EFFECT OF SUBSTRATE TO BIOMASS RATIO (S_0/X_0) ON KINETIC AND STOICHIOMETRIC PARAMETERS OF *Pseudomonas putida* F1

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Introduction. Respirometry is the measurement of the biological oxygen consumption rate under well-defined conditions (1). This technique is commonly used to determine the main kinetic and stoichiometric parameters of biological processes; namely, the substrate affinity constant (K_S), the maximum growth rate (μ_{max}) and the cell growth yield ($Y_{X/S}$), within others. According to the literature, there is a large variability in the estimation of these kinetic and stoichiometric parameters, attributed to three key factors (2): the culture history, the parameter identifiability and the ratio between substrate and biomass concentration (S_0/X_0**)**. The effect of S_0/X_0 ratio on kinetic and stoichiometric parameters has only been partially studied and to the best of our knowledge, its effect on K_S, μ_{max} and $Y_{X/S}$ has never been systematically studied. The goal of this study was to assess respirometrically the effect of the S_0/X_0 ratio on the kinetic and stoichiometric parameters of a model culture of *Pseudomonas putida* F1.

Methods. The model strain chosen was *Pseudomonas putida* F1 cultured on glucose as sole carbon source. Cultures used during respirometry tests were prepared in a 1 L Erlenmeyer flasks with 600 mL of culture medium containing (g L^{-1}); $C_6H_{12}O_6$, 5; $(NH_4)_2SO_4$, 1.32; KH_2PO_4, 1.25; $MgSO_4 \cdot 7H_2O$, 0.10; $FeSO_4 \cdot 7H_2O$, 0.075; yeast extract, 1; trace elements, 1 mL L^{-1}. Cultures were incubated for 48 h at 30 ºC and 200 rpm orbital shaking. Respirometry tests were done in a microbioreactor system (24-microwell plate, Presens, Applikon, Mexico). Each well included a pre-calibrated fluorometric dissolved oxygen sensor. The respirometric method chosen was pulse dynamic respirometry which consists in measuring on-line the dissolved oxygen profile after the injection of a substrate pulse of a known concentration. This method has been exhaustively described for standard respirometers (4). The experimental strategy consisted in injecting 7 different glucose pulse concentrations in microwells containing variable biomass concentrations in such manner S_0/X_0 ratio was kept constant. 5 different S_0/X_0 ratios were tested.

Results. Figure 1 shows an example of respirograms observed after the injection of pulses of increasing concentration (8 – 128 mg of soluble Chemical Oxygen Demand (COD_S) L^{-1}). In all cases, OUR increased sharply immediately after pulse injection and then, progressively returned to zero. Table 1 shows the values obtained for K_S, μ_{max} y $Y_{X/S}$ parameters determined for each S_0/X_0 ratio. As shown, K_S increased linearly with S_0/X_0 ratio, while μ_{max} presented a "Monod type" in regards to S_0/X_0 ratio. $Y_{X/S}$ did not present significant changes with S_0/X_0 ratio increases.

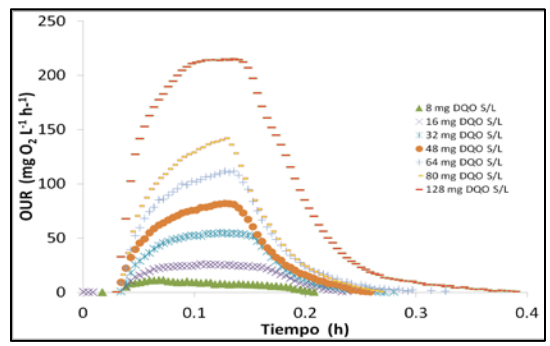

Fig.1 Example OUR profile for a S_0/X_0 ratio of 0.025, pulses glucose (mg COD L^{-1}).

Table 1. K_S, μ_{max} y $Y_{X/S}$ parameters determined from respirometry for different S_0/X_0 ratio.

S_0/X_0 (mgCOD$_S$ mgCOD$_X^{-1}$)	K_S (mg COD L^{-1})	μ_{max} (h^{-1})	$Y_{X/S}$ (mgCOD$_X$ mgCOD$_S^{-1}$)
0.0075	0.77 ± 0.12	0.059 ± 0.0020	0.73 ± 0.08
0.0150	1.20 ± 0.13	0.124 ± 0.0017	0.74 ± 0.05
0.0250	3.22 ± 0.16	0.166 ± 0.0006	0.79 ± 0.03
0.0500	8.32 ± 1.62	0.158 ± 0.0054	0.74 ±0.05
0.1000	27.12 ± 0.87	0.183 ± 0.0035	0.94 ± 0.02

Conclusions. S_0/X_0 ratio is an important factor to be considered in the determination of kinetic parameters of *Pseudomonas putida* F1

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