BIOFILM AND BIOFOULING DEVELOPMENT ON NOVEL SENSING SURFACES IN A MARINE RECIRCULATED AQUACULTURE SYSTEM

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Introduction

The control of the aquaculture systems is actualized with a variety of monitoring sensors that are operating 24/7 and providing measurements of water quality parameters. Once these sensors are introduced into the water, they serve as a new niche for microbial colonization and subsequent installation of macroorganisms. The development of biofouling on sensing surfaces and electrodes can affect their operation, leading to acquisition of inaccurate measurements.
The aim of the present study was to assess the biofilm and biofouling development on a new sensing surface developed by Tyndall, under real aquaculture conditions. The study was performed in a marine recirculated aquaculture system (RAS), so as to allow natural biofilm/biofouling development. The methods employed included microbiological analysis on the developed biofilms implemented with molecular techniques, as well as epi-fluorescent microscopy to monitor the biofouling installed on the surface of the sensing material.

Methods

Folded plastic nets containing coupons in triplicates of sensor's material and stainless steel as reference were placed into an aquarium tank, part of a marine RAS, where sea bass adults were reared.
Silicon chip sensor devices were fabricated using common microelectronic processes as described previously (Wahl et al., 2018). In brief, interdigitated gold microbands were patterned in resist by photolithography followed by metal evaporation (Ti/Au 5/50 nm) and standard lift-off. Similarly, photolithography, metal evaporation (Ti/Au 10/90), and lift-off procedures were then employed to overlay electrical interconnection tracks including peripheral probe pads. Macroscale gold and platinum counter and pseudo-reference electrodes, respectively, was also deposited during this process. Finally, 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 and windows selectively opened with a dry etch to allow exclusive contact between the working, reference and counter electrodes with the solution of interest.
At 14, 28 and 42 days after placement, microbiological analysis was conducted for planktonic bacteria in the water and biofilm cells. For the latter, sessile cells on coupons (in dublicates) were sampled following a bead vortexing method. Enumeration of viable biofilm and planktonic cells was performed on Marine Agar (MA) plates for marine heterotrophic bacteria and on TCBS agar plates for the detection of presumptive *Vibrio* species.
Temporal changes of the bacterial community composition of the water and biofilms' comparison were assessed by a PCR-DGGE method. Following a culture dependent technique, the total amount of bacterial colonies grown on MA plate of the minimum dilution was collected for both materials and for the water sample at each time point. Cells were lysed using a lysozyme based protocol to extract bacterial DNA. PCR-DGGE was performed as previously described (Schoina et al., 2019), targeting the hypervariable V3-V5 region of the 16S rRNA gene.

Biofilm/biofouling development monitoring was performed by fluorescent microscopy. At every sampling coupons were retrieved, biofouling was fixed with methanol and stained with Acridine Orange dye, to be observed under the microscope.

Results and Discussion

The population of planktonic marine heterotrophic bacteria throughout the experimental period ranged from 10^4 to 10^5 CFU ml^{-1}, in accordance with previous studies in similar marine RAS setups (Schoina et al. 2019). The presence of presumptive *Vibrio* species in the water samples was high, representing 32-55% of the population of cultured marine heterotrophs. The population of biofilm cells on both materials was comparable at each time point, being stable at 10^6 CFU cm^{-2}. Presumptive *Vibrio* species were also detected in the biofilms. Their participation in the biofilm increased through time, starting from 10^3 CFU cm^{-2} at day 14 and reaching 10^4 CFU cm^{-2} at days 28 and 42. This observation confirms that biofilms may act as a reservoir for potentially pathogenic bacteria in RAS setups, as previously reported (Bourne et al., 2006).

The DGGE profiles revealed that the RAS water microbial association remained unchanged during the experimental period, in accordance with previous reports (Attramadal et al. 2012). Regarding the biofilm communities, DGGE fingerprinting showed high similarity of predominant operational taxonomic units (OTUs) sampled at the same time points, being independent of the type of material, as previously described when comparing stainless steel and glass (Schoina et al., 2020), while observing a dynamic succession of biofilm predominant OTUs. Similar findings of biofilm dynamics has been previously shown in other types of surface materials (Bourne et al., 2006).

Epi-fluorescent images revealed that after 14 days of immersion in the water, coupons were equally covered by biofouling, regardless of the material type. Accordingly, at days 28 and 42 biofouling was predominant on both types of surfaces. Even though the experiment was performed in a RAS where natural seawater is introduced after UV treatment, biofouling organisms were installed on the test surfaces.

Conventional microbiological analysis and molecular techniques, in combination with microscopy demonstrated the dynamics of biofilms/biofouling development on novel sensing surfaces in a Mediterranean RAS. The tested sensing surface was subjected to biofilm adherence with high contribution of *Vibrio* species, as well as to biofouling installation, with unknown effects on its function.

This work is part of a series of *in situ* studies for prolonged time periods, as an approach for better assessment of the biofilm and biofouling formation on novel sensing surfaces.

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