A methodology for coupling DGGE and mathematical modelling: Application in bio-hydrogen production

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Abstract
Molecular biology techniques provide valuable information in terms of microbial dynamic and evolution. DGGE analysis is one of the most popular methods, which has been used in bioprocess assessment. A novel procedure that combines common experimental measurements, DGGE and image analysis is presented as a new methodology for preliminary assessment. In this study, the methodology was applied as an example to the start up of a hydrogen bioreactor, in order to have a preliminary estimation of the actual concentration of the different microbial species. The obtained results are used for determining the kinetic parameter by using a simple mathematical model focusing on species dynamic.

Keywords anaerobic digestion, biomass, DGGE, image analysis, model.

1. Introduction

Several molecular techniques have been applied in the case of biohydrogen production by anaerobic digestion, among them the DGGE technique stands out. The DGGE enabled to identify the dominant bacterial populations involved in the biohydrogen production process under various operating conditions (Mariakakis et al., 2011).
Most of the mathematical models in anaerobic digestion consider several microbial population as state variables, however the specific species concentration are unknown variables due to difficulties in performing measurements. This issue may trigger some identification problems since some parameters cannot be determined independently (Bernard et al., 2001).
The aim of this study is to develop and validate a simple procedure to convert a typical DGGE fingerprinting in a quantitative measurement of the individual ribotypes evolution in a bioreactor, for use in mathematical modeling. This approach was applied during the start-up of an anaerobic reactor aiming to produce hydrogen, where significant changes of the population characteristics are expected to take place.

2. Material and Methods

2.1. Experimental set up

A glass-made reactor operating as continuous stirred tank reactor (CSTR) of 2 L was used for the experiment. A hydraulic retention time (HRT) of 10 h was set for population selection of Hydrogen Producing Bacteria (HPB). Glucose (5 $g.L^{-1}$) was used as the sole carbon source. The biogas production was measured by liquid displacement, and the composition of biogas was measured by gas chromatography,

using GC Perkin Elmer Clarus 500, with nitrogen as the carrier. Biomass concentration was measured by volatile suspended solids (VSS) through gravimetric method.

2.2. 16S rDNA PCR-DGGE fingerprinting

Aliquots of well-homogenized biomass samples (120 mL) were taken from the CSTR every day during the seven first days of operation. DNA was extracted from biomass sample pellet using Powersoil DNA Isolation Kit, MO BIO Laboratories Inc (Carlsbad, CA, US). Bacterial 16S rRNA genes was amplified by PCR using the primers U968-f and L1401-r (Nübel et al., 1996). A 40-base GC clamp was attached to the primer U968-f at the 5' end. PCR products were separated by Denaturing Gradient Gel Electrophoresis (DGGE) with a linear gradient ranging from 30% to 60%, according to the protocol of Muyzer et al., 1993. The migration was carried out for 16h at 85V and 60°C, in 0.5X TAE buffer, using the DCode System (Bio-Rad Laboratories Inc, Hercules, CA, US).

2.3. Estimation of OTUs concentration

DGGE gel was analysed by the program *Image J* (National Institutes of Health, USA). Each DGGE profile was converted in a densitometric curve where each band was represented by a peak of given width and intensity. According to the classical DGGE postulate, a single band was related to a single sequence, called ribotype or Operational Taxonomic Unit (OTU), and the ratio between the area of the peak and the total area of the pattern (rather than the peak height) was assumed to be an estimator of the ribotype relative abundance in the community (Loisel et al 2006).

2.4. Mathematical model with several species groups

2.4.1. Model description

A simple species-based chemostat model was developed, based on two main hypotheses: (1)The microorganisms mass balance is respected and we assume that each species has the same biomass yield coefficient *y*. (2) Interactions between species only result from the competition for the common substrate. We have chosen Monod functions to represent the microbial growth rate. Under these assumptions, we consider the simple chemostat model.

2.4.2. Model identification

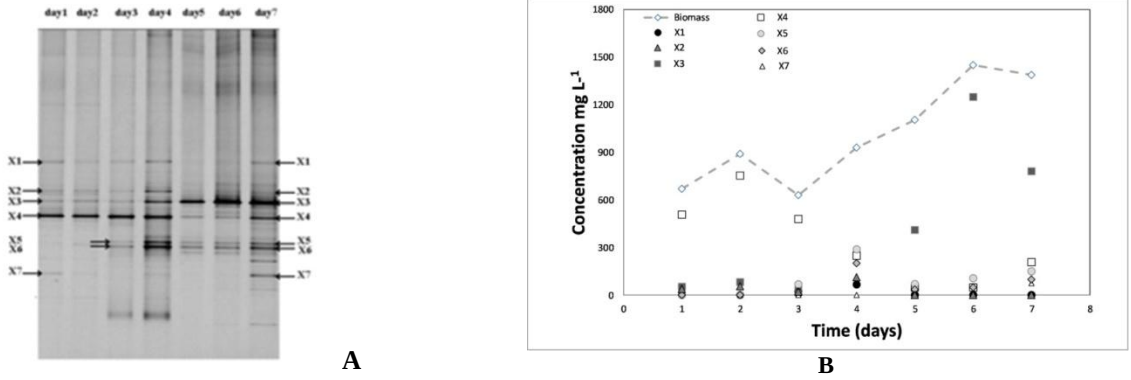
Estimating separately the yield coefficient and the maximum growth rates is quite complicated since these parameters are highly correlated (Batstone 2006). Therefore the experimental data were fitted to this model, to identify the parameter *y* and pairs μ_i^{max} and K_i for each species i, using a least square method. First of all the coefficient *y* has been estimated with the measurement of the total biomass *X* and substrate *S*.

3. Results and discussion

3.1 Evolution of reactor performance and community structure

The evolution of the bacterial community structure is reflected in the DGGE pattern (Figure 1A). Based on densitometric peak areas, the seven most intense bands were selected and each was assumed to correspond to a single ribotype (X1 to X7) whose concentration was estimated on the basis of DGGE band relative intensity and the total biomass concentration (Figure 1B). According to this quantitative interpretation of the

DGGE fingerprint, there was a clear selection for X_3 ribotype, whose relative abundance exhibited the highest increase during the evaluation period (from day 4 on).



A B

Figure 1. A) DGGE pattern of bacterial 16S rDNA during the first week of operation. The bands are identified from X1 to X8.B) Concentration estimation of the seven dominant bacterial ribotypes from the total measured biomass and DGGE band intensities, during the first 7 days of reactor operation.

Nevertheless, it is important to point out that our quantitative interpretation of DGGE profile is based on an assumption which can be subjected to conceptual limits and potential methodological biases, both implying that one DGGE band does not always correspond to one single ribotype and that relative band intensity is not always related to ribotype concentration (Muyzer & Smalla, 1998). However DGGE still remains convenient to compare the dynamics of major populations in a large number of samples undergoing the same methodological treatment, and, as previously reported, DGGE fingerprintings can be revisited by simulation and used as a tool to measure microbial diversity (Loisel et al., 2006).

3.2. Model parameter determination and calibration

For parameter identification purposes, and taking into account the experimental data, only the three dominant ribotypes were considered: X_2, X_3 and X_4. All the other ribotypes were pooled in a sole group (X*). Figure 3a presents the chemostat-model fit with the actual experimental data of total biomass and substrate concentration, from which the yield coefficient (y). Knowing that the applied dilution rate was 2 d^{-1} and the glucose concentration of the inlet was 5 g L^{-1}, the estimated value of the biomass-substrate yield was 0.3 $g_{Biomass}$ $g_{Glucose}^{-1}$.

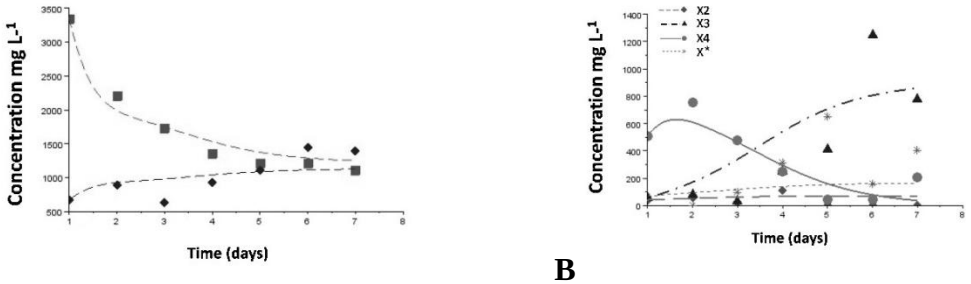


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Figure 3. (A) Evolution of substrate concentration (square) and biomass concentration (diamond) during the first 7 days of reactor operation. (B) Evolution of ribotypes concentrations during the first 7 days of reactor operation.

Figure 3b presents the multi-species chemostat-model fit for each ribotype concentration, from which the individual growth parameters could be identified from Equations (1)-(2) (see Table 1). The X4 ribotype was the most abundant during the first three days but its abundance decreased after day 4. Its kinetic parameters (high affinity

constant) are characteristic of a slow competitor (Table 1). X4 got quickly out competed by X3 whose abundance increased from day 4 and became dominant from day 5. Ks values are in agreement with those found using the same substrate (Sharma and Li 2009). However the X_3 parameters values are similary to a study by Nath et al., 2006, reported for *E. clocae*, a bacterium that can produce hydrogen at a substantially high rate (the maximum specific growth rate was 0,398 h^{-1} and Ks 5.5 gL^{-1} with glucose).

Table 1. Estimation of maximal growth rate (μmax) and affinity constant (Ks) of the four main groups of ribotypes identified from DGGE profiles.

Ribotype	Parameters	
	μ^{max} (h^{-1})	Ks (g L^{-1})
X*	0.225	5.5
X_2	0.68	18
X_3	0.45	5.8
X_4	0.45	10

Conclusion

A novel procedure that combines common experimental measurements and molecular biology technique, in this case the DGGE, with image analysis allow us to count with a quantitative approximation of the most important microbial species of a hydrogen bioreactor. The proposed method has allowed fitting the results with a simple chemostat model based on the assumptions that each species has the substrate yield and their specific growth rate follow a Monod function. Because the growth curves that have been identified intersect (more precisely, the set of non-dominant species is expected to be less efficient under larger dilution rate), further experimental investigations with other dilution rates and more measurements are necessary to validate thoroughly the assumptions.

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