Biochemical Engineering Journal 128 (2017) 168–177

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Regular article

Microrespirometric model calibration applied to wastewater processes

Miguel Vital-Jacome [a], Denis Dochain [b], Frederic Thalasso [a,*]

[a] Department of Biotechnology and Bioengineering, Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (Cinvestav-IPN), Av. IPN 2508, 07360 Ciudad de México, México
[b] ICTEAM Institute, Université catholique de Louvain, 4 Avenue Georges Lemaître, Louvain-la-Neuve, Belgium

ARTICLE INFO

Article history:
Received 16 May 2017
Received in revised form 22 August 2017
Accepted 1 October 2017
Available online 4 October 2017

Keywords:
Microrespirometry
Microreactor
Microbioreactor
Kinetics
Activated sludge
Parameter estimation

ABSTRACT

Microrespirometry is a recently developed method that combines classical respirometry and microreactor systems, for the characterization of microbial cultures. This method, which allows multiple simultaneous replicates from a single low-volume biomass sample, can improve and simplify the model calibration exercise by providing more and better experimental data. To follow up on the interest in microrespirometry, two model wastewater treatment processes were set up and used for model calibration: an aerobic degradation of 4-chlorophenol by acclimated sludge, and a synthetic wastewater treatment by activated sludge, which were well described by a Haldane model and a modified ASM3 model, respectively. For each process, the model parameters were estimated by model fitting, minimizing the objective function for each replicate independently, and minimizing the objective function for all replicates simultaneously (multiresponse approach). Parameter confidence intervals were determined for all parameter estimates, and the impacts of measurement errors, number of replicates, and parameter correlation were assessed. It was observed that the multiresponse approach presents several advantages, including single-step parameter estimation, independent of the number of replicates, and determination of confidence intervals that include the main sources of uncertainty. It was concluded that microrespirometry is a high-throughput and convenient method for model calibration.

1. Introduction

Estimation of kinetic and stoichiometric parameters of biokinetic models, also known as model calibration, is essential in the model building exercise [1]. In biotechnology, where complex biological systems are used, one of the main obstacles for model calibration is the acquisition of reliable experimental data, and the complexity of that task is further increased by the limited availability of methods to measure the key variables of the processes. In this regard, respirometry is a well-established experimental method, which is used to provide data for model calibration and for control strategies in activated sludge and other biological processes [2–4]. Respirometry is defined as the measurement of the exogenous oxygen uptake rate (OUR) in the liquid phase, under well-defined conditions [5]. In aerobic systems, the OUR reflects the microbial metabolism, including microbial growth, substrate uptake, maintenance, storage of polymers, among others.

Traditional respirometric methods, usually performed in batch stirred reactors on a liter scale, are susceptible to errors, resulting in high variability between replicates of experiments [6]. Several reasons for such variability have been suggested, among which the most important are the difficulty in maintaining accurate and constant mass transfer in the respirometer and the difficulty in reproducing the actual conditions prevailing in the biological system under study. For instance, Chu et al. [7] reported that low oxygen transfer rates in mechanical stirred respirometers can cause mass transfer limitations whereas high oxygen transfer rates obtained through high mixing can change the microbial aggregation; in both cases the OUR is modified, leading to errors in parameter estimation. Another common drawback of traditional respirometry is the use of electrochemical dissolved oxygen (DO) sensors, which are still the primary option for DO measurements. Electrochemical DO sensors (i) have a significant response time, which must be considered for data interpretation [8]; (ii) consume oxygen, which is undesirable when the samples are small

* Corresponding author.
E-mail addresses: mvital@cinvestav.m (M. Vital-Jacome), denis.dochain@uclouvain.be (D. Dochain), thalasso@cinvestav.mx (F. Thalasso).

https://doi.org/10.1016/j.bej.2017.10.002
1369-703X/

or oxygen-deficient; (iii) require frequent calibration and maintenance; (iv) are susceptible to drift; and (v) are sensitive to several compounds, including H_2S, O_3, and Cl_2 [9].

Since the late 1990s, dynamic fluorescence quenching sensors have been developed. These sensors present several advantages, making them attractive for respirometry; these include: very short response time, low signal-to-noise ratio, no oxygen consumption, small size, and capability of non-invasive measurement through transparent vessel walls. The development of these sensors promoted the development of microreactor systems, which allow parallel cultivations in multiple independent wells. Microreactor systems have attracted much interest in high-throughput selection of culture media, strain screening, and process optimization [10]. The advantages of microreactors over bench-scale reactors have been listed by Kim et al. [11]. Recently, Esquivel-Rios et al. [12] and Ramirez-Vargas et al. [13] showed that the application of respirometry in microreactor systems – called microrespirometry – generates a high quantity and quality of respirometric data with potential for model calibration.

Whatever the determination method, including respirometry or microrespirometry, model calibration is potentially subject to identifiability problems because of the nonlinear nature of the microbial kinetics. The concept of identifiability refers to the possibility of finding unique parameter values for the calibration of a given model. Identifiability can be structural, i.e., related to the structure of the model; or practical, i.e., related to the information of the experimental data available for calibration [1]. An analysis of practical identifiability can reveal whether the information provided by the experiments leads to the accurate estimation of parameters; in addition, it provides information about the uncertainty of the estimated parameters in terms of parameter correlation.

During the model calibration, it is important to determine the confidence intervals of the estimated parameters, with the aim of quantifying the uncertainties associated with the estimation method. Parameters reported in literature rarely include confidence intervals, and when these are included, they often do not consider all sources of uncertainty, such as parameter correlation, measurement errors, and differences among replicates [14].

Different methods have been developed to determine the uncertainty caused by parameter correlation [1,15], and the Fisher Information Matrix (FIM) is the most common approach to evaluate this issue. Petersen et al. [16] showed that through optimal experimental design (OED) and a combination of respirometric and titrimetric data, the parameter correlation can be reduced substantially. With respect to the uncertainty caused by measurement errors, Liu and Zachara [17] as well as Guisasola et al. [18] have shown the importance of minimizing the measurement errors and increasing the data acquisition frequency to decrease parameter uncertainty. The uncertainty caused by the number of replicate experiments has received less attention, mainly due to the difficulty of achieving reproducible respirometric experiments. Some authors report the arithmetic mean of the parameter estimates from different replicates [19,20], while other authors report parameter estimates considering all replicates at the same time [21]. A third approach, called PRAMUS, was suggested by Magbanua et al. [6], who combined information retrieved from several parameters estimates in a single dataset prior to a new parameter estimation.

The use of a high-throughput method, such as microrespirometry, can simplify the model calibration task. This method can improve parameter identifiability by increasing the quality and quantity of the experimental data, obtained under reproducible conditions, during a single experiment. The aim of our work was to evaluate microrespirometry by comparing two methods of data analysis and applying an exhaustive error analysis. We selected two wastewater treatment processes: aerobic degradation of 4-chlorophenol and synthetic complex wastewater by activated sludge, which were described by a substrate inhibition model and a standard activated sludge model (ASM3), respectively. For each of these models, we compared two methods of data analysis for parameter estimation: one considering each replicate independently and the other one using all replicates simultaneously (the multi-response approach). We assessed the impact of measurement errors, number of replicates, and parameter correlation on the uncertainty of the parameters, by determining the parameter confidence intervals under different simulated conditions.

2. Materials and methods

2.1. Microbial cultures

Two processes were selected and set up (Table 1). The activated sludge degrading 4-chlorophenol as a single xenobiotic substrate was obtained from a sequencing batch reactor (SBR), previously described by Vital-Jacome et al. [22], and operated under steady-state conditions; i.e., the reactor was maintained under stable operation with the effluent substrate concentration remaining unchanged over time. The activated sludge process degrading complex synthetic wastewater was obtained from a continuous reactor operated under steady-state conditions, previously described by Esquivel-Rios et al. [12].

2.2. Microrespirometric method

The two selected cultures were characterized by microrespirometry, performed in a microreactor system (Micro-24, Pall Corporation), as previously described by Ramirez-Vargas et al. [13]. In brief, the Micro-24 uses a 24-well cassette fixed on an orbital shaker. This system allows control of agitation speed, airflow, temperature, pH, and DO. The device uses DO fluorescence quenching sensors with a maximum data acquisition capacity of 2000 readings per hour. Cassettes designed for animal cell culture and with surface aeration were used (PRC, Pall Corporation, USA). Each well of the cassettes included a DO sensor, which are factory pre-calibrated. However, the sensors must be compensated for temperature and atmospheric pressure. As such, an additional calibration was done by injecting oxygen-free nitrogen and air at 0 and 100% saturation, respectively. The experimental conditions were the following: liquid volume, 4 mL; temperature, 25 °C; no airflow (surface aeration); agitation speed, 600 rpm for both activated sludge cultures. The system was not sealed and the filter of the caps (Type-D, Pall Corporation, USA) was removed to ensure the gas exchange between the headspace and the environment. A better surface aeration was thus ensured. Details of surface aeration in the microreactor system can be found in Ramirez-Vargas et al. [13]. The DO concentration was continuously recorded during the experiments, but not controlled, as usually done in dynamic respirometry.

Dynamic pulse respirometry was performed according to previously described methods for microreactors [12,13]. In brief: (i) biomass samples from each culture were centrifuged at 7000 rpm for 10 min (Centrifuge 5810, Eppendorf) and resuspended in culture media without a carbon source; (ii) each well of the microreactor cassette was filled with 3.9 mL of the biomass sample and aerated until endogenous respiration state was reached [23]; (iii) a pulse of 0.1 mL of known substrate concentration was injected in each well; (iv) DO readings were recorded until the system returned to endogenous respiration state; and (v) the oxygen mass transfer coefficient (K_La) was determined according to the method described by Badino et al. [24]. For each culture, the experiment was performed with 23 simultaneous replicates plus one negative control in which the substrate was replaced by a mineral