Abundance and dynamics of viruses within Activated Sludge Processes M. R. Brown[*], D. L. Swan[*], E. P. Kurdziel[****], J. C. Baptista[*], M. Lunn[**], R. J. Davenport[*], W. T. Sloan[***], L. F. Read[*], T. P. Curtis[*]

*School of Civil Engineering and Geosciences, Newcastle University, NE1 7RU, UK; **Department of Statistics, University of Oxford, OX1 3TG, UK; ***Department of Civil Engineering, University of Glasgow, G12 8LT, UK, ****Department of Biology, University of Bergen, N-5020, Norway.

Abstract

Viral abundance and dynamics were examined over time, in conjunction with the abundance of general bacteria and ammonia oxidising bacteria, within a suite of laboratory scale activated sludge reactors. Virus concentrations ranged from $1.63 \times 10^7 - 1.31 \times 10^9$ cells/mL and underwent large fluctuations through time, a pattern also observed within both bacterial communities. The preliminary data suggests that viruses are abundant and variable; however no direct link was evident between virus and bacterial populations and/or reactor performance. Sinusoidal shifts in temperature also showed no clear association to viral abundance.

Keywords: Viruses; Activated Sludge; Wastewater Treatment

Introduction

Heterotrophic bacteria are an integral part of wastewater treatment processes (WWTP), dozens, perhaps hundreds, of different species play key roles in nutrient removal and the transformation and mineralisation of organic matter (Shapiro & Kushmaro, 2011). Thus factors controlling bacterial abundance, diversity and activity are central to understanding and developing such processes. Among these factors, top down control through viral lysis could have an important and largely unrecognised role. Bacteriophages (viruses that infect prokaryotes) are the most abundant and diverse biological entities on earth, typically in the order of 10^7 mL^{-1} in most studied ecosystems, and are known to continuously regulate microbial ecology and activity by affecting carbon and nutrient fluxes, food web dynamics and microbial diversity and diversification (Suttle, 2007; Shapiro & Kushmaro, 2011). Whilst viruses, including bacteriophages, are known to be found at high abundance and diversity in WWTP such as activated sludge (AS) ($10^8 - 10^9$ virus like particles (VLP)/mL), they have proven difficult to study (Otawa *et al*., 2007; Tamaki *et al*., 2012). Consequently our knowledge and understanding of phage ecology in WWTP, and their potential influence, is limited.

Here we attempt to address this inadequacy. The abundance of viruses, general bacteria (GB) and Ammonia Oxidising Bacteria (AOB), an integral functional group to the AS process, are determined and observed over time in a suite of laboratory scale AS reactors, to investigate the potential impact viral activity has on the processes microbial ecology and performance. The effect of temperature, the single most important parameter governing bacterial community structure in AS plants and a known environmental stress factor to AOB, on viral abundance is also examined.

Materials and Methods

Twelve identical 1L laboratory scale continuous flow bioreactors (CFBs) were assembled and seeded with AS from the aeration basin of a nitrifying domestic wastewater treatment plant. Settled sewage was continuously fed to each CFB maintaining a hydraulic retention time of approximately 4 days, with dissolved oxygen (DO) concentrations kept above 4mg/L, pH left to stabilise naturally and the CFBs stirred at 200rpm. All CFBs were initially operated under steady state conditions, where possible, for a period of 72 days, allowing for an acclimatisation phase. During this period temperature was maintained at 14.5^{o}C. Once stable conditions were achieved, 6 reactors were subjected to a temperature sine wave over 130 days, with temperature's ranging from 8^{o}C to 21^{o}C. The remaining 6 reactors were exposed to a constant temperature of 14.5^{o}C.

Viral abundance was determined every two days, with samples collected, preserved and prepared for analysis as described by Brown et al. (unpublished, see supporting information). Samples were analysed using a FACScan flow cytometer (Becton Dickinson, San Jose, Calif.) equipped with a 15m-mW 488-nm air cooled argon-ion laser and a standard filter setup. The trigger was set on green fluorescence. Quantitative polymerase chain reaction was used to measure the abundance of the GB and AOB communities using primer sets 338F/1046R and amoA-1F/amoA-2R (Huber et al., 2007; Rotthawe *et al.*, 1997; Yu et al., 2005), respectively. The temperature, pH and

DO concentration within each reactor was monitored in real time using individual probes. Analyses of reactor influent and effluent for other environmental parameters, including COD, NH_3, anions and trace metals, were also performed every two days according to Standard Methods, high performance Ion Chromatography (Dionex ICS-1000) and inductively coupled plasma optical emission spectroscopy (Vista-MPX CCD) (APHA, 1998).

Preliminary Results (1 of 12 CFBs with sinusoidal temperature variation)

Viral abundance varied greatly during the 202 day experiment, with large temporal oscillations evident and cell numbers ranging from $1.63 \times 10^7 – 1.31 \times 10^9$ cells/mL (Fig. 1). As expected total virus abundance was greater than bacterial abundance, averaging 1.67×10^8 cells/mL in comparison to 1.47×10^8 and 6.04×10^7 cells/mL for GB and AOB respectively. Large temporal fluctuations in abundance were also evident within the bacterial populations, with fluctuations generally, but not always, mimicked across both communities (Fig. 1). No significant impact on either viral or bacterial abundances was associated with the sinusoidal change in temperature, although natural shifts in pH did seem to indicate increased viral numbers at higher pH levels (data not shown). Preliminary statistical analysis suggests a correlation between GB and total viral abundances could exist, however the extent of the correlations is not yet known.

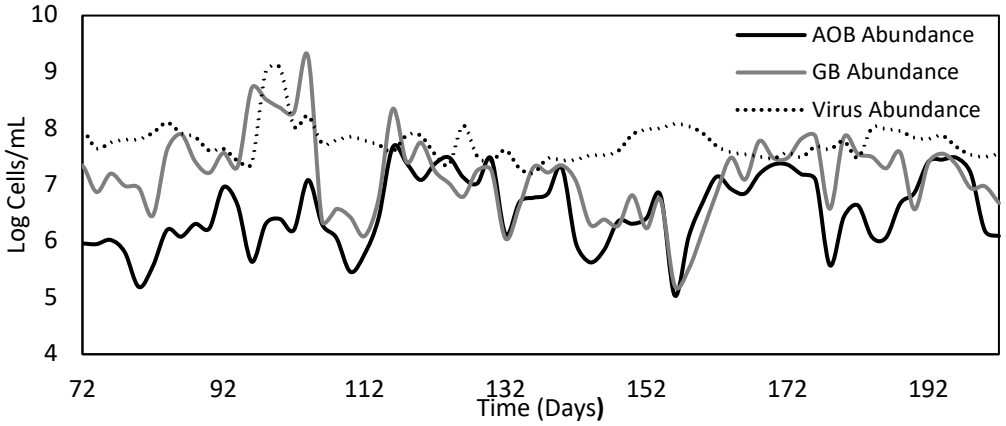

Figure 1.1 – Virus, GB and AOB abundances over the 130 day period. Before day 72 the reactor was undergoing acclimatisation.

Note throughout the 202 day experiment the CFB performed as expected, with effluent COD and NH_3 concentrations reduced significantly from those in the influent. No failure event was evident, thus no link between viral abundance and plant performance could be determined.

References

American Public Health Association (1998). Standard Methods for the Examination of Water and Wastewater. APHA.

Huber, J. A., D. Mark Welch, H. G. Morrison, S. M. Huse, P. R. Neal, D. A. Butterfield and M. L. Sogin (2007). "Microbial population structures in the deep marine biosphere." Science **318**(5847): 97-100.

Otawa, K., S. H. Lee, A. Yamazoe, M. Onuki, H. Satoh and T. Mino (2007). "Abundance, diversity, and dynamics of viruses on microorganisms in activated sludge processes." Microbial Ecology **53**(1): 143-152.

Rotthauwe, J. H., K. P. Witzel and W. Liesack (1997). "The ammonia monooxygenase structural gene amoA as a functional marker: Molecular fine-scale analysis of natural ammonia-oxidizing populations." Applied and Environmental Microbiology **63**(12): 4704-4712.

Shapiro, O. H. and A. Kushmaro (2011). "Bacteriophage ecology in environmental biotechnology processes." Current Opinion in Biotechnology **22**(3): 449-455.

Suttle, C. A. (2007). "Marine viruses - major players in the global ecosystem." Nature Reviews Microbiology **5**(10): 801-812.

Tamaki, H., R. Zhang, F. E. Angly, S. Nakamura, P.-Y. Hong, T. Yasunaga, Y. Kamagata and W.-T. Liu (2012). "Metagenomic analysis of DNA viruses in a wastewater treatment plant in tropical climate." Environmental Microbiology **14**(2): 441-452.

Yu, Y., C. Lee, J. Kim and S. Hwang (2005). "Group-specific primer and probe sets to detect methanogenic communities using quantitative real-time polymerase chain reaction." Biotechnology and Bioengineering **89**(6): 670-679.