Contents lists available at ScienceDirect

Food and Bioproducts Processing

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

Application of a dynamic metabolic flux algorithm during a temperature-induced lag phase

*D. Vercammen, D. Telen, P. Nimmegeers, A. Janssens, S. Akkermans, E. Noriega Fernandez, F. Logist, J. Van Impe**

KU Leuven, Department of Chemical Engineering, BioTeC+ & OPTEC, Gebroeders De Smetstraat 1, 9000 Ghent, Belgium

ARTICLE INFO

Article history:
Received 20 May 2016
Received in revised form 22 September 2016
Accepted 11 October 2016
Available online 14 November 2016

Keywords:
Dynamic metabolic flux analysis
B-spline parameterization
Experimental validation
Predictive microbiology
Dynamic optimization
Lag phase

ABSTRACT

In predictive microbiology, the (induced) lag-phase is a phenomenon of specific interest, as it has a large impact on the assessment of safety and quality of food products. This lag phase has been studied mostly on a macroscopic level. However, a quest for more mechanistically-based predictive models has started, for example, through the integration of a metabolic reaction network into widely used macroscopic model structures. This multi-scale modeling approach is called dynamic metabolic flux analysis (dMFA). In this contribution, a recently developed algorithm for dMFA is used to estimate the metabolic fluxes in *Escherichia coli* K12 during an experimentally induced lag phase through a sudden shift in temperature. To study this phenomenon, controlled bioreactor experiments were performed: on the one hand at a fixed and optimal temperature for growth (37 °C), and on the other hand starting at 20 °C, with a sudden temperature shift to 37 °C during the exponential growth, inducing an intermediate lag phase. The evolution of biomass and metabolite concentrations was monitored during these experiments. After dMFA analysis of the gathered measurements, some interesting patterns in metabolic activity during the different growth phases are revealed. The described case study is a first practical test case to assess the capabilities of the recently developed dMFA methodology in an experimental predictive microbiology setting.

1. Introduction

The concept of predictive microbiology entails that a detailed knowledge of the behavior, i.e., growth, survival and inactivation, of microorganisms in food products can be expressed in mathematical models, which enable an objective evaluation of the microbiological safety and quality of foods (McMeekin et al., 1997). An important phenomenon studied in predictive microbiology is the *lag phase*, which is a period in which no growth occurs as microorganisms have to adapt to a (sudden) change in environmental conditions. This can happen because of inoculation of the organism in a new medium, in which case the phase is called *initial lag*, or due to a change in one or more environmental variables, e.g., temperature or pH, during an exponential growth phase, in which case the period is called an *intermediate* or *induced lag phase*. Because of the frequent changes in environmental conditions taking place during the production, distribution and consumption of food products, a good understanding of the lag phase is of vital importance for the assessment of microbial safety and quality of food products. Once the influence of environmental conditions on the occurrence and the length of the lag phase is determined, shelf-life of food products can be determined more accurately, and strategies can be developed to inhibit the growth of microorganisms by keeping them in this lag phase for extended periods of time.

For these reasons, the lag phase has been studied extensively in recent years in predictive food microbiology (Swinnen

* *Corresponding author.*
E-mail address: jan.vanimpe@kuleuven.be (J. Van Impe).
http://dx.doi.org/10.1016/j.fbp.2016.10.003
0960-3085/

Nomenclature

List of symbols and abbreviations

3PG	3-phosphoglycerate
6PG	6-phosphogluconate
AA	amino acid
Ac	acetic acid
AcCoA	Acetyl-Coenzyme A
AIC	akaike information criterion
AIC_c	corrected AIC criterion value
AKG	alpha-ketoglutarate
ATP	adenosine triphosphate
Ala	alanine
Arg	arginine
Asn	asparagine
Asp	aspartate
CFU	total viable plate count (colony forming units)
Cit	citrate
c_{in}	vector of inlet tank concentrations
c_{int}	vector of intracellular metabolite concentrations
CTR	carbon dioxide transfer rate
Cys	cysteine
d	number of free fluxes
DHAP	dihydroxyacetone-phosphate
dMFA	dynamic metabolic flux analysis
DW	dry weight
E4P	erythrose-4-phosphate
F	objective function
F^{in}	incoming gas flow rate
F6P	fructose-6-phosphate
FBP	1,6-fructose-biphosphate
FTHF	formyltetrahydrofolate
Fum	fumarate
g	number of internal spline knots
G3P	glyceraldehyde-3-phosphate (G3P)
G6P	glucose-6-phosphate
GAP	glyceraldehyde-3-phosphate
Gln	glutamine
Glu	glutamate
Gluc	glucose
Gly	glycine
His	histidine
HPLC	high performance liquid chromatography
Icit	isocitrate
Ile	isoleucine
$\mathbf{I}$	identity matrix
$\mathbf{I}_{irr}$	irreversibility matrix
$\mathbf{K}$	basis for the null space of the intracellular stoichiometric matrix
k	spline degree
l	minimum number of time points for starting the free flux estimation
L-DAP	L-diaminopimelate
Leu	leucine
Lys	lysine
Mal	malate
METHF	methyltetrahydrofolate
MEETHF	methylentetrahydrofolate
Met	methionine
m	total number of metabolites/concentration states
m_{ext}	number of extracellular metabolites
m_{ij}	average measurement for output j at time point t_i
m_{int}	number of intracellular metabolites
n	total number of reactions
NAD	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
n_g	total number of spline knots over all free fluxes
n_{irr}	number of irreversible reactions
n_{meas}	number of measurements
n_{out}	number of outputs
n_p	number of parameters
n_{rev}	number of reversible reactions
n_{time}	number of measurement time points
n_x	number of states
n_y	number of outputs
OAC	oxaloacetate
OUR	oxygen uptake rate
$\mathbf{p}$	parameter vector
Phe	phenylalanine
Pro	proline
P5P	pentose-5-phosphate
PEP	phosphoenolpyruvate
PP	pentose phosphate
Pyr	pyruvate
p_u	vector of spline parameters
$\mathbf{q}_{bio}$	biomass selection vector
R5P	ribose-5-phosphate
RID	refractive index detector
Ru5P	ribulose-5-phosphate
$\mathbf{S}$	full stoichiometric matrix
S7P	seduheptulose-7-phosphate
Ser	serine
Suc	succinate
SucCoA	succinyl-coenzyme A
$\mathbf{s}_{bio}$	row of the stoichiometric matrix corresponding to the biomass pseudometabolite
$\mathbf{S}_e$	combined extracellular and biomass stoichiometric matrix
$\mathbf{S}_{ext}$	rows of the stoichiometric matrix corresponding to extracellular metabolites
σ_{ij}	measurement standard deviation for output j at time point t_i
$\mathbf{S}_{int}$	rows of the stoichiometric matrix corresponding to intracellular metabolites
T	temperature
TA	transaldolase
TCA	tricarboxylic acid
Thr	threonine
TK	transketolase
Trp	tryptophane
Tyr	tyrosine
t_0	initial time
t_f	final time
t_{knot}	vector containing the knot locations for each free flux
$\mathbf{u}$	vector of free fluxes
V	reactor volume
V_m	ideal gas standard molar volume
$\mathbf{x}$	vector of (concentration) states
$\mathbf{x}_0$	vector of initial values for the states