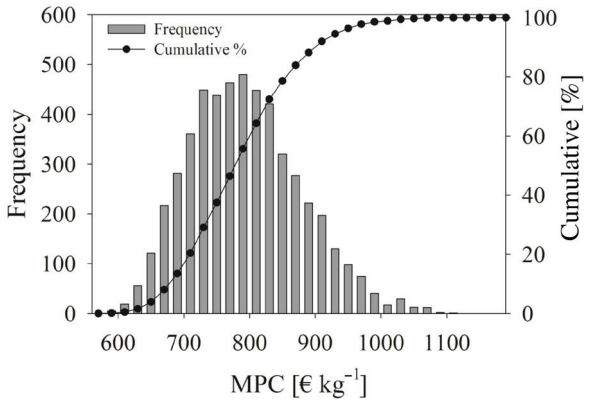

Fig. 6 – *Histogram and cumulative probability diagram of minimum product costs (MPC)*

Conclusions

It was shown how process model based on reliable kinetic data could be used for process optimization as well as a sound basis for preliminary calculation of process costs. An optimal operating point that was selected based on the compromise of different process metrics enabled obtaining 62.4 g dm^{-3} of product, volume productivity of 87.1 g dm^{-3} d^{-1}, product yield of 96 %, and biocatalyst productivity of 25.8 kg_P kg^{-1}_{MenD}. These numbers were a prerequisite for further process consideration, and based on the selected solution, an early-stage economic analysis was conducted. The most probable product price which could guarantee the return of the investment after 8 years was estimated at 790 € kg^{-1}, which is significantly lower than a similar product on the market. Even with additional costs for labour and agitation taken into consideration, preliminary cost analysis confirms the potential viability of the process at this early stage of process development.

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List of symbols

BP – biocatalyst productivity, $kg_{product}$ kg^{-1}_{enzyme}
c – molar concentration, mmol dm^{-3}
C_T – total capital investment, €

f – coefficient that quantifies the effect of ThDP and Mg^{2+} concentration (Eq. 3)
k – annuity factor (Eq. 14)
k – kinetic constant of the first order, min^{-1}
k_d – operational stability decay rate constant, h^{-1}
K_i – inhibition constant, mmol dm^{-3}
K_m – Michaelis constant, mmol dm^{-3}
m – production capacity per year, kg
NPV – net present value, €
O_N – yearly operating cost, €
q – volume flow rate, $cm^3\ min^{-1}$
q_1 – volume flow rate of acrylonitrile in the fed-batch experiment, $cm^3\ min^{-1}$
q_2 – volume flow rate of the second feed in the fed-batch experiment containing ThDP, α-ketoglutaric acid and Mg^{2+} salt, $cm^3\ min^{-1}$
Q_P – volume productivity, g $dm^{-3}\ d^{-1}$
r – required internal rate of return, years
r – reaction rate of enzymatic reaction, mmol $dm^{-3}\ min^{-1}$
r_1 – reaction rate of acrylonitrile polymerization, mmol $dm^{-3}\ min^{-1}$
$S.A.$ – specific enzyme activity, U mg^{-1}
t – reaction time, min
t – economic lifetime of the project, year
T_N – tax, %
V_m – maximum reaction rate, U mg^{-1}
V – reactor volume, m^3
Y – product yield, %
γ – mass concentration, mg cm^{-3}
Φ – product price, €

Abbreviations

ACN – acrylonitrile
COHA – 6-cyano-4-oxohexanoic acid
KGA – α-ketoglutaric acid
MCA – Monte Carlo analysis
MenD – 2-succinyl-5-enolpyruvyl-6-hydroxy-3-cyclohexene-1-carboxylate synthase
MPC – minimum product cost
PMP – *p*-methoxyphenol
RCA – raw materials cost
ThDP – thiamine diphosphate

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