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Concerning the role of cell lysis-cryptic growth in anaerobic side-stream reactors: The single-cell analysis of viable, dead and lysed bacteria

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ABSTRACT

In the Anaerobic Side-Stream Reactor (ASSR), part of the return sludge undergoes alternating aerobic and anaerobic conditions with the aim of reducing sludge production. In this paper, viability, enzymatic activity, death and lysis of bacterial cells exposed to aerobic and anaerobic conditions for 16 d were investigated at single-cell level by flow cytometry, with the objective of contributing to the understanding of the mechanisms of sludge reduction in the ASSR systems.

Results indicated that total and viable bacteria did not decrease during the anaerobic phase, indicating that anaerobiosis at ambient temperature does not produce a significant cell lysis. Bacteria decay and lysis occurred principally under aerobic conditions. The aerobic decay rate of total bacteria (b_{TB}) was considered as the rate of generation of lysed bacteria. Values of b_{TB} of 0.07–0.11 d^{-1} were measured in anaerobic + aerobic sequence. The enzymatic activity was not particularly affected by the transition from anaerobiosis to aerobiosis. Large solubilisation of COD and NH_4^+ was observed only under anaerobic conditions, as a consequence of hydrolysis of organic matter, but not due to cell lysis.

The observations supported the proposal of two independent mechanisms contributing equally to sludge reduction: (1) under anaerobic conditions: sludge hydrolysis of non-bacterial material, (2) under aerobic conditions: bacterial cell lysis and oxidation of released biodegradable compounds.

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1. Introduction

Activated sludge is an efficient and reliable process in treating wastewaters; however, the process produces a large amount of excess sludge, incurring high costs for treatment and disposal. Various technologies have been proposed for the reduction of excess sludge production directly within the wastewater treatment plant (WWTP), based on mechanical, physical–chemical or biological processes (Foladori et al., 2010a).

Among the biological techniques, the Oxic-Settling-Anaerobic (OSA) process is based on an anaerobic reactor operating at ambient temperature inserted in the return sludge line between the secondary settler and the aeration tank (Chudoba et al., 1992a,b). In this process, part of the return sludge undergoes alternating oxic (in the activated sludge reactor) and anaerobic (in the additional anaerobic reactor) conditions. Various modifications of the original OSA process have recently been proposed, in which the settled sludge is fed in the anaerobic side-stream reactor (ASSR) intermittently rather than continuously, as in the OSA process (Semblante et al., 2014). The OSA and ASSR systems are promising techniques for reducing sludge production, also having additional benefits such as low operational costs, good process stability and easy management (Wang et al., 2008). Reduction of sludge production of up to 60% was found, but the highest reductions have been obtained in lab-scale plants using synthetic wastewater (Foladori et al., 2010a; Semblante et al., 2014), while the plants fed with real wastewater demonstrate lower sludge reduction (Coma et al., 2013).

Despite the increasing interest in the application of OSA or ASSR systems, the present level of understanding of the mechanisms underlying sludge reduction in these processes is still limited (Semblante et al., 2014). Some hypotheses have been proposed in the literature to explain the possible mechanisms of sludge reduction, such as uncoupling metabolism (Chudoba et al., 1992b; Troiani et al., 2011) or cell lysis-cryptic growth (Wei et al., 2003; Quan et al., 2012), but these processes have not been fully demonstrated to date. In the study of An and Chen (2008) sludge decay in the anaerobic reactor was indicated as the main mechanism of the OSA system. However, sludge is a complex matrix composed of both bacterial biomass and non-bacterial material and sludge decay is the result of how each part is affected.

Microbiological aspects of sludge seem to play an important role in the OSA and ASSR systems. It is well known that cultivation-dependent analysis of microbial populations in sludge produces partial and heavily biased results and therefore this approach has never been applied. To obtain a more accurate view of bacteria populations and dynamics, molecular methods would be advised, but the application of these approaches in the OSA and ASSR systems is still being researched.

Amongst these methods, flow cytometry (FCM) is a powerful single-cell analysis that allows for obtaining a rapid and precise quantification of bacteria in environmental samples (*inter alia* Steen, 2000; Tracy et al., 2010). When coupled with the fluorescent molecular staining of cells, various functions of bacterial cells can be investigated in just few minutes at single-cell level.

This paper aims to investigate the viability, activity, death and lysis of bacterial cells exposed to aerobic and anaerobic conditions, with the objective of contributing to the understanding of the mechanisms of sludge reduction in the ASSR systems. Viability, activity, death and lysis of bacterial cells were investigated in this research by FCM according to the physiological status presented in Fig. 1.

Viability was assessed by membrane integrity, which demonstrates the protection of constituents in intact cells classified as viable cells (Nebe-von-Caron et al., 2000). Cells without an intact membrane are considered as permeabilised and are classified as dead cells. Viable and dead cells can be identified simultaneously by applying Propidium Iodide (a dye able to enter only permeabilised cells) and SYBR-Green I (able to enter all cells) (Ziglio et al., 2002).

As the structures of dead cells are freely exposed to the environment, they will eventually undergo subsequent cell lysis and decomposition of constituents (Nebe-von-Caron et al., 2000). Lysed cells are no longer detectable by FCM, because their components are released in the bulk liquid; therefore they can be quantified by difference of total cells at two different points in time (Fig. 1).

Cellular activity is a more restrictive condition than membrane integrity (Fig. 1), because it requires that cells be intact and able to demonstrate one of the following functions:

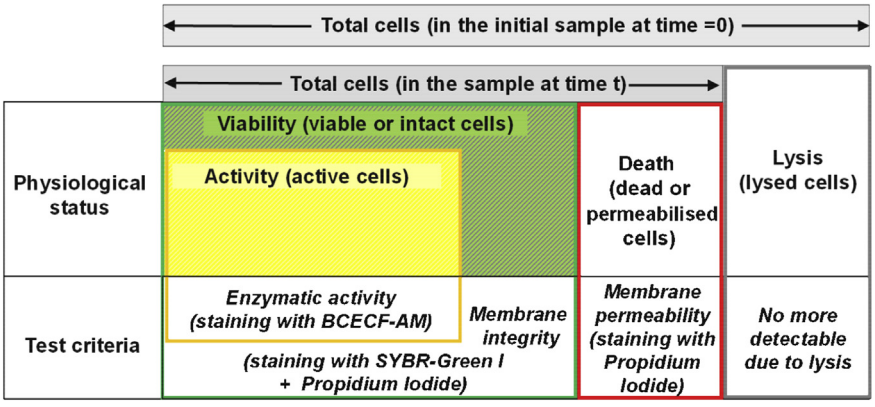

Fig. 1 – Physiological status of bacterial cells and test criteria for identification. Lysed cells are quantified by difference of total cells at two different times (0, t).