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# Investigation of natural antimicrobial compounds for prevention of microbiologically influenced corrosion (MIC)



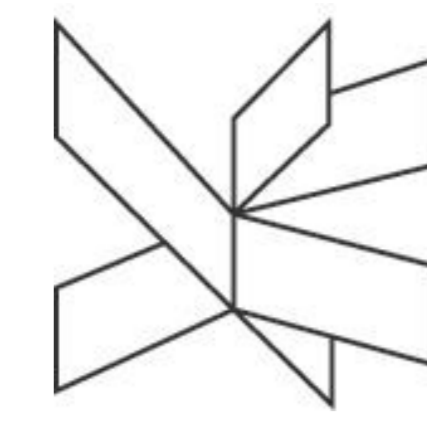
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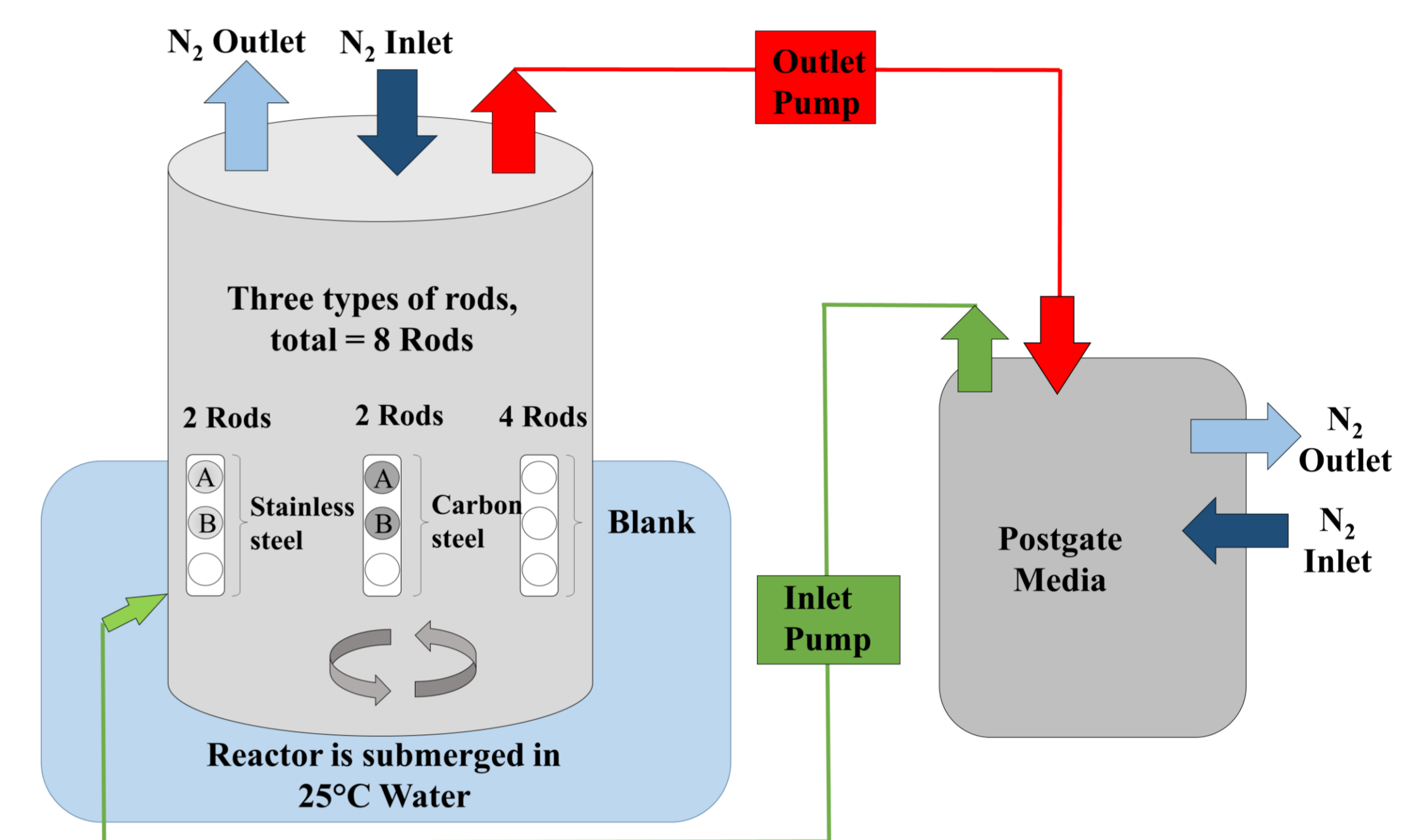
## BACKGROUND AND NEEDS

### Current options and the necessity to apply natural antimicrobial compounds

- Eliminating sulfates through filtration of seawater is a possible way to control sulfides - however it is expensive.
- The addition of nitrate to injection water could abate H<sub>2</sub>S production - but it requires repeated treatments and is associated with high chemical costs. Addition of nitrate might also increase the biomass of microorganisms in the water, causing plugging and even increased corrosion rates in some fields.
- The current industrial methods of controlling MIC are maintenance pigging and chemical treatment (biocides and corrosion inhibitors).
- A combination of mechanical cleaning and chemical treatment are the most efficient techniques currently. Chemical controls are generally considered to be the most effective in performance and cost. While biocides readily destroy planktonic cells, biofilm cells located on the pipeline surfaces are protected by a polysaccharides covering and ward off the effects of toxic biocides.

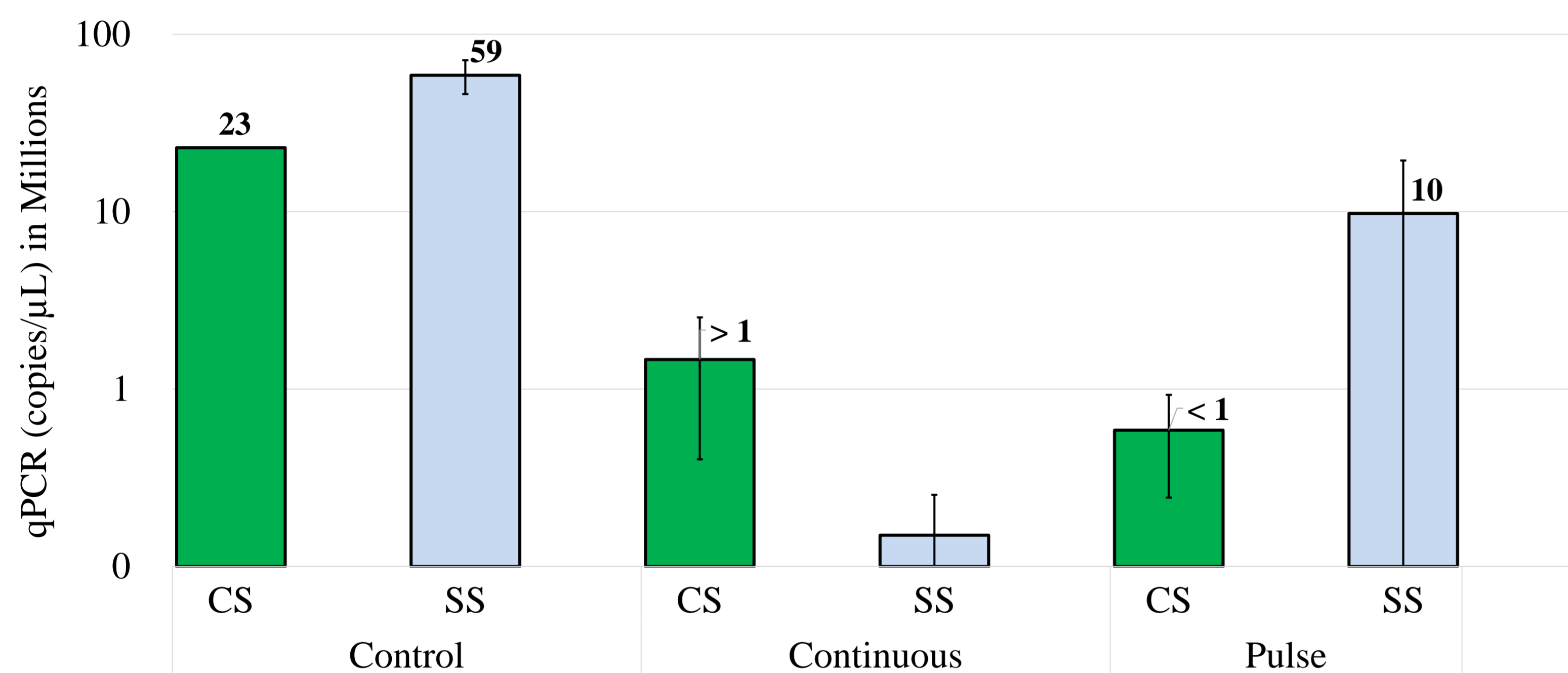
## EXPERIMENTAL SETUP

- 3 reactors (Control, Continuous and Pulse)** inoculated with sediments from Wadden Sea.
- Nitrogen was flushed to maintain anaerobic conditions.
- Each reactor has 8 rods, and 3 coupons in each rod, allowing for 24 coupon positions. Out of the 24 available positions, 4 stainless steel (SS) and 4 carbon steel (CS) coupons were used in EACH reactor.
- Extractives mixture is added to Continuous and Pulse reactors. The extractives mixture is obtained from halophytes (*Patent pending*)
- 10% (v/v) extract mix is added to the Continuous reactor
- 3 pulses, of 5, 10, and 15 % (v/v) are added on Day 14, 20, and 26 respectively to the Pulse reactor
- Coupons are rinsed and sent for analysis at the end of the experiments.

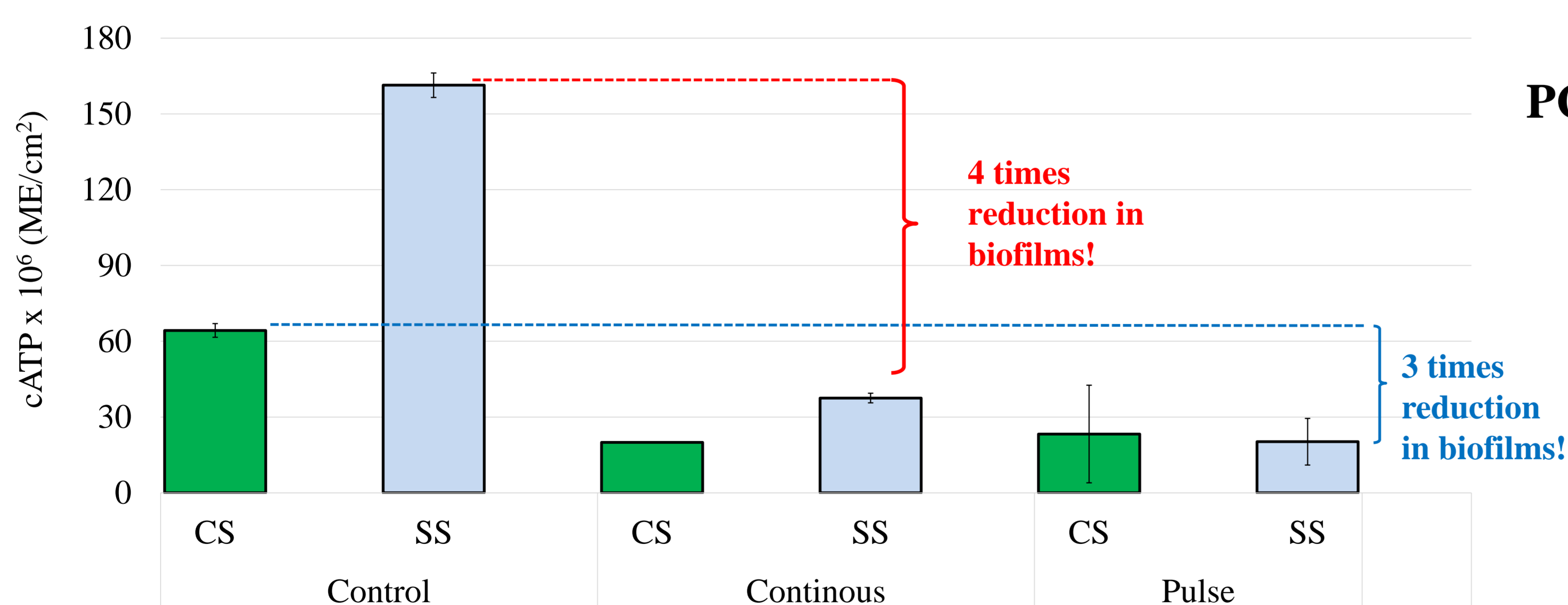


## RESULTS

### qPCR (Bacteria) of biofilm microorganisms:



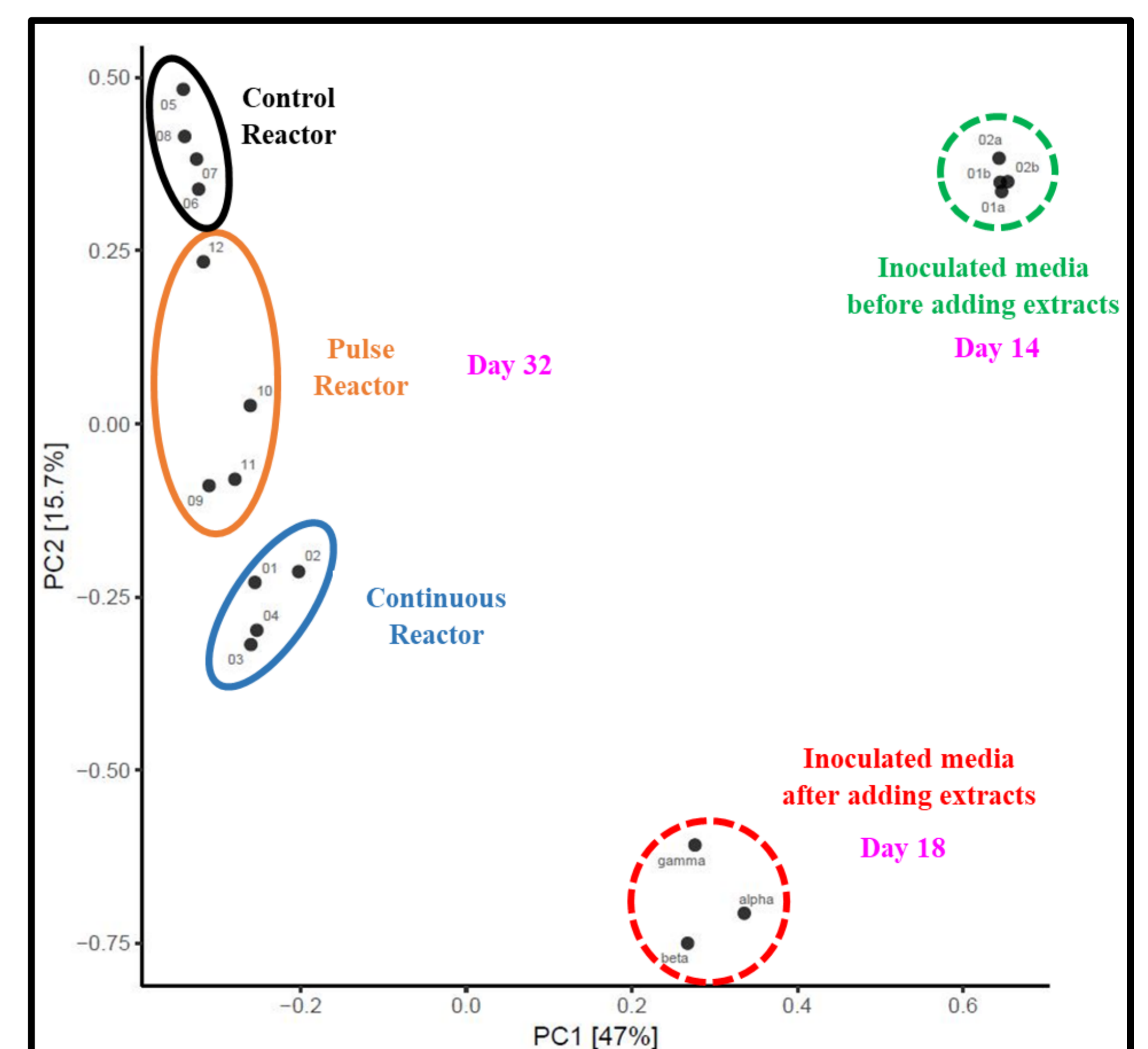
### ATP measurement of biofilm collected on coupon surface:



### Heat-map of key genera:

	Continuous Reactor				Control Reactor				Pulse Reactor			
Gammaproteobacteria; Marinobacterium	22.3	16.8	22.9	23	16.1	16.5	19.4	24.5	18.6	10.1	16.5	12.3
Deltaproteobacteria; Desulfobivrio	28.1	19.2	32	33.5	5.3	9	6	6.6	11.7	9.6	10.8	1.8
Synergistetes; Dethiosulfobivrio	11	11.4	12.2	9.6	1.7	3.5	1.4	1.8	35.4	25.7	22.9	14.8
Gammaproteobacteria; Vibrio	2.7	4.5	2.2	2.5	15.1	17.3	7.2	11.9	2.8	7.8	3.6	38.5
Bacteroidetes; Carboxylicivirga	16.2	16.6	7.8	5.6	4.5	6	6.4	6.4	9.1	10.9	10.8	5.8
Gammaproteobacteria; Shewanella	1.6	1.3	1.7	2.1	15.1	1.9	20.9	4.9	2.9	1.2	4.6	17.4
Firmicutes; Proteinclasticum	0.6	0.7	1.1	1.3	10.5	15.7	6.2	9.5	2.3	2.6	2.8	0.8
Gammaproteobacteria; Pseudomonas	0.2	0.2	0.3	0.3	9.9	4	15	8.2	1.3	1	2.4	0.9
Gammaproteobacteria; Halomonas	0	0	0	0	8.7	7.2	3.7	11.3	0	6.1	0	0.1
Firmicutes; Fusibacter	1.3	1.6	1.6	1.6	5.7	6.9	3.5	4.6	1.4	2.5	1.4	1
Fusobacteria; Fusobacterium	3.4	4.7	4.5	4.9	0.2	0.2	0.2	0.1	1.4	1.8	1.7	0.5
Bacteroidetes; Bacteroides	2.2	4.3	2.1	1.9	1.3	1.9	0.9	0.9	0.9	2.2	1.4	0.4
Epsilonbacteraeota; Arcobacter	0.5	0.7	1.7	1.3	0.4	1	1.8	1.1	0.9	1.2	3.7	0.5
Deltaproteobacteria; Desulfobacter	3.7	3.4	1	1.4	0	0.1	0	0	1.6	1.8	1	0.2
Firmicutes; f_Family_XIII_OTU_22	0.6	0.8	0.7	2	0.5	1.6	0.4	1.2	1.3	2.5	1.4	0.5
Alphaproteobacteria; Cohaesibacter	0.7	0.7	0.4	0.5	0.2	0.3	0.2	0.1	0.8	1	1.5	0.8
Bacteroidetes; Sunxiuqinia	0.2	1	0.5	0.6	0.5	0.6	0.5	0.5	0.3	0.7	0.4	0.1
Fusobacteria; Propionigenium	2	2.9	0.2	0.2	0.1	0.1	0	0	0.1	0.1	0	0
Firmicutes; Tyzzerella	0.4	1.1	0.6	0.9	0.1	0.1	0.1	0	0.4	1.3	0.3	0.1
Deltaproteobacteria; Halodesulfobivrio	0.5	1.1	0.1	0.1	0	0	0	0	0.5	1.8	0.6	0.3

### PCA plot of all samples:



## MAJOR FINDINGS

- Significant inhibition of MIC bacteria was observed in liquid samples from the reactors treated with **Halophytic Plant Extracts**.
- Biofilm formation was reduced by three times on carbon steel and four times on stainless steel coupons in reactors treated with **Halophytic Plant Extracts**.
- Visible and measurable reduction in pitting and corrosion damages on carbon steel coupons was observed with the help of 3D surface scanning (data not shown).
- Addition of **Halophytic Plant Extracts** reduce the microbiological *abundance* (number of microorganisms measured by qPCR) by **20 times for Carbon steel** and by **at least 5 times for Stainless steel**.
- Halophytic Plant Extracts DO** reduce the microbiological *diversity*, which was measured for top 40 genera (diversity index, data not shown).

## WHAT'S NEXT

- Characterization of halophytic plant extracts and formulations.
- Compare pulse versus continued flow, variation in concentrations, contact time, and frequency of extractive addition.
- Techno-economic evaluation of the natural biocide production.