Desalination and Water Treatment
www.deswater.com

doi: 10.1080/19443994.2015.1040852

57 (2016) 2719–2725
February

Taylor & Francis
Taylor & Francis Group

Changes in enzymatic and microbiological activity during adaptation of a conventional activated sludge (CAS) to a CAS-oxic-settling-anaerobic (OSA) adapted process

Pablo Maria Romero Pareja[a,*], Alvaro Real Jiménez[b], Carlos Alberto Aragón[b], Jose Maria Quiroga[a], Maria Dolores Coello[a]

[a]*Department of Environmental Technology, Universidad de Cadiz, Plg. Río San Pedro s/n, Puerto Real, Cádiz, Spain, Tel. +34 659621623, +34 95601658; Fax: +34 956016040; emails: pablo.romero@uca.es (P.M. Romero Pareja), josemaria.quiroga@uca.es (J.M. Quiroga), dolores.coello@uca.es (M.D. Coello)*
[b]*Foundation Centre for New Water Technologies (CENTA), Autovía Sevilla-Huelva (A-49) km. 28. Carrión de los, Céspedes, Seville, Spain, Tel. +34 954759020; Fax: +34 954755295; emails: areal@centa.es (A. Real Jiménez), caragon@centa.es (C.A. Aragón)*

Received 22 October 2014; Accepted 12 March 2015

ABSTRACT

The production of excess sludge in the conventional activated sludge (CAS) process is an important aspect of the operation of wastewater treatment plants. The oxic-settling-anaerobic (OSA) process is one of the most promising strategies among those to achieve a reduction in the excess sludge produced. Cell decay occurs at a low oxidation–reduction potential and subsequent degradation reactions seem to be the major causes of sludge reduction in the OSA process. As a consequence of previous exposure of the sludge to anaerobic conditions in the sludge holding tank, an increase of enzymatic and microbiological activity in the aeration reactor could enhance the degradation of the released material. In this study, the results of a pilot plant running sequentially as a CAS and a CAS-OSA system are shown not only in the terms of the crucial aspect of excess sludge reduction, but also in terms of enzymatic and microbiological activities in the sludge from the aeration tank. Specifically, the application of the OSA strategy with a sludge anaerobic exposure time (SAET) of 5.5 h achieved a 33.6% reduction of the produced sludge in comparison with the CAS approach. Similarly, this system showed an average increase of 23.2, 22.6 and 7.59% in the specific oxygen uptake rate (SOUR), dehydrogenase, and protease activity, respectively, and a negligible change in glucosidase activity compared to the conventional process. At the same way, the OSA process with a SAET of 8 h led to a reduction of 38.9% of produced sludge and an average increase of 27.9, 11.31, 28.7 and 26.57% in SOUR, dehydrogenase, protease, and glucosidase activity, respectively, compared to control unit.

Keywords: Sludge reduction; OSA; Activated sludge; Enzymatic activities; Dehydrogenase

*Corresponding author.

Presented at the International Congress on Water, Waste and Energy Management 16–18 July 2014, Porto, Portugal.

1. Introduction

The activated sludge process is the most widely used wastewater treatment being the core process at about 90% [1] of the municipal wastewater treatment plants (WWTP). The main drawback of this technology is the generation of excess sludge. The costs associated with sludge management account for up to 65% of the total operative costs of a typical WWTP [2]. The minimization of excess sludge production in the water line of the wastewater treatment process, rather than post-treatments of the produced sludge, is the most preferable way to deal with the problem. To date, several strategies addressing this objective have been presented in the literature [3]. These strategies have been classified in the literature in four groups: cell lysis-cryptic growth, endogenous metabolism, uncoupled metabolism, and microbial predation [2,4]. Westgarth et al. [5] suggested the OSA (oxic-settling-anaerobic) process as a technique for reducing sludge production. The invention consists of insertion of an anaerobic tank in the recirculation line resulting in an economical method, which requires neither addition of chemical products, nor application of physical treatment. A general layout of OSA process is shown in Fig. 1.

Yet, a clear explanation of the sludge reduction mechanism in the OSA process has not been established [6]. Initially, the mechanism of uncoupling metabolism was proposed as the predominant mechanism in sludge reduction in the OSA process [7,8]. Later studies pointed to sludge decay, sludge hydrolysis in the anaerobic tank, and subsequent metabolic reactions in the anaerobic environment as the main causes for the reduction of produced sludge [9,10]. The influence of slow grower organisms is reported not to be relevant for the sludge reduction in the OSA process [9]. The redox potential (ORP) in the anaerobic tank is a key parameter for evaluating the degree of anaerobiosis in the unit, being a useful indicator for the expected reduction of produced sludge [11,12]. A pool of OSA experiences has been already reported, which points to a reduction of produced sludge between 15 and 65%, depending chiefly on the values of sludge anaerobic exposure time (SAET), ORP, and temperature [6]. The OSA process does not affect the performance of the WWTP and improves, in the most cases, the chemical oxygen demand (COD) removal and sludge settleability [10,12,13]. The denitrification process and phosphorous removal are also enhanced in the OSA process [13,14], although it is important to highlight the absence of a supply of an external substrate in the anaerobic tank unlike in the typical anoxic-denitrification tank.

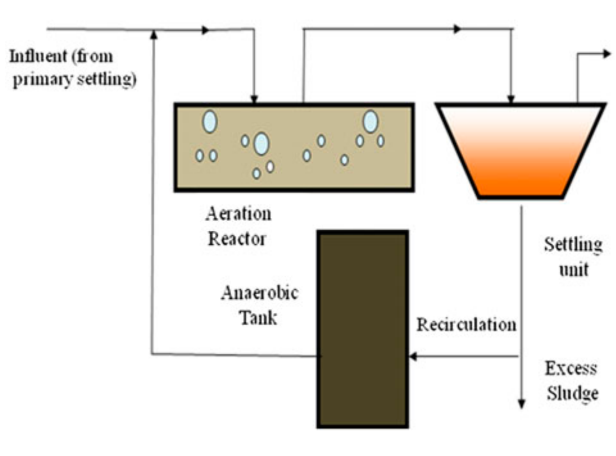

Fig. 1. Oxic-settling-anaerobic (OSA) process.

Despite a certain increase of dehydrogenase activity (DHA) and specific oxygen uptake rate (SOUR) in OSA process has been reported [6], specific data on changes in enzymatic and microbial activity are difficult to be found [3]. The goal of this study was to study changes in enzymatic and metabolic activity of a pilot plant which has shifted from a CAS process to a CAS-OSA process scheme, with an emphasis on the reduction of the produced sludge achieved and on COD removal rate.

2. Materials and methods

The pilot plant used in this study is made of methacrylate. The aerobic reactor, settling unit, and anaerobic tank (OSA) have a volume of 13.2, 6, and 14 l, respectively. All the units are equipped with agitators to avoid scumming and biofouling. A programmable logic controller (PLC, Schneider SR2B202B) was used to impose operating conditions (SAET and influent flow rate). Also, the agitator of settling unit was timely controlled not to damage the settling process, 15 s of activation for every 8 min, prior to the operation of the recirculation pump. A smartphone application (IPCam Viewer Pro by Robert Chou) is used for 24 h visual control.

Prior to the phases of study, a first stage was operated to achieve the stabilization of the pilot plant during 20 d. Inoculum of sludge from WWTP of Jerez de La Frontera was taken to seed the system. After this period, the pilot plant worked in CAS mode and after 70 d of steady operation, the unit configuration was set as a CAS-OSA process by inserting an intermediate anaerobic tank in the recirculation line. In addition, two stages could be distinguished during the OSA period: a SAET of 5.5 h was set during the first stage (60 d long) and a SAET of 8 h during the second one (50 d long). The solid concentration in the aeration reactor and the anaerobic tank (OSA process only) was kept around 3.0 and 6.5 g/l, respectively, throughout study. Specifically, the values of MLSS concentration were 2.84 g/l ± 0.17 in the aerobic tank and 6.37 g/l ± 0.24 in the anaerobic tank. Dissolved oxygen was kept at 2.5 mg O_2/l throughout the study by monitoring DO display with the 24 h IPCam. Six diffuser stones were fixed with plastic bridles in the bottom of the aerobic reactor to assure the homogeneous air diffusion, paying special attention to the corners. The sludge withdrawal was carried out every two days, and considered as excess sludge produced.

TSS, VSS, MLSS, MLVSS, and COD were measured every two days according to the Standard Methods [15]. The SOUR test was carried out according to Awong et al. [16]. The DHA was tested using the method described by Hongwei et al. [17]. The α-glucosidase and protease activities were measured following the methods described by Goel et al. [18] and Cadoret et al. [19], respectively. Previous works have employed these enzymatic activities, SOUR, and DHA for characterization of the activated sludge [20–23]. These measurements were carried out twice a week on sludge from the aeration reactor. SOUR test was done every time the plant was fed (5–6 d a week). Also ORP was measured using an ORP probe provided with a SD memory card for continuous data recording.

The influent of the process was composed by 90% synthetic wastewater (recipe according to [24] with extra 70 mg/l glucose) and 10% real wastewater taken from the WWTP of Jerez de la Frontera. The hydraulic residence time (HRT) was kept at 9 h throughout the study, with an imposed influent flow rate of 1.47 l/h. The influent was characterized every week, and average values and standard deviation obtained were 247.15 ± 19.61 mg O_2/l, 9.24 ± 1.16 mg P/l, 39.15 ± 3.41 mg N/l, 28.64 ± 2.97 mg N/l, and 23.46 ± 1.34 mg TSS/l in terms of COD, TP, TN, NH_4-N, and TSS, respectively. The average value of F/M was 0.45 d^{-1}.

3. Results and discussion

3.1. Reduction of excess sludge produced and ORP values

The mass balance carried out, including the solid loss in effluent, pointed to a reduction of sludge production of 33.6 and 38.9% compared to CAS system when the OSA process is operating with a SAET of 5.5 and 8 h, respectively. The values of accumulative sludge produced led to a solid retention time in the plant of 64, 95, and 103 d for the conventional process and OSA system with 5.5 and 8 h SAET, respectively. The values of the reduced sludge obtained are in agreement with those from the bibliography [4] and follow the general trend of higher sludge reduction at higher SAET [6]. The ORP value in the anaerobic tank is inversely proportional to value of SAET (when solid concentration is stable). In fact, average values of ORP during OSA process were 200.9 ± 10.9 mV for stage with SAET of 5.5 h and −245.5 ± 14.8 mV when a SAET of 8 h is imposed.

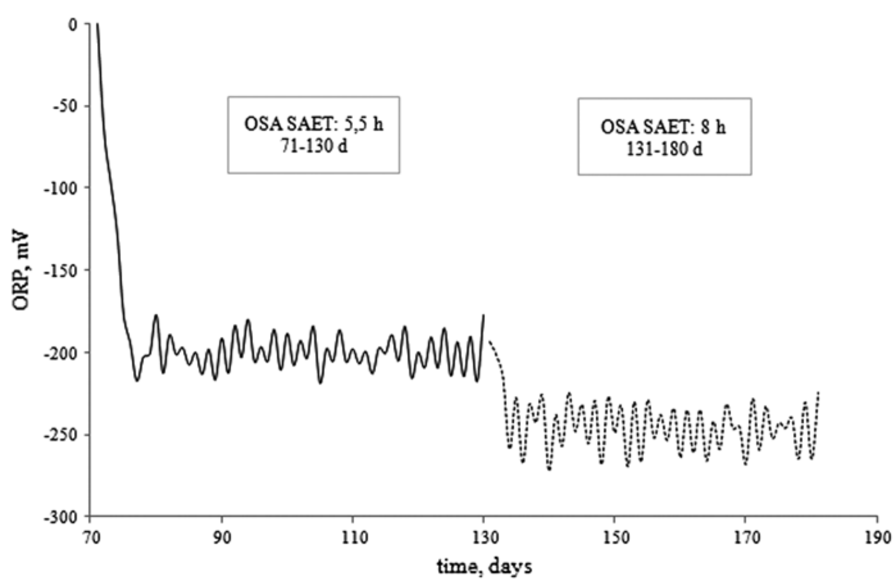

Fig. 2. Evolution of ORP in the anaerobic tank during the OSA stages.

During OSA stages, the ORP values showed a fairly stable trend without the need of a nitrogen gas supply, as proposed by Saby et al. [12]. Fig. 2 shows the continuous values of ORP in the anaerobic tank recorded throughout study.

3.2. COD removal

The rate of COD removal improved slightly after the addition of the anaerobic tank. During the initial CAS process, the average COD removal rate was 89.7%. During the stages of CAS-OSA process, this rate increased to 94.2% for a SAET of 5.5 h and to 94.0% for a SAET of 8 h. The variation between these values is negligible, even though some authors [25] relate the increase in SAET with a slightly worse COD removal rate under a certain value. It is concluded from that study that ORP values below 100 mV in the anaerobic tank lead to sludge decay and a slight increase in COD in the medium. This phenomenon is linked to the sludge reduction, as this extra COD is used in endogenous maintenance and in biochemical reactions in the anaerobic tank, which lead to sludge reduction [9].

3.3. Biomass activity: SOUR and DHA

The results of SOUR and DHA tests are depicted in Fig. 3.

During the CAS process, the average value of SOUR was 146.8 mg O_2/(d g VSS) ± 15.1, while during OSA, this parameter increased to 180.9 mg O_2/(d g VSS) ± 8.7 and 187.7 mg O_2/(d g VSS) ± 7.4 for a SAET of 5.5 and 8 h, respectively, thus implying an increase in 23.23 and 27.86%. A similar increase of SOUR was observed in the previous work [14]. This enhancement in the aerobic activity is also linked to the higher COD removal rate [26], as the active biomass shows an enhanced ability to substrate assimilation after being submitted to starvation conditions in the anaerobic tank.

The DHA test quantifies the activity through metabolic pathways using both oxygen and non-oxygen as the last electron acceptor. As in the case of SOUR, an increase in DHA was registered after the plant started to operate in the OSA mode. During CAS process, an average value of DHA of 32.6 mg O_2/(gVSS d) ± 1.50 was measured, increasing to the average values of 40.01 and 36.31 mg O_2/(gVSS d) during the OSA stages of SAET of 5.5 and 8 h, respectively (SD: 2.31 and 1.12). The increase is of 22.6 and 11.3%, respectively, compared to CAS. The higher value of DHA activity for 5.5 h is related to the pronounced increase of the activity occurred after changing the pilot plant configuration to the OSA mode with a maximum measurement of 43.5 mg O_2/(gVSS d) for a few days after including the anaerobic tank. After that, the DHA value stabilized to around 38 mg O_2/(gVSS d). An increase of DHA was also observed when sludge

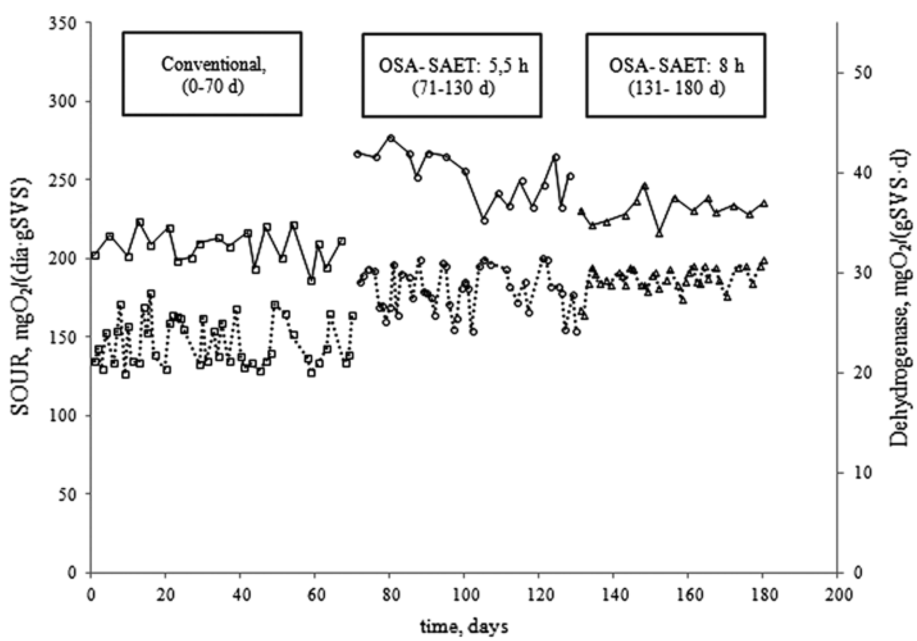

Fig. 3. Biomass activity measured SOUR (dotted line) and DHA tests (solid line) during study.

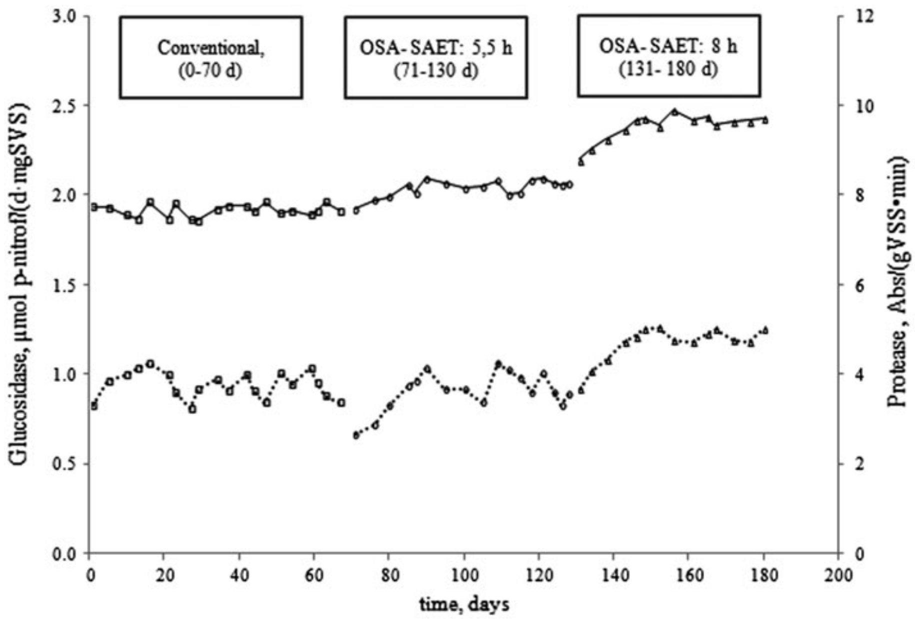

Fig. 4. Measured activities during study (dotted line—α-Glucosidase activity; solid line—Protease activity).

taken from an anoxic–aerobic system was submitted to anaerobic incubation [18].

3.4. Enzymatic Activities: α-Glucosidase and protease

Glucosidase enzymes are responsible for the degradation of such important components of wastewater as glycogen and cellulose. Protease activity is directly related to the degradation of proteins. The results of α-glucosidase and protease activity tests are depicted in Fig. 4

The average value of the glucosidase activity is the same for the both operation modes: CAS mode and OSA mode with a 5.5 h SAET (0.94 p-nitrof/(min gVSS), and SD of 0.08 and 0.11, respectively). However, when the SAET was set to 8 h, the glucosidase activity was by 28.7% higher, reaching a value of 1.21 μmol p-nitrof/(min gVSS) ± 0.10.

This could be explained by the fact that the degradation of the internal glycogen reserves as an energy source is not so intense for shorter anaerobic exposures as for longer SAET [27], because of the shorter anaerobic exposure period itself and low abundance of the organisms capable of using glycogen as an internal reserve (GAO). Another reason is that the release of carbohydrates under anaerobic conditions is not as extreme as for proteins at short SAET [28]. The correlation between concentration of carbohydrates and α-glucosidase has been reported by Yu et al. [29]. Furthermore, higher sludge decay itself under longer SAET could also increase the presence of bulk enzymes.

In contrast to the results of the glucosidase activity, protease activity of the sludge from the stage running OSA process with shorter SAET (5.5 h) is higher than the protease in the sludge from CAS mode. Specifically, the average activity was higher by 7.59%, increasing the average values from 7.64 ABS/(g VSS min) ± 0.13 to 8.22 ABS/(g VSS min) ± 0.18. When SAET was set to 8 h, protease activity was 26.57% higher than in CAS mode, reaching 9.67 ABS/(gVSS min) ± 0.27. Under alternating aerobic–anaerobic conditions, sludge decay is associated with a higher presence of lipases and protease-segregating bacteria [30], related also to the breakage of the floc matrix [18] after the reduction of the iron ion, from Fe^{3+} to Fe^{2+}, which acts as bond in the matrix.

4. Conclusions

A 33.6% reduction in produced sludge is achieved when operating in OSA mode with a SAET of 5.5 h. For a SAET of 8 h, the reduction reaches 38.9% with respect to CAS operation. Low and stable ORP levels are achieved in both OSA periods without the need for a nitrogen supply. Longer SAET during the OSA process results in an increase in enzymatic activity. Different steps can be distinguished in the enhancement of enzymatic activity during the OSA process, as glucosidase shows a similar value in CAS mode and in OSA with lower SAET. Sludge decay is the main cause of sludge reduction. However, the more intensive this phenomenon is, the greater enzyme release is observed, both being, concomitant effects promoting sludge reduction.

Acknowledgments

The authors greatly thank for the financial support provided by the Consejería de Economía, Innovación, Ciencia y Empleo de la Junta de Andalucía, Spain (Proyecto Excelencia No. P09-TEP-4539 financed by ERDF funds). The authors are also grateful to Aqualia Company and Jerez WWTP employees for collaboration in this study.

References

[1] Y. Liu, Chemically reduced excess sludge production in the activated sludge process, Chemosphere 50 (2003) 1–7.
[2] Y. Wei, R.T. Van Houten, A.R. Borger, D.H. Eikelboom, Y. Fan, Minimization of excess sludge production for biological wastewater treatment, Water Res. 37 (2003) 4453–4467.
[3] E. Paul, Y. Liu, Biological Sludge Minimization and Biomaterials/Bioenergy Recovery Technologies, Wiley, Hoboken, NJ, 2012
[4] A. Khursheed, A.A. Kazmi, Retrospective of ecological approaches to excess sludge reduction, Water Res. 45 (2011) 4287–4310.
[5] W.C. Westgarth, F.T. Sulzer, D.A. Okun, Anaerobiosis in the activated sludge process [C], Proceedings of the Second IAWPRC Conference, 1964, Tokyo, 43–55 (not published in journal).
[6] Q. Yuan, H.Y. Wang, Y.X. Zhou, H. Zhao, The research progress on oxic-settling-anaerobic sludge reduction process, Adv. Mater. Res. 838–841 (2013) 2726–2734.
[7] P. Chudoba, J. Chudoba, B. Capdeville, The aspect of energetic uncoupling of microbial growth in the activated sludge process—OSA system, Water Sci. Technol. 26 (1992) 2477–2480.
[8] E. Low, H.A. Chase, M.G. Milner, T.P. Curtis, Uncoupling of metabolism to reduce biomass production in the activated sludge process, Water Res. 34 (2000) 3204–3212.
[9] G.-H. Chen, K.-J. An, S. Saby, E. Brois, M. Djafer, Possible cause of excess sludge reduction in an oxic-settling-anaerobic activated sludge process (OSA process), Water Res. 37 (2003) 3855–3866.
[10] K.-J. An, G.-H. Chen, Chemical oxygen demand and the mechanism of excess sludge reduction in an oxic-settling-anaerobic activated sludge process, J. Environ. Eng. 134 (2008) 469–477.
[11] B. Zhang, F.-Y. Ji, C. Guan, X.-P. Gao, C.-J. Ding, Research on municipal wastewater sludge reduction in OSA process and its influencing factors, J. Chongqing Jianzhu. Univ. 28 (2006) 92–95.
[12] S. Saby, M. Djafer, G.-H. Chen, Effect of low ORP in anoxic sludge zone on excess sludge production in oxic-settling-anoxic activated sludge process, Water Res. 37 (2003) 11–20.
[13] J.-F. Wang, W.-B. Jin, Q.-L. Zhao, Z.-G. Liu, J.-K. Lin, Performance of treating wastewater and anti-shock-loading in oxic-settling-anaerobic (OSA) process for minimization of excess sludge, Huanjing Kexue/Environ. Sci. 28 (2007) 2488–2493.
[14] K.-J. An, Reduction of excess sludge in an oxic-settling-anaerobic (OSA) system: A modified activated sludge process, PhD from The Hong-Kong University of Science and Technology, 2004.
[15] American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation, Standard Methods for the Examination of Water and Wastewater, American Public Health Association, Washington, DC, 2005.
[16] J. Awong, G. Bitton, B. Koopman, ATP, oxygen uptake rate and INT-dehydrogenase activity of actinomycete foams, Water Res. 19 (1985) 917–921.
[17] Y. Hongwei, J. Zhanpeng, S. Shaoqi, W.Z. Tang, INT–dehydrogenase activity test for assessing anaerobic biodegradability of organic compounds, Ecotoxicol. Environ. Saf. 53 (2002) 416–421.
[18] R. Goel, T. Mino, H. Satoh, T. Matsuo, Enzyme activities under anaerobic and aerobic conditions in activated sludge sequencing batch reactor, Water Res. 32 (1998) 2081–2088.
[19] A. Cadoret, A. Conrad, J.-C. Block, Availability of low and high molecular weight substrates to extracellular enzymes in whole and dispersed activated sludges, Enzyme Microb. Technol. 31 (2002) 179–186.
[20] G.-H. Yu, P.-J. He, L.-M. Shao, D.-J. Lee, Enzyme activities in activated sludge flocs, Appl. Microbiol. Biotechnol. 77 (2007) 605–612.
[21] K. Calderón, P. Reboleiro-Rivas, F.A. Rodríguez, J.M. Poyatos, J. González-López, B. Rodelas, Comparative analysis of the enzyme activities and the bacterial community structure based on the aeration source supplied to an MBR to treat urban wastewater, J. Environ. Manage. 128 (2013) 471–479.
[22] D. Nabarlatz, F. Stüber, J. Font, A. Fortuny, A. Fabregat, C. Bengoa, Extraction and purification of hydrolytic enzymes from activated sludge, Resour. Conserv. Recycl. 59 (2012) 9–13.
[23] C. Aragón, M.D. Coello, J.M. Quiroga, Effect of manganese(II) on the respiratory activity of biological sludge from wastewater treatment plant, Chem. Eng. Res. Des. 88 (2010) 641–646.
[24] C. Aragón, J.M. Quiroga, M.D. Coello, Comparison of four chemical uncouplers for excess sludge reduction, Environ. Technol. 30 (2009) 707–714.
[25] F.-X. Ye, R.-F. Zhu, Y. Li, Effect of sludge retention time in sludge holding tank on excess sludge production in the oxic-settling-anoxic (OSA) activated sludge process, J. Chem. Technol. Biotechnol. 83 (2008) 109–114.
[26] G.-H. Chen, W.-K. Yip, H.-H. Mo, Y. Liu, Effect of sludge fasting/feasting on growth of activated sludge cultures, Water Res. 35 (2001) 1029–1037.

[27] R.J. Zeng, M.C.M. van Loosdrecht, Z. Yuan, J. Keller, Metabolic model for glycogen-accumulating organisms in anaerobic/aerobic activated sludge systems, Biotechnol. Bioeng. 81 (2003) 92–105.

[28] P. Foladori, G. Andreottola, G. Ziglio, Sludge Reduction Technologies in Wastewater Treatment Plants, IWA Publishing, London, 2010

[29] G. Yu, P. He, L. Shao, D. Lee, Extracellular enzymes in sludge flocs collected at 14 full-scale wastewater treatment plants, J. Chem. Technol. Biotechnol. 83 (2008) 1717–1725.

[30] S.-J. Jung, K. Miyanaga, Y. Tanji, H. Unno, Effect of intermittent aeration on the decrease of biological sludge amount, Biochem. Eng. J. 27 (2006) 246–251.