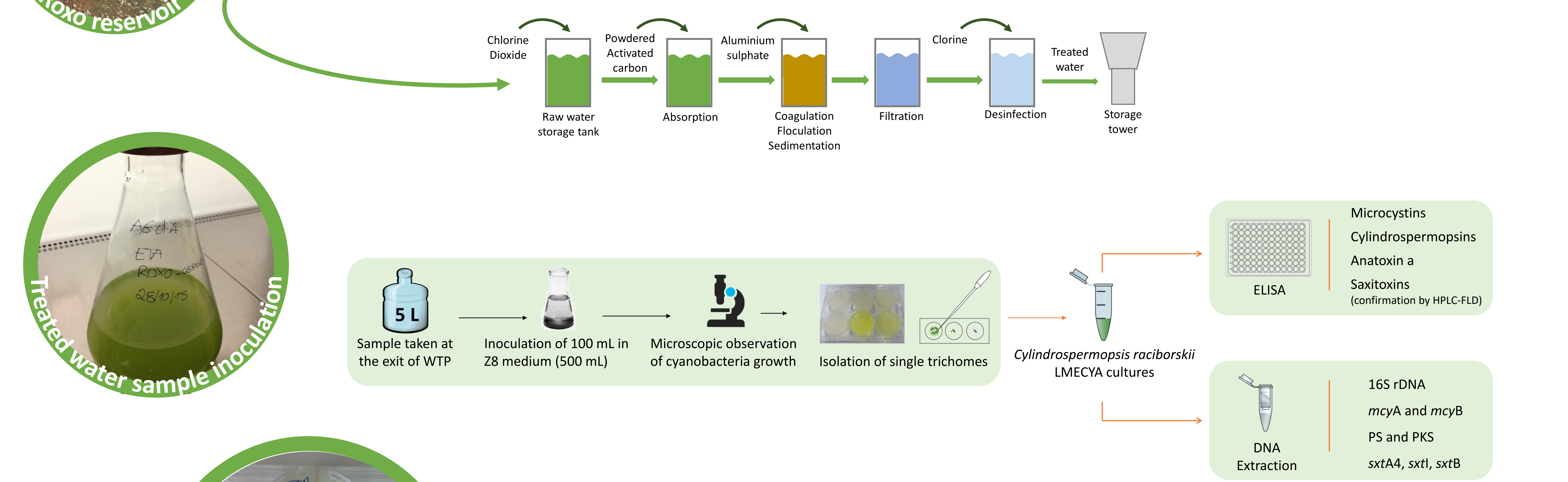


C. Menezes¹, E. Valério¹, M. J. Botelho², O. Martins³, E. Dias¹
¹Department of Environmental Health, National Institute of Health Dr. Ricardo Jorge, Avenida Padre Cruz, 1649-016 Lisbon, Portugal
²Department of Sea and Marine Resources, Portuguese Institute for the Sea and Atmosphere, Rua Alfredo Magalhães Ramalho, 6,1495-006 Lisbon, Portugal
³AgdA – Águas Públicas do Alentejo, S.A., Rua Dr. Aresta Branco nº51, 7800-310 Beja, Portugal

Background

This work presents the successful establishment of *Cylindrospermopsis raciborskii* cultures isolated from water samples collected at the exit point of Water Treatment Plant (WTP). An intense bloom dominated by filamentous cyanobacteria (*Aphanizomenon* spp., *Planktothrix* spp., *Cylindrospermopsis raciborskii*, *Anabaena* spp.) occurred in the summer of 2015 in Roxo reservoir (south Portugal). Several cyanotoxins (microcystins, saxitoxins, cylindrospermopsin) were detected in raw and treated water, although at levels below the corresponding regulatory and/or guideline values. Nevertheless, this bloom caused intense unpleasant odour and taste in the water supplied to the populations and cyanobacterial cells (up to 1000 cells.mL⁻¹) were detected in finished water samples collected at the exit point of WTP.



Treated water sample inoculation

Strains isolation

Toxin production

	LMECYA324	LMECYA325	LMECYA326
Microcystins	Negative	Negative	Negative
Cylindrospermopsin	Negative	Negative	Negative
Saxitoxin	ELISA: Positive HPLC: Negative	ELISA: Positive HPLC: Negative	ELISA: Positive HPLC: Negative
Anatoxin-a	Negative	Negative	Negative

Strains characterization

Taxonomic classification

- Three monoalgal isolates were established in culture.
- These isolates were identified as *Cylindrospermopsis raciborskii*, according to morphometric determinations [1].
- Taxonomic identification was confirmed by phylogenetic analyses.
- These strains are maintained in the “Estela Sousa e Silva Algae Culture Collection” (ESSACC) [2] as LMECYA324, LMECYA 325 and LMECYA 326.

	Akinetes		Heterocytes		Vegetative cells	
	Lenght (µm)	Width (µm)	Lenght (µm)	Width (µm)	Lenght (µm)	Width (µm)
LMECYA324	12,08±3,01	2,94±0,41	5,91±1,62	1,90±0,37	10,83±3,22	1,74±0,16
LMECYA325	15,16±3,36	3,68±0,50	9,35±2,54	3,49±0,61	14,75±2,61	2,29±0,19
LMECYA326	13,71±4,08	3,50±0,54	6,03±1,95	1,98±0,37	9,05±2,25	1,73±0,16

Toxin genes

	Microcystins (<i>mcyA</i> / <i>mcyB</i>)	Cylindrospermopsin (PS / PKS)	Saxitoxin (<i>sxtA4</i> , <i>sxtI</i> , <i>sxtB</i>)
LMECYA324	Negative	Negative	Negative
LMECYA325	Negative	Negative	Negative
LMECYA326	Negative	Negative	Negative

Conclusions

- The *Cylindrospermopsis raciborskii* cells remaining after the conventional water treatment processes at the WTP, maintained the ability to grow and produce healthy cultures.
- Three strains (LMECYA 324, 325 and 326) of *C. raciborskii* were isolated from treated water samples and are being maintained as monoalgal cultures at the “Estela Sousa e Silva Algae Culture Collection” (ESSACC) [2].
- Although these strains are not producers of cylindrospermopsin, microcystins, anatoxin-a and saxitoxin, their ability to resist to water treatment and proliferate downstream of the WTP should not be disregarded, considering the invasiveness potential and the cosmopolitan status of *C. raciborskii* [3].
- To our knowledge, this is the first report on the establishment of successful cultures of *C. raciborskii* isolated from finished treated water.

References:
[1] Komárek J, Anagnostidis K. 1998. Cyanoprokaryota, 3. Teil: Heterocystous Genera. Susswasseefflora von Mitteleuropa (19/3). Stuttgart: Gustav Fisher.678-685
[2] Paulino S,Sam-Bento F, Churro C, Alverca E, Dias E, Valério E, Pereira P. 2009. The Estela Sousa e Silva Algal Culture Collection: a resource of biological and toxicological interest. Hydrobiologia. 636:489
[3] Antunes JT, Leão PN, Vasconcelos VM. 2015. *Cylindrospermopsis raciborskii*: review of the distribution, phylogeography, and ecophysiology of a global invasive species. Front Microbiol. 6: 473.

Acknowledgements:
Fundação para a Ciência e Tecnologia (Portugal), for research funding Grant SFRH/BPD/77981/2011 attributed to Elsa Dias. Iberlab, for the offer of Beacon ELISA kits for saxitoxin and cylindrospermopsis.