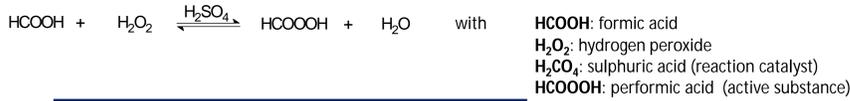


Assessment of the impact of a wastewater treatment process including Advanced Oxidation Technique on environment using chemical and biological indicators

Context

In a context of several European directives for transitional and coastal waters masses (WFD, MSFD, BWD), the good ecological status of water but also conservation of marine ecosystem should be quickly achieved. The high density of population over the year, especially during summer, on the very touristic Basque coastline (SE of the Bay of Biscay) can occasionally generate high nutrient discharge and water and sediment pollution and, in fine, impacts the quality of the marine environment. Reducing anthropogenic release is the main concern of authorities. An alternative wastewater treatment process was applied locally for one year and a half to reduce bacterial load of treated water. A strong oxidizing agent, performic acid (PFA), was continuously mixed with treated water at a mean concentration of 1.2 g m⁻³ (i.e. 50.4 kg.d⁻¹ for a daily flow of 42 000 m³.d⁻¹). Injection point of PFA occurred in a concrete pipe 1000 meters before the discharge point into the sea.

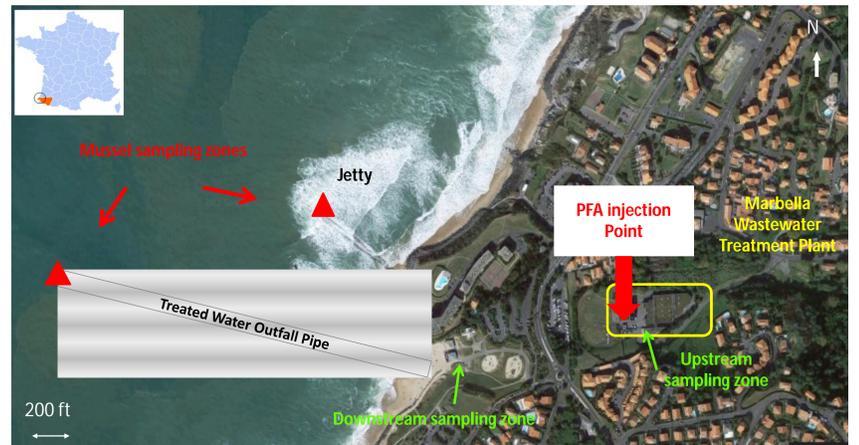


Equation for the chemical synthesis of performic acid (PFA)

A project aiming at 1) assessing the bactericide efficiency of PFA; 2) monitoring a potential impact of PFA treatment on the receiving environment and 3) considering the concrete pipes response to this new treatment is currently undertaken.

This poster focuses on the first results obtained for the points 1) and 2).

Sampling sites



Marbella is located in the French Basque coast. Aerial view of Marbella WasteWater Treatment Plant and WWTP outlet. Location of the point of injection of PFA and the upstream and downstream sampling points.

Methodology

To assess the resulting water quality

Physico-chemical characteristics



➢ Evolution of the water physico-chemical characteristics following injection of PFA:

- Covered period: 2014-01 to 2015-06
- 81 weekly samplings upstream and downstream the injection point
- Different rainfall conditions

- systematically measured parameters: pH; conductivity; turbidity; Suspended Particulate Matter (SPM); dissolved and particulate organic carbon (DOC, POC)

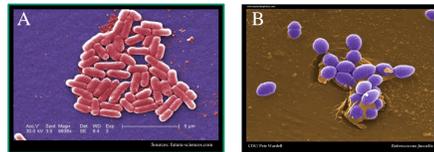
➢ Impact of PFA on water quality regarding metal concentrations:
- 3 samplings date in April 2015
- 24 h flow-weighted samples, upstream and downstream the tertiary treatment
- 13 elements considered (e.g. cadmium, chromium, copper, nickel, lead, zinc)
- metal concentrations in un-filtered and filtered water (<0.45 µm) to assess their biodisponibility

Bacteriological survey



➢ Bactericide efficiency of PFA assessed by enumeration of bacteria:

- two bacteria considered: *Escherichia coli* (A) and *Enterococcus spp* (B)



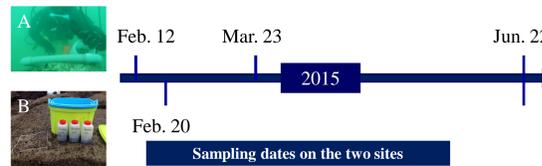
- removal or inactivation of the microorganisms defined as log (N_t/N₀), with N_t: enumeration after a processing time (t) and N₀: enumeration before treatment

To assess the impact on the surrounding biological system

Morphological and genetic variations of a sedentary benthic population



➢ Selected species: *Mytilus spp.*
➢ Sampling on two different environments: the sewage outfall (A); a close undisturbed reference site (rocky jetty) (B)



➢ Per site: 3*0.1 m² quadrats
➢ Biological response was investigated in laboratory by:
- the relative quantification of the expression of genes (2^{-ΔΔCT} method) involved in mitochondrial metabolism (*cox1*, *12S*), oxidative stress (*Sod* *Cu/Zn*, *Cat*) and detoxification process (*mt1*) in gills and digestive gland of 5 controlled (jetty site) and exposed (sewage outfall) mussels

- shell morphometric analysis and condition index (> 50 ind.)
* measured parameters: shell length (L) and height (H) (accuracy +/- 0.1 mm), dry weight of shell (Ws) and of soft tissues (Wt) and maturity
* synthetic indicators: ratios H/L and Ws/L, gravimetric condition index (CI) (Walne, 1976)

Ecological inventory



➢ Qualitative and quantitative assessment of the benthic communities present on the sewage outfall
➢ Scuba dive: 2015-03-23 only for sampling *Mytilus sp.* and images
➢ Ecological sampling date: 2015-06-22
➢ Sampling of *Mytilus sp.* for toxicological analyses
➢ Observation of the global colonization of the environment
➢ Biological description of the site at the species level or groups of species level: cnidarians (anthozoans and hydrozoans); molluscs (bivalves, gastropods...); crustaceans; bryozoans; annelids polychaetes ; sponges; echinoderms; algae; fishes...
➢ Photos of colonization and species



Octopus, gastropod with eggs and hydrozoans

Physico-chemical characteristics

Tab.1 Water status according to WQES-river for metal concentrations (µg L⁻¹) in un-filtered water upstream and downstream PFA injection (sampling date: 2015-04-08).

Metal	Concentration (µg L ⁻¹)		Status	Un-filtered/Filtered concentration ratios	
	Upstream	Downstream		Upstream	Downstream
Cd	0.064	0.057	Moderate	1.0	1.0
Cr	0.39	0.37	Good	1.7	1.6
Cu	4.1	4.2	Moderate	2.1	1.2
Ni	4.6	4.8	Good	1.0	1.0
Pb	0.16	0.17	High	8.0	8.5
Zn	31	32	Moderate	0.9	1.1

✓ PFA did not impact particulate/dissolved partition for most metal except Cu in some cases (high affinity for organic matter)
✓ Heterogeneity of suspended particulate matter in wastewater and difficulty in sampling a same water mass upstream and downstream the tertiary treatment (PFA) could induce bias in measured parameters
➔ On-going lab experiments will complete field observations

Bacteriological survey

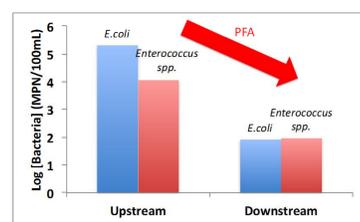


Fig.1 Concentrations of viable bacteria (*E. coli* and *Enterococcus spp.*) estimated by the most probable number (MPN) method upstream and downstream the PFA injection point.

✓ PFA succeeded in suppressing 3.4 log u. of *E. coli* cells and 2.0 log u. of *Enterococcus spp.* cells under normal operating conditions of the WWTP
✓ Treatment efficiency was sensitive to the load of SPM in treated water

First results

Morphological and genetic variations

✓ Mussels collected on the sewage outfall did not show any cytoplasmic oxidative stress
✓ An increase of the mitochondrial metabolism in the visceral gland of mussels from the sewage outfall was probably related to higher environmental pressure (e.g. organic and inorganic compounds in wastewater)

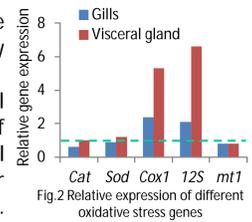


Fig.2 Relative expression of different oxidative stress genes

✓ Mussels (for 2<L<4 cm) collected on the sewage outfall were less elongated than the individuals collected on the jetty
✓ Similar CI for immature individuals of the common class (2<L<4 cm) between sites in February, different in June

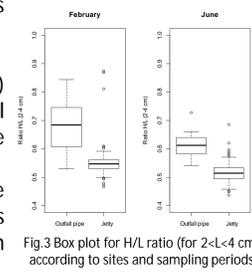


Fig.3 Box plot for H/L ratio (for 2<L<4 cm) according to sites and sampling periods

Ecological inventory

✓ Bad sampling conditions (high currents, waves 1.4 m, 13 s of period, turbidity...)
✓ Definition of depth (zero of marine maps) sandy bottom at 6 m depth stand up at 3 m high
✓ Qualitative inventory: mainly tubeworms; hydrozoans and bryozoans
✓ Their location on the structure
✓ Individual sampling for examination in laboratory mainly tubeworms, hydrozoans and bryozoans
✓ Photos of samples collected

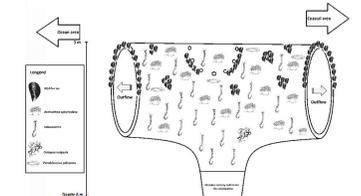
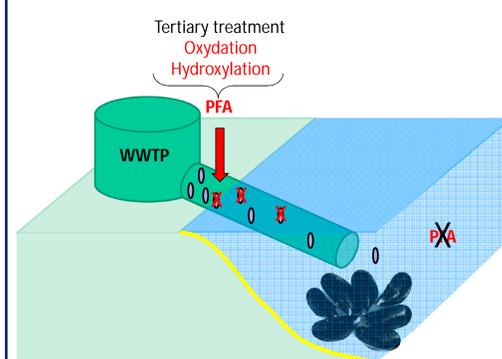


Fig.4 Preliminary results of the main species present on the structure

References:

Reimer O., Tedengren M., 1996. Phenotypical improvement of morphological defences in the mussel *Mytilus edulis* induced by exposure to predator *Asteria rubens*. *Oikos* 75, 383 – 390.
Walne P.R., 1976. Experiments on the culture in the sea of the butterflyfish *Venerupis decussata* L. *Aquaculture* 8, 371 – 381.

Discussion/conclusion



✓ Removal of 3.4 log u. of *E. coli* cells and 2.0 log u. of *Enterococcus spp.*
✓ No oxydative stress induced in mussels
✓ Very short half life (< 20 mn in marine water), no persistence in the receiving environment
✓ Morphological differences between sites but no consistent patterns concerning CI
✓ Next dive in September (with the high of waves < 1 m) for compilation of ecological survey: seasonal variability, identification of samples, quantitative data...
First results presently does not show any modification of the biological indicators in relation to the new process. Those results will be complemented with upcoming samplings. It could be interesting to work on shell thickness to complete the CI information (with regards to relative allocation of resources to either tissues or shell growth).