WATER RESEARCH 46 (2012) 5085–5101

Available online at www.sciencedirect.com

SciVerse ScienceDirect

journal homepage: www.elsevier.com/locate/watres

Structural, physicochemical and microbial properties of flocs and biofilms in integrated fixed-film activated sludge (IFFAS) systems

Basuvaraj Mahendran [a], *Lori Lishman* [b], *Steven N. Liss* [a,*]

[a] School of Environmental Sciences, University of Guelph, Guelph, ON, Canada N1G 2W1

[b] Wastewater Science and Technology Directorate, Environment Canada, Burlington, ON, Canada L7R 4A6

ARTICLE INFO

Article history:
Received 27 February 2012
Received in revised form
25 May 2012
Accepted 26 May 2012
Available online 13 July 2012

Keywords:
Integrated fixed film activated sludge
Floc
Biofilm
Physicochemical properties
Extracellular polymeric substances
Microbial community

ABSTRACT

Integrated fixed-film activated sludge systems (IFFAS) may achieve year-round nitrification or gain additional treatment capacity due to the presence of both flocs and biofilms, and the potential for multiple redox states and long solids retention time. Flocs and biofilms are distinctive microbial structures and characterization of the physicochemical and structural properties of these may provide insight into their respective roles in wastewater treatment and contaminant removal in IFFAS. Flocs and biofilms were examined from five different pilot media systems being evaluated for potential full scale implementation at a large municipal wastewater treatment plant. Flocs and biofilms within the same system possessed different surface characteristics; flocs were found to have a higher negative surface charge (−0.35 to −0.65 meq./g VSS) and are more hydrophobic (60%–75%) than biofilms (−0.05 to −0.07 meq/g VSS; 19–34%). The EPS content of flocs was significantly higher (range of 2.1–4.5 folds) than that of biofilms. In floc-derived extracellular polymeric substances (EPS), protein (PN) was clearly dominant; whereas in biofilm-derived EPS, PN and polysaccharide (PS) were present in approximately equal proportions. Biofilm EPS had a higher proportion of DNA when compared to flocs. Biofilm growth was preferential on the protected internal surfaces of the media. Colonization of the external surfaces of the media was evident by the presence of small microcolonies. The structural heterogeneity of the biofilms examined was supported by observed differences in biomass content, thickness and roughness of biofilm surface. The biofilm on the interior surface of media was found to be patchy with clusters of cells connected by an irregular arrangement of interconnecting EPS projections. Biofilm thickness ranged between 139 μm and 253 μm. The pattern of oxygen penetration is expected to be complex. Nitrifiers and denitrifiers were predominantly associated with the biofilms, and the latter were found to be dispersed throughout the film and arranged in micro-clusters, suggesting partial oxygen penetration.

* *Corresponding author*. Present address: School of Environmental Studies and Department of Chemical Engineering, Richardson Hall, Room 251, Queen's University, Kingston, ON, Canada K7L 3N6. Tel.: +1 613 533 6933; fax: +1 613 533 6934.
E-mail address: steven.liss@queensu.ca (S.N. Liss).

0043-1354/$ – see front matter
http://dx.doi.org/10.1016/j.watres.2012.05.058

1. Introduction

Integrated fixed-film activated sludge systems (IFFAS) are hybrid suspended growth- biofilm systems that incorporate a high surface area of fixed or free floating media in oxygen-deprived or enriched zones of the conventional activated sludge process (CAS). Moving bed bioreactors (MBBRs) are configured similarly but the process does not accumulate mixed liquor (Hem et al., 1994). Supports for the biofilm growth include fixed (i.e. based on looped cords) or free floating media (i.e. neutrally buoyant plastic formed into finned cylinders or chips). The applicability of IFFAS, based on free floating media, to create processes suited to North America is on-going. Previous evaluations occurring at full-scale occurred at the Region of Peel (Stricker et al., 2009; Ross et al., 2004) and Broomfield, Colorado (Johnson and Haegh, 2005; Onnis-Hayden et al., 2007; Rutt et al., 2006). Creating a hybrid reactor by the addition of floating media addresses key issues limiting the efficiency of CAS systems (Stricker et al., 2009). Primarily IFFAS configurations have been implemented to achieve year-round nitrification and gain additional treatment capacity (Rutt et al., 2006; Stricker et al., 2009; Kim et al., 2010); although other applications such as nitrogen removal (nitrification/denitrification) (Cheng et al., 1997; Aesoy et al., 1998; Zammataro et al., 2004; Di Trapani et al., 2010) and combined biological nitrogen and phosphorus removal processes are being evaluated (Guang Hao et al., 1997; Chuang et al., 1997; Jones et al., 1998; Van et al., 1998).

Nitrification, a two step process, involves the conversion of ammonia nitrogen to nitrite nitrogen and the subsequent conversion of nitrite to nitrate. Traditionally, *Nitrosomonas europea* and *Nitrobacter* sp., have been identified as the predominant AOB and NOB, respectively, in activated sludge – a view that has changed due to community analysis using fluorescent in-situ hybridization (FISH). There are FISH based studies indicating bacteria belonging to the *Nitrosomonas* genus and not the *Nitrosospiras* genus are primarily responsible for ammonia oxidation in nitrifying wastewater treatment plants (Wagner et al., 2002; Persson et al., 2002); however, there are also studies that suggest the contrary (Coskuner and Curtis, 2002). The literature suggests that the dominant AOB may be a function of the experimental system. A clearer picture emerges for NOB community composition as members of the genus Nitrospira are found most frequently in both lab-scale studies, which were mainly carried out in biofilm reactors (Daims et al., 2001a, b; Gieseke et al., 2001) and in nitrifying wastewater treatment plants (Juretschko, 2000; Daims et al., 2001a, b). In hybrid reactors, it is possible to have compositionally different AOB and NOB bacterial communities due to differences in substrate concentration and retention time.

There are clear process advantages to implementing IFFAS in preference to CAS; however, they are more complex and poorly understood in comparison. In hybrid systems, flocs and biofilms make very different contributions to overall performance (Sriwiriyant and Randall, 2005). Based on studies done on other systems, the physio–chemical properties of the flocs taken from IFFAS systems are expected to be comparable to found in CAS (Liao et al., 2001; Wilen et al., 2003); similarly, media-associated biofilms should be comparable to those derived from other hybrid bioreactor processes (Lydmark et al., 2007; van den Akker et al., 2010). However, Zhang et al. (2006) proposed that flocs originating from hybrid reactors could be fundamentally different than those derived from comparable suspended-growth systems. Further characterization is required to test whether the structural and physiochemical properties are unique to IFAS hybrid structures. The objective of this study is to provide this information.

In this study, five different IFFAS trains treating municipal wastewater and operated in parallel are used as a model system. Processes were operated under comparable conditions but had a unique combination of media type, reactor fill ratio and process configuration (i.e. sequencing and volume of aerobic, media and anoxic zones). Floc and biofilm samples from the reactors were characterized in terms of their physiochemical properties, morphological features and microbial community composition. To the best of our knowledge there is no study characterizing flocs and biofilms derived from IFFAS systems, treating municipal wastewater, in this way. A better understanding of these systems will be useful for modeling and optimizing systems.

2. Materials and methods

2.1. *IFFAS design and performance*

Pilot operation was done to evaluate the performance of media systems for potential full-scale implementation at Lakeview WWTP (Mississauga, ON). Five pilots operated in a dedicated building with minimal heat. The pilot design flow was 4.0 L/min with an instantaneous peaking factor of 2.2. The IFFAS aeration tank had a total liquid volume of 1.4 m^3 with partitioning of the reactor being left to the discretion of the media supplier. All of the suppliers decided to create three cells within the tank. Specifics such as cell volume, oxidation state (i.e. aerobic or anoxic), media placement, and media fill ratio are supplier specific (Table 1 and Fig. 1). An aeration manifold was fashioned out of a drilled piece of pipe to mimic coarse bubble aeration. A small submersible pump provided mixing in the anoxic zone.

For the first few months, the IFFAS systems were initially fed clarified raw sewage using a pilot scale clarifier. Later primary effluent from a full scale clarifier was used and this resulted in the pilots receiving a feed comparable to the main plant. Iron addition occurred throughout the study as it was used for phosphorus control and enhanced settling in the primary clarifier, an interim operational requirement of the main plant. Median concentrations of iron in the influent are 13.8 mg/L with values reaching as high as 41 mg/L (95 percentile) during the period of enhanced settling.

Pilots operated for approximately 18 months with an intensive sampling period occurring over the last 12 months. Dissolved oxygen readings (DO), using a manual DO probe, were taken in each aerobic compartment of the pilot to ensure the DO was greater than 4 mg/L. The air supply was adjusted daily as required to maintain 4 mg/L.

Staff at the treatment facility, collected 24-hr composite samples from the effluent of each pilot, three times a week.