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C/N ratio-induced structural shift of bacterial communities inside lab-scale aquaculture biofilters

Luigi Michaud[a,*], Angelina Lo Giudice[a], Filippo Interdonato[a], Sebastien Triplet[b], Liu Ying[c], Jean Paul Blancheton[b]

[a] Department of Biological and Environmental Sciences (DiSBA), University of Messina, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy
[b] IFREMER Palavas-les-Flots, Chemin de Maguelone, 34250 Palavas-les Flots, France
[c] Institute of Oceanology, Chinese Academy of Sciences, Qingdao 266071, China

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ABSTRACT

In recirculating aquaculture systems (RASs) various chemical compounds (mainly nitrates and organic carbon) accumulate in the rearing water. These chemical substrata regulate the ecophysiology of the bacterial communities of the biofilter and have an impact on its nitrification efficiency and reliability.

In the present study chemical and microbiological parameters in static mineral bed (SBB) and moving plastic bed (MBB) biological filters were monitored at increasing C/N ratios ranging from 0 (pure nitrification) to 4 (combined nitrification and organic carbon removal), with the aim to investigate the shift of the bacterial community structure and major taxa relative abundances.

Results suggest that the MBB are less subjected to the nitrification reduction than the SBB, probably due to their self-cleaning characteristic. Moreover, the dynamics and flexibility of the bacterial community to adapt to influent water changes seemed to be linked with the biofilter performance. The increase of the C/N ratio resulted in a shift of the bacterial community structure in terms of reduction of taxa richness and diversity indices, and in a positive selection of the *Gammaproteobacteria* (especially in the SBB).

One of the key aspects for improving the reliability and sustainability of RASs is a proper management of the biofilter bacterial populations, which is directly linked to the C availability. Nevertheless, it is a pertinent question whether it is possible to modify the composition of a microbial community in an environment like a biological filter, using direct microbe controlling systems (e.g. water exchange and UV disinfection).

1. Introduction

Recirculating aquaculture systems (RASs) employ various strategies in order to purify and reuse rearing water, thus dramatically reducing its make up water consumption compared to traditional systems. RAS technology provides the option of rearing fish at high densities under controlled conditions, leading to a potentially reduced environmental impact from the fish production (Piedrahita, 2003; Badiola et al., 2012). The understanding of the system is one of the key factors in the management of RASs, as this requires interaction between engineering and organism biology and husbandry. All the key biological mechanisms involved in the functioning of RAS therefore need to be understood and mastered. This is particularly the case processes determining the development of bacterial populations and their interactions with fish (Blancheton et al., 2013).

The efficient removal of TAN and nitrites is an essential issue in relation to the commercial fish production. TAN is oxidized to nitrate in biofilters by nitrifying bacteria attached to a solid inert support medium, internally in pore spaces or directly on the surface, forming a fixed biofilm (Michaud et al., 2006, 2009; Prehn et al., 2012).

The C/N (organic carbon/inorganic nitrogen) ratio has often been used as a link between the availability and competition for organic carbon mainly composed of fish faeces and uneaten feed, and ammonium (Hu et al., 2009). At high C/N ratios the heterotrophic bacteria reduce the diffusion of nitrogenous substrate and DO to the autotrophic nitrifying bacteria, thus negatively affecting the nitrification rate (Nogueira et al., 2002; Chen et al., 2006). Reduction in TAN removal rates as high as 70% has been reported at C/N ratios above 1 for dissolved carbon (Zhu and Chen, 2001; Ling and Chen, 2005), while a reduction of 73% has been reported at a C/N ratio of 2 for particulate carbon (Michaud et al., 2006). Improved feed quality and assimilation, in addition to a better removal or finer mechanical filtration of waste solids, are the two primary means of reducing particulate and dissolved organic concentrations in

* Corresponding author. Tel.: +39 090 6765533; fax: +39 090 6765526.
E-mail address: lmichaud@unime.it (L. Michaud).

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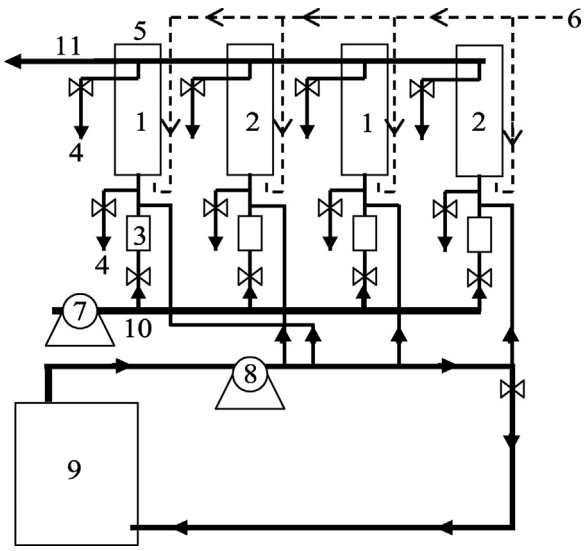

Fig. 1. Schematic diagram of the lab-scale biofilter system. (1) Static bed biofilters (×2, Biogrog packing media); (2) moving bed biofilters (×2, Acui T media); (3) flow metres (×4); (4) sampling ports for waters analyses (×2 for each filter); (5) sampling port for packing media; (6) air line; (7) circulation pump; (8) peristaltic pump for nutrients enrichments; (9) enrichment reservoir; (10) water inlet; and (11) water outlet.

recirculating systems (Guerdat et al., 2011). Fast growing bacteria (r-strategists) are the first to exploit an increase in substrate supply but, if the resources are consumed, they can be gradually outcompeted by slower growing specialists (K-strategists) (Hansen and Olafsen, 1999).

The effect of organic carbon on biofilters has been mainly studied with respect to either the nitrifying reactor performance or the microbial spatial and quantitative distributions (Ohashi et al., 1995; FDZ-Polanco et al., 2000). The present study was aimed at investigating the effect of increasing C/N ratios on the bacterial community structure and major taxa relative abundances in aquaculture lab-scale biofilters. In particular, two biofilter configurations (mineral static bed and plastic moving bed) were tested.

2. Materials and methods

2.1. Experimental system and procedures

2.1.1. Lab-scale biofilter system

The system was constituted by four 9.5 L biofilters that were continuously filled with heated (20 ± 2 °C), sand-filtered and UV disinfected seawater (salinity 37 ± 1, pH 7.5 ± 0.5) (Fig. 1). Two biofilters (replicates) were submerged reactors (defined as static bed biofilter, SBB) and filled with 8 L of a mineral packing media (cooked clay with a high specific surface usable by bacteria of about 800 m^2/m^3, Biogrog, Argiles et Mineraux, Montguyon, France). The other two biofilters (replicates) were set up as moving bed reactors (defined as moving bed biofilter, MBB) and 2/3 filled with a plastic packing media (Acui T, specific surface of 800 m^2/m^3, Nantes, France).

The system was equipped with a 300 L tank for the enrichment mixture (inorganic N and organic C, as described below) and with a 1 m^3 tank used as a buffer for the make-up water. The raw seawater was pumped into each biofilter at a constant flow rate of 2 L/min and the concentrated enrichment mixture was pumped at a constant flow rate of 0.2 L/min. The effluent was not re-injected in the buffer tank in order to keep constant the inlet water composition.

2.1.2. Experimental procedures

Inlet water was enriched with particulate organic matter (POM) and ammonium chloride (see below). Theoretical C/N levels were fixed to 0, 0.5, 0.8, 2 and 4. Each C/N ratio step was set up in duplicate (two filters for MBB and two for SBB) and run for at least four weeks to allow the formation of a steady-state biofilm. Chemical and physical parameters (pH, oxygen concentration, temperature, redox potential) were daily measured.

At the end of each C/N ratio step, samples were collected for chemical and microbiological analyses (see below), and the system was subsequently set up and run again for 4 more weeks.

2.1.3. Enrichment mixture

The input of ammonium chloride (Sigma, France) was set to achieve an ammonia concentration of 2 mg/L at the inlet of each biofilter. This concentration was kept constant for all the experiment and the C/N ratio was modified through the change of the carbon concentration.

The organic carbon used for the experiment was composed of fish faeces and unconsumed feed collected from particle separators at the outlet of various experimental seabass rearing systems. Such mixture (94 ± 0.1% dry matter) was sterilized by autoclaving, freeze-dried and grinded in a fine homogenous powder as described in Michaud et al. (2006). The resulting powder was chemically analysed with an auto-analyser Carlo Erba Instruments 1500 CHN for determination of the average carbon content (42 ± 0.8% of total organic carbon, data not shown), and stored at −20 °C during the entire experiment.

2.2. Chemical analyses

Ammonia, nitrites and nitrates were analysed with a Technicon® Autoanalyser II as described by Treguer and La Torre (1974). Biofilters were routinely monitored (twice a week) for TAN oxidation ($TAN_{In} - TAN_{Out}$). At every sampling time, nitrification efficiency was evaluated by connecting each biofilter in batch mode and following the decreasing of the TAN in the batch for one hour. The batch mode consisted in a tank filled with 50 L of water containing 2 mg/L of ammonia and the correct amount of POC to maintain the desired C/N ratio. Water was collected from the tank at fixed intervals (0, 20, 40 and 60 min).

2.3. Microbiological analyses

2.3.1. Sampling procedures

Bacterial communities fixed on two Biogrog and 10 Acui T, packing media subunits were detached following the procedure described in Michaud et al. (2006). Briefly, the packing media subunits were placed in a detachment buffer (0.1% of sodium pyrophosphate in a phosphate buffer saline, PBS: 130 mM NaCl, 10 mM Na_2HPO_4, and 10 mM NaH_2PO_4; pH 7.4), manually scraped with a sterile brush and placed in an ultrasonic iced bath for 10 min at 20 kHz. This collected mixture was divided in aliquots for microbiological analyses. All chemicals were purchased by Sigma, France.

2.3.2. Bacterial enumeration

Samples for direct enumeration of free living bacteria were fixed in formalin and stored at −20 °C until processing. Sample aliquots were filtered on 25 mm diameter, 0.2 μm pore size black polycarbonate filters, and stained with DAPI (4′,6-diamidino-2-phenilindole, Sigma, France) (Porter and Feig, 1980). Cells were visualized by epifluorescence microscope (Axioplan, Zeiss). Results are expressed in cells/mL.

Cultivable heterotrophic bacteria associated with packing media were enumerated by colony forming units (CFU/mL) on