AGRICULTURAL UNIVERSITY OF ATHENS

Department of Food Science and Human Nutrition

Laboratory of Microbiology and Biotechnology of Foods

aquaculture europe 20

Biofilm and Biofouling Development on Novel Sensing Surfaces in a Marine Recirculated Aquaculture System

Eirini Schoina*[1], Robert Daly[2], Pierre Lovera[2], Alan O'Riordan[2], Brendan O'Flynn[2] and George-John Nychas[1]

[1]Laboratory of Microbiology and Biotechnology of Foods, Department of Food Science and Human Nutrition, Agricultural University of Athens, Athens, Greece

[2]Tyndall National Institute, University College Cork, Cork T12 R5CP, Ireland

*eschoina@aua.gr

INTRODUCTION & OBJECTIVE

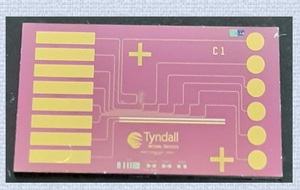
As soon as water monitoring sensors are introduced into the water, they serve as a new niche for microbial colonization and subsequent installation of fouling macroorganisms.

The development of biofouling on sensing surfaces and electrodes can affect their operation, thus, leading to acquisition of inaccurate measurements.

The aim of the present study was to assess the biofilm and biofouling development on a novel sensing surface under real aquaculture conditions.

MATERIALS AND METHODS

Silicon chip sensor devices were fabricated using common microelectronic processes as described previously (Wahl et al., 2018). A silicon nitride passivation layer (500 nm thick) – representing the majority of the surface of the sensors chip - was deposited to passivate the entire chip.



Triplicates of sensor devices (17* 9.5* 0.5 mm) and stainless steel coupons (30* 10* 1 mm) were placed into an aquarium tank of a marine RAS, where sea bass adults were reared

Samplings were performed after **14**, **28** and **42** days of submersion:

- Microbiological analysis in water and biofilms (in duplicates)
  - bead vortexing method
  - enumeration of viable cells on Marine Agar (heterotrophic bacteria) and on TCBS agar plates (detection of presumptive Vibrio species)
- Temporal changes of the bacterial community composition (culture dependent) of the tank water and of the biofilms were assessed by denaturing gradient gel electrophoresis (DGGE) based on the 16S rRNA gene (V3-V5)
- Biofilm/biofouling development was monitored with fluorescent microscopy (Acridine Orange staining)

DISCUSSION

- Planktonic marine heterotrophic bacteria ranged from 10^4 to 10^5 CFU ml^{-1}, as previously reported in similar RAS setups (Schoina et al. 2019), while presumptive Vibrio represented 32-55% of the total population of culturable marine heterotrophic bacteria - Table 1
- The population of biofilm cells on both materials was comparable at each time point -Table 1 and Figure 1
- Presumptive Vibrio species were detected in the biofilms, confirming that biofilms may act as a reservoir for potentially pathogenic bacteria in RAS setups (Bourne et al., 2006) – Table 1 and Figure 1
- DGGE profiles of water samples revealed that the RAS water microbial association remained unchanged during the experimental period, in accordance with previous reports (Attramadal et al. 2012) – Figure 2
- DGGE fingerprinting of biofilms revealed high similarity of predominant operational taxonomic units (OTUs) at each time point, being independent of the type of material, as previously described (Schoina et al., 2020), while observing a dynamic succession of predominant OTUs – Figure 2
- Fluorescent microscopy revealed that even after 14 days of water submersion, surfaces were covered by biofouling, regardless of the material. At days 28 and 42 biofouling coverage remained at the same levels – Table 2

ACKNOWLEGMENT

IMPAQT Intelligent Management System for Integrated Multi-trophic Aquaculture

This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 774109

RESULTS

Table 1: Evolution of the planktonic and biofilm marine heterotrophic bacteria (MA) and presumptive Vibrio species (TCBS) during the experimental period

	Water (log cfu*ml^{-1})		Novel Sensor (log cfu*cm^{-2})		Stainless steel (log cfu*cm^{-2})	
Bacterial group	MA	TCBS	MA	TCBS	MA	TCBS
Day 0	3.7	3.0	-	-	-	-
Day 14	4.0	3.8	6.0	3.3	5.9	2.9
Day 28	5.0	4.4	6.2	4.1	6.0	4.2
Day 42	4.9	4.4	6.3	4.6	5.8	4.5

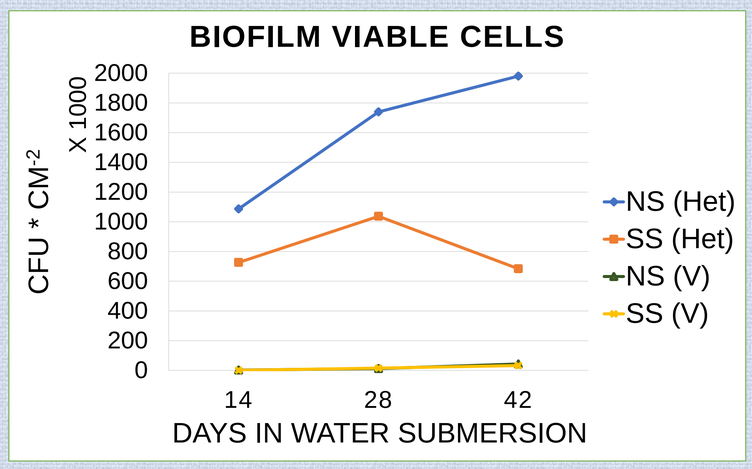


Figure 1: Development of the number of biofilm cells on (marine heterotrophs) on novel sensor (NS-Het for heterotrophic bacteria and NS-V for presumptive vibrio) and on stainless steel (NS-Het for heterotrophic bacteria and NS-V for presumptive vibrio). Values are expressed in CFU*cm^{-2}

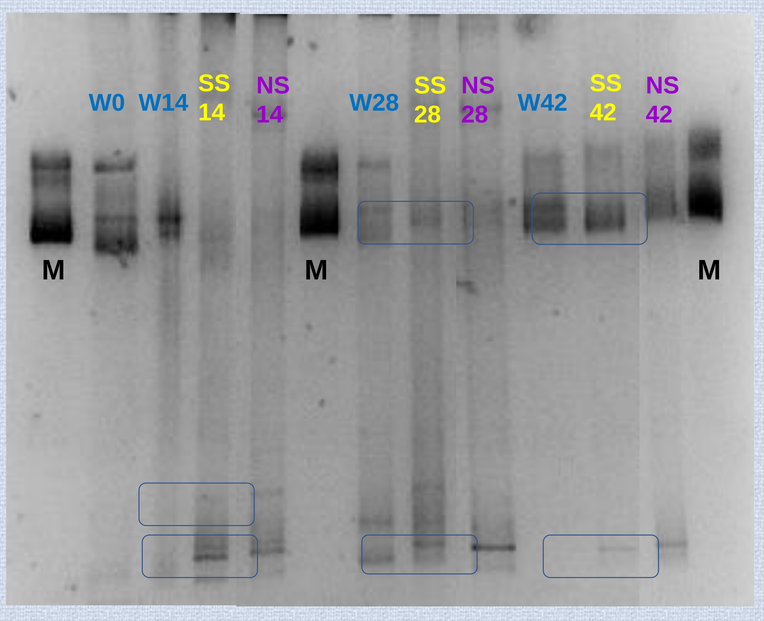


Figure 2: DGGE fingerprint of water and biofilm cells collected from MA plates. Bands with same position are considered as the same OTU. Blue rectangles indicate similar OTUs between the 2 different surfaces. (water samples W, biofilm on stainless steel coupons SS and on novel sensor NS)

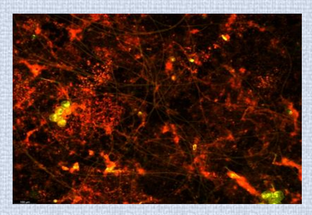
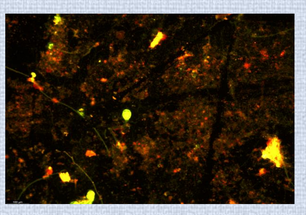
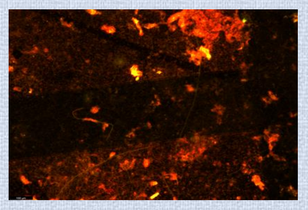
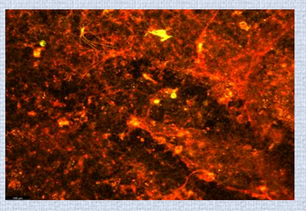
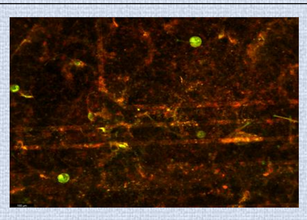
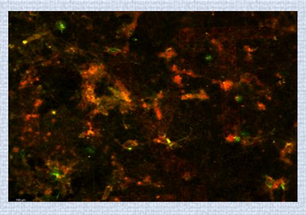
Days in submersion	Novel sensor	Stainless steel
14		
28		
42		

Table 2: Indicative images from epi-fluorescent microscope of biofouling developed on different surface materials at 3 time points after staining with Acridine Orange (excitation at 450-490 nm and emission at 515 nm) Magnification 10 x

REFERENCES

•Attramadal K.J.K., I. Salvesen, R. Xue, G. Øie, T.R. Størseth, O. Vadstein and Y. Olsen. 2012. Recirculation as a possible microbial control strategy in the production of marine larvae. Aquaculture Engineering 46: 27–39.

•Bourne D.G., L. Høj, N.S. Webster, J. Swan and M.R. Hall. 2006. Biofilm development within a larval rearing tank of the tropical rock lobster, *Panulirus ornatus*. Aquaculture 260: 27–38.

•Schoina E., E. Miliou . and G-J Nychas. 2019. Evaluation of the biofilm formation on stainless steel surfaces in a marine recirculated system. In: Aquaculture Europe 19 conference, Berlin, Germany, pp1389-1390.

•Schoina E., I. Bru and G-J Nychas. 2020. Dynamics of Biofilm Formation in a Mediterranean RAS. In: ASM Microbe 2020 conference (online).

•Wahl, A., Seymour, I., Moore, M., Lovera, P., O'Riordan, A., Rohan, J. Diffusion profile simulations and enhanced iron sensing in generator-collector mode at interdigitated nanowire electrode arrays, Electrochimica Acta, 277(2018) 235-43