

# Enrichment of bacterial strains for the biodegradation of endocrine disrupting compounds from sediments of the Pearl River Delta (PRD)

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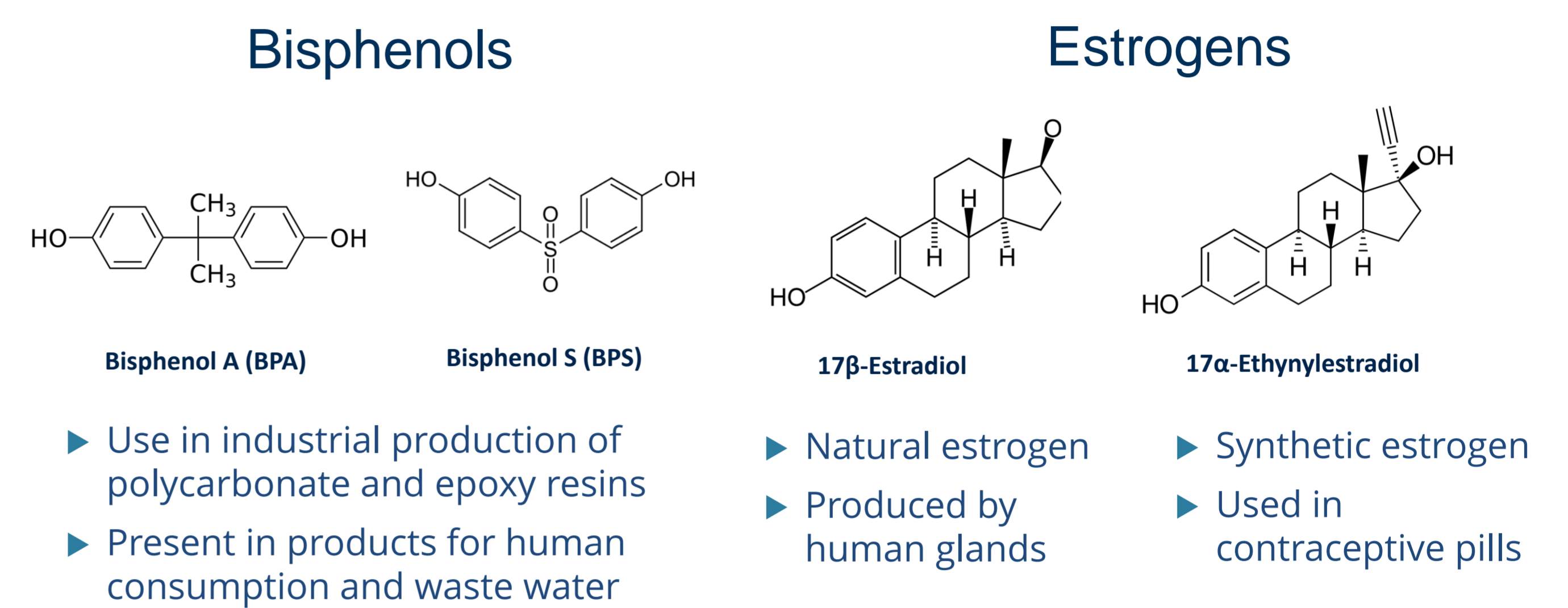
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## Introduction

Endocrine disrupting chemicals (EDCs) are those which alter the homeostasis, reproduction, development and/or behavior of organisms. Negative effects have been reported on aquatic species, wildlife, and humans due to exposure to very low (ng L<sup>-1</sup>) concentrations. Wastewater treatment plants are not able to completely remove these chemicals, contributing to the contamination of receiving water bodies. The situation of The Pearl River Delta (PRD) is of particular concern due to the high industrialization and dense urbanization.

In the present study, bacteria degrading estradiols and bisphenols were isolated from sludge from an aeration tank and sediments obtained near a discharge site of a WWTP located in Coloane, Macao.

## Target Endocrine Disrupting Compounds



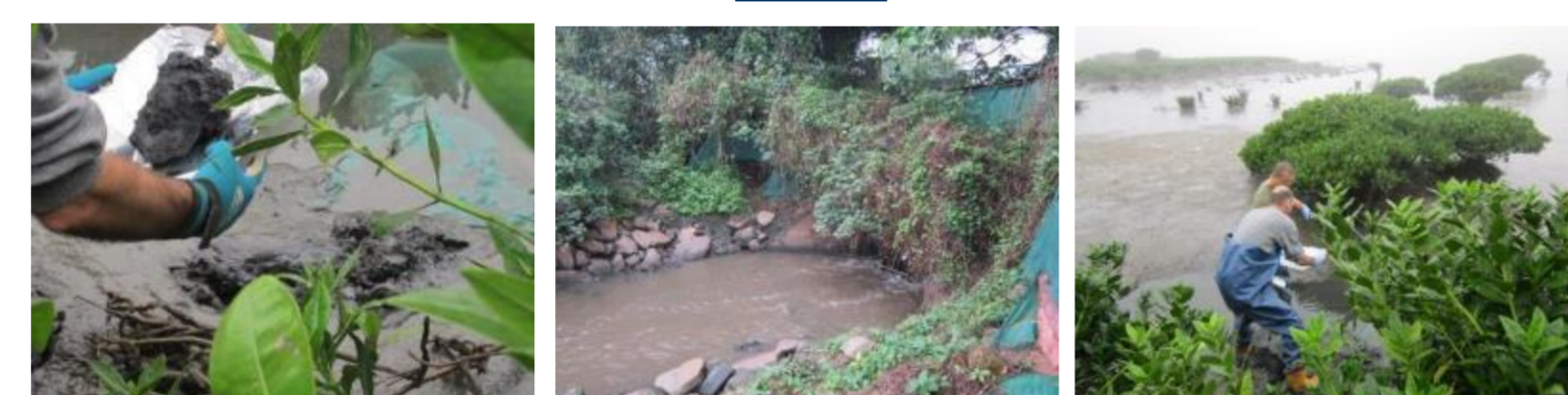
## Methods and Results

### SAMPLING

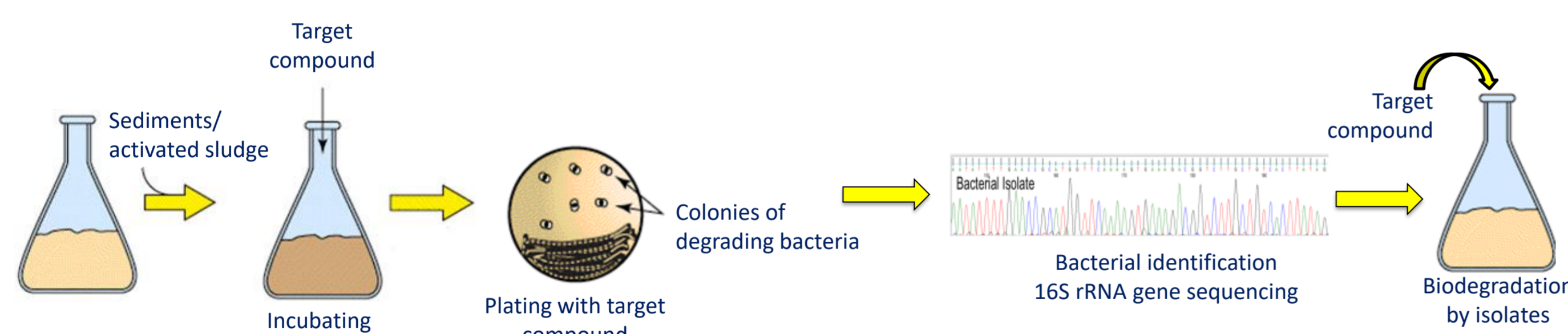
Activated sludge from an aeration tank of a sewage treatment plant



Composite of 3 individual sediment samples near the discharge point of the WWTP



### SELECTIVE ENRICHMENTS



### EDCs REMOVAL DURING ENRICHMENT

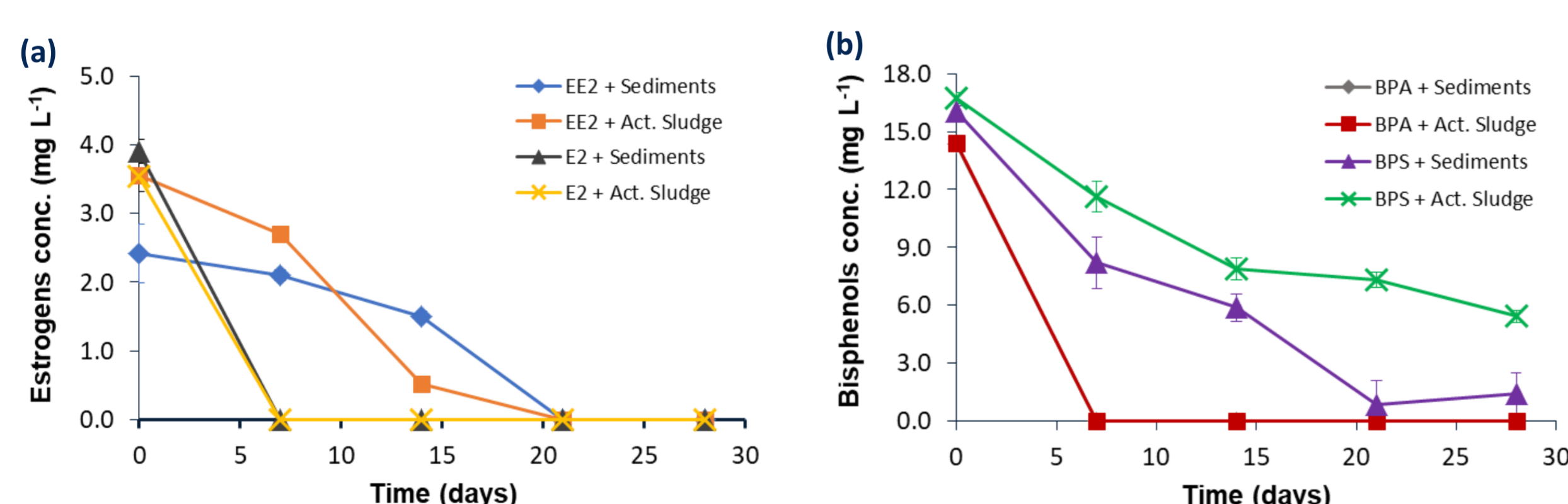


Figure 1. Removal of estrogens (a) and bisphenols (b) during enrichment of sediments and activated sludge samples. Experiments were performed in triplicate.

- Total removal of E2 and EE2 (3,5 mg L<sup>-1</sup>) and BPA (15 mg L<sup>-1</sup>)
- Removal of 91% of BPS in the enrichment from sediments (15 mg L<sup>-1</sup>)
- Removal of 68% of BPS in the enrichment from activated sludge (15 mg L<sup>-1</sup>)

**Removal includes degradation and adsorption to particles from sediments and activated sludge**

### EDCs DEGRADATION BY ENRICHED CONSORTIA

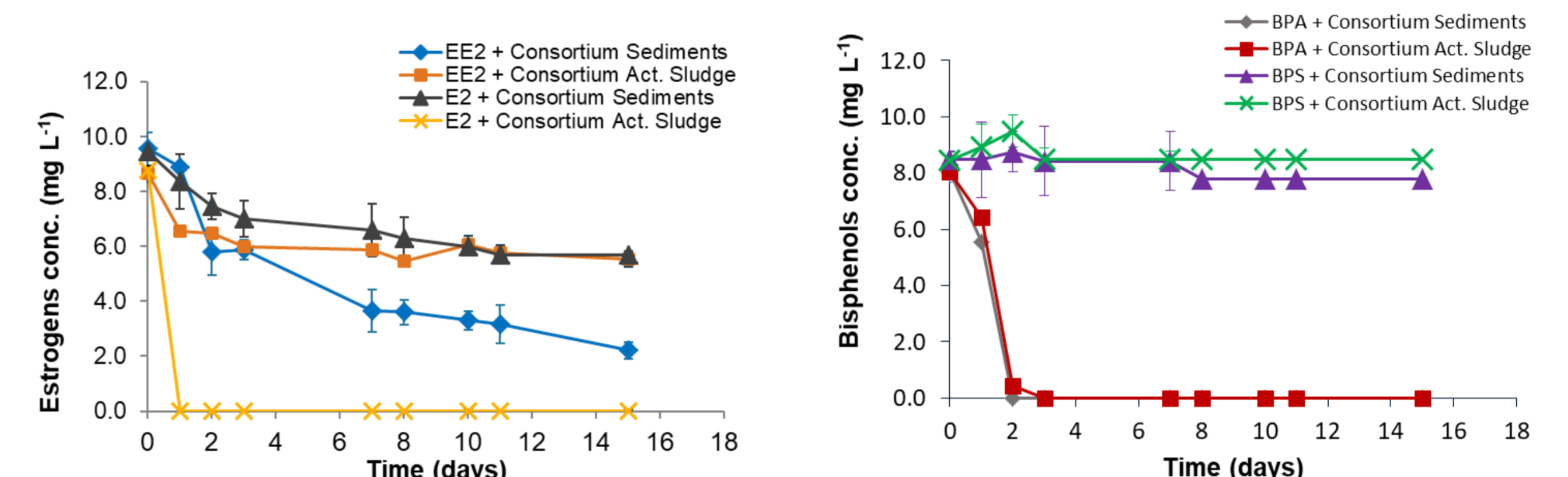


Figure 2. Degradation of estrogens (a) and bisphenols (b) by enriched consortia. Experiments were performed in triplicate.

- Degradation of EE2 varied between 36 – 77%
- 100% degradation of E2 in 1 day with consortia from activated sludge
- Only 40% degradation of E2 with consortia from sediments
- 100% degradation of BPA in 3 days
- BPS was not degraded

### EDCs DEGRADATION BY ISOLATED BACTERIA

Isolate	Phylogenetic affiliation	Closest relative (accession no.)	Similarity (%)	Growth on agar plates				Degradation on liquid media (%)			
				EE2	E2	BPA	BPS	EE2	E2	BPA	BPS
15	<i>β-proteobacteria</i>	<i>Achromobacter</i> sp. BR3 clone 2 (JN196541)	99	++	++	++	++	34.0	54.9	1.0	5.5
17	<i>γ-proteobacteria</i>	<i>Stenotrophomonas</i> sp. enrichment culture clone 2	99	-	-	-	-	-	-	-	-
19	<i>γ-proteobacteria</i>	<i>Pseudomonas</i> sp. J2.1D12	100	++	++	++	+++	28.0	53.7	1.78	10.2
24	<i>Actinobacteria</i>	<i>Mycobacterium austroafricanum</i> strain IFP 2009	99	++	+++	+++	+++	35.1	52.8	0.8	5.9
55	<i>β-proteobacteria</i>	<i>Castellaniella denitrificans</i> strain RS-MT7	98	++	+++	+++	+++	67.4	79.4	23.2	33.5
63	<i>Firmicutes</i>	<i>Staphylococcus</i> sp. CCM 8730	99	++	+++	+++	+++	0	27.7	20.4	19.2
64	<i>β-proteobacteria</i>	<i>Achromobacter denitrificans</i> strain SMV191#5	99	-	-	-	++	-	-	-	19.0
70	<i>β-proteobacteria</i>	<i>Alicyciphilus</i> sp. UF5H	99	++	++	+	++	52.2	61.9	3.1	17.6
73	<i>Firmicutes</i>	<i>Paenibacillus glycanilyticus</i> strain S178a	99	-	-	-	-	-	-	-	-
86	<i>β-proteobacteria</i>	<i>Castellaniella denitrificans</i> strain RS-MT7	98	++	+	++	+++	67.4	55.2	8.5	11.1
89	<i>β-proteobacteria</i>	<i>Castellaniella</i> sp. TCOB-5	99	++	+	++	+	25.2	55.7	7.4	10.2
95	<i>γ-proteobacteria</i>	<i>Pseudomonas putida</i> strain F1-1-2	99	++	+	++	+	30.9	72.6	0	9.5
96	<i>γ-proteobacteria</i>	<i>Pseudomonas</i> sp. strain PHE14	99	-	-	-	-	-	-	-	-
106	<i>α-proteobacteria</i>	<i>Ochrobactrum anthropi</i> strain DE2010	99	+++	+++	+++	+	6.3	49.3	2.8	7.2
113	<i>β-proteobacteria</i>	<i>Cupriavidus</i> sp. USMAHM13	99	+++	++	++	+	60.9	100	0	0
115	<i>β-proteobacteria</i>	<i>Alicyciphilus</i> sp. R-24604	99	-	-	-	-	-	-	-	-
117	<i>β-proteobacteria</i>	<i>Castellaniella hirudinis</i> strain E103	99	++	++	++	++	26.8	76.8	2.8	14.3
136	<i>Bacteroidetes</i>	<i>Chitinophaga arvensicola</i>	97	-	-	-	-	-	-	-	-
138	<i>Actinobacteria</i>	<i>Microbacterium oxydans</i> strain NSB-43	99	-	-	-	-	-	-	-	-
141	<i>Firmicutes</i>	<i>Bacillus oleronius</i> strain LZ1306-2-19	86	-	-	-	-	-	-	-	-
158	<i>γ-proteobacteria</i>	<i>Citrobacter freundii</i> strain 1-N-1-1-1	99	-	-	-	-	-	-	-	-
174	<i>γ-proteobacteria</i>	<i>Pseudomonas</i> sp. Ammsd-3	99	+	+	++	+	33.1	65.7	9.6	11.4
185	<i>β-proteobacteria</i>	<i>Alcaligenes</i> sp. A23	99	++	++	++	+	64.7	62.0	4.4	11.4
190	<i>β-proteobacteria</i>	<i>Achromobacter</i> sp. QUEBA08	99	++	++	++	+	61.0	63.2	0	2.4
196	<i>β-proteobacteria</i>	<i>Achromobacter</i> sp. Ir-12.2	99	+	++	++	++	54.6	59.8	0	6.4
201	<i>β-proteobacteria</i>	<i>Achromobacter</i> sp. QUEBA08	99	++	++	++	+	35.9	59.0	0	9.2
206	<i>β-proteobacteria</i>	<i>Alcaligenes</i> sp. S3P179	99	+++	+++	+	+	58.9	65.3	1.8	3.3
209	<i>Firmicutes</i>	<i>Paenibacillus</i> sp. SCH-2	99	+	++	-	+	39.0	43.8	-	11.4
210	<i>Actinobacteria</i>	<i>Microbacterium azadiractae</i>	99	-	-	-	-	-	-	-	-

## Conclusions

- ✓ The isolated strains represent valuable candidates for in situ bioremediation of contaminated soils and waters.
- ✓ Promising results were obtained by strain *Castellaniella* sp. ED55, able to degrade the four compounds at different extents.
- ✓ Further studies are ongoing to optimize the degradation of the compounds and to deepen the knowledge about the mechanisms of degradation including genome sequencing and transcriptomic experiments. .

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