

Additional file 1

Developing a single-stage continuous process strategy for Vitamin B₁₂ production with *Propionibacterium freudenreichii subs shermanii*

Álvaro Calvillo¹, Teresa Pellicer², Marc Carnicer^{1,*}, Antoni Planas^{1,*}

¹ Laboratory of Biochemistry, Institut Químic de Sarrià, University Ramon Llull, 08017 Barcelona, Spain; ² HealthTech Bio Actives, 08029 Barcelona, Spain

* Corresponding authors: marc.carnicer@iqs.url.edu and antoni.planas@iqs.url.edu

1. Determination of Dry Cell Weight (DCW)/OD₆₀₀ ratio

In order to determine the DCW/OD₆₀₀ ratio for *P. freudenreichii* three independent cultures were performed where DCW and OD₆₀₀ were evaluated at different culture times (See Table S1).

Table S1: Evolution of DCW and OD₆₀₀ in three independent cultures.

Time (h)	Culture 1		Culture 2		Culture 3	
	OD ₆₀₀	DCW (g/L)	OD ₆₀₀	DCW (g/L)	OD ₆₀₀	DCW (g/L)
0	0.05	-	0.05	-	0.05	-
24	1.85	0.78	1.75	0.71	1.71	0.75
48	10.0	3.98	10.3	4.08	10.0	4.0
72	22.0	9.07	21.5	9.0	20.9	8.1
96	29.0	11.2	29.5	11.7	29.0	11.9

The mean DCW/OD₆₀₀ ratio for each culture was 0.404, 0.404 and 0.408 respectively for cultures 1, 2 and 3. The established value for DCW/OD₆₀₀ ratio was the mean of each culture value, 0.405 ± 0.02.