Multiphase compartment modelling and systems identification of a U-loop reactor for continuous single cell protein production

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Abstract

In this study, a multiphase compartment model for U-loop bioreactors is proposed and applied to a 5.5 [] pilot scale U-loop. The developed compartment model that includes the material balance of multiple compounds in the gas and liquid phases as well as interfacial mass transfer is coupled with the microbial biokinetics to simulate the fermentation process. The model inputs and outputs are identified in terms of control systems, and the model is linearized around industrially relevant operating conditions. The linearized system is identified in terms of relative gain and a linear state-space model is fitted.

**Keywords**: Compartment model, Multiphase, Bioreactor, Process control.

* 1. Introduction

Single Cell Protein (SCP) stands as a promising alternative to traditional protein sources to meet the growing global demand for sustainable and high-quality proteins. With a growing world population and increasing environmental concern, the exploration of alternative protein sources has gained significant attention. SCP offers an attractive solution, using cheap and available energy and carbon sources, and having a relatively low environmental footprint compared to traditional protein sources (animal, vegetal) [1].

It has been shown that SCP can be produced through the fermentation of *Methylococcus* *Capsulatus*, using methane as the main carbon source [2]. Although SCP has the potential to be used as a more sustainable protein source, the manufacturing of such proteins presents some engineering challenges related to the complex metabolism of methanotrophic microorganisms [3], and the high heat and mass transfer demand, that cannot be reached using conventional bioreactors [2]. Recent work has provided insight into the complex reaction kinetics through the study of cometabolic processes and full genome scale metabolic model of the microorganism [4,5], and it has been shown that U-loop bioreactors can be used to overcome the heat and mass transfer limitations [6,7]. The operation of continuous large-scale productions is, however, limited by the complex process dynamics arising from the inhomogeneous process conditions in a U-loop reactor.

This study presents a dynamic numerical model of *Methylococcus Capsulatus* cultivation in a U-loop reactor, including a reduced biokinetic model of the microorganism and a gas-liquid hydrodynamic model of a U-loop. The purpose is to use the combined model to simulate the complex process dynamics and understand how to maintain the desired operating conditions during continuous production.

* 1. Materials & Methods
     1. Biological Model

The applied biological model is a simplified Monod type kinetic model describing the growth rate accounting for the substrate limitations. The production and consumption rates of products and substrates determined from the reaction yields. The overall reaction is described in eq. (1), and the microbial specific growth rate mu in eq. (2).

|  |  |
| --- | --- |
|  | (1) |
|  | (2) |

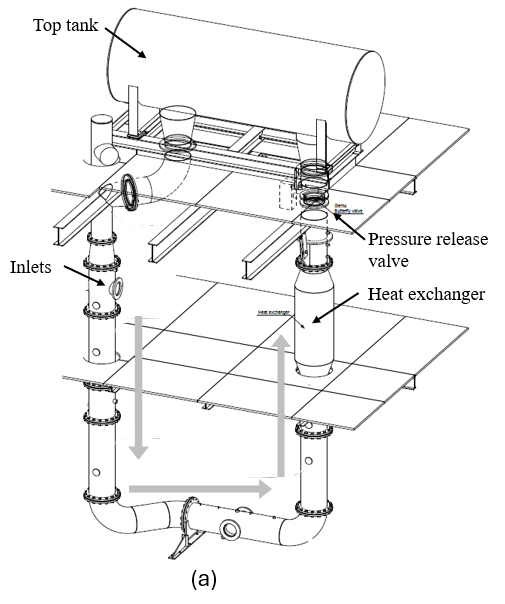
The volumetric rates of formation of the different species involved in the reaction can be expressed as shown in eq. (3). The model parameters are summarized in Table 1.

|  |  |
| --- | --- |
| ; ; ; ; | (3) |
| *Table 1: Biokinetic model parameters* |  |
| |  |  |  | | --- | --- | --- | | Parameter | Value | Unit | |  | 0.37 | [] | |  | 9.6e-6 | [] | |  | 2.09e-5 | [] | |  | 5.274e-4 | [] | |  | 0.651 | [] | |  | 1.995 | [] | |  | 2.099 | [] | |  | 0.106 | [] | |  |

* + 1. Compartment Modelling

Compartment models are a way of representing volumes as discretized regions with known volumes and exchange flow rates. The compartment models can be used to carry out dynamic simulations of the mixing between compartments. The purpose is to include the gas-liquid hydrodynamics and the inhomogeneous conditions of the U-loop reactors to the final model. To this extent, a multiphase compartment mode including interfacial mass transfer is required. A simplified sketch of a U-loop reactor is shown in figure 1 (a). The top tank (or degassing tank) is considered as a single compartment, and the loop section is discretized along the length (assuming plug flow). Gas and liquid inlets are located in the down-going leg, and the outlets are all located in the top tank. The pressure profile in the loop is determined from the height, as well as the contribution from the pump and pressure release valve. For each compartment, a mass balance of the gas and liquid phases is performed to solve for the gas and liquid composition. The volume fraction of each phase in each compartment is determined based on the relative volumetric flowrates of gas and liquid entering the compartment and the assumed superficial gas velocity according to eq. (4). The respective volume fractions are then determined by solving the system of equations (5).

|  |  |
| --- | --- |
|  | (4) |
|  | (5) |

A diagram of a machine

Description automatically generatedWhere and are respectively the liquid and gas volumetric flow rates, is the cross sectional area of the loop section, is the superficial gas velocity and and are the liquid and gas volume fractions, respectively. The bulk liquid phase is assumed to be in steady-state, so the total mass balance in compartment can be expressed as shown in eq. (6). The specie material balance for component in the liquid phase is expressed as eq. (7) including a reaction term and a mass transfer term. The material balance in the gas phase in compartment (eq. (9)) is expressed as function of the gas residence time in each compartment, corresponding to the gas velocity divided by the length of the compartment , (eq. (8)). It is assumed that no reactions are taking place in the gas phase.

|  |  |
| --- | --- |
|  | (6) |
|  | (7) |
|  | (8) |
|  | (9) |

Where is the liquid concentration of component in compartment , and the molar amount of component in compartment , is the volumetric rate of formation of component in compartment , and is the interfacial mass transfer rate of component in compartment . The biomass is assumed to stay in the liquid phase; therefore the mass transfer term is set to zero. Static pressure is assumed to be the main contributor to the overall pressure profile in the loop, so the pressure is set by the height of the liquid added to the pressure created by the pump and valve located respectively at the beginning and at the end of the loop. The pressure in the top tank is assumed atmospheric. The pressure drop along the loop due to friction with the pipe wall is assumed negligible, however, the pressure drop related to the static mixers located in the loop are added manually based on the position, size, and pressure drop of each mixer. A constant temperature of 30°C is assumed throughout the reactor. The interfacial mass transfer rates are expressed in eq. (10) according to the Film Theory [8].

*Figure 1: (a) Sketch of the pilot-scale U-loop (grey arrows show the direction of the flow in the loop), (b) Sketch of the multiphase compartment model of the U-loop.*

|  |  |
| --- | --- |
|  | (10) |

Where is the volumetric mass transfer coefficient in compartment , and the saturation concentration of component in compartment . The is assumed constant throughout the loop, more advanced models may be implemented in further work. The saturation concentration of each component is expressed in eq. (11) and the Henrys coefficients according to [9].

|  |  |
| --- | --- |
|  | (11) |

Where is the partial pressure of component in compartment , based on the relative amount of component in the gas phase, and the total pressure in compartment . The resulting system of ordinary differential equations (one balance equation for each component for each compartment for each phase, excluding the biomass balance equation in the gas phase) is solved in Python using the *solve\_ivp()* function from the *scipy.integrate* library, with the LSODA solver. Initial conditions are reported in table 2.

*Table 2: Initial conditions for the numerical model.*

|  |  |  |
| --- | --- | --- |
| Component | Initial value | Unit |
|  | 20 | [] |
| ; | 70 ; 0.1 | [] ; [mol] |
| ; | 1e-3 ; 7e-3 | [] ; [mol] |
| ; N | 1 ; 3e-3 | [] ; [mol] |
| ; | 1e-5 ; 1e-8 | [] ; [mol] |

The multiphase compartment model is constructed for a 5.5 [] pilot-scale U-loop, with a liquid height of 6 [] and a loop pipe diameter of 0.4 []. The system is discretized into 51 compartments (1 for the top-tank, and 50 for the loop).

* + 1. Systems Identification

Based on the main actuators, and the key process conditions to control, a list of 6 inputs and 6 outputs is identified and reported in table 3. The nominal input values are taken from industrial operating conditions. At first, a simulation with the given initial condition is performed over 200 h simulation time, to determine the steady-state output values. The local steady-state sensitivity of the outputs to changes in the inputs is quantified using the Monte Carlo approach. A set of 5000 input values are randomly sampled from normal distributions around the nominal value with standard deviation of 5%. For each set of input values, the steady-state responses of the model are evaluated. Finally, a linear model is fitted on the generated input/output data, normalized relative to the nominal values.

*Table 3: Inputs and outputs considered in the systems identification.*

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | # | Symbol | Nominal Value | Unit |
| Inputs |  |  |  |  |
| Gas inlet flowrates | Input 1 |  | 6.033e-3 | [] |
|  | Input 2 |  | 1.666e-3 | [] |
|  | Input 3 |  | 7.083e-3 | [] |
|  | Input 4 |  | 3.333e-4 | [] |
| Liquid inlet flowrate | Input 5 |  | 1.000e-4 | [] |
| Liquid circulation rate | Input 6 |  | 1.256e-1 | [] |
| Outputs |  |  |  |  |
| Liquid concentrations in the top-tank | Output 1 |  | 1.763e+1 | [] |
|  | Output 2 |  | 8.243e-6 | [] |
|  | Output 3 |  | 3.417e-2 | [] |
| Offgas component flowrates | Output 4 |  | 1.599e-3 | [] |
|  | Output 5 |  | 2.676e-3 | [] |
|  | Output 6 |  | 2.558e-3 | [] |

* 1. Results and Discussion

The nominal steady-state output values are reported in table 3. The standardized regression coefficients are represented in figure 2. In can be noted that the inlet flowrate has the largest impact om the model outputs. The magnitude of the regression coefficients can be used to pair the inputs to the outputs in a control structure. The numerical model is used to determine the coefficients for a linear state-space model around the nominal operating point. Perturbations in the inputs and the states allow for quantification of the linear model coefficients in the matrices , and (eq. (12)).

|  |  |
| --- | --- |
|  | (12) |

A diagram of a computer component

Description automatically generated with medium confidenceWhere is the normalized state vector, is the derivative of the normalized state vector, is the normalised input vector and the normalized output vector. For the investigated system, the total of 459 states made the computation of the observability and controllability matrices infeasible computationally. The implementation of a reduced order state space model (with less states) e.g. using the eigen value realization algorithm [10], the balanced truncation or the balanced proper orthogonal decomposition [11] method could allow for the computation of the observability and controllability matrices. Although the pressure is taken into account for calculating the solubility of the gaseous compounds, further work will focus on implementing more advanced models for the , to account for the changing bubble sizes and mass transfer coefficient along the loop.

*Figure 2: Standardized regression coefficient (log10 of the absolute value)*

* 1. Conclusions

The proposed multiphase compartment model was used to simulate the *Methylococcus Capsulatus* fermentation for single cell protein production. The coupling of the microbial biokinetic model with the gas-liquid hydrodynamic model made it possible to simulate the effects of mass transfer and mixing limitations on biological growth. Industrially relevant inputs and outputs are identified, and the numerical model was linearized around nominal operating conditions. The linear model may be used in further work to develop and tune control systems and model-based monitoring for the fermentation process. Monitoring and control are key to maintaining desired process conditions, making continuous production possible. Optimal control is also crucial for reproducibility of the process and ensuring “Quality by Control”.

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