

Research progress of *in-situ* reduction technology of sludge

Jiating Wu^{a,b}, Shaomin Liu^{a,b,*}

a School of Earth and Environment, Anhui University of Science and Technology, Huainan 232001, China, Tel.: +8613855456687; email: shmliu1@163.com (S. Liu), Tel.: +8615155442086; email: 905333120@qq.com (J. Wu) b State Key Laboratory of Mining Response and Disaster Prevention and Control in Deep Coal Mines (Anhui University of Science and Technology), Huainan 232001, China

Received 24 April 2023; Accepted 26 July 2023

abstract

The activated sludge method is currently the most widely used biological wastewater treatment process in the world. It operates stably, the effluent quality is better, and part of the generated sludge is effectively disposed of in a concentrated form. However, due to the status quo of emphasizing water over sludge and the gradual increase of wastewater treatment rate, residual sludge becomes an unavoidable by-product of the treatment process. This by-product not only significantly increases the total operating cost of wastewater treatment plants, but also carries a large amount of toxic biochemical residues in the environment. The issue of how to treat and dispose of sludge safely and economically has begun to receive attention. The *in-situ* reduction technology for the simultaneous treatment of wastewater and sludge has many advantages over conventional sludge disposal. Through the analysis and comparison of various treatment technologies of thermal, chemical, mechanical, and other biological processes, their ability to enhance sludge hydrolysis and their advantages and disadvantages are summarized.

Keywords: Residual sludge; *In-situ* reduction; Cytolysis; Uncoupling; Biological predation

1. Introduction

With the acceleration of industrialization and urbanization in China, the number and scale of wastewater treatment plants (WWTPs) are increasing. The activated sludge method has been widely used to treat domestic and industrial wastewater and plays an irreplaceable role in WWTPs. However, due to the proliferation of the scale of WWTPs and the increasing tightening of environmental protection policies, the sludge problem is subsequently becoming increasingly severe. At present, approximately 60 million·tons of residual sludge (RS) is produced every year in China, and it has been growing [1]. The high RS production and strict environmental quality standards have gradually become the biggest problems in the operation process, and the operating costs and environment problems of processing sludge are increasing [2,3]. Sludge produced in the wastewater

treatment process without proper treatment and disposal has a complex composition, long-term toxicity, and non-degradability of some pollutants. Its original form contains not only water, sediment, and fibrous plant and animal residues, but also a large amount of organic matter, heavy metals, nitrogen, phosphorus, and many other toxic and harmful substances. It emits bad odors and attracts disease-causing agents [4]. If the sludge is not adequately treated in time, it is easy to cause secondary pollution of water quality, threatening human life and health [5]. Therefore, for the treatment of sludge, the principles of reduction, resourcefulness and harmlessness should be observed to promote a virtuous cycle in the water treatment process.

In recent years, the available sludge disposal methods are becoming less and less. The traditional end-of-pipe urban sludge disposal methods, such as incineration, composting, landfill, and agricultural use, still occupy a dominant

^{*} Corresponding author.

position in various countries. Although part of methane gas can be recovered from sludge landfill sites, its energy recovery efficiency is lower than municipal solid waste incineration due to the limited organic content in sludge, and high concentration of leachate is produced [6]. Incineration of sludge can reduce volume, recover energy, and destroy pathogens, but requires high standards of pollution emission control [7]. Strict legislation on the presence of heavy metals and toxic non-biodegradable organic matter has resulted in only a small amount of sludge being used as fertilizer in agriculture [8]. Traditional methods are not only costly but the valuable phosphate resources in sludge ash are lost during the treatment process [9,10], In addition, traditional sludge disposal methods produce secondary pollution, such as entering the food chain through the media of the atmosphere, soil, surface water, and groundwater, and eventually entering the human body, endangering human health. Furthermore, it faces a series of policy, environmental and technical problems.

Therefore, *in-situ* sludge reduction technology, a method to minimize excess sludge from the source, has attracted widespread attention in order to reduce the RS production generated by the activated sludge method and the subsequent transportation and treatment, and disposal costs [11]. This is considered a promising and economical method to minimize the reduction of sludge content, which can effectively reduce sludge production during wastewater treatment and reduce toxic components in the system [12]. It can reduce the dry content of the sludge by affecting the process of microbial metabolism, improve the sludge settling properties, and recover useful energy and components from the sludge while controlling the effluent to meet the standards [13,14]. It not only reduces the environmental impact and economic burden of the sludge treatment process, but also does not require extensive modification of the original process [15]. In recent years, many scholars have conducted research in this field. Based on the mechanisms of stealth growth, maintenance metabolism, uncoupling metabolism and microbial predation, various feasible and novel *in-situ* sludge reduction technologies have been developed (Fig. 1), and some remarkable results have been achieved.

2. Uncoupling metabolism

Uncoupling metabolism is a decrease in the phosphorylation of adenosine diphosphate (ADP) to adenosine triphosphate (ATP), leading to an increase in energy differences and energy uncoupling between catabolism and anabolism, limiting the energy supply, and releasing the energy produced not for cellular synthesis but in other forms. As a result, when energy uncoupling occurs, a corresponding decrease in biomass growth production and a significant reduction in the apparent yield of sludge can be observed [16]. Adding chemical uncoupling agents or inducing uncoupling under abnormal conditions can be a cost-effective and efficient method to reduce the high cost and difficulty of WWTPs operation [17].

2.1. Injection of the uncoupling agent

Uncoupling agents function by entering the aqueous phase and combining with H^* and destroying the normal proton gradient on both sides of the cell membrane. This interference inhibits the production of activated sludge, hinders the synthesis of ATP, reduces the total energy of biomass synthesis, and leads to the dissipation of the energy produced in the form of heat, thereby reducing sludge production [16].

The reduction effect of uncoupling agent is shown in Table 1. Yang et al. [17,18] and Ferrer-Polonio et al. [19] achieved sludge reduction by adding 3,3',4',5-tetrachlorosalicylanilide (TCS) to the sequencing batch reactor (SBR). Compared to the blank group, the dosing group with a TCS concentration of 0.8 mg/L can reduce the sludge growth rate by 40%. Continuous operation at this dose for a short period did not affect the effluent water quality. Thus, TCS has been recognizes and utilized as a mild and environmentally benign metabolic uncoupling agent [20]. Fang et al. [21] and Wang et al. [22] showed that dosing the system with 17–62 mg/L of o-chlorophenol (oCP) leading to a sludge reduction rate of 17.4%–56.1%. Metabolic uncoupling, changes in electron distribution and cell lysis were the main reasons for the decrease in sludge yield after oCP addition. The sludge reduction effect brought by the decoupling agent

Fig. 1. Method for *in-situ* reduction of sludge.

Reduction effect	References
40%	[18, 19]
$17.14\% - 56.1\%$	[21, 22]
45%	$[23]$
75%	[24]
49%	$[25]$
87%	$[17]$

Table 1 Sludge reduction with different uncoupling agents

is closely related to the reagent's acidity coefficient pKa. In generally, decoupling agents with lower acidity coefficients have a better reduction effect [17]. Experiments by Feng et al. [23] found that mixing and dosing 2,4,5-trichlorophenol (TCP) and TCS reagents produced a good coupling effect and effectively inhibited sludge growth. Under the experimental conditions of $T = 25^{\circ}$ C and $pH = 7$, the optimal result was achieved when the uncoupling agent TCS was dosed at 0.8 mg/L and TCP at 2.8 mg/L, leading to the reduction of activated sludge yield from 0.72 to 0.398. During the study, Ma et al. [24] found that combining copper ions and uncoupling agents can effectively reduce the sludge yield. The results showed that 2,6-dichlorophenol (DCP) had a significant synergistic effect with divalent copper. When the DCP concentration was 20 mg/L, and $Cu²⁺$ concentration was 1 mg/L, the chemical oxygen demand (COD) removal efficiency decreased by only 7%, while the sludge yield was reduced by up to 75%. In addition, the measured average concentration of DCP in water decreased to 0.28 mg/L, which was easier to remove, and the removal rate of $\tilde{C}u^{2+}$ reached more than 90%.

However, the dosage of an uncoupling agent should be reasonably controlled, if the dosage is too high or frequently used, microorganisms will have a domestication effect and the number and diversity of aquatic communities will be changed. Moreover, most of the uncoupling agents are toxic and harmful substances, which are costly and difficult to degrade, which will cause some side effects. Therefore, further research and solution are necessary to address these issues.

2.2. High S_o/X_o

The initial substrate concentration/initial sludge concentration is an essential parameter in sludge culture (S_0/X_0) is COD/biomass). As the S_0/X_0 ration increase, the growth yield decreases significantly, and energy is dissipated to the environment in the form of work and heat [26]. To achieve high S_0/X_0 uncoupling, an S_0/X_0 ratio greater than 8 is required. However, the actual S_0/X_0 values for domestic wastewater are 0.01 to 0.13 mg·COD/mg·MLSS. Therefore, the high S_{0} / X_0 conditions differ significantly from the actual treatment plant requirements and cannot be applied to the actual process due to high loading at present [27].

2.3. Aerobic-sedimentation-anaerobic (OSA) process

The transformation of organisms in aerobic and anaerobic environments can contribute to the production of uncoupling and consequently to the reduction of sludge yield. The OSA process is a modified activated sludge system based on the principle of sludge reduction by adding an anaerobic reactor to the sedimentation return section of the conventional activated sludge process. In this process, microorganisms are exposed to the aerobic and anaerobic sections alternately to minimize substrate residues and food storage in the effluent effect [26]. This method has been proven to be promising for sludge reduction in wastewater treatment processes, with the advantages of no chemical dose, easy construction, and improved nutrients [28].

Vitanza, Martins, Demir et al. [29–31] showed that the OSA process could reduce sludge yield by 50% and improve COD removal and sludge settling properties with no deterioration in effluent quality compared to the conventional activated sludge method. Since then, the OSA process has been widely used for RS reduction. By adding TCS to the aeration tank in the OSA process, Ye, Li, and Saini et al. [32–34] minimized the residual sludge while minimizing the adverse effects, which in turn led to the combination of TCS and OSA process. As a result, sludge production was reduced by 21% to 56%.

3. Maintenance metabolism

Maintenance metabolism refers to the priority of microorganisms to supply the energy needed for normal cell growth during wastewater treatment rather than supplying additional biomass synthesis, which can be achieved by extending the sludge retention time (SRT) or reducing the sludge loading rate (F/M) in aerobic wastewater biological treatment processes to achieve a reduction in sludge production [35]. Microorganisms use a portion of their cellular self-oxidation to generate energy to maintain metabolism and the rest to proliferate the organism.

Membrane bioreactor (MBR) offer several advantages, such as the ability to easily control the sludge age extension in the system, enhance the maintenance metabolism and endogenous metabolism of microorganisms in the system, and reduce the proliferation of sludge microorganisms to achieve RS reduction or even residual sludge-free discharge. Zhao and Liu [36,37] used MBR technology to achieve a longterm sludge reduction. The sludge reduction rate reached 80% during operation, and biological predation played a great influence in sludge reduction due to the stability of protozoan and postbiotic species and numbers in the system, resulting in 73.9% of the total cumulative sludge reduction. Yeom, Wang et al. [38,39] combined MBR and ozone pretreatment to accelerate sludge degradation, with an optimal ozone dosing of 0.04 g \cdot O₃/g \cdot SS, and the degree of sludge fragmentation was observed with a cumulative sludge reduction of more than 70%. Zheng, Cheng et al. [40,41] combined the anaerobic side-stream reactor (ASSR) and MBR in their research to build a process that can effectively remove pollution and simultaneously reduce sludge production, with a reduction rate of about 40%, which is a promising method. Under the optimal sludge reduction condition, sludge setting ability and biological maintenance can be improved.

Further studies have been conducted on membrane reactors, but their high cost of membrane equipment and problems such as membrane contamination remains a challenge. Therefore, many researchers have begun to put metabolic uncoupling agents into MBR systems to control the formation of membrane contamination [42–44].

4. Lysis-cryptic growth

Lysis-cryptic growth is the release of cellular contents after cell decay for the metabolic growth of the substrate microorganism, and the whole process includes two main steps: lysis and cryptic growth [45] (Fig. 2). A portion of the carbon source is released as respiration products during metabolism, resulting in a reduction in overall biomass production, followed by cryptic growth due to the inability to separate from the original organic matter biomass growth on the primary substrate.

4.1. Biolysis

Biolysis can be carried out by adding bacteriological agents and enzymes so that the secretable extracellular enzymes can lyse the bacteria. It has the advantages of simple operation and a good sludge reduction effect. Enzymes can not only lyse the cells during anaerobic digestion but also decompose large molecules of organic matter that are not easily biodegradable into small molecules [46], thereby improving sludge digestibility and facilitating the secondary use of the substrate by bacteria [47].

In a specific study, Li and Wang et al. [48,49] investigated the effect of multifunctional compound micro-organisms preparation (MCMP) reagent injection on sludge reduction in the aerobic section of the wastewater treatment process. The study revealed that the best sludge reduction effect was achieved when the amount of MCMP bacterial agent injection reached 0.02%. During two months of continuous operation of the system, no residual sludge was discharged. Wu et al. [50] studied the parameters of effective microorganisms (EM) bacterial agent dosage and temperature to obtain the optimal operating conditions, which can effectively reduce sludge production when the dosage of the bacterial agent is 0.005%, the experimental temperature is 30°C, and the contact time reaches 6~18 h. Song et al. [51] investigated the effect of adding hydrolytic lysozyme to the SBR system on the treatment effect of the system. The results showed that the lysozyme treatment of raw sludge had higher hydrolysis efficiency. With 0.8 g of hydrolytic lysozyme in a 12 L treatment system, the sludge reduction effect reached 76.3% and the effluent COD and total phosphorus (TP) removal rates reached 88% and 54%, respectively. Bai, Yang, Zou et al. [46,52,53] found that due to the strong specificity of the biological enzymes themselves, a specific type of enzyme can only catalyze a certain substrate. Due

Fig. 2. Sludge lysis process in water treatment.

to the complexity of the sludge composition, a mixture of two enzymes (protease:amylase = 1:3) was administered at 50°C, and multiple enzymes were essential for the hydrolysis of different substrates. The best hydrolysis efficiency of 68.43% was achieved at 50°C. To achieve the best treatment effect, amylase was added before protease. When the ration of lysozyme and protease is 4:1, the extracellular polymeric substances (EPS) increase greatly, but it should be added back-to-back, otherwise it will interfere with each other and reduce the hydrolysis level. In addition, after enzyme treatment, EPS was more extractable, and the floc structure was looser. Wawrzynczyk, Kavitha, and Nguyen et al. [47,54,55] achieved reduced adsorption and more effective and lower enzyme doses by pretreating sludge with cationic binding agents. Citric acid was the most effective of the three cationic binding agents tested, and it is biodegradable. It can be produced endogenously by microorganisms in the sludge and also has the most significant potential for practical application in improving biogas production.

Biolysis is environmentally friendly and does not produce secondary pollution. However, long-term use may change the native microbial composition of the system. It may make the original microorganisms dependent and is expensive, so further research is still needed to enable its widespread implementation in domestic wastewater treatment.

4.2. Chemical lysis

Chemical cytolysis methods include ozonolysis, chlorine cytolysis [56], and the use of acids and bases [57]. Among them, ozonolysis has been the most widely studied [58]. Ozone, as a green reactive oxidant, is one of the effective means of sludge solubilization due to its favorable performance in destroying microbial cell walls and releasing intracellular compounds with compact preparation, simplified operation and no secondary pollution [59,60]. There is good evidence that the organic matter in the sludge can be converted to biodegradable substances, and partial mineralization of activated sludge into carbon dioxide and water can be achieved by partial ozonation of the returned sludge in the activated sludge process [61]. As the ozone dose increase, the water content of the sludge decreases, and the scum and swelling were reduced [62]. Ozonolysis cells were studied in Japan as early as the 1990s [63]. Sludge was decomposed in biological treatment after passing through the ozonation cell, so that the intracellular material was dissolved in water and recirculated to the aeration tank, making it more accessible to microorganisms. Chu, and Qiang [64,65] explored the application of ozone in the A2O pilot scale and found that when the ozone doses ranged from 0.03 to 0.05 $\text{g-O}_{\text{s}}/\text{g}\cdot\text{TSS}$, a balance between sludge reduction efficiency and the cost was achieved, and approximately 70%~90% of the sludge was inactivated [66]. The optimal sludge residence time for ozone treatment was 75 d. Under this condition, the reduction of organic matter in sludge was 41.53% and the total amount of remaining sludge was reduced by 25.92% compared to the control group. Since ozone is an expensive reagent, optimizing of the ozonation process is a critical point of process engineering. Chu and Hashimoto et al. [67,68] used ozone oxidation technology combined with micro and nanobubbles to improve ozone

utilization and sludge dissolution. The sludge dissolution efficiency of the microbubble system reached 25%~40% at ozone doses of $0.06 \sim 0.16$ g $\cdot O_3/g \cdot TSS$. For a contact time of 80 min, the ozone utilization efficiency was over 99% in the microbubble system. However, even for the microbubble ozone system, there was no significant change in sludge dissolution and solubilization efficiency when the ozone dose was higher than $0.16 \text{ g} \cdot \text{O}_3/\text{g} \cdot \text{TSS}$. Li et al. [11] adopted Venturi aerator to achieve sludge reduction, and the sludge dissolution efficiency was 0–100mg/g·MLSS. The device transformed ozone into many tiny bubbles to promote the self-decomposition of ozone, destroyed the surface components of cell membranes and dissolved organic matter into the supernatant, thus reducing energy consumption and improving utilization efficiency. These two methods improve the limitation of traditional ozone technology in water treatment by reducing the cost of sludge ozone treatment and expanding the application range of ozone.

4.3. Physical lysis

Physical lysis refers to the use of physical methods such as ultrasound [69,70], mechanical crushing [71], and heat treatment [72,73] (heating the sludge at 40° C \sim 180 $^{\circ}$ C) to destruct the original cell structure [74]. The organic material released from the crushed cells is rapidly increased and used by the active organisms in the system to promote the implicit growth of microorganisms. Physical lysis of cells does not pose a risk of secondary contamination by by-products but requires additional facilities to the original treatment equipment. Among them, ultrasonic treatment is to decompose sludge effectively by inducing ultrasonic waves to emit the frequency of sludge cracking, and to disinfect wastewater [75,76]. It involves decomposing microbial cells to extract intracellular substances and is most effective at low ultrasonic frequencies by generating large cavitation bubbles. When these cavitation bubbles collapse, it will trigger powerful jets that exert strong shear forces in the liquid resulting in sludge hydrolysis, but will not affect the wastewater treatment and sludge settling performance [77]. Gao et al. [78] discussed the feasibility of reducing sludge production in SBR reactors under low intensity ultrasound, and the results showed that the sludge production rate was reduced by 53.2%. The dehydrogenase activity, effluent quality and sedimentation performance of sludge were not affected. Tahmasebian et al. [79] applied ultrasonic technology to the moving bed biofilm reactor, and achieved a reduction of 61.7% of the sludge, and 42.2% of the excess sludge was cracked and converted into soluble COD. Parandoush et al. [80] combined ultrasonic wave with ozonation, and used the ability of ultrasonic wave to destroy floc structure and the oxidation capacity of ozone gas to reduce the volume of sludge produced. The sludge output in the system was reduced by 54%, and the wastewater treatment efficiency was still maintained at the standard level. Niu [72] et al. adopted thermal hydrolysis to improve the dehydration and digestibility of sludge and accelerate the cell lysis to achieve the reduction of sludge mass. By analyzing the contents of protein and polysaccharide, the damage of the flocculation structure of sludge and the process of sludge reduction could be determined.

5. Biological predation

Microbial predation is used to extend the food chain in the wastewater treatment process, leading to a longer food chain that loses more nutrients and energy [81]. In the wastewater environment, there are not only microorganisms but also protozoa and post-zoa [82] that can feed on sludge flocs. It is a promising technology due to its advantages of high efficiency, environmental protection as well as high-quality effluent, and low sludge yield [83]. However, nitrogen and phosphorus removal is poor, so the research on nitrogen and phosphorus removal should be carried out simultaneously in combination with other methods (Fig. 3).

5.1. Inoculation of microscopic organisms

The method of inoculation of microfauna is to inject the reactor directly with micro-protozoa and post-protozoa for sludge reduction. The drawback of this technique is that the culture of protozoa is changing the control and may also cause the collapse of the nitrification system.

Worm technology based on predation to reduce RS has received increasing research attention because of its low cost and eco-friendliness [82,84]. In different types of worm reactors, sludge reduction efficiency can reach 30%–50% [85]. Worm predation leads to not only the release of soluble microbial products (SMP) but also the changes in EPS characteristics, including EPS content and carbohydrate/ protein ratio [86]. Tamis, Zheng, Emamjomeh et al. [87–89] found that the presence of earthworms led to a 56.6%–75% reduction in sludge in the control system, improving the sludge settling possibility in continuous flow processes and significantly enhancing sludge reduction. Hendrickx, Wei, Ding et al. [90–92] utilized some novel worm reactors in order to promote worm growth and improve sludge

Table 2

Advantages and disadvantages of different treatment effects

reduction rates, sludge dewatering performance and setting performance are improved. In their study, the amount of total suspended solids (TSS) was reduced by about 75%, and worm predation resulted in significant sludge reduction, almost as much as in the blank experiment three times.

5.2. Two-stage method

The two-stage method uses a two-stage system to treat wastewater. The first stage is the dispersive bacteria cultivation stage, without biomass retention, and the growth of dispersive bacteria is induced under short sludge age conditions. The second stage is the stimulation of protozoa and the post-zoa predation stage with long sludge age. In this stage, the front-end sludge is predated by microscopic

Fig. 3. Degradation and reduction of sludge worms during predation.

animals and converted into $CO_{2'}$ water, and energy to promote the growth of protozoa using a biofilm process or membrane bioreactor [93].

Ratsak et al. [94] first conducted a study on the dosing of ciliates in a two-stage reactor for biological predation to minimize sludge yield and reduce biomass. Compared to the first stage, the sludge production in the second stage was significantly reduced by 60%–80%. The better the growth of microfauna in the water column, the less RS production. Ghyoot et al. [82] used a two-stage system for treating aerobic wastewater with synthetic wastewater and used cultured protozoa-rich sludge to achieve a reduction in RS production with stable effluent quality. The cultivation of predators in the two-stage MBR system not only reduced the nitrification capacity but also led to an increase in the effluent N and P concentrations. The advantages and disadvantages of various methods are shown in Table 2.

6. Conclusion

Currently, new stringent regulations on sludge treatment and disposal and social as well as environmental issues are making a significant contribution to the development of strategies to reduce excess sludge production, and *in-situ* sludge reduction technology is an effective way to solve the current problem of sludge yield. The variety of this technology is complex and can be selected according to the actual situation, to achieve the optimal combination of sludge reduction and wastewater purification without comparing effluent quality. In addition, the technology transforms various substances during the process, making effective use of nitrogen and phosphorus-rich in sludge. Currently, ozonolysis, ultrasonic crushing, and the addition of bacterial or enzymatic agents are widely used in experimental studies, but not applied in large-scale systems. In subsequent experimental studies, sludge lysis and subsequent hidden growth can be promoted by combining physical, chemical, and multiple processes to reduce sludge production and avoid its existing drawbacks and shortcomings.

Acknowledgements

We appreciate the financial support from Scientific Research Foundation of Anhui Universities (KJ2021A0442), Start-up Foundation for High-level Introduction Talents of Anhui University of Science and Technology (2021yjrc04) and State Key Laboratory of Pollution Control and Resource Reuse Foundation (NO. PCRRF21040).

Abbreviations

Symbols

References

- [1] Y. Cheng, K. Tian, P. Xie, X.H. Ren, Y. Li, Y.Y. Kou, K.M. Chon, M.H. Hwang, M.H. Ko, Insights into the minimization of excess sludge production in micro-aerobic reactors coupled with a membrane bioreactor: characteristics of extracellular polymeric substances, Chemosphere, 292 (2022) 133434, doi: 10.1016/j.chemosphere.2021.133434.
- [2] J. Zhang, Y. Tian, J. Zhang, Release of phosphorus from sewage sludge during ozonation and removal by magnesium ammonium phosphate, Environ. Sci. Pollut. Res., 24 (2017) 23794–23802.
- [3] Z. Zhou, Y.Y. Sun, L. Fu, Y. Zuo, Y.J. Shao, L.H. Wang, C.T. Zhou, Y. An, Unraveling roles of the intermediate settler in a microaerobic hydrolysis sludge *in-situ* reduction process, Bioresour. Technol., 384 (2023) 129228, doi: 10.1016/j. biortech.2023.129228.
- [4] C. Cheng, J.J. Geng, Z. Zhou, Q.M. Yu, R.W. Gao, Y.H. Shi, L.Y. Wang, H.Q. Ren, A novel anoxic/aerobic process coupled with micro-aerobic/anaerobic side-stream reactor filled with packing carriers for *in-situ* sludge reduction, J. Cleaner Prod., 311 (2021) 127192, doi: 10.1016/j.jclepro.2021.127192.
- [5] C. Cheng, J.J. Geng, H.D. Hu, Y.H. Shi, R.W. Gao, X. Wang, H.Q. Ren, *In-situ* sludge reduction performance and mechanism in an anoxic/aerobic process coupled with alternating aerobic/anaerobic side-stream reactor, Sci. Total Environ., 777 (2021) 145856, doi: 10.1016/j.scitotenv.2021.145856.
- [6] A. Iqbal, X.M. Liu, G.H. Chen, Municipal solid waste: review of best practices in application of life cycle assessment and sustainable management techniques, Sci. Total Environ., 729 (2020) 138622, doi: 10.1016/j.scitotenv.2020.138622.
- [7] Y. Liang, D.H. Xu, P. Feng, B.T. Hao, Y. Guo, S.Z. Wang, J.J. Klemes, Municipal sewage sludge incineration and its air pollution control, J. Cleaner Prod., 295 (2021) 126456, doi: 10.1016/j.jclepro.2021.126456.
- [8] A. Hanc, I. Komorowicz, K. Sek, D. Baralkiewicz, Test of the relationships between the content of heavy metals in sewage sludge and source of their pollution by chemometric methods, J. Environ. Sci. Health. Part A Toxic/Hazard. Subst. Environ. Eng., 44 (2009) 1441–1448.
- [9] Y. Liu, J.H. Tay, Strategy for minimization of excess sludge production from the activated sludge process, Biotechnol. Adv., 19 (2001) 97–107.
- [10] M. Boehler, H. Siegrist, Potential of activated sludge disintegration, Water Sci. Technol., 53 (2006) 207–216.
- [11] H.L. Li, Q.L. Zhang, M. Zeng, J.G. Cao, Q.Y. Zhao, L.L. Hao, Insights into gas flow behavior in venturi aerator by CFD-PBM model and verification of its efficiency in sludge reduction

through O_3 aeration, J. Water Process Eng., 54 (2023) 103960, doi: 10.1016/j.jwpe.2023.103960.

- [12] Z.Y. Wang, T. Liu, H.R. Duan, Y.R. Song, X. Lu, S.H. Hu, Z.G. Yuan, D. Batstone, M. Zheng, Post-treatment options for anaerobically digested sludge: current status and future prospect, Water Res., 205 (2021) 117665, doi: 10.1016/j.watres.2021.117665.
- [13] S.S. Yang, W.Q. Guo, G.L. Cao, H.S. Zheng, N.Q. Ren, Simultaneous waste activated sludge disintegration and biological hydrogen production using an ozone/ultrasound pretreatment, Bioresour. Technol., 124 (2012) 347-354.
- [14] E. Paul, H. Debellefontaine, Reduction of excess sludge produced by biological treatment processes: effect of ozonation on biomass and on sludge, Ozone Sci. Eng., 29 (2007) 415–427.
- [15] J. Jiang, Z. Zhou, L.Y. Jiang, Y. Zheng, X.D. Zhao, G. Chen, M.Y. Wang, J. Huang, Y. An, Z.C. Wu, Bacterial and microfauna mechanisms for sludge reduction in carrier-enhanced anaerobic side-stream reactors revealed by metagenomic sequencing analysis, Environ. Sci. Technol., 55 (2021) 6257–6269.
- [16] M. Mayhew, T. Stephenson, Biomass yield reduction: is biochemical manipulation possible without affecting activated sludge process efficiency?, Water Sci. Technol., 38 (1998) 137–144.
- [17] X.F. Yang, M.L. Xie, Y. Liu, Metabolic uncouplers reduce excess sludge production in an activated sludge process, Process Biochem., 38 (2003) 1373–1377.
- [18] X.F. Yang, X.P. Xu, X.Y. Wei, J.C. Li, J. Wan, Assessment of the sludge reduction of the metabolic uncoupler 3,3',4',5-tetrachlorosalicylanilide (TCS) in activated sludge culture, Int. J. Environ. Res. Public Health, 16 (2019) 1686, doi: 10.3390/ijerph16101686.
- [19] E. Ferrer-Polonio, J. Fernandez-Navarro, J.L. Alonso-Molina, A. Bes-Pia, I. Amoros, J.A. Mendoza-Roca, Changes in the process performance and microbial community by addition of the metabolic uncoupler 3,3',4',5-tetrachlorosalicylanilide in sequencing batch reactors, Sci. Total Environ., 694 (2019) 133726, doi: 10.1016/j.scitotenv.2019.133726.
- [20] Y. Li, A.M. Li, J. Xu, B. Liu, L.C. Fu, W.W. Li, H.Q. Yu, SMP production by activated sludge in the presence of a metabolic uncoupler, 3,3',4',5-tetrachlorosalicylanilide (TCS), Appl. Microbiol. Biotechnol., 95 (2012) 1313–1321.
- [21] F. Fang, S.N. Wang, K.Y. Li, J.Y. Dong, R.Z. Xu, L.L. Zhang, W.M. Xie, J.S. Cao, Formation of microbial products by activated sludge in the presence of a metabolic uncoupler o-chlorophenol in long-term operated sequencing batch reactors, J. Hazard. Mater., 384 (2020) 121311, doi: doi: 10.1016/j. jhazmat.2019.121311.
- [22] S.N. Wang, F. Fang, K.Y. Li, Y.R. Yue, R.Z. Xu, J.Y. Luo, B.J. Ni, J.S. Cao, Sludge reduction and microbial community evolution of activated sludge induced by metabolic uncoupler o-chlorophenol in long-term anaerobic-oxic process, Environ. Manage., 316 (2022) 115230, doi: doi: 10.1016/j. jenvman.2022.115230.
- [23] X.C. Feng, Study on Characteristics of Complex Uncoupling Agents for Sludge Process Reduction and Their Effects on Treatment Efficiency, Harbin Institute of Technology, 2013 (in Chinese).
- [24] Z.K. Ma, Y. Tian, H.F. Cheng, Sludge reduction under the synergistic effect of copper ions and uncoupling agents, Environ. Sci., (2007) 1697–1702 (in Chinese).
- [25] E.W. 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.
- [26] Z. He, H.Y. Wang, H.H. Tian, L. Wang, Y.X. Zhou, Research progress in sludge reduction water treatment technology, China Water Supply Drain., 25 (2009) 1–7 (in Chinese).
- [27] P. Chudoba, B. Capdeville, J. Chudoba, Explanation of biological meaning of the S_0/X_0 ratio in batch cultivation, Water Sci. Technol., 26 (1992) 743–751.
- [28] G.U. Semblante, F.I. Hai, H.H. Ngo, W.S. Guo, S.J. You, W.E. Price, L.D. Nghiem, Sludge cycling between aerobic, anoxic and anaerobic regimes to reduce sludge production during wastewater treatment: performance, mechanisms, and implications, Bioresour. Technol., 155 (2014) 395–409.
- [29] C.L. Martins, V.F. Velho, B.S. Magnus, J.A. Xavier, L.B. Guimarães, W.R. Leite, R.H.R. Costa, Assessment of sludge reduction and microbial dynamics in an OSA process with short anaerobic retention time, Environ. Technol. Innovation, 19 (2020) 101025, doi: 10.1016/j.eti.2020.101025.
- [30] R. Vitanza, A. Cortesi, M.E. De Arana-Sarabia, V. Gallo, I.A. Vasiliadou, Oxic settling anaerobic (OSA) process for excess sludge reduction: 16 months of management of a pilot plant fed with real wastewater, J. Water Process Eng., 32 (2019) 100902, doi: 10.1016/j.jwpe.2019.100902.
- [31] O. Demir, A. Filibeli, The investigation of the sludge reduction efficiency and mechanisms in oxic–settling–anaerobic (OSA) process, Water Sci. Technol., 73 (2016) 2311–2323.
- [32] F.X. Ye, Y. Li, Oxic-settling-anoxic (OSA) process combined with 3,3',4',5-tetrachlorosalicylanilide (TCS) to reduce excess sludge production in the activated sludge system, Biochem. Eng. J., 49 (2010) 229–234.
- [33] F.X. Ye, Y. Li, Uncoupled metabolism stimulated by chemical uncoupler and oxic-settling-anaerobic combined process to reduce excess sludge production, Appl. Biochem. Biotechnol., 127 (2005) 187–200.
- [34] G. Saini, Technical comments on "Oxic-settling-anoxic (OSA) process combined with 3,3′,4′,5-tetrachlorosalicylanilide (TCS) to reduce excess sludge production in the activated sludge system" by Fenxia Ye and Ying Li, Biochem. Eng. J., 50 (2010) 150–151.
- [35] J.S. Guo, F. Fang, P. Yan, Y.P. Chen, Sludge reduction based on microbial metabolism for sustainable wastewater treatment, Bioresour. Technol., 297 (2020) 122506, doi: 10.1016/j. biortech.2019.122506.
- [36] B.X. Zhao, L.J.H. Huang, W.F. Huang, Y.J. Liu, R.H. Li, Study on mechanism of excess sludge reduction in MBR technology, Membr. Sci. Technol., 39 (2019) 73–80+87 (in Chinese).
- [37] B.J. Liu, H.J. Fan, L. Feng, L.Q. Zhang, Study on sludge reduction effect based on biological predation in membrane bioreactor process, Environ. Pollut. Prev., 34 (2012) 30–33+39 (in Chinese).
- [38] I.T. Yeom, K.R. Lee, Y.G. Choi, H.S. Kim, J.H. Kwon, U.J. Lee, Y.H. Lee, A pilot study on accelerated sludge degradation by a high-concentration membrane bioreactor coupled with sludge pretreatment, Water Sci. Technol., 52 (2005) 201–210.
- [39] Z. Wang, L. Wang, B.Z. Wang, Y.F. Jiang, S. Liu, Bench-scale study on zero excess activated sludge production process coupled with ozonation unit in membrane bioreactor, J. Environ. Sci. Health., Part A, 43 (2008) 1325–1332.
- [40] Y. Zheng, C. Cheng, Z. Zhou, H. Pang, L.Y Chen, L.M. Jiang, Insight into the roles of packing carriers and ultrasonication in anaerobic side-stream reactor coupled membrane bioreactors: sludge reduction performance and mechanism, Water Res., 155 (2019) 310–319.
- [41] C. Cheng, Z. Zhou, T.H. Niu, Y. An, X.L. Shen, W. Pan, Z.H. Chen, J. Liu, Effects of side-stream ratio on sludge reduction and microbial structures of anaerobic side-stream reactor coupled membrane bioreactors, Bioresour. Technol., 234 (2017) 380–388.
- [42] X.C. Feng, W.Q. Guo, H.S. Zheng, S.S. Yang, J.S. Du, Q.L. Wu, H.C. Lou, X. Zhou, W.B. Jin, N.Q. Ren, Inhibition of biofouling in membrane bioreactor by metabolic uncoupler based on controlling microorganisms accumulation and quorum sensing signals secretion, Chemosphere, 245 (2020) 125363, doi: 10.1016/j.chemosphere.2019.125363.
- [43] Y.J. Shao, Z. Zhou, J. Jiang, L.M. Jiang, J.P. Huang, Y. Zuo, Y.Q. Ren, X.D. Zhao, Membrane fouling in anoxic/oxic membrane reactors coupled with carrier-enhanced anaerobic side-stream reactor: effects of anaerobic hydraulic retention time and mechanism insights, J. Membr. Sci., 637 (2021) 119667, doi: 10.1016/j.memsci.2021.119657.
- [44] Y. Zuo, Y.J. Shao, L.H. Wang, Y.Y. Sun, Y. An, L.M. Jiang, N. Yu, R.J. Hao, C.T. Zhou, J. Tao, Z. Zhou, Simultaneous sludge minimization and membrane fouling mitigation in membrane bioreactors by using a microaerobic - settling pretreatment module, J. Environ. Manage., 328 (2023) 116977, doi: 10.1016/j. jenvman.2022.116977.
- [45] W.Q. Guo, S.S. Yang, W.S. Xiang, X.J. Wang, N.Q. Ren, Minimization of excess sludge production by *in-situ* activated sludge treatment processes — a comprehensive review, Biotechnol. Adv., 31 (2013) 1386–1396.
- [46] Q. Yang, K. Luo, X.M. Li, D.B. Wang, W. Zheng, G.M. Zeng, J.J. Liu, Enhanced efficiency of biological excess sludge hydrolysis under anaerobic digestion by additional enzymes, Bioresour. Technol., 101 (2010) 2924–2930.
- [47] J. Wawrzynczyk, M. Recktenwald, O. Norrlow, E.S. Dey, The function of cation-binding agents in the enzymatic treatment of municipal sludge, Water Res., 42 (2008) 1555–1562.
- [48] J. Li, Z. Zhu, G.Z. Zhu, D.T. Xie, C.F. Wei, Z.S. Pang, Study on reducing excess sludge production by using MCMP microbial preparation, J. Environ. Eng., (2007) 92–95 (in Chinese).
- [49] M. Wang, L.A. Wang, L. Bao, Z. Zhu, Research on multifunctional microbial agents for sludge reduction, China Water Supply Drain., 23 (2007) 16–19 (in Chinese).
- [50] B.L. Wu, Z.M. Huang, X. Wang, L. Qian, *In-situ* sludge reduction by EM bacteria and its impact on sludge yield, Ind. Saf. Environ. Prot., 42 (2016) 99–102 (in Chinese).
- [51] Y. Song, Z. Shi, Evaluation of sludge reduction in an activated sludge process using lysozymes, Fresenius Environ. Bull., 25 (2016) 1988–1996.
- [52] R.Y. Bai, K. Chen, W.J. Zhang, Study on the kinetic mechanism of activated sludge dissolution by complex enzyme treatment, J. Environ. Eng., 10 (2016) 5840–5846 (in Chinese).
- [53] X. Zou, J.G. He, P.F. Zhang, X.L. Pan, Y.J. Zhong, J. Zhang, X.W. Wu, B.Q. Li, X. Tang, X.N. Xiao, H.L. Pang, Insights into carbon recovery from excess sludge through enzymecatalyzing hydrolysis strategy: environmental benefits and carbon-emission reduction, Bioresour. Technol., 351 (2022) 127006, doi: 10.1016/j.biortech.2022.127006.
- [54] S. Kavitha, S.A. Kumar, K.N. Yogalakshmi, S. Kaliappan, J.R. Banu, Effect of enzyme secreting bacterial pretreatment on enhancement of aerobic digestion potential of waste activated sludge interceded through EDTA, Bioresour. Technol., 150 (2013) 210–219.
- [55] M.T. Nguyen, N.H.M. Yasin, T. Miyazaki, T. Maeda, Enhancement of sludge reduction and methane production by removing extracellular polymeric substances from waste activated sludge, Chemosphere, 117 (2014) 552–558.
- [56] S. Saby, M. Djafer, G.H. Chen, Feasibility of using a chlorination step to reduce excess sludge in activated sludge process, Water Res., 36 (2002) 656–666.
- [57] S. Tanaka, T. Kobayashi, K. Kamiyama, M.L.N. Signey Bildan, Effects of thermochemical pre-treatment on the anaerobic digestion of waste activated sludge, Water Sci. Technol., 36 (1997) 209–215.
- [58] G.U. Semblante, F.I. Hai, D.D. Dionysiou, K. Fukushi, W.E. Price, L.D. Nghiem, Holistic sludge management through ozonation: a critical review, Environ. Manage., 185 (2017) 79–95.
- [59] A. Chiavola, C. Salvati, S. Bongirolami, C. Di Marcantonio, M.R. Boni, Techno-economic evaluation of ozone-oxidation for sludge reduction at the full-scale. Comparison between the application to the return activated sludge (RAS) and the sludge digestion unit, J. Water Process Eng., 42 (2021) 102114, doi: 10.1016/j.jwpe.2021.102114.
- [60] S. Cosgun, N. Semerci, Combined and individual applications of ozonation and microwave treatment for waste activated sludge solubilization and nutrient release, J. Environ. Manage., 241 (2019) 76–83.
- [61] Z.M. Qiang, L. Wang, H.Y. Dong, J.H. Qu, Operation performance of an A/A/O process coupled with excess sludge ozonation and phosphorus recovery: a pilot-scale study, Chem. Eng. J., 268 (2015) 162–169.
- [62] Y.X. Zhao, J. Yin, H.L. Yu, N. Han, F.J. Tian, Observations on ozone treatment of excess sludge, Water Sci. Technol., 56 (2007) 167–175.
- [63] H. Yasui, M. Shibata, An innovative approach to reduce excess sludge production in the activated sludge process, Water Sci. Technol., 30 (1994) 11–20.
- [64] L.B. Chu, S.T. Yan, X.H. Xing, X.L. Sun, B. Jurcik, Progress and perspectives of sludge ozonation as a powerful pretreatment

method for minimization of excess sludge production, Water Res., 43 (2009) 1811–1822.

- [65] Z.M. Qiang, L. Wang, H.Y. Dong, J.H. Qu, Operation performance of an A/A/O process coupled with excess sludge ozonation and phosphorus recovery: a pilot-scale study, Chem. Eng. J., 268 (2015) 162–169.
- [66] W. Saktaywin, H. Tsuno, H. Nagare, T. Soyama, J. Weerapakkaroon, Advanced sewage treatment process with excess sludge reduction and phosphorus recovery, Water Res., 39 (2005) 902–910.
- [67] L.B. Chu, S.T. Yan, X.H. Xing, A.F. Yu, X.L. Sun, B. Jurcik, Enhanced sludge solubilization by microbubble ozonation, Chemosphere, 72 (2008) 205–212.
- [68] K. Hashimoto, N. Kubota, T. Okuda, S. Nakai, W. Nishijima, H. Motoshige, Reduction of ozone dosage by using ozone in ultrafine bubbles to reduce sludge volume, Chemosphere, 274 (2021) 129922, doi: 10.1016/j.chemosphere.2021.129922.
- [69] W. Li, N.J.W. Yu, Q. Liu, Y.R. Li, N.Q. Ren, D.F. Xing, Enhancement of the sludge disintegration and nutrients release by a treatment with potassium ferrate combined with an ultrasonic process, Sci. Total Environ., 635 (2018) 699–704.
- [70] E. Zielewicz, M. Tytla, Effects of ultrasonic disintegration of excess sludge obtained in disintegrators of different construction, Environ. Technol., 36 (2015) 2210–2216.
- [71] M. Zubrowska-Sudol, J. Podedworna, K. Sytek-Szmeichel, A. Bisak, P. Krawczyk, A. Garlicka, The effects of mechanical sludge disintegration to enhance fullscale anaerobic digestion of municipal sludge, Therm. Sci. Eng. Prog., 5 (2018) 289–295.
- [72] C.X. Niu, Y. Pan, X.Q. Lu, S.S. Wang, Z.Y. Zhang, C.T. Zheng, Y.J. Tan, G.Y. Zhen, Y.C. Zhao, Y.Y. Li, Mesophilic anaerobic digestion of thermally hydrolyzed sludge in anaerobic membrane bioreactor: long-term performance, microbial community dynamics and membrane fouling mitigation, J. Membr. Sci., 612 (2020) 118264, doi: 10.1016/j.memsci.2020.118264.
- [73] L.F. Wang, C. Qian, J.K. Jiang, X.D. Ye, H.Q. Yu, Response of extracellular polymeric substances to thermal treatment in sludge dewatering process, Environ. Pollut., 231 (2017) 1388–1392.
- [74] P. Camacho, P. Ginestet, J.M. Audic, Understanding the mechanisms of thermal disintegrating treatment in the reduction of sludge production, Water Sci. Technol., 52 (2005) 235–245.
- [75] J. Zhang, Y.L. Dong, Q.W. Wang, D.Y. Xu, L.Y. Lv, W.F. Gao, L. Sun, G.M. Zhang, Z.J. Ren, Effects of ultrasonic lysis frequency on sludge lysis-cryptic growth: sludge reduction, microbial community, and metabolism, Chem. Eng. J., 469 (2023) 144000, doi: 10.1016/j.cej.2023.144000.
- [76] B.A. Madge, J.N. Jensen, Disinfection of wastewater using a 20-kHz ultrasound unit, Water Environ. Res., 74 (2002) 159–169.
- [77] M. Zheng, Y.C. Liu, J. Xin, H. Zuo, C.W. Wang, W.M. Wu, Ultrasonic treatment enhanced ammonia-oxidizing bacterial (AOB) activity for nitritation process, Environ. Sci. Technol., 50 (2015) 864–871.
- [78] J.L. Gao, Y. Liu, Y.X. Yan, J.F. Wan, F. Liu, Promotion of sludge process reduction using low-intensity ultrasonic treatment, J. Cleaner Prod., 325 (2021) 129289, doi: 10.1016/j. jclepro.2021.129289.
- [79] S. Tahmasebian, S.M. Borghei, M. Torkaman, H.H. Goudarzi, Influence of ultrasonic cell disintegration on excess sludge reduction in a moving bed biofilm reactor (MBBR), J. Environ. Chem. Eng., 7 (2019) 102997, doi: 10.1016/j.jece.2019.102997.
- [80] S. Parandoush, N. Mokhtarani, Reducing excess sludge volume in sequencing batch reactor by integrating ultrasonic waves and ozonation, J. Environ. Manage., 317 (2022) 115405, doi: 10.1016/j.jenvman.2022.115405.
- [81] X.Q. Zhang, H.Y. Zeng, Q. Wang, J.M. Li, C.R. Ma, Sludge predation by aquatic worms: physicochemical characteristics of sewage sludge and implications for dewaterability, J. Cleaner Prod., 258 (2020) 120612, doi: 10.1016/j.jclepro. 2020.120612.
- [82] W. Ghyoot, W. Verstraete, Reduced sludge production in a two-stage membrane-assisted bioreactor, Water Res., 34 (2000) 205–215.
- [83] L.P. Li, Y. Tian, J. Zhang, W. Zuo, H. Li, A.R. Li, D.P. Huang, J. Liu, Y.H. Liu, Z.M. Sun, Y.S. Liu, Insight into the roles of worm reactor on wastewater treatment and sludge reduction in anaerobic-anoxic-oxic membrane bioreactor (A2 O-MBR): performance and mechanism, Chem. Eng. J., 330 (2017) 718–726.
- [84] A. Khursheed, A.A. Kazmi, Retrospective of ecological approaches to excess sludge reduction, Water Res., 45 (2011) 4287–4310.
- [85] Y.S. Wei, J.X. Liu, Sludge reduction with a novel combined worm-reactor, Hydrobiologia, 564 (2006) 213–222.
- [86] Y. Tian, Z.P. Li, Y.B. Lu, Changes in characteristics of soluble microbial products and extracellular polymeric substances in membrane bioreactor coupled with worm reactor: relation to membrane fouling, Bioresour. Technol., 122 (2012) 62–69.
- [87] J. Tamis, G. van Schouwenburg, R. Kleerebezem, M.C.M. van Loosdrecht, A full scale worm reactor for efficient sludge reduction by predation in a wastewater treatment plant, Water Res., 45 (2011) 5916–5924.
- [88] Y.D. Zheng, M.Y. Xing, L.Z.Y. Cai, T. Xiao, Y.F. Lu, J.Z. Jiang, Interaction of earthworms-microbe facilitating biofilm dewaterability performance during wasted activated sludge reduction and stabilization, Sci. Total Environ., 581–582 (2017) 573–581.
- [89] M.M. Emamjomeh, M. Tahergorabi, M. Farzadkia, E. Bazrafshan, A review of the use of earthworms and aquatic worms for reducing sludge produced: an innovative ecotechnology, Waste Biomass Valorization, 9 (2017) 1543–1557.
- [90] T.L.G. Hendrickx, H. Temmink, H.J.H. Elissen, C.J.N. Buisman, Aquatic worms eating waste sludge in a continuous system, Bioresour. Technol., 100 (2019) 4642–4648.
- [91] Y.S. Wei, Y.M. Wang, X.S. Guo, J.X. Liu, Sludge reduction potential of the activated sludge process by integrating an oligochaete reactor, J. Hazard. Mater., 163 (2009) 87–91.
- [92] W.Q. Ding, X. Zhou, W.B. Jin, Z.C. Zhao, S.H. Gao, Y.D. Chen, W. Han, H. Liu, Q.L. Wang, A novel aquatic worm (*Limnodrilus hoffmeisteri*) conditioning method for enhancing sludge dewaterability by decreasing filamentous bacteria, Sci. Total Environ., 849 (2022) 157949, doi: 10.1016/j.scitotenv.2022.157949.
- [93] N.M. Lee, T. Welander, Use of protozoa and metazoa for decreasing sludge production in aerobic wastewater treatment, Biotechnol. Lett., 18 (1996) 429–434.
- [94] C.H. Ratsak, B.W. Koi, H.W. van Verseveld, Biomass reduction and mineralization increase due to the ciliate *Tetrahymena pyriformis* grazing on the bacterium *Pseudomonas fluorescens*, Water Sci. Technol., 29 (1994) 119–128.