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Research article

Changes in the process performance, sludge production and microbial activity in an activated sludge reactor with addition of a metabolic uncoupler under different operating conditions

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ABSTRACT

Sludge production in wastewater treatment plants is nowadays a big concern due to the high produced amounts and their characteristics. Consequently, the study of techniques that reduce the sludge generation in wastewater treatment plants is becoming of great importance. In this work, four laboratory sequencing batch reactors (SBRs), which treated municipal wastewater, were operated to study the effect of adding the metabolic uncoupler 3,3′,4′,5-tetrachlorosalicylanilide (TCS) on the sludge reduction, the SBRs performance and the microbial hydrolytic enzymatic activities (MHEA). In addition, different operating conditions of the SBRs were tested to study the effect of the TCS on the process: two dissolved oxygen (DO) concentrations (2 and 9 mg L^{-1}) and two F/M ratio (0.18 and 0.35 g COD·g $MLVSS^{-1}·d^{-1}$). The sludge production decreased under high DO concentrations. At the same time, the DNA and EPS production increased in the four SBRs. After these stress conditions, the performance of the reactors were recovered when DO was around 2 mg L^{-1}. From that moment on, results showed that TCS addition implied a reduction of the adenosine triphosphate (ATP) production, which implied a decrease in the sludge production. In spite of this reduction, the SBRs performances did not decay due to the increase in the global MHEA. Additionally, the sludge reduction was enhanced by the increase of the F/M ratio, achieving 28% and 60% of reduction for the low and the high F/M ratio, respectively.

1. Introduction

In recent years, different techniques for the reduction of the sludge production in activated sludge processes have been studied. Thus, lysis and cryptic growth, uncoupling metabolism, reactor operation under high dissolved oxygen concentrations, oxic/settling/anaerobic (OSA) process and enzymatic hydrolysis have been reported in the literature (Khursheed and Kazmi, 2011; Liu, 2003; Low and Chase, 1999; Song and Feng, 2011). The mechanisms of most of these processes are still being discussed and contradictory results can be found. As an example, the mechanisms of sludge reduction of the OSA processes are not clear until now (Chen et al., 2003; Khursheed et al., 2015).

Focusing on uncoupled metabolism, it occurs when respiration does not control the bacteria metabolism and anabolism is the rate-limiting process, which implies energy in excess. Hao et al., 2010 discussed about its mechanisms. These authors revised both the theories supporting that uncoupling is related to maintenance energy and theories refusing it, considering that uncoupling is a separate intracellular metabolic activity. These authors supported the last one. Uncoupling can be achieved either under substrate-sufficient conditions (this is not the case in activated sludge reactors treating municipal wastewater) or by addition of some chemicals (uncouplers).

Different uncouplers have been used successfully at laboratory and pilot plant scales for the reduction of the sludge production. The main disadvantage of the uncouplers use is the loss of process performance in terms of reduction of the organic matter removal efficiency. The first utilized uncouplers were phenolic compounds as *para*-nitrophenol, 2-4-5 trichloro-phenol (TCP) and 2–4 dinitrophenol (DNP). However, the results reported about their application have been contradictory. Meanwhile Low and Chase,

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1999 reported a 49% of sludge reduction working at concentrations between 100 and 120 mg L^{-1} of *para*-nitrophenol, Qiong et al., 2013 obtained a 62% of sludge reduction with only 3 mg L^{-1} of *para*-nitrophenol. In a recent study, Zuriaga-Agustí et al., 2016 obtained sludge reductions below 10% with 25 mg L^{-1} of *para*-nitrophenol.

Another metabolic uncoupler, which has been studied in this work, is 3,3′,4′,5-tetrachlorosalicylanilide (TCS), whose use has been also successful for the reduction of the sludge production (Alexandre et al., 2016; Chen et al., 2002; Feng et al., 2014; Saini and Wood, 2008). The hazardous associated to the use of this chemical is lower than that of the phenolic uncouplers. In addition, lower concentrations of TCS are used since solubility in water at the pH of an activated sludge reactor is between 0.8 and 1 mg L^{-1}.

Once it is clarified that metabolic uncouplers are effective for the sludge reduction, their use at a commercial scale should be based on a deep knowledge about the consequences of their addition on the biomass and, consequently, on the process performance. Thus, the literature about metabolic uncouplers has evolved from studying the percentage of sludge reduction to wondering why it is produced (Feng et al., 2014). Important issues like the appropriate food to microorganisms ratio (F/M) for uncoupling and the consequences of different dissolved oxygen concentrations are still to be discussed.

One of the indicators that could explain what happens in the process of sludge reduction by uncoupled metabolism is the cell activity. For its measurement, different parameters like the microbial hydrolytic enzymatic activities (MHEA) have been considered. They offer valuable information about the organic matter hydrolysis in activated sludge systems (Anupama et al., 2008; Molina-Muñoz et al., 2010). Chen et al. (2002) observed that the microbial activity and the cell respiratory activity increased significantly in the reactors where TCS was added. Feng et al. (2014) used two tetrazolium salts for the activity measurement to studying the effect of TCS on the electronic transported system (ETS). Contrary to the work by Chen et al. (2002), Feng et al., 2014 reported that ETS activities decreased significantly after ten days from the beginning of TCS addition.

Summarizing, it can be stated that contradictory results about how the addition of a metabolic uncoupler affects the biomass of an activated sludge process have been reported until now. Mechanisms are not clear and going deeper on the biomass characterization by analysis of microbial activity among other parameters is fundamental in order to know the viability of the sludge reduction by chemical uncouplers. This work has the aim of increasing the knowledge on this theme by the study of the effects of the addition of TCS under different operating conditions (two levels of dissolved oxygen concentration and two F/M ratios) on the biomass of four in parallel operated laboratory SBRs. In addition, soluble microbial products (SMP), extracted extracellular polymeric substances (eEPS), adenosine triphosphate (ATP) and MHEA were measured to study the influence of the TCS addition on the biomass and on the process performance. These parameters contributed to the understanding of the phenomena occurring after addition of TCS under different operating conditions in a sequencing batch reactor (SBR). Moreover, COD removal efficiency and deoxyribonucleic acid (DNA) measurements were carried out to evaluate the reactors performance and cell lysis, respectively.

2. Materials and methods

2.1. Wastewater

SBRs were fed with municipal wastewater (MWW). Samples were taken twice a week at the outlet of the primary treatment. Table 1 shows the average values and the standard deviations (SD) of several parameters for the wastewater samples used in the experiments. Analysis procedures are explained in section 2.3.

Table 1
Wastewater characterisation.

Parameter	Average ± SD
pH	7.7 ± 0.1
Conductivity (mS cm^{-1})	2.2 ± 0.1
Turbidity (NTU)	70.8 ± 16.7
COD (mg L^{-1})	321 ± 49
N_T (mg L^{-1})	60 ± 13
P_T (mg L^{-1})	49 ± 11
NH_4^+-N (mg L^{-1})	6 ± 1
SS (mg L^{-1})	122 ± 109
VSS (mg L^{-1})	69 ± 46

2.2. Sequencing batch reactors

The experiments were carried out in four identical sequencing batch reactors, named SBR-1, SBR-2, SBR-3 and SBR-4. Fig. 1 shows a scheme of each SBR.

Each SBR consisted of a cylindrical tank (diameter of 10 cm and height of 30 cm). The main components were: mechanical stirrer (Velp Scientifica), air compressor 550 R Plus (Sera Precision), two air diffusers located on the reactor bottom, an oximeter OXI 49 (Crison) and two peristaltic pumps (Aiguapres) to fill the reactor with the aimed wastewater volume and to draw the treated effluent.

All the reactors worked with 4.5 L of volume reaction, three cycles per day and at a hydraulic retention time (HRT) of 1 day. In each cycle a wastewater volume of 1.5 L was fed into the reactor starting at this moment the aerobic reaction time. The stirrer and the air compressor worked throughout the reaction phase, stopping in the sedimentation phase. After this time, a volume of 1.5 L of treated wastewater was drawn and finally idle phase occurred. For all the reactors, the aerobic reaction and the sedimentation times were 6 and 1.5 h, respectively.

2.3. Operation strategy

All the SBRs worked during 42 days. The effect of two F/M ratios

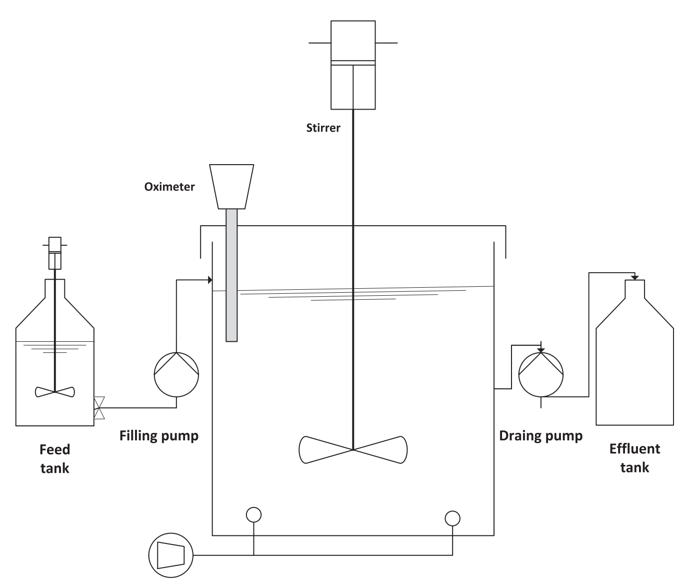

Fig. 1. SBR scheme.