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DOI
10.1016/j.bej.2022.108796

Publication date
2023

Document Version
Final published version

Published in
Biochemical Engineering Journal

Biochemical Engineering Journal 191 (2023) 108796

Contents lists available at ScienceDirect

Biochemical Engineering Journal

journal homepage: www.elsevier.com/locate/bej

Modelling of end-product inhibition in fermentation

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ARTICLE INFO

Keywords:
Batch mode
Chemostat
Pirt equation
Growth rate
Substrate consumption
Kinetics

ABSTRACT

Microbial conversions are generally inhibited by their products. This product inhibition is particularly dependent on the product's hydrophobicity and on its acid dissociation behaviour, hence on pH. Dependence on the microbial strain is relatively modest according to many published data. Since product inhibition has various mechanistic backgrounds, one requires an empirical model for simulation of fermentation processes. For obtaining a modelling framework that is consistent for various bioreactor operation modes, literature data for the glucose to ethanol fermentation were used to elaborate a relatively simple case. The Pirt equation and hyperbolic substrate uptake equation were used with three parameters that depend linearly on the inhibiting product concentration. This model type should facilitate model-based optimization of bioreactor operation.

1. Introduction

To optimize a particular microbial production process, a model that properly describes the microbial kinetics can be very useful. Such a model allows comparison of the theoretical performance of operational settings in all bioreactor modes, such as batch, fed-batch, and chemostat, optionally including multistage operation, cell retention/recycling, and in-situ product removal. The model may reduce the number of experiments required for achieving a production process with an attractive performance. This is one of the main reasons why developing models of microbial kinetics is the topic of numerous studies. To increase the probability that the model predicts the performance of a microbial conversion in a bioreactor operation mode that was not experimentally accessed, the model should incorporate mechanistic knowledge up to the detail supported by experimental data and required for optimizing the microbial conversion [1].

In this paper, the focus is on describing the influence of extracellular microbial products that slow down the progress of the microbial conversion ("fermentation") in which they are produced, i.e. inhibiting products. No distinction will be made between inhibition and toxicity. Product inhibition generally increases with increasing concentration c_p of the product. A key challenge in bioprocess development is to achieve a high concentration of inhibiting products but still a high volume-specific productivity.

Previous reviews in this field have focused on product inhibitions mechanisms [2] selecting models for describing the dependency of biomass-specific rates on inhibiting product concentration [3], or on correlation models for inhibition parameters [4]. For lactic acid fermentation, used inhibition models have been reviewed, but without reanalysing data [5]. For ethanol fermentation, batch data were reanalysed in a review [6], but it is not clear how to use the selected model for simulating continuous fermentation. The aim of the current review is to indicate how to simulate product-inhibited fermentations consistently for different bioreactor operation modes. Hence, a model is desired that describes reaction kinetics over the range of operation conditions of practical applicability. Kinetics of lag time in batch mode conversions are kept out of scope, though, and growth systems rather than biotransformations will be considered. There will be no focus on ways to decrease product inhibition.

2. Mechanisms of product inhibition

Numerous mechanisms can cause product inhibition [4,7–10], and these might be grouped as follows:

- Accumulation of (hydrophobic) products in cellular membranes leads to loss of membrane integrity, such that efficiency of membrane potential dependent ATP production decreases.
- Product that is actively excreted and is partly dissociated at extracellular pH, may diffuse back into the cell as non-dissociated species, and dissociate intracellularly, affecting intracellular pH. Then, ATP is again required to pump it out, hence dissipating proton motive force.
- Unspecific or specific binding of product to intracellular proteins, nucleic acids, and other macromolecules may decrease catalytic rates or increase ATP spent on repairing damaged macromolecules/

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https://doi.org/10.1016/j.bej.2022.108796
Received 19 September 2022; Received in revised form 21 December 2022; Accepted 22 December 2022
Available online 31 December 2022

Nomenclature

a_s	Amount of substrate per biomass in the hypothetical absence of maintenance (mol_s/mol_x).
c	Concentration (g/L or mol/L).
K_s	Michaelis-like constant for substrate uptake (g_s/L).
m_s	Substrate consumption rate for maintenance (mol_s/(mol_x h)).
q_i	Biomass-specific production rate of i (mol_i/(mol_x h)).
β_a	Constant for describing increase of a_s (g_p/L).
β_m	Constant for describing increase of m_s (g_p/L).
μ	Biomass-specific growth rate, i.e. q_x (h^{-1}).
Subscripts and superscripts	
0	in the absence of the inhibiting product.
crit	critical for biomass growth (μ), product formation (qp), or substrate consumption (qs).
feed	in the initial medium.
max	at maximum rate, in case of no substrate or nutrient limitation.
p	for product.
s	for substrate.
x	for dry biomass.

structures. For example, the product may inhibit an enzyme or trigger a regulation mechanism that decreases metabolic rates.
- Product excretion may require more ATP at higher extracellular product concentrations.

In addition, a high product concentration makes the thermodynamic equilibrium of the overall metabolic reaction less favourable, and hence slows down the reaction. Incorporation of reaction equilibria in kinetic models requires not only high product concentrations but also very low substrate concentrations [11] and has been performed successfully for microbes [12]. The aforementioned "product inhibition" mechanisms in this paper do not rely on a low substrate concentration.

3. Critical concentrations for growth

Numerous publications report effects of initially added fermentation products or related inhibiting compounds on microbial growth, substrate consumption or product formation.

A key issue is how to obtain and process experimental inhibition data. Some growth inhibition studies report the relative concentration of (grown) cells after some specific incubation time, as function of the concentration of added inhibiting product. This is less informative than providing maximum biomass-specific growth rates (μ^{max}) values, which are derived from the concentration of cells at *several* incubation times. The traditional literature usually presents μ^{max} values without error range, but it is advised to use an automated protocol for data processing that incorporates the error range [13].

Typically, μ^{max} is determined in batch experiments as function of concentration c_p of inhibiting product. It is generally observed that cell growth completely stops at a critical product concentration for growth, $c_p^{crit,\mu}$, despite sufficient nutrients of all types. Table 1 shows a non-exhaustive list of publications in this field, with occasionally inaccurate values due to extrapolation of few data points. The data are for completely dissolved organic products, and for comparison purposes data are included for inhibiting organic compounds that may occur as contaminant. Substrate inhibition is out of scope in this paper, so Table 1 does not include the effect of compounds that will serve as substrate under the applied conditions. Some effects may be seen in Table 1:

- Effect of product type: For non-dissociating products, the value of $c_p^{crit,\mu}$ correlates with the hydrophilicity of the product. This is an important and well-known correlation, and for correlation models one is referred to the literature [4,14]. A more hydrophobic product typically shows a larger extent of accumulation in cellular membranes, and it causes stronger inhibition by affecting the membrane's integrity.
- Effect of pH: If a compound such as a carboxylic acid shows acid-base dissociation in the pH range of interest, product inhibition depends not only on product concentration and product type, but also on pH. A pH-dependent fraction of the compound will be undissociated outside the cell. This species may diffuse relatively easily back into the cell and affect intracellular pH. ATP required for pumping protons to keep intracellular pH has been calculated [15], and maintenance requirements must be coupled to this [16]. This understanding has been used to quantify acetic acid inhibition [17], but not, for example, for describing lactic acid inhibition [5].
- Effect of substrate type: Data on the effect of changing substrate (electron donor) type, while keeping product type, pH and microorganism, are only given for [18] in Table 1. In that case, product inhibition was usually larger in case of using xylose, as compared to glucose. Potentially, the cells obtain less ATP from xylose than from glucose. No generic conclusion can be drawn from the presented data, though.
- Effect of microbe type: An effect of changing the microbe type is certainly present, but Table 1 contains only a few cases with a larger than twofold effect. Hence, it is a small effect in comparison to the aforementioned effects of product type and pH. This is surprising, because microbes can evolve to become more tolerant to inhibiting products [19,20], and metabolic engineering can increase tolerance to inhibiting products [21]. On the other hand, a microbe's $c_p^{crit,\mu}$ does not seem to be increased more than twofold in studies on this topic. Mind that the literature has a bias to include microbes that have a relatively high tolerance as wild type already, because their wild-type metabolism leads to a certain product of interest, such that they become subject of product inhibition studies. Sensitive microbes may remain out of scope. Another factor is that different types of microbes may have a very different tolerance to low pH and carboxylic acids [22], leading to a covariance between multiple factors, but Table 1 is not suited to identify such covariance.
- Effect of temperature. For glucose to ethanol as main fermentation product, the data at 30 °C from Table 1 lead to $c_p^{crit,\mu}$ = 89 g/L on average, with 14 g/L standard deviation. At higher temperatures, $c_p^{crit,\mu}$ decreases. For *K. marxianus*, $c_p^{crit,\mu}$ was 65 g/L at 37 °C [23]. For *Z. mobilis*, $c_p^{crit,\mu}$ decreased stepwise from 72 g/L at 30 °C to 29 g/L at 40 °C [24]. Lower values of $c_p^{crit,\mu}$ will be due to increasing maintenance requirements at higher temperatures [25].

Aeration, nutrients, and additives are additional factors that may affect $c_p^{crit,\mu}$. It is interesting to consider if machine learning approaches [26] can help to establish the contribution of all factors. A very strong dataset will be required, and Table 1 may facilitate building it.

A minority of studies does not only report $c_p^{crit,\mu}$ but also the critical product concentration $c_p^{crit,qs}$ or $c_p^{crit,qp}$ beyond which no substrate consumption or product formation, respectively, occurs. For glucose to ethanol fermentation, reported $c_p^{crit,qp}$ values [24,29–31,36–38] are on average 49% higher than $c_p^{crit,\mu}$ from the same study. For glucose to ethanol fermentation, only a single study [35] reported $c_p^{crit,qs}$, and it was 23% higher than the reported $c_p^{crit,\mu}$ value. No study reports all three critical concentrations. Section 8 will elaborate on the relation between these three critical concentrations of a fermentation.

4. Modelling the influence of product concentration on μ^{max}

The decrease from $\mu^{max} = \mu_0^{max}$ (for $c_p = 0$) to $\mu^{max} = 0$ (for $c_p = c_p^{crit,\mu}$) often has been successfully described by Eq. (1), a simple, linear model.

Table 1
Representative data on $c_p^{crit,\mu}$.

Inhibiting compound	pH	T (°C)	Substrate	Microbe	$c_p^{crit,\mu}$ (g/L)	Source
Alcohols						
Methanol	?	30	Glycerol	*Cupriavidis necator*	> 120	[27]
Ethanol	3.6–4.0	21	Glucose	*Saccharomyces cerevisiae*	68	[28]
Ethanol	4	30	Glucose	*S. cerevisiae*	94	[29]
Ethanol	4	30	Glucose	*S. cerevisiae*	100	[30]
Ethanol	4	30	Glucose	*S. cerevisiae*	87	[31]
Ethanol	4	30	Glucose	*S. cerevisiae*	84	[32]
Ethanol	4	30	Glucose	*S. cerevisiae*	120	[33]
Ethanol	4.0–4.8	30	Glucose	*S. cerevisiae*	107	[34]
Ethanol	5	30	Glucose	*S. cerevisiae*	78	[35]
Ethanol	5	30	Glucose	*Saccharomyces carlsbergiensis*	95	[36]
Ethanol	4.5	30	Glucose	*Saccharomyces uvarum*	73	[37]
Ethanol	?	37	Glucose	*Kluyveromyces marxianus*	65	[23]
Ethanol	5	30	Glucose	*Zymomonas mobilis*	72	[24]
Ethanol	5	35	Glucose	*Z. mobilis*	61	[24]
Ethanol	5	37	Glucose	*Z. mobilis*	55	[24]
Ethanol	5	40	Glucose	*Z. mobilis*	29	[24]
Ethanol	5	30	Glucose	*Z. mobilis*	85	[38]
Ethanol	5	30	Glucose	*Z. mobilis*	85	[39]
Ethanol	5	30	Glucose	*Z. mobilis*	72	[40]
Ethanol	5.8	30	Glucose	*Z. mobilis*	90	[41]
Ethanol	5.8	30	Glucose	*Z. mobilis*	100	[18]
Ethanol	5.8	30	Xylose	*Z. mobilis*	100	[18]
Ethanol	7.2	37	Glucose	*Actinobacillus succigenes*	42	[42]
Ethanol	6.5	37	Glucose	*Escherichia coli*	35	[43]
Ethanol	6.5	37	Glucose	*Klebsiella oxytoca*	36	[44]
Ethanol	6.8	37	Glycerol	*K. pneumoniae*	26	[45]
Ethanol	6.8	37	Glycerol	*K. pneumoniae*	17	[45]
Ethanol	5	30	CO	*Clostridium carboxidivorans*	45	[46]
Isopropanol	7.0	30	Glucose	*Cupriavidus necator*	20	[13]
1-Butanol	> 5.6	?	Glucose	*Clostridium acetobutylicum*	17	[47]
1-Butanol	?	37	Glucose	*Clost. acetobutylicum*	20	[48]
1-Butanol	6.8	37	Glucose	*Clostridia* mix	18	[49]
1-Butanol	5.0	30	CO	*Clost. carboxidivorans*	15	[46]
1-Butanol	5.8	25	Syngas	*Clost. ljungdahlii*	15	[50]
1-Butanol	5.8	25	Syngas	*Butyribacterium methylotrophicum*	15	[50]
1-Butanol	~7	37	Glucose	*E. coli*	15	[14]
2-Butanol	4.5	30	Glucose	*S. cerevisiae*	36	[51]
2-Butanol	6.5	37	Glucose	*Bacillus subtilis*	20	[51]
2-Butanol	6.5	37	Glucose	*E.coli*	21	[51]
Isobutanol	~7	37	Glucose	*E. coli*	15	[14]
1-Pentanol	~7	37	Glucose	*E. coli*	5	[14]
Isopentanol	~7	37	Glucose	*E. coli*	5	[14]
3-Methylbut-3-en-1-ol	~7	37	Glucose	*E. coli*	7	[20]
1-Hexanol	~7	37	Glucose	*E. coli*	2.5	[14]
Furfuryl alcohol	5.8	30	Glucose	*Z. mobilis*	22	[18]
Furfuryl alcohol	5.8	30	Xylose	*Z. mobilis*	20	[18]
2-Phenylethanol	5	30	Glucose	*S. cerevisiae*	2	[52]
1,3-Propanediol	7.0	33	Glycerol	*Clost. diolis*	65	[53]
1,3-Propanediol	6.8	35	Glycerol	*Clost. butyricum*	~100	[54]
1,3-Propanediol	7.0	37	Glycerol	*Clost. butyricum*	65	[55]
1,3-Propanediol	6.8	37	Glycerol	*K. pneumoniae*	69	[45]
1,3-Propanediol	6.5	?	Glycerol	*Clost. butyricum*	66	[56]
1,3-Propanediol	6.5	32	Glycerol	*Clost. butyricum*	60	[57]
2,3-Butanediol	6.5	37	Glucose	*K. oxytoca*	74	[44]
2,3-Butanediol	6.5	30	Glucose	*B. subtilis*	130	[58]
2,3-Butanediol	5.5	37	Xylose	*K. oxytoca*	105	[59]
Aldehydes						
Acetaldehyde	5.6	30	Glucose	*S. cerevisiae*	0.8	[60]
Furfural	5.8	30	Glucose	*Z. mobilis*	5.0	[18]
Furfural	5.8	30	Xylose	*Z. mobilis*	4.0	[18]
Furfural	5.5	30	Glucose	*S. cerevisiae*	5.1	[61]
HMF	4.5	30	Glucose	*S. cerevisiae*	2.2	[51]
HMF	6.5	37	Glucose	*B. subtilis*	1.9	[51]
HMF	6.5	37	Glucose	*E. coli*	2.2	[51]
HMF	5.8	30	Glucose	*Z. mobilis*	7.9	[18]
HMF	5.8	30	Xylose	*Z. mobilis*	7.9	[18]
4-Hydroxybenzaldehyde	5.8	30	Glucose	*Z. mobilis*	3.1	[18]
4-Hydroxybenzaldehyde	5.8	30	Xylose	*Z. mobilis*	3.1	[18]
Vanillin	4.5	30	Glucose	*S. cerevisiae*	1.08	[51]
Vanillin	6.5	37	Glucose	*B. subtilis*	1.84	[51]
Vanillin	6.5	37	Glucose	*E. coli*	2.2	[51]
Vanillin	5.8	30	Glucose	*Z. mobilis*	3.0	[18]
Vanillin	5.8	30	Xylose	*Z. mobilis*	2.0	[18]

(*continued on next page*)

Table 1 (*continued*)

Inhibiting compound	pH	T (°C)	Substrate	Microbe	$c_P^{crit,\mu}$ (g/L)	Source
Syringaldehyde	4.5	30	Glucose	*S. cerevisiae*	2.5	[51]
Syringaldehyde	6.5	37	Glucose	*B. subtilis*	2	[51]
Syringaldehyde	6.5	37	Glucose	*E. coli*	2.7	[51]
Syringaldehyde	5.8	30	Glucose	*Z. mobilis*	7.3	[18]
Syringaldehyde	5.8	30	Xylose	*Z. mobilis*	5.1	[18]
Ketones						
2-Butanone	4.5	30	Glucose	*S. cerevisiae*	45	[51]
2-Butanone	6.5	37	Glucose	*B. subtilis*	31	[51]
2-Butanone	6.5	37	Glucose	*E. coli*	17.8	[51]
Acetoin	6.5	37	Glucose	*K. oxytoca*	24	[44]
Acetoin	6.5	30	Glucose	*B. subtilis*	45	[58]
Dihydroxyacetone	6.0	28	Glycerol	*Gluconobacter oxydans*	82	[62]
Dihydroxyacetone	4.5	28	Glycerol	*G. oxydans*	80	[63]
Carboxylic acids						
Formate	7.2	37	Glucose	*A. succigenes*	16	[42]
Formate	6.5	37	Glucose	*K. oxytoca*	14.5	[44]
Formate	5.8	30	Glucose	*Z. mobilis*	11	[18]
Formate	5.8	30	Xylose	*Z. mobilis*	3.0	[18]
Formate	7	37	Glucose	*A. succigenes*	35	[64]
Formate	7	37	Glucose	*E. coli*	35	[64]
Acetate	5		Glucose	*Z. mobilis*	10	[16]
Acetate	6.5	37	Glucose	*K. oxytoca*	22	[44]
Acetate	7.2	37	Glucose	*A. succigenes*	46	[42]
Acetate	4.5	30	Glucose	*S. cerevisiae*	10.5	[65]
Acetate	6.4	66	Glucose	*Acetogenium kivui*	42	[66]
Acetate	6.4	66	Glucose	*A. kivui*	35	[67]
Acetate	7.0	55	Glucose	*C. thermocellum*	65	[68]
Acetate	6.8	37	Glycerol	*K. pneumoniae*	15	[45]
Acetate	6.5	32	Glycerol	*Clost. butyricum*	27	[57]
Acetate	6.5	?	Glycerol	*Clost. butyricum*	22	[56]
Acetate	> 5.6	?	Glucose	*Clost. acetobutylicum*	12	[47]
Acetate	5.8	30	Glucose	*Z. mobilis*	22	[18]
Acetate	5.8	30	Xylose	*Z. mobilis*	6.6	[18]
Acetate	7	37	Glucose	*A. succigenes*	20	[64]
Acetate	7	37	Glucose	*E. coli*	60	[64]
Acetate	6.5	30	Glucose	*Acetobacter aceti*	~50	[19]
Butyrate	6.5	32	Glycerol	*Clost. butyricum*	19	[57]
Butyrate	6.5	?	Glycerol	*Clost. butyricum*	11	[56]
Butyrate	6.0	37	Glucose	*Clost. tyrobutylicum*	100	[69]
Butyrate	> 5.6	?	Glucose	*Clost. acetobutylicum*	11	[47]
Hexanoate	5.8	30	Glucose	*Z. mobilis*	3.9	[18]
Hexanoate	5.8	30	Xylose	*Z. mobilis*	2.0	[18]
Octanate	~7	37	Glucose	*E. coli*	5	[14]
Lactate	6.8	37	Glycerol	*K. pneumoniae*	26	[45]
Lactate	6.8	37	Glycerol	*K. pneumoniae*	19	[45]
Lactate	6.5	30	Lactose	*Lactococcus lactis*	50	[70]
Lactate	?	42	Glucose	*Lactobacillus casei*	70	[71]
Lactate	5.9	42	Lactose	*Lactobacillus helveticus*	75	[72]
Lactate	5.8	30	Glucose	*Z. mobilis*	54	[18]
Lactate	5.8	30	Xylose	*Z. mobilis*	36	[18]
Lactate	7	37	Glucose	*A. succigenes*	18	[64]
Lactate	7	37	Glucose	*E. coli*	18	[64]
Lactate	5.3	45	Glucose	*Lactobacillus delbrueckii*	47	[73]
D-Lactate	6.5	37	Glucose	*L. delbrueckii*	52	[74]
Pyruvate	7.2	37	Glucose	*A. succinogenes*	74	[42]
Levulinate	5.8	30	Glucose	*Z. mobilis*	55	[18]
Levulinate	5.8	30	Xylose	*Z. mobilis*	10	[18]
Acrylate	~7	37	Glucose	*E. coli*	2.5	[75]
Oxalate	5.8	30	Glucose	*Z. mobilis*	8.1	[18]
Oxalate	5.8	30	Xylose	*Z. mobilis*	9.5	[18]
Succinate	7	39	Glycerol	*Mannheimia succiniciproducens*	45	[76]
Succinate	7.2	37	Glucose	*A. succinogenes*	104	[42]
Succinate	6.5	37	Glucose	*K. oxytoca*	72	[44]
Succinate	5.8	30	Glucose	*Z. mobilis*	40	[18]
Succinate	5.8	30	Xylose	*Z. mobilis*	40	[18]
Succinate	7	37	Glucose	*A. succigenes*	50	[64]
Succinate	7	37	Glucose	*E. coli*	80	[64]
Malate	6.0	25	Glycerol	*Aspergillus niger*	115	[77]
Itaconate	5.8	30	Glucose	*Z. mobilis*	42	[18]
Itaconate	5.8	30	Xylose	*Z. mobilis*	50	[18]
2-Furoate	5.8	30	Glucose	*Z. mobilis*	34	[18]
2-Furoate	5.8	30	Xylose	*Z. mobilis*	20	[18]
Benzoate	5.8	30	Glucose	*Z. mobilis*	15	[18]
Benzoate	5.8	30	Xylose	*Z. mobilis*	4.0	[18]
4-Hydroxybenzoate	5.8	30	Glucose	*Z. mobilis*	20	[18]
4-Hydroxybenzoate	5.8	30	Xylose	*Z. mobilis*	12	[18]

(*continued on next page*)

Table 1 (*continued*)

Inhibiting compound	pH	T (°C)	Substrate	Microbe	$c_p^{crit,\mu}$ (g/L)	Source
Vanillate	5.8	30	Glucose	*Z. mobilis*	24	[18]
Vanillate	5.8	30	Xylose	*Z. mobilis*	12	[18]
4-Hydroxycinnamate	5.8	30	Glucose	*Z. mobilis*	17	[18]
4-Hydroxycinnamate	5.8	30	Xylose	*Z. mobilis*	10	[18]
Ferulate	5.8	30	Glucose	*Z. mobilis*	23	[18]
Ferulate	5.8	30	Xylose	*Z. mobilis*	23	[18]
Esters						
Methyl propionate	4.5	30	Glucose	*S. cerevisiae*	23	[51]
Methyl propionate	6.5	37	Glucose	*B. subtilis*	21	[51]
Methyl propionate	6.5	37	Glucose	*E. coli*	14	[51]
Ethyl acetate	7	32	Lactose	*K. marxianus*	17	[78]
Ethyl acetate	4.5	30	Glucose	*S. cerevisiae*	22	[51]
Ethyl acetate	6.5	37	Glucose	*B. subtilis*	30	[51]
Ethyl acetate	6.5	37	Glucose	*E. coli*	19	[51]
Ethyl propionate	~7	37	Glucose	*E. coli*	15	[14]
Ethyl isobutyrate	~7	37	Glucose	*E. coli*	7.5	[14]
Ethyl butyrate	~7	37	Glucose	*E. coli*	7.5	[14]
Propyl propionate	~7	37	Glucose	*E. coli*	5	[14]
Propyl butyrate	~7	37	Glucose	*E. coli*	2	[14]
Isopropyl propionate	~7	37	Glucose	*E. coli*	5	[14]
Isopropyl butyrate	~7	37	Glucose	*E. coli*	2	[14]
Butyl acetate	~7	37	Glucose	*E. coli*	4	[14]
Butyl propionate	~7	37	Glucose	*E. coli*	2	[14]
Butyl butyrate	~7	37	Glucose	*E. coli*	1	[14]
Isobutyl acetate	~7	37	Glucose	*E. coli*	4	[14]
Isobutyl propionate	~7	37	Glucose	*E. coli*	2.5	[14]
Isobutyl butyrate	~7	37	Glucose	*E. coli*	1	[14]
Amino acids						
L-Glutamate	7	30	Glucose	*Corynebacter glutamicum*	12	[79]

$$\mu^{max} = \mu_0^{max}\left(1 - \frac{c_p}{c_p^{crit,\mu}}\right) \quad (1)$$

This equation was originally presented by Ghose and Tyagi in 1979 [31], but a mathematically analogous equation was proposed by Dagley and Hinshelwood already in 1938 [80]. Since deviations occur between the model and experimental data, in particular when many data points are available, many alternative equations have been tried [6]. Here, two popular extensions of Eq. (1) are given, which both introduce one additional parameter, exponent n [3,30,32]. Whereas Eq. (1) leads to a straight line in a plot of μ^{max} vs. c_p, Eqs. (2) or (3) might fit data points showing a positive or negative curvature, as illustrated by Fig. 1.

$$\mu^{max} = \mu_0^{max}\left(1 - \left(\frac{c_p}{c_p^{crit,\mu}}\right)^n\right) \quad (2)$$

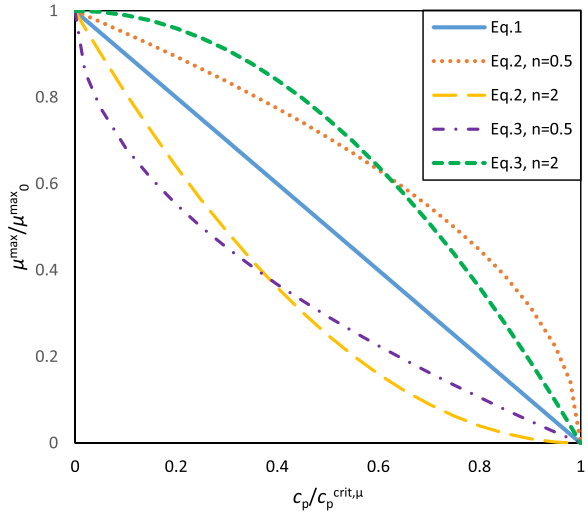

Fig. 1. Dependence of maximum specific growth rate on product concentration according to Eqs. (1–3).

$$\mu^{max} = \mu_0^{max}\left(1 - \frac{c_p}{c_p^{crit,\mu}}\right)^n \quad (3)$$

Exponential and hyperbolic inhibition models such as Eqs. (4–5), using only $K_{i,p}$ as inhibition parameter, follow the same trend as the first part of the lower curved lines in Fig. 1, that need two parameters with Eqs. (2–3). This has led to some successful applications of Eqs. (4–5) at relatively low product concentrations. However, Eqs. (4–5) do not manifest a critical product concentration at which growth stops, and therefore should be rejected for modelling product inhibition if high product concentrations are involved as well [3].

$$\mu^{max} = \mu_0^{max}\exp\left(\frac{-c_p}{K_{i,p}}\right) \quad (4)$$

$$\mu^{max} = \mu_0^{max}\left(\frac{K_{i,p}}{K_{i,p} + c_p}\right) \quad (5)$$

Some additional remarks when fitting Eqs. (1–3) to experimental data:

- One should include μ_0^{max} as fit parameter, instead of assuming that the μ^{max} value measured in the absence of product addition equals μ_0^{max}. Normalizing of measured μ^{max} values by dividing by such an assumed μ_0^{max} should be avoided, unless the measurement error in all values is propagated.
- Eqs. (1–3) predict negative growth rates at $c_p > c_p^{crit,\mu}$. However, negative growth rates are hardly detectable in traditional growth experiments, and are probably usually reported as "no growth". A product concentration at which experimentally no growth is observed may be higher than $c_p^{crit,\mu}$ and therefore should not be assumed to be equal to $c_p^{crit,\mu}$. Instead, $c_p^{crit,\mu}$ should be one of the fit parameters. A product concentration at which no growth was observed should not be included as data point in the fit procedure.
- To determine the significance of the extra parameter n, it is recommended to perform a model reduction test (F test), in which the improvement of adding this extra parameter in a progressive model is quantified as the difference in the resulting sum of squares [51].

- In practice, fitting the two aforementioned parameters (μ_0^{max} and $c_p^{crit,\mu}$) to the linear model (Eq. 1) leads to inaccurate parameter estimates when less than five data points are available. Data points at $c_p = 0$ and at c_p approaching $c_p^{crit,\mu}$ contribute in particular to accurate prediction of μ_0^{max} and $c_p^{crit,\mu}$.

As alternative to reporting $c_p^{crit,\mu}$, many inhibition studies report the product concentration $c_p^{50\%\mu}$ at which the maximum specific growth rate is reduced by 50%. Determination of $c_p^{50\%\mu}$ requires fitting a model equation through experimental μ^{max} data, and exactly the same issues occur as just mentioned for determination of $c_p^{crit,\mu}$. In case linear inhibition in product concentration is adopted according to Eq. (1), it is obvious that $c_p^{50\%\mu} = ½c_p^{crit,\mu}$, allowing a quick switch between these two types of inhibition metrics. If a more complex growth inhibition equation is selected for describing the experimental data, a more complex relation between $c_p^{50\%\mu}$ and $c_p^{crit,\mu}$ applies. Overall, choosing between $c_p^{50\%\mu}$ and $c_p^{crit,\mu}$ seems largely a matter of taste and tradition.

Note that Eqs. (1–3) do not have any mechanistic basis. Incorporation of the product inhibition mechanisms mentioned in Section 2 with all their details in a mechanistic model would likely need numerous intracellular measurements and lead to parametrization problems [81, 82].

5. An unstructured kinetic model for incorporation of product inhibition

Structured kinetic models can be very useful to understand microbial metabolism, guide metabolic engineering or to design and control a specific fermentation process [81,82]. However, unstructured kinetic models, with much less equations and parameters, are usually adequate for the early stages of designing fermentation processes. Unstructured kinetic models that still take into account ATP availability [15,83] are attractive, because the main mechanistic feature of the aforementioned product inhibition mechanisms seems to be a decrease in ATP availability, due to a decrease in ATP production and an increase in ATP requirement.

For simplicity, the following description applies to fermentation leading to a single catabolic product. Section 10 shortly discusses some more complicated fermentations.

The unstructured kinetic model that is used here is explained first without addressing product inhibition. The kinetic equations are a rewritten Pirt equation (Eq. (6)), and an equation for hyperbolic substrate uptake (Eq.(7)). The biomass-specific substrate consumption rate is indicated by "$-q_s$", because q_i is used here to indicate the biomass-specific *production* rate of compound i. Eq. (6) indicates that consumed substrate is used either for growth (biomass-specific growth rate μ) or for maintenance (biomass-specific rate m_s). Parameter a_s indicates the amount of substrate consumed per amount of biomass produced in the hypothetical absence of maintenance, and can be called a maximum yield parameter. Growth and maintenance both need a certain specific rate of ATP generation, which is provided by the catabolic reaction. Hence, substrate uptake and catabolic product formation are stoichiometrically coupled to growth and maintenance.

$$-q_s = a_s\mu + m_s \tag{6}$$

$$-q_s = -q_s^{max}\frac{c_s}{K_s + c_s} \tag{7}$$

The background of Eq. (7) is gradual saturation of a limiting enzyme or transport protein, similar as in Michaelis-Menten kinetics. Here, Eq. (7) is preferred to the more popular Monod equation (Eq. (8)). In case of depleted substrate ($c_s = 0$), the Monod equation predicts $\mu = 0$, and then Eq. (6) predicts that there should still be substrate uptake due to maintenance, which is inconsistent. Eq. (7), however, correctly describes that there is no substrate consumption in the absence of substrate. Then, Eq. (6) predicts negative growth (decrease of biomass) due to maintenance. The different consequences of Eqs. (7) and (8) will be unobservable in most batch growth experiments, but become amplified when the relative importance of maintenance increases at the product inhibition conditions described later.

$$\mu = \mu^{max}\frac{c_s}{K_s + c_s} \tag{8}$$

The used unstructured kinetic model is assumed to be valid during exponential growth (such as in batch mode conversion) and during single-nutrient limited growth. In case of $c_s >> K_s$, such as during exponential growth in batch mode, the maximum specific substrate consumption rate is called $-q_s^{max}$ such that Eq. (6) can be rewritten to include the maximum specific growth rate μ^{max}:

$$-q_s^{max} = a_s\mu^{max} + m_s \tag{9}$$

In summary, the model so far contains 5 parameters:

1. μ^{max}, the maximum specific growth rate
2. $-q_s^{max}$, the maximum specific substrate consumption rate
3. K_s, the Michaelis-like constant for substrate uptake
4. a_s, the maximum yield parameter
5. m_s, the specific substrate consumption rate for maintenance

Only four of these parameters are independent because of Eq. (9).

The overall microbial reaction will contain additional compounds, for example:

$$q_sC_6H_{12}O_6 + q_nNH_3 + \mu X + q_pC_2H_6O + q_cCO_2 + q_hH_2O = 0 \tag{10}$$

If q_s and μ have been determined, the four remaining q-values at that experimental condition can be calculated from Eq. (10) using the four elemental balances of C, H, N, and O and an elemental composition for dry biomass X such as $CH_{1.8}N_{0.2}O_{0.5}$. Thus, in case of a simple stoichiometry such as Eq. (10), q_s and μ determine q_p. Then, only 2 of these 3 parameters are independent. Some other dependent parameters (q_n, q_c and q_h) will play no role in subsequent modelling and discussions. During batch exponential growth, when $q_s = q_s^{max}$ and $\mu = \mu^{max}$, the value of q_p thus obtained will be called q_p^{max} here.

Product inhibition will not change Eqs. (6,7) or (9) of the unstructured kinetic model, but only these equations' parameters. Thus, a complete kinetic model will be found by finding the joint dependency on product concentration of four independent parameters. The next sections describe how this works out for anaerobic fermentation of glucose to ethanol, if K_s, μ^{max}, a_s, and m_s are taken as independent parameters, and q_s^{max} and q_p^{max} are calculated from these independent parameters.

6. Traditional approaches to obtain q_s^{max} and q_p^{max}

The aforementioned dependency of q_s^{max} and q_p^{max} on four other kinetic parameters differs from the traditional approach in which analogues of Eqs. (1–3) have been used [35]. For example, Eq. (11) shows a dependency of q_s^{max} on its value in the absence of product ($q_{s,0}^{max}$) and on a critical product concentration at which substrate consumption stops ($c_p^{crit,qs}$):

$$q_s^{max} = q_{s,0}^{max}\left(1 - \frac{c_p}{c_p^{crit,qs}}\right) \tag{11}$$

By analogy, Eq. (12) has been used to describe the maximum specific product formation rate q_p^{max} [84], using a critical product concentration at which product formation stops ($c_p^{crit,qp}$):

$$q_p^{max} = q_{p,0}^{max}\left(1 - \frac{c_p}{c_p^{crit,qp}}\right) \tag{12}$$

Later, it will be shown that using Eqs. (11,12) can lead to inconsistency.

7. Influence of ethanol on parameters of ethanol fermentation

The most comprehensive data on product inhibition can be found for fermentation of glucose fermentation to ethanol as main product, using various yeast species and *Z. mobilis*. Data from this fermentation type will be used for a deeper discussion. Since substrate inhibition can obscure product inhibition, data at inhibiting substrate concentrations [38,85] will not be taken into account for the following analysis of product inhibition by ethanol.

7.1. Influence of ethanol concentration on K_s

The value of K_s for glucose uptake by *Z. mobilis* did not depend on ethanol concentration, and for *Saccharomyces cerevisiae* it did not depend on concentrations of ethanol, isopropanol, 1-propanol and 1-butanol that decreased $-q_s^{max}$ by more than 50% [29,31,84,86]. Apparently, glucose binding by the protein(s) that determine K_s is not influenced by the used alcohol concentrations.

7.2. Influence of ethanol concentration on μ^{max}

Visual inspection was performed on data in papers that measured the influence of added ethanol on batch specific growth and product formation rates. For growth, Eq. (1) is adequate for most cases in Table 2. There are clear exceptions, but these include positive as well as negative curvatures of typically only 5–20%, such that there is no clear justification for taking a more complex equation than Eq. (1) as generic model for describing ethanol inhibition of maximum specific growth rates. For the biomass-specific ethanol production rate, less batch data are available, but the main trend is again linear inhibition by ethanol (Table 2). No analogous data were found for biomass-specific substrate consumption rate.

The decrease of μ^{max} with increasing ethanol concentration may originate from decreasing maximum catalytic rates of enzymatic reactions involved in the overall growth reaction. For analogous reasons, q_p^{max} and $-q_s^{max}$ will decrease due to product inhibition.

7.3. Influence of ethanol concentration on a_s and m_s

In chemostat experiments at different dilution rates, the slope of the plot of $-q_s$ versus dilution rate gives a_s, and the intercept gives m_s. To study the influence of ethanol concentration on these parameters, several plots are required with each a different, fixed ethanol concentration. For most references given in Table 3, this has been achieved to a reasonable extent by using different glucose feed concentrations. Adding ethanol to the feed has also been used [29,31]. Generally, an increase in a_s and an increase in m_s were found. These trends can be explained by an increased ATP demand for growth and for repair mechanisms, respectively. Besides, less ATP might be produced per catabolized glucose. The individual references in Table 3 use 2–4 different ethanol concentrations, and the slope and intercept from plots of $-q_s$ vs. dilution rate show a relatively high variance. Therefore, it is not probable that a complex dependency of a_s and m_s on ethanol concentration can be extracted from these data.

Instead, a simple dependency was assumed, in which a_s and m_s both increase linearly with ethanol concentration, using the parameters β_a and β_m for a_s and m_s, respectively. Such an m_s dependency has been drawn in Fig. 2, which will be discussed later.

$$a_s = a_{s,0}\left(1+\frac{c_p}{\beta_a}\right) \tag{13}$$

$$m_s = m_{s,0}\left(1+\frac{c_p}{\beta_m}\right) \tag{14}$$

Superscript "0" indicates the value at $c_p = 0$. One can consider β_a and β_m to be the product concentrations at which a_s and m_s, respectively, would have doubled, such that twice as much substrate would be required for growth and maintenance, respectively.

Consequently, Eq. (6) becomes Eq. (15) in case of inhibiting product ($c_p > 0$).

$$-q_s = \mu a_{s,0}\left(1+\frac{c_p}{\beta_a}\right) + m_{s,0}\left(1+\frac{c_p}{\beta_m}\right) \tag{15}$$

Published chemostat studies on glucose to ethanol fermentation, including studies in which the ethanol concentration was systematically varied, were used to assess the validity of incorporating the β-terms in the aforementioned equation. Original steady-state data sets of q_s, μ and c_p were retrieved (WebPlotDigitizer 4.5). Multiple linear regression was

Table 2
Dependency of batch anaerobic fermentation on ethanol addition. Per reference, the number of evaluated rate values is given between curved brackets.

Batch specific rate	Studies showing a linear dependency on ethanol concentration	Studies showing positive curvature	Studies showing negative curvature
μ^{max}	[80](7);[86](21);[31](3); [42](11);[24](19);[46](6); [23](5);[43] (6)	[30](6);[36](8); [87] (10)	[34](43);[33] (14)
q_p^{max}	[86](34);[85](7);[88](7)	[30](6)	

Table 3
Influence of increasing ethanol concentration on a glucose to ethanol fermentation in chemostat experiments, as derived from literature data.

Strain	Used ethanol range (g/L)	Increase in a_s (%)	Increase in m_s (%)	Data source
Z. mobilis ATCC 10988	9–27	~25	~200	[89]
Z. mobilis Z1	49–60	~7	~90	[90]
Z. mobilis Z4	65–80	~5	~4	[39]
Z. mobilis ATCC 29191	10–60	~20	~30	[91]
S. cerevisiae NRRL-Y-132	6–60	~20[a]	[a]	[31]
S. cerevisiae ATTC 4126	4–78	~80[a]	[a]	[29]

[a] Parameters were obtained by plotting data that were not analysed in the original paper. Negative maintenance coefficients, close to zero, were obtained for all four ethanol concentrations studied.

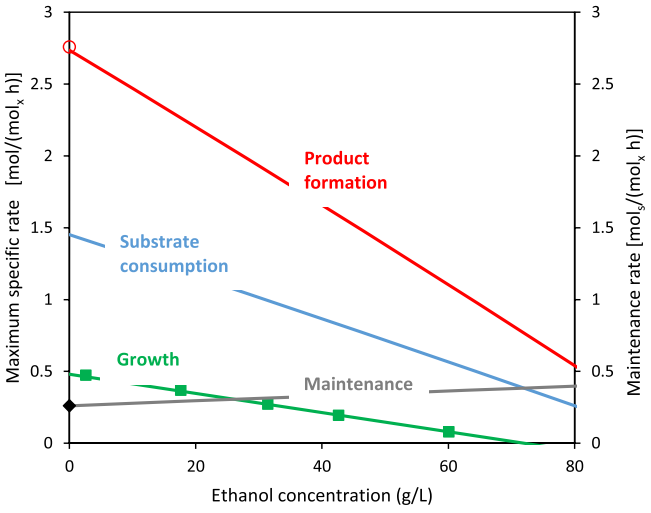

Fig. 2. Dependence of maximum specific rates (for growth, substrate consumption and product formation) and of maintenance rate, calculated for glucose fermentation to ethanol. Lines are calculations using kinetic parameters from Table 5 in Eqs. (1,9,10,13,14,16). Markers are literature data for growth and product formation [24] and for maintenance [91].

performed on models with and without the β-terms. By performing partial F-tests, the *P*-values of Table 4 were obtained. These indicate that in more than half of the cases there is sufficient support (>95% probability) to include the β_a-term, in half of the cases there is sufficient support to include the β_m-term in the model, and in half of the cases there is sufficient support to include both in the model. The references with the lowest support for including the β-terms used a relatively narrow range of μ and c_p values [40] or used strongly correlated μ and c_p values [35] such that the individual influence of these variables on the observed q_s values was unobservable. Therefore, it was decided to progress with Eq. (15) as a reasonable description of chemostat rates, even though not all data provide support for inclusion of the β-terms. Alternative functions might exist that can describe the data better, but finding those will require a deeper study. If such functions are nonlinear, they will complicate process simulation.

8. Relations between the different maximum specific rates and critical concentrations

If no nutrient limits growth, Eq. (15) becomes (cf. Eq. (9)):

$$-q_s^{max} = \mu_0^{max}\left(1-\frac{c_p}{c_p^{crit,\mu}}\right)a_{s,0}\left(1+\frac{c_p}{\beta_a}\right)+m_{s,0}\left(1+\frac{c_p}{\beta_m}\right) \quad (16)$$

Such a dependency of q_s^{max} on c_p is shown in Fig. 2, which will be discussed later. The structure of Eq. (16) is more complicated than Eq. (11). In literature, Eq. (11) is used to determine the product concentration $c_p^{crit,qs}$ at which $q_s^{max} = 0$, but Eq. (11) is not part of the current framework. Instead of assuming Eq. (11), Eq. (16) is used to relate $c_p^{crit,qs}$ and q_s^{max}. This is preferred, because Eq. (16) follows from prior assumptions, unlike Eq. (11). After deriving from Eq. (16) the dependency of q_s^{max} on c_p, one may derive an equation for relating q_p^{max} to c_p using Eq. (10), without having to assume Eq. (12), which is also not part of the current framework. The q_p^{max} equation is not shown because it incorporates the parameters from the element formulas of biomass and ethanol used in Eq. (8), hence it would be different per fermentation case. However, a specific case is plotted in Fig. 2.

Now, as $q_s^{max} = f(c_p) = 0$ when $c_p = c_p^{crit,qs}$, and $q_p^{max} = f(c_p) = 0$ when $c_p = c_p^{crit,qp}$, one can obtain equations in which $c_p^{crit,qs}$ and $c_p^{crit,qp}$ depend on both β_a and β_m. The latter equations can be rewritten to obtain equations in which β_a and β_m depend on both $c_p^{crit,qs}$ and $c_p^{crit,qp}$. Therefore, instead of determining β_a and β_m values from a large series of chemostat experiments, one may determine $c_p^{crit,qs}$ and $c_p^{crit,qp}$ (together with $c_p^{crit,\mu}$) from batch experiments, and subsequently calculate β_a and β_m. Either approach should lead to a complete set of independent parameters, but batch experiments with product addition may be easier than chemostat experiments.

For simulating glucose fermentation to ethanol, a complete set of independent kinetic parameters is required. Table 5 gives a complete set,

Table 4
Evaluation of the statistical significance of including the β-terms in Eq. (15) for describing published chemostat data on ethanolic fermentation. *P*-values above 0.05 indicate that there is less than 95% probability that including the β-term(s) significantly improves the fit.

Data source	*P*-value with β_a term (no β_m term)	*P*-value with β_m term (no β_a term)	*P*-value with β_a term and β_m term
[86]	0.05	0.11	0.14
[29]	0.0004	0.004	0.002
[31][a]	0.004	0.0009	0.005
[39]	0.003	0.36	0.012
[89]	0.0000	0.0000	0.0000
[40]	0.51	0.31	0.62
[91]	0.11	0.038	0.12
[35]	0.66	0.15	0.37

[a] The published data [31] do not include q_s values. They were calculated from these published data.

Table 5
Values of independent parameters used for simulations of glucose fermentation to ethanol by *Z. mobilis* ATCC29191 at 30 °C and pH 5.0 in complex media.

Parameter	Value	Unit	Source
K_s	0.5	g_s/L	[24]
μ_0^{max}	0.48	h^{-1}	[24,91]
$a_{s,0}$	19	g_s/g_x	Calculated in this work from[91]
$m_{s,0}$	2.0	$g_s/(g_x$ h)	Calculated in this work from[91]
$c_p^{crit,\mu}$	72	g/L	[24]
β_a	2700	g_p/L	Calculated in this work from[91]
β_m	154	g_p/L	Calculated in this work from[91]
Ash-free dry biomass formula	$CH1_{.73}O_{0.41}N_{0.23}$	-	[91,92]

originating from experiments at slightly different conditions. As just mentioned, $c_p^{crit,qs}$ and $c_p^{crit,qp}$ could have been given instead of β_a and β_m. One can calculate from the values in Table 5 that $c_p^{crit,qs} = 97$ g/L and $c_p^{crit,qp} = 99$ g/L. The literature data in Section 3 already indicated that $c_p^{crit,\mu} < c_p^{crit,qs} < c_p^{crit,qp}$.

These parameters allow calculation of values of maximum specific rates as function of inhibiting product concentration, using Eqs. (1,9,10,13,14). Fig. 2 illustrates how inhibition decreases maximum specific rates. While the ethanol concentration increases from zero to the critical value for growth ($c_p^{crit,\mu} = 72$ g/L) in this simulated case, the substrate use for maintenance increases from 0.26 to 0.38 $mol_s/(mol_x$ h), equalling 2.0–2.9 $g_s/(g_x$ h). At the $c_p^{crit,\mu}$ value, all consumed substrate is used for maintenance, since growth stops, whereas at $c_p = 0$ only 18% of the consumed substrate is used for maintenance. For the used parameter set, a_s hardly increases with ethanol concentration (not plotted). Therefore, there is hardly any curvature visible in the plots of substrate consumption and product formation, such that Eqs. (11) and (12) happen to be useful approximations in this case.

At ethanol concentrations beyond $c_p^{crit,\mu}$, Eq. (1) predicts negative growth (not shown in Fig. 2), which might be considered as death rate. Biomass might be metabolized for maintenance use if there is no longer sufficient substrate consumption to cover the maintenance requirement. Because of biomass wash-out, such ethanol concentrations beyond $c_p^{crit,\mu}$ cannot be reached in a chemostat; they might be reached in batch mode, or in a continuous culture with cell recycle. However, it is not clear if the model will describe kinetics beyond $c_p^{crit,\mu}$ realistically, because metabolism such as occurring at minimal energy [93] has been kept out of scope.

9. Course of batch and chemostat fermentations

Mass balances of batch and continuous reactor systems were solved in conventional ways using kinetic Eqs. (6) and (7), in which μ^{max}, a_s and m_s depend on product concentrations according to Eqs. (1, 13, 14). With ethanol as the only catabolic product, the rate of ethanol production was obtained from the reaction stoichiometry. The results are shown in Figs. 3–4.

At the feed glucose concentration of the batch simulation of Fig. 3 (0.55 mol/L), inhibition by the gradually produced ethanol (47 g/L) is modest, because during most of the batch the ethanol concentration is much lower than its critical concentration for growth (72 g/L). To validate the product inhibition part of the batch model, additional independent experimental data with the same strain at comparable conditions are required. However, published batch data were either at initial glucose concentrations too low for observing an effect of the produced ethanol, or at initial concentrations so high (>0.6 mol/L glucose [24]) that substrate inhibition occurred, thus complicating validation.

For the same conditions, Fig. 4 illustrates the dependence of

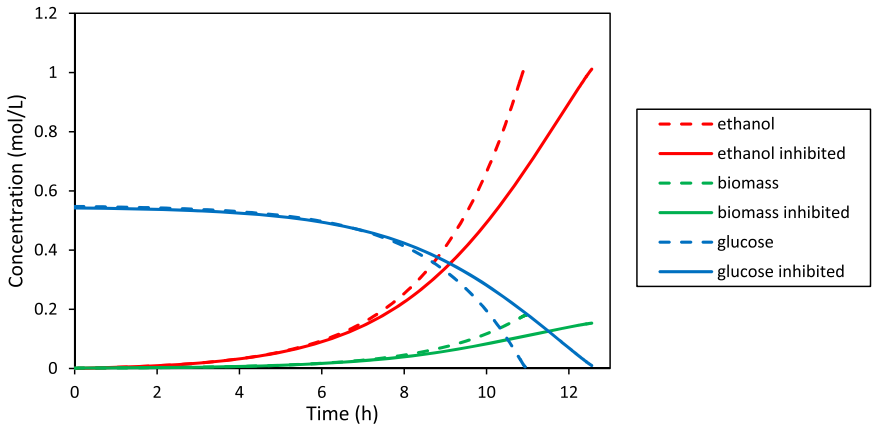

Fig. 3. Calculated batch reaction progress for glucose fermentation to ethanol, using the model given in the text, the parameters of Table 5, $c_{s,feed}$ = 0.542 mol/L, and $c_{x,feed}$ = 1 mmol/L. Dashed lines indicate the situation if product inhibition would not occur.

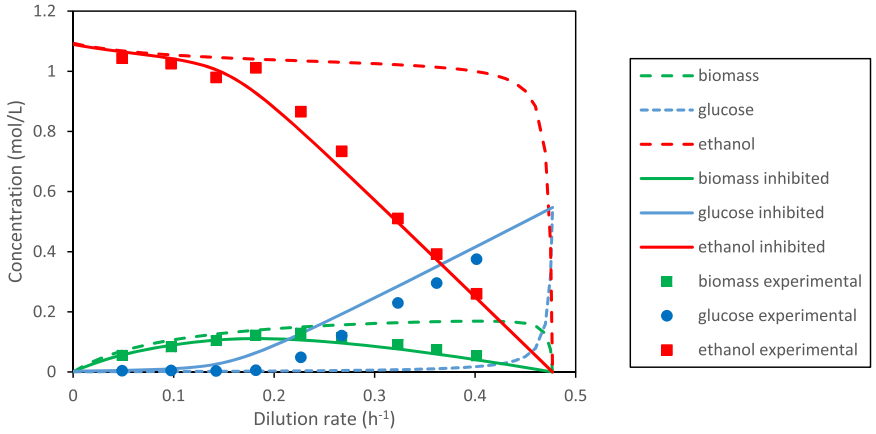

Fig. 4. Chemostat concentrations for glucose fermentation to ethanol for $c_{s,feed}$ = 0.542 mol/L. Markers are experimental data [91]. Full lines are simulations using the model given in the text with the parameters of Table 5. Dashed lines indicate the simulated situation if product inhibition would not occur.

concentrations on dilution rate in a chemostat. In the absence of inhibition, available substrate would be almost completely converted until the dilution rate approaches the maximum growth rate. However, due to product inhibition, less substrate is converted into biomass and product, especially at high dilution rates. Unconverted glucose leaves the chemostat. The plots for the inhibited case are in line with experimental data [91] that were used for estimation of some of the model parameters, and resemble experimental data for related cases [31,39].

The literature has been confused by plots of μ vs. c_p for chemostat studies [39,91]. In chemostats, μ equals the imposed dilution rate and consequently does not depend on the achieved product concentration. On the contrary, c_p depends on μ. Since μ equals the dilution rate in a chemostat, there is no linear dependency between μ on c_p in Fig. 4. At dilution rates below 0.15 h^{-1}, growth is substrate-limited. For high dilution rates, though, $c_s >> K_s$, such that $q_s = q_s^{max}$ and $\mu = \mu^{max}$. The latter depends on c_p according to Eq. (1). Therefore, the ethanol line of Fig. 4 has a diagonal part at dilution rates beyond 0.2 h^{-1}. Extrapolation of that line to zero dilution rate would lead to c_p = 1.57 mol/L = 72 g/L = $c_p^{crit,\mu}$. This implies that the $c_p^{crit,\mu}$ value found in batch experiments can also be found by plotting μ vs. c_p for a series of chemostat experiments at different dilution rates [39,91], provided that a significant part of the substrate concentration remains unconsumed ($c_s >> K_s$).

This case illustrates that product inhibition models for use in various operation mode need quite some experimental data sets for training and validation, while taking into account observability [94]. Obviously, product inhibition parameters are better observable at high product concentrations. These occur toward the end of batch experiments with high initial substrate concentrations; in chemostat experiments with high substrate feed concentrations; and in batch or chemostat experiments when product has been added to the feed.

Non-chemostat continuous ethanol fermentations, for example involving cell retention [35,37] or in-situ product removal [95], will show different features than chemostats and require a specific evaluation.

10. Growth inhibition patterns for other fermentations

Trends seen with the glucose to ethanol fermentation can also be found in reports for other fermentations. For example, $c_p^{crit,\mu} < c_p^{crit,qp}$ [59,62,66,70]. Frequently, a linear dependence of μ^{max} on inhibiting product concentration is an adequate model, but also many publications contain data that are described better by another model, such as the other ones shown in Fig. 1. When more complicated dependencies on product concentrations are used than in the previous sections, the unstructured kinetic model can still be used as framework.

Situations really different from the glucose to ethanol fermentations can be expected in case of fermentation leading to multiple inhibiting products, or in case of inhibiting anabolic product.

In case multiple inhibiting products are formed (P1 and P2, for example), they might independently contribute to the inhibition such that their effects are additive, as described by:

$$\mu^{max} = \mu_0^{max}\left(1 - \frac{c_{p1}}{c_{p1}^{crit,\mu}} - \frac{c_{p2}}{c_{p2}^{crit,\mu}}\right) \quad (17)$$

Instead, it is more common to multiply inhibition terms per product, implying that the products strengthen each other's inhibition [56,59]:

$$\mu^{max} = \mu_0^{max}\left(1 - \frac{c_{p1}}{c_{p1}^{crit,\mu}}\right)\left(1 - \frac{c_{p2}}{c_{p2}^{crit,\mu}}\right) \quad (18)$$

Similarly, a_s and m_s will depend in some way on concentrations of multiple products, but no systematic research on this topic was found. Another complication is that the additional product has to be added to the stoichiometric equation, such that elemental balances do not allow anymore to obtain q_p from q_s and μ, such as was the case with Eq. (10). An additional assumption will be required to model such a fermentation.

In case of anabolic products, i.e. those requiring ATP for their formation, an additional term should be added to the Pirt equation (Eq. (6)) to account for substrate use for product formation. Again, at least one extra kinetic parameter will be involved, and it will depend in some way on the concentration of inhibiting product. Obviously, inhibition will slow down the specific rate of formation of the anabolic product, and potentially increase the amount of substrate required for this production. Since an electron acceptor such as O_2 will be involved, also in this case the stoichiometric coupling between rates will become more complicated than merely Eq. (10), and q_p^{max} becomes an independent kinetic parameter.

11. Conclusions and outlook

The dependency of product inhibition on microbial strain seems much more modest than the well-known dependencies on hydrophobicity of the product and, in case of dissociating products, pH. This triggered merging datasets on fermentations that are inhibited by ethanol.

To model product inhibition in fermentation, it is recommended to use the Pirt equation, the hyperbolic substrate uptake equation, and the stoichiometric relation between maximum specific rates. Parameters of these equations depend on the product concentration if the product is inhibiting. Finding these dependencies is required for a consistent product inhibition model. The simplest dependency, which is in line with many product inhibition studies, assumes that μ^{max} decreases linearly to zero with increasing c_p. If, for simplicity, a_s and m_s are assumed to increase linearly with product concentration, a complete model is obtained in case a fermentation leads to a catabolic product. This should facilitate model-based optimization of bioreactor operation for such fermentations.

Still, product inhibition models remain a weak part of fermentation models, because insufficient experimental data are available to obtain values of product inhibition parameters. One can expect a sharp rise, though, in the amount of data and data analysis in this field, because high-throughput experimentation and machine learning have become common research tools. Using statistical tools for parameter estimation and model discrimination will have to become standard practice in the field. It is likely that empirical product inhibition equations will remain prominent because of the diverse mechanisms that cause product inhibition.

Funding

This research received no external funding.

Declaration of Competing Interest

The author declares that he hasve no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data Availability

Excel and Matlab files with source data and calculations are available at 4TU.ResearchData at https://doi.org/10.4121/21762986.

Acknowledgments

Robbert Kleerebezem provided useful comments and Stan van der Lans critically reviewed fitting procedures of μ^{max}.

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