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IN SITU RHEOLOGY OF THE BIOFILM FROM A NITRYFYING BIOREACTOR RELEVANT FOR RAS – A PROOF OF PRINCIPLE STUDY

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ABSTRACT

Nitrifying bioreactors are a vital component of recirculating aquaculture systems (RAS) to maintain the required water quality. Bioreactors contain suspended plastic carriers on which nitrifying microorganisms grow. Understanding the rheology of biofilms that form on these carriers can provide useful information that can be correlated with other analyses to monitor the development and stability of the microbial communities, both over the lifetime of the bioreactor and in response to environmental changes, such as the salinity changes typical of salmonid aquaculture. The standard carriers have an open patterned geometry for biofilm formation and good fluid flow through but is not convenient for *in situ* rheological measurements. In this study, we included carriers in the same material (HDPE) and with the same external dimensions but with a 20mm diameter flat surface area suitable for placement within the rheometer. The carriers were placed in stainless steel "tea-ball" cages in two lab-scale bioreactors with different configurations for five weeks and then retrieved. After securing the carrier within the rheometer (Kinexus Ultra+ with a 20mm roughened parallel plate upper geometry) the upper plate was lowered to a normal force of 0.05N. Oscillatory measurements (frequency sweep and amplitude sweep) and creep compliance measurements confirmed the biofilm on the carrier surface as a solid dominant viscoelastic solid at low strain. This is comparable with the reported behaviour of microbial biofilms.

INTRODUCTION

A biofilm is a complex community of micro-organisms, such as bacteria and fungi, embedded in an extracellular matrix composed of self-excreted polymeric substances, which provides adhesion to a growing surface, cohesion to the community, and a barrier against external agents as well as protection from shear forces and mechanical disruption[1, 2]. Biofilms may be problematic if they grow in medical or hygiene settings and provide pathogenic bacteria with protection against antibiotics and disinfectants, but in other circumstances, such as in recirculating aquaculture systems (RAS) bioreactors, they can be beneficial, and provide stability for a microbial community that performs an industrial function[3]. The secreted extracellular polymeric substances (EPS) of the biofilm may variably include polysaccharides, protein, lipids and DNA. As with other cellular systems imbedded in extracellular matrices, the rheology of both the EPS itself and the total biofilm is important for functionality.

In RAS, nitrifying microorganisms are grown in a biofilm on solid phase carriers within a bioreactor. The biofilm microbial community is essential for removing the ammonia produced by the fish from the recirculating water through the process of nitrification. These are not static systems, and the microbial communities are dynamic, developing over time and responding to resource competition and changes in environmental conditions. For salmonid aquaculture, the transition from freshwater to saltwater conditions represents a significant environmental change which can influence the performance of the bioreactor and where the EPS matrix may play a role in the robustness of the microbial community [4].
It is well established that biofilm rheology can be influenced by the mechanical perturbations involved in removal from the growth surface[5-7] so it is desirable to measure rheology *in situ*. The standard biofilm carriers used in bioreactors have an open patterned geometry for biofilm formation and good fluid flow through that is not convenient for *in situ* rheological measurements. In this proof of principle study, we included within the bioreactor carriers in the same material (HDPE) and with the same external dimensions but with a 20mm diameter flat surface area suitable for *in situ* rheological characterisation of the biofilm.

MATERIALS AND METHODS

Carriers were produced at the Mechanical Workshop of the Faculty of Natural Sciences, NTNU, in HDPE in cylindrical form with external dimensions 5mm height, 25mm diameter as shown in **Fig. 1** to match the standard carriers.

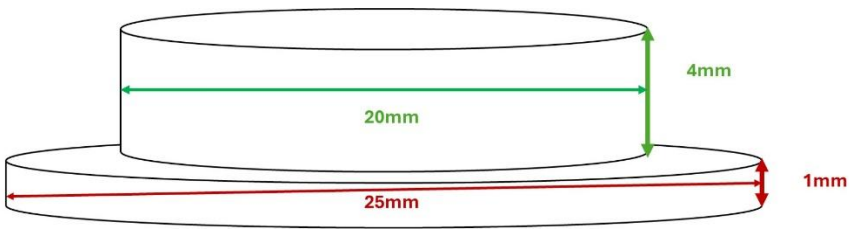

FIGURE 1. design of the rheometer compatible bioreactor carrier

Carriers were submerged for five weeks in either a lab-scale activated sludge bioreactor or a moving bed biofilm reactor (MBBR). The bioreactors were operated under environmental conditions that selected for nitrifying microbes, including continuous aeration and addition of required substrates. To prevent interference from other carriers and ensure consistent orientation, the carriers were enclosed in a metal mesh to protect the surface that would be used for rheology, (the smaller disc as seen in **Fig. 1**). Both bioreactors were operated under continuous flow with a hydraulic retention time (HRT) set to 25-26h, a pH range of 7-8, and room temperature. The media fed to the reactors were synthetic wastewater composed of ammonium sulphate ($(NH_4)_2SO_4$, 0.2 g/L), potassium dihydrogen phosphate (KH_2PO_4, 0.3 g/L), sodium carbonate (Na_2CO_3, 1 g/L), and 10 mL/L trace metal solution. pH of the media was adjusted to 7.5. During the experimental period, the system was challenged by decreasing HRT and/or increasing the ammonium concentration in the media. Spectrophotometric analyses showed that both reactors had a full conversion of ammonium to nitrate, indicating that the nitrification process was established. The carriers were stored in the fridge for 2 days before rheology measurements.

Rheology measurements were carried out using a Kinexus Ultra + rheometer (Netzsch), fitted with a 20mm diameter roughened parallel plate upper geometry at room temperature. The lower surface of the biofilm covered carrier was cleaned with 70% ethanol and the carrier was secured to the lower plate of the rheometer, and the upper plate was lowered to a normal force of 0.05N before commencing measurements. The following rheological tests were performed in the order given:
Frequency sweep 10-0.1Hz, logarithmic sampling (10 points per decade) with a target strain of 0.1%. Creep compliance repeated cycle 3x (20 Pa stress for 30s, with 2 minutes recovery time) followed by 3x (50 Pa stress for 30s, with 2 minutes recovery time). Amplitude sweep 0.1-100% strain logarithmic sampling (10 points per decade) at a frequency of 1 and 0.1Hz.

RESULTS

Oscillatory measurements showed the biofilm behaved as a viscoelastic solid dominant material at low strains, transitioning to flow dominant behaviour at higher strains (see **fig. 2 A & B**). Within the linear viscoelastic region, the absolute moduli were frequency dependent with a trend towards slightly higher phase angle at lower frequency (see **fig.2 C**). As the amplitude sweep is a potentially destructive test (if the material is not rheologically reversible) these were carried out last on the test biofilm matrix, after both the frequency sweep and the creep compliance measurements. However, the second amplitude sweep at lower frequency carried out after a rest period of 10 minutes shows a recovery of viscoelastic properties with broadly comparable moduli values to the frequency sweep.

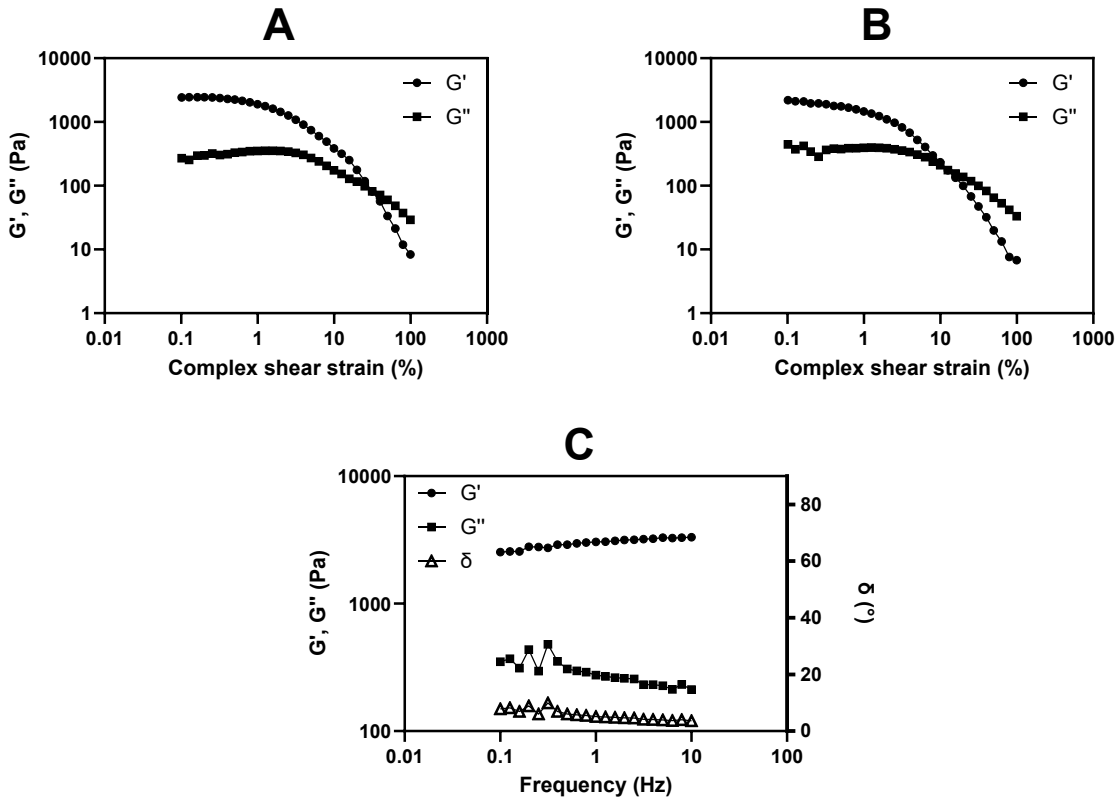

FIGURE 2. oscillatory rheology of the biofilm *in situ* on the bioreactor carrier showing an amplitude sweep at 1Hz (A), an amplitude sweep at 0.1Hz (B), and a frequency sweep in the linear viscoelastic region (0.1% strain) (C)

Creep compliance measurements also showed the biofilm behaved as a viscoelastic material with recovery of initial properties during the 2 minute recovery time (**fig. 3**). The compliance values were higher for 50Pa stress (**fig. 3 D-F**) than for 20Pa stress (**fig. 3 A-C**) indicating the stress was outside the linear viscoelastic regime and the *in situ* biofilm was more prone to deformation, however the 3 repeats showed similar behaviour suggesting that the material was not permanently weakened by the applied stress despite exceeding the limits of the linear regime.

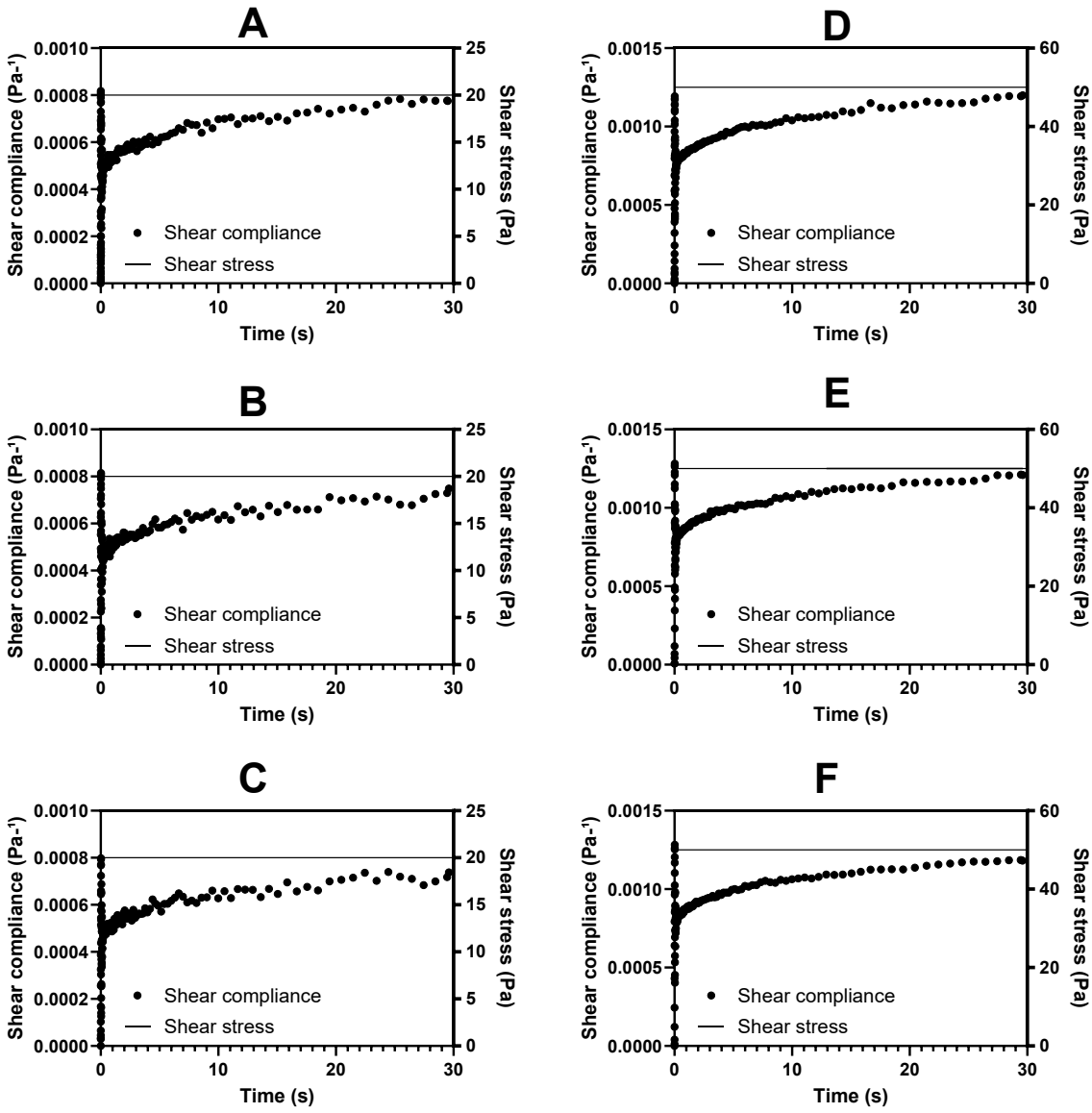

FIGURE 3. creep compliance of the *in situ* biofilm with 3 cycles of 20Pa constant stress for 30 seconds with 2 minutes recovery time (A-C), followed by 3 cycles of 50Pa constant stress for 30 seconds with 2 minutes recovery time (D-F)

DISCUSSION

This proof of principle study was intended to investigate the possibility of *in situ* measurements of rheology in biofilms present RAS bioreactors. To approach this question, we utilised laboratory scale nitrifying bioreactors and adapted carriers for initial testing. Visually, examination of the carrier surface suggested the presence of microbial biofilm material and the rheological testing carried out indicted a thin layer of viscoelastic material present on the carrier surface. The rheological data obtained indicate the biofilm was reasonably robust to perturbations, with repeated measures outside the linear viscoelastic regime generating similar data with relatively short recovery times (**fig. 2 A&B, fig. 3**). However, the *in situ* measurement system avoided the major perturbations linked to scraping and compression during loading onto the rheometer for testing that have been shown to significantly influence rheological results [5-7]. Whilst reported rheology of biofilms varies widely based on the microorganisms involved and the growing conditions [6-10], the data reported here show similar fundamental viscoelastic behaviours to previous reports, particularly for biofilms grown on solid supports [5, 6]. Based on these data, we believe the method has potential for monitoring aspects of biofilm stability in RAS.

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