Supplementary material

A pilot study combining ultrafiltration with ozonation for the treatment of secondary urban wastewater: organic micropollutants, microbial load and biological effects

Cátia A.L. Graça[1], Sara Ribeirinho-Soares[2], Joana Abreu-Silva[3], Inês I. Ramos[4], Ana R. Ribeiro[1], Sérgio M. Castro-Silva[5], Marcela A. Segundo[4], Célia M. Manaia[3,*], Olga C. Nunes[2,*] and Adrián M.T. Silva[1,*]

[1] Laboratory of Separation and Reaction Engineering - Laboratory of Catalysis and Materials (LSRE-LCM), Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal; catiaalgraca@fe.up.pt (C.A.L.G); ritalado@fe.up.pt (A.R.R); adrian@fe.up.pt (A.M.T.S)

[2] LEPABE - Laboratory for Process Engineering, Environment, Biotechnology and Energy, Faculdade de Engenharia, Universidade do Porto, Rua Dr. Roberto Frias, 4200-465 Porto, Portugal; saramariasoares@hotmail.com (S.S.S); opnunes@fe.up.pt (O.C.N)

[3] Universidade Católica Portuguesa, CBQF - Centro de Biotecnologia e Química Fina – Laboratório Associado, Escola Superior de Biotecnologia, Rua Diogo Botelho 1327, 4169-005 Porto, Portugal; jsilva@porto.ucp.pt (J. A-S); cmanaia@porto.ucp.pt (C. M. M.)

[4] LAQV, REQUIMTE, Departamento de Ciências Químicas, Faculdade de Farmácia, Universidade do Porto, Rua de Jorge Viterbo Ferreira 228, 4050-313 Porto, Portugal; iibmramos@gmail.com (I.I.R); msegundo@ff.up.pt (M. A. S)

[5] Adventech-Advanced Environmental Technologies, Centro Empresarial e Tecnológico, Rua de Fundões 151, 3700-121 São João da Madeira, Portugal; sergio.silva@adventech.pt (S. M. C-S)

***** Correspondence: adrian@fe.up.pt (A.M.T. S); opnunes@fe.up.pt (O.C. N); cmanaia@porto.ucp.pt (C.M. M).

Table S1. Quantitative PCR conditions used in the present study for absolute gene quantification in all WW, TWW_0, and TWW_7 samples.

Gene	Primer sequences (5′ to 3′)			Reference DNA		Accuracy (R^2)	Efficiency (%)	Cycling conditions †
16S rRNA	1114	CGGCAACGAGCGCAACCC	Denman et al., 2006 [41]	*E. coli* ATCC 25992	Sousa et al., 2017 [15]	1.0	97.0	Initial denaturation step at 95 °C for 10 min; 15 s at 95 °C, 20 s at 55°C, and 10 s at 72 °C (35 cycles) ¹
	1275	CCATTGTAGCACGTGTGTAGCC						
***intI*1**	*intI*1-LC1	GCCTTGATGTTACCCGAGAG	Barraud et al., 2010 [42]	pNORM1	Rocha et al., 2018 [44]	0.999	91.0	Initial denaturation step at 95 °C for 10 min; 15 s at 95 °C, and 1 min at 60 °C (40 cycles) ²
	*intI*1-LC5	GATCGGTCGAATGCGTGT						

† StepOnePlus™ Real-Time PCR System (Life Technologies, USA).

¹ KAPA SYBR® FAST qPCR kit, ABI Prism®, 200 nM of primer in a reaction volume of 20 μL.

² *Power* SYBR™ Green PCR Master Mix, Applied Biosystems™, 200 nM of primer in a reaction volume of 20 μL.