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Dynamic metabolic modelling of volatile fatty acids conversion to polyhydroxyalkanoates by a mixed microbial culture

Research Paper

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In this work, we present a dynamic metabolic model that describes the uptake of complex mixtures of volatile fatty acids (VFA) and respective conversion into PHA by mixed microbial cultures (MMC). This model builds upon a previously published flux balance analysis model [1] that identified the minimization of TCA cycle activity as the key metabolic objective to predict PHA storage fluxes and respective composition. The model was calibrated either with experimental data of PHA production from fermented sugar cane molasses or from synthetic mixtures of VFA. All PHA production experiments were performed using a MMC selected with fermented sugar cane molasses under feast and famine regimen. The model was able to capture the process dynamics denoted by an excellent fit between experimental and computed time profiles of concentrations with the regression coefficients always above 0.92. The introduced VFA uptake regulatory factor reflects the decrease of acetyl-CoA and propionyl-CoA available to TCA cycle in conformity with the hypothesis that the minimization of TCA cycle is a key metabolic objective for MMC subjected to feast and famine regimen for the maximization of PHA production.

Background

Polyhydroxyalkanoates (PHA) are biopolymers that fulfil the requirements of a green bioplastic as opposed to conventional petroleum-based plastics. Their physico-chemical properties are similar to polypropylene and polystyrene with the advantages of being biodegradable, biocompatible and produced from renewable sources.

Nowadays, commercial PHAs are produced using pure or recombinant cultures and well-defined synthetic media with single substrates at high production costs. The use of waste materials as substrate is a strategy to decrease PHA production costs pursued by many researchers [1 and reviewed in 2] and [3]. Several authors investigated different reactor configurations and operating conditions [4–7], while others focused on waste-based substrates and their influence in the process and/or in the PHA properties [8–11]. Other studies addressed the microbial characterization of the MMC [12–14].

Mathematical modelling of PHA production by MMC has also received considerable attention [15–18]. Over the past years the complexity of models has increased considerably. Beun and co-workers [19] developed the first consistent metabolic model, stemming from [20,21], which was able to describe polyhydroxybutyrate (PHB) production from acetate. Other studies [15,17] proposed modifications of [18] but always for the single substrate scenario.

The production of co-polymers of PHA, namely P(HB-co-HV), is of high interest because a more complex substrate could be used and its mechanical properties are better or similar than the homopolymer PHB [8,22]. Dias *et al.* [16] extended their previous single substrate (acetate) model for the case of two substrates (acetate + propionate) to describe the production of P(HB-co-HV) co-polymers. Later on, Jiang *et al.* [18] improved the model [15] to describe cell growth and co-polymers production simultaneously.

Waste-based feedstocks normally have complex composition and comprise several substrates that can be metabolized by MMC

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GLOSSARY

Φ Regulation factor
Φ′ Regulation factor affecting substrates ($\Phi' = \Phi$)
Φ″ Regulation factor affecting products ($\Phi'' = 1/\Phi$)
Ac acetate
ATP Adenosine-5′-triphosphate
But Butyrate
DOC Dissolved organic carbon
E_FA Fatty acids with even number of carbon atoms
FA Fatty acids
f_i Intracellular PHA, PHB and PHV contents (C-mol/C-mol)
$f_{PHA,max}$ Maximum intracellular PHA contents (C-mol/C-mol)
h consistency index
HB Hydroxybutyrate
HV Hydroxyvalerate
K_N Ammonia half-saturation constant (N-mmol/L)
K_S Substrate half-saturation constants (C-mmol/L)
m_{ATP} Maintenance on ATP
MMC Mixed microbial cultures
$NADH_2$ Reduced form of nicotinamide adenine dinucleotide (phosphate)
O_FA Fatty acids with odd number of carbon atoms
P/O ratio, δ Oxidative phosphorylation efficiency (mol-ATP/mol-$NADH_2$)
PHA Polyhydroxyalkanoates
PHB Polyhydroxybutyrate
PHV Polyhydroxyvalerate
Prop Propionate
r Vector of metabolic fluxes
$r_{i,max}$ Maximum specific rate of reaction on compound *i* [C-mol/(C-mol h)]
r_i Specific rate of reaction on compound *i* [C-mol/(C-mol h)]
SBR Sequencing batch reactor
TCA Tricarboxylic acid
TOC Total organic carbon
Val Valerate
VFA Volatile fatty acids
α Saturation order constant
χ^2 Chi-square distribution

to PHA. Currently, there is no dynamic metabolic model that describes multiple VFA conversion to PHA. Our previous model [16] cannot be directly extended for more than two VFA. With that model structure, more than 2 VFAs would result in an undetermined system of material balances, which would require the modelling of additional kinetic laws for VFAs uptake. In a previous study, Pardelha *et al.* [1] applied flux balance analysis (FBA) to describe the conversion of complex mixtures of volatile fatty acids (VFA) to PHA, namely fermented sugar cane molasses. FBA is a constraint-based modelling approach that can be applied to underdetermined metabolic networks based on the optimization of a predefined objective function under the constraints of reactions stoichiometry, reactions irreversibility and experimental flux data. On the basis of published results about PHA regulation (summarized in [1]), the objective function defined was the minimization of TCA cycle fluxes to optimize the carbon flux available for PHA production. This approach enabled to accurately predict PHA storage fluxes and also the respective HB:HV composition, from measurements of VFA uptake fluxes. This result corroborates the hypothesis that MMC selected under the feast and famine feeding regimen maximizes carbon usage for PHA storage.

FBA is a static modelling method that cannot be used to predict PHA production dynamics. The aim of the present study is to extend the previous FBA model to a dynamic formulation able to describe the uptake of complex mixtures of volatile fatty acids (VFA) and respective conversion to PHA along batch time. To accomplish this, the minimization of TCA cycle activity [1] was implemented through a regulation factor that controls the flux of carbon through the main metabolic pathways [1]. The definition of this regulation factor was based on the cybernetic approach proposed by Venkatesh *et al.* [23] and Ramakrishna *et al.* [24] for multiple substrates uptake regulation.

Metabolic model

Metabolic network

The metabolic network adopted in this study is schematized in Fig. 1. VFA molecules (divided as VFA with odd or even number of carbons) are assimilated by the cells through active transport (1 mol ATP/1 mol VFA) [25] following their activation to acyl-CoA molecules. Simpler VFA (acetate and propionate) are activated directly to acetyl-CoA and propionyl-CoA; meanwhile the remaining VFA pass through β-oxidation pathway to be converted to acetyl-CoA and propionyl-CoA with consumption of one more ATP mol. This assumption holds because net material, energetic and reducing power balances are identical for both pathways available (details in [1]). These reactions are included for each VFA, namely R_{Ac}, R_{But}, R_{Prop}, R_{Val}, R_{E_FA} and R_{O_FA}.

Furthermore, one needs to consider the portion of the propionyl-CoA that is converted to acetyl-CoA (R_3).

Acetyl-CoA, produced via VFA activation or propionyl-CoA decarboxylation, is catabolised to CO_2 through the TCA cycle (R_4) [25]. Additionally to the reactions described above, cell maintenance also requires energy (R_5). The production of energy occurs via oxidative phosphorylation and the amount of ATP generated per mole of $NADH_2$ oxidized is expressed by the P/O ratio, δ, which represents the efficiency of oxidative phosphorylation (R_6).

Acetyl-CoA and propionyl-CoA are converted into PHA monomers (acetyl-CoA* and propionyl-CoA*, respectively) according with reactions R_7 and R_8. Then, they are condensed to form the biopolymer (R_{PHB} and R_{PHV}).

For a detailed description of all reactions see Pardelha *et al.* [1] and Dias *et al.* [16].

Connectivity, thermodynamic and kinetic constraints

Similarly with previous published work [1], the metabolic network is defined by 10 + k reactions (k stands for acetate, propionate, butyrate, valerate, even and odd unknown carbon sources (E_FA and O_FA)), 6 intracellular metabolites (acetyl-CoA, propionyl-CoA, acetyl-CoA*, propionyl-CoA*, $NADH_2$, ATP), 2 + k input substrates (O_2, ammonia, all carbon sources) and 4 end-products (biomass, PHB, polyhydroxyvalerate (PHV) and CO_2) (Table A1). The steady-state material balances for the 6 intracellular metabolites (represented by a system of algebraic equations published in [1]) were considered as connectivity constraints.

All metabolic reactions but R_9 and R_{10} are irreversible. These two reactions comprise the β-oxidation pathway of longer chain VFA into acetyl-CoA and propionyl-CoA. In case of PHA precursors