Desalination and Water Treatment
www.deswater.com
doi: 10.1080/19443994.2014.943062

55 (2015) 1683–1689
July

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A study on TOC and nutrients removal in SBR and CFSTR systems in relation to sludge EPS during granulation process

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Received 25 September 2013; Accepted 18 May 2014

ABSTRACT

The aim of this study was to develop a reactor system which is more efficient for removing TOC and nutrients (nitrogen, phosphorous) from waste water and also to study the role of extracellular polymeric substances (EPS) in their removal rates during aerobic sludge granulation process. Two reactors, including R-1 and R-2 were operated as sequencing batch reactor and continuous flow stirred tank reactor, respectively, for 35 days. Different patterns of food supply and aeration period were maintained in the two reactors. The sludge granule size was around 750 μm in R-1 as compared to R-2 which had average granule size about 340 μm at the end of experiment. EPS contents and TOC removal rates were very high in R-1 than R-2. Nitrogen and phosphorous removal efficiencies were 71 and 56%, respectively, in R-1 and 83 and 64% in R-2. The enhanced nutrients removal rate in R-2 was due to its larger sludge surface area (small granular size) than R-1. Moreover, due to small size particles of R-2, the boundary proteins of EPS were more exposed to nitrogen, therefore electrophilic–nucleophilic interactions between proteins and nitrogen atom of ammonia or nitrate were more profound, and thus increased its removal efficiency, even though R-1 had higher protein contents.

Keywords: Extracellular polymeric substances; TOC; Nitrogen; Phosphorous

1. Introduction

The presence of high concentration of organics and nutrients like ammonia and phosphate in the wastewater can seriously pollute the environment. The discharge of such type of wastewater without any treatment badly affects the receiving water bodies. Many physical and chemical methods [1] have been applied successfully at the present for this purpose. But these are very expensive and also environment unfriendly at the same time. From economical prospective and reusability, biological methods dominate to all other available treatment systems [2]. During the biological treatment of wastewater, the micro-organisms breakdown and accumulate the

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Presented at IMSTEC 2013 – 8th International Membrane Science and Technology Conference, Organized by the Membrane Society of Australia, 25–29 November, 2013, Melbourne, Australia

nutrients during the granulation process. It allows the efficient recycling of these nutrients and, as a result, helps to remove pollution from the wastewater.

Biological removal of pollutants from the waste water mainly depends upon the nature and composition of microbial population and their products present in it [3,4]. The designing of reactor and supply of food are very important factors as they yield different types and compositions of micro-organisms and microbial products [3] like extracellular polymeric substances (EPS) which affect the physicochemical characteristics of granulated sludge. Studies have been made for the biological treatment of wastewater in the sequencing batch reactor (SBR) system as it involves a high bioactivity. The biomass in SBR produces large size sludge granules with excellent settling and dewatering characteristics [5,6]. These granules include microbial cells, degradable particles and EPS [7]. This microbial aggregation may help in degradation of nutrients, thus increasing their removal rates. EPS molecules have excellent property to remove inorganic and organic contaminants due to the presence of hydrophilic and hydrophobic regions in their structures [8].

Presently, focused has been made in the SBR style of operation for nutrients removal and less work has been done in continuous flow stirred tank reactor (CFSTR) system. But the problem is that, SBR system can only be applied to small communities. Furthermore, no significant studies have shown the correlation of nutrients removal with EPS contents during the sludge granulation process. Therefore, the main purpose of the present studies was to investigate the nutrient removal efficiencies of SBR and CFSTR systems for their best use in wastewater treatment systems and also to find out the effects of EPS contents on these removal rates during aerobic sludge granulation process.

2. Materials and methods

2.1. Configuration and operation of reactors

Two reactors, R-1 and R-2, were operated as SBR and CFSTR, respectively, for 35 days. R-1 was operated on 6-h cycle and 4 cycles/day basis. One cycle included 10 min filling, 280 min aeration, 50 min settling, 5 min drainage, and 15 min idle time. The total volume of reactor was 3.0 L and the influent dischargeable volume was 1.6 L. All the unit operations like feeding, aeration, and drainage of effluent were controlled by fixing the timers to each of them. R-2 had two principle compartments called aeration and settling tanks, which were separated by divider but not joined from the bottom so that the effluent can move to the settling tank for its onward discharge into the effluent storage tank. The workable volume of CFSTR was also 3.0 L. The influent was continuously pumped into the aeration tank (reaction tank) at a predetermined F/M (food/micro-organism) ratio where it was mixed with the sludge and aerated constantly. The clear effluent was discharged from the top of the settling tank into the effluent storage tank. The important parameters maintained during the operation are listed in Table 1, whereas the schematic diagrams of SBR and CFSTR are shown in Fig. 1.

2.2. Inoculated seed sludge and waste water characteristics

The same activated seed sludge was inoculated in both the reactors. Mixed liquor suspended solids (MLSS) concentration of seed sludge was 2,500 mg/L with SVI_{30} value of 210 mL/g. The influent COD concentration in the reactors was maintained at 350 mg/L by adding glucose. The contents of nitrogen and phosphorous in the lab-synthesized wastewater were 25 and 15 mg/L by injecting 95 and 66 mg/L of NH_4Cl and KH_2PO_4, respectively. The composition of trace element solution was same as given by [9].

2.3. Extracellular polymeric substances (EPS)

Boundary EPS of sludge samples of both the reactors were extracted by following Frolund et al. [10] method by using sodium cation exchange resin (CER) from Fluka (44445). The sludge samples were shaken at 250 rpm for 2.5 h at 4 ℃ in shaking incubator. The extracted EPS were centrifuged at 20,000 × g by using GYROZEN 1580 MGR centrifuge machine at 4 ℃ for 20 min and filtered by using Whatman filter paper.

Proteins and polysaccharides are the main EPS contents, therefore only these two were determined in the present studies. Proteins were measured by modified Lowery method, Frolund et al. [11], and bovine

Table 1
Characteristics of influent waste water and reactor parameters

Reactor	R-1 (SBR)	R-2 (CFSTR)
MLSS (mg/L)	2,500	2,500
COD Concentration (mg/L)	350	350
Number of cycles/d	4	Continuous
pH	7.0	7.0
Temperature (℃)	22	22
Nitrogen (mg/L)	25	25
Phosphorous (mg/L)	15	15

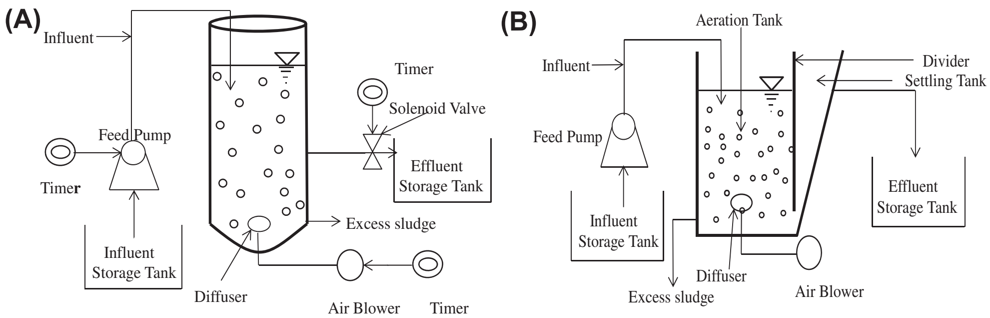

Fig. 1. Schematic diagrams of (A) SBR and (B) CFSTR.

serum albumin was the standard solution. Polysaccharides were determined by phenol sulphuric acid of Dubois et al. [12] using glucose as the standard solution.

2.4. TOC, nitrogen, and phosphorous determinations

TOC (total organic carbon) was measured by TOC-V_{CPH} (Total Organic Carbon Analyzer, Schimadzu Japan) instrument. High purity air was used as carrier gas at 300 kPa pressure. It operated on 680 ℃ combustion catalytic oxidation method with Non-Dispersive Infra-Red (NDIR) as detecting sensor. Automatic internal acidification and sparging took place. The sparge gas was bubbled through the sample to eliminate the IC (inorganic carbon) component and remaining TC (total carbon) was referred as TOC. Total nitrogen and phosphorous were determined by HACH spectrophotometer (DR/4000) using program 2558 and 3040, respectively.

2.5. Others

The particle size of sludge samples was noted using optic laser diffraction HELOS/KF-MAGIX (Sympa Quixel Germany) particle size analyzer with a detecting limit up to 3,500 μm. The analysis for MLSS, pH, and SVI_{30} values of sludge and influent samples were done by following the standard methods [13].

3. Results and discussion

3.1. Granular sludge characteristics

The flocculation, settleability, and shape of granules were very different in two reactors. Aerobic granulation was well achieved in R-1, while the sludge mostly appeared as floc-like particles in R-2. In R-1, small granules were first appeared on day six, while full granulation was achieved around the 30th day of studies. Due to the increase in granule size and speed in R-1, the SVI_{30} (sludge volume index) values were significantly lower than the seed sludge and appeared as 45 ml/g on day 30 to that of 210 ml/g of seed sludge. The SVI_{30} of R-2 on the same day was 91 ml/g and remained almost same till the end. These high SVI values in R-2 indicated the poor compressibility of sludge in this reactor as compared to R-1. MLSS were determined on daily basis in SBR system; much higher concentrations of MLSS were observed, while CFSTR showed less increase in its MLSS concentration. The extra sludge from both reactors discharged to ensure a constant F/M ratio for comparable studies.

The particle size distributions for both reactors on day 35 of experiment are shown in Fig. 2. At the beginning, the particles were very fine in both the reactors but with time, these increased remarkably in R-1 as compared to R-2. On day 18, 52% granules of R-1 had particle size of about 500 μm, and only 19% were less than 250 μm. While on the same day in R-2, approximately 48% particles were of 230 μm, while the size of 32% was lower than 160 μm. This trend continued with the progress of experiment and on day 30, the majority granules of R-1 were found to have

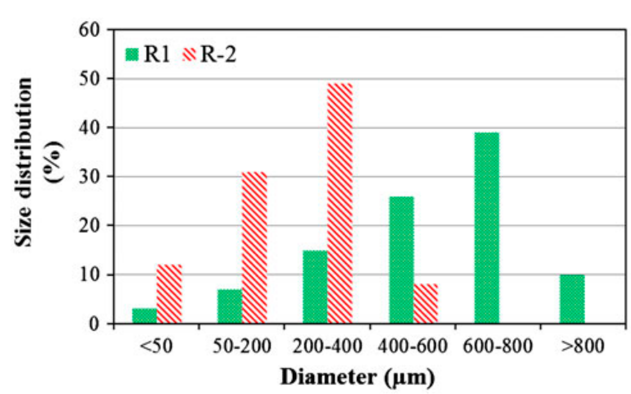

Fig. 2. Particle size distribution of R-1 and R-2 on day 35.

660 μm size in comparison to R-2 where the average floc size was about 300 μm. The results at the close of studies (day 35) highlighted that 750 μm was the dominant sludge granule size in R-1, while 360 μm was the mean particle size in R-2. It is very evident that different reactor conditions resulted in very different particle size of sludges. The reason for increased particle size in R-1 was its high concentrations of polysaccharides than R-2. Polysaccharides are considered as bioglues, holding the particles together resulting in the increase in floc size. Another reason for bigger particles in R-1 than R-2 was the inclusion of settling period in R-1. During the settling phase of SBR system, the sludge particles had sufficient contact time with each other and the EPS substances helped to bind the particles due to electrostatic interactions, which resulted in the increase in their size. While in CFSTR, there was no settling period and the sludge was continuously dispersed in the reactor and no significant interactions of particles took place, therefore low sludge particle size was found.

3.2. EPS during granulation process

The changes in EPS components in both reactors are shown in Fig. 3. Many researchers have found that proteins and polysaccharides are the main constituents of EPS molecules, therefore only these two were investigated and the sum of their values was described as total EPS contents. EPS contents augmented consistently during the operation but the increase was much lower towards the end of experiment. EPS of R-1 and R-2 raised to 124 and 91 mg/g SS, respectively, (62 mg/g SS seed sludge) on day 20 and further increased to 145 and 103 mg/g SS on the last day of experiment. R-2 behavior was very different from R-1, and the increase in EPS values was much lower in R-2 than R-1. It clearly showed that the composition and concentration of EPS was very much dependent upon the reactor operating conditions. This was due to the reason that each cycle of SBR system had several stages like influent injection, aeration, settling, and drainage of effluent, which took place periodically. As a result, the conditions for micro-organisms inside the reactor were continuously changed which eventually led them to produce more EPS contents to compete in the varying environment. These results were in good agreement with the finding of [14–16]. It is also reported in several studies that EPS help to maintain the integrity and stability of biofilms and also protect the micro-organism under severe conditions. Ogawa et al. [17] found that substances which possess shielding roles are secreted in higher amounts under changing conditions. On the other hand, in CFSTR system, the whole process was uniform, therefore the micro-organisms did not experience such changes there, and hence the production of EPS was much lower. Protein contents of R-1 and R-2 were increased to 99 and 72 mg/g SS to that of 40 mg/g SS of seed sludge, while the polysaccharides were 46 and 31 mg/g SS, respectively, (seed sludge 22 mg/g SS) on day 35 of experiment.

In the case of R-1, whole of the food in terms of influent COD was supplied at once in the beginning of each cycle, therefore the organic loading rate was very high at that stage. This high loading rate stimulated micro-organisms to produce more EPS (microbial product) due to increased metabolic activities. But in R-2, such feast period was never achieved in the reactor due to same F/M ratio as constant feeding rate was maintained in the whole experimental period, and thus suppressed the production of EPS. Therefore, the higher values of EPS in R-1 were noted, and then in R-2. As all food was supplied at the start of SBR cycle as mentioned above, this created a condition of starvation towards the end of each cycle because micro-organisms consumed almost all food at the start. This was further supported by its TOC removal results which indicated that 80% TOC was removed

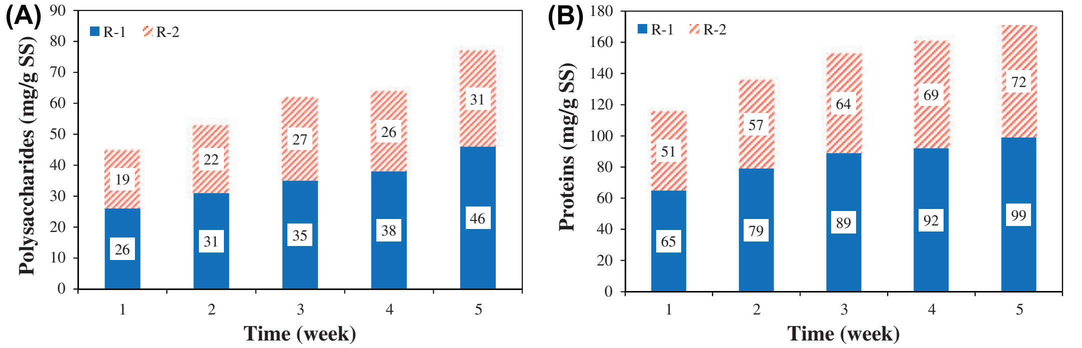

Fig. 3. The trend of variations of (A) polysaccharides, (B) proteins contents in R-1 (SBR) and R-2 (CFSTR) with time.

even at the end of first hour of aeration period. Bossier and Verstreate [18] highlighted that under starvation conditions micro-organisms become hydrophobic and in turn enhanced microbial adhesions and aggregation, therefore higher particle size in R-1 was observed. Yang et al. [19] also noted that the metabolic blocking of polysaccharides had adverse effects on the granulation. The results of our experiment showed that SBR system produced much profound contents of polysaccharides than CFSTR. Theses were the reasons for the enhanced particle size of R-1 compared to R-2.

3.3. TOC, nitrogen and phosphorous removal efficiencies and their relationship with EPS

Fig. 4(A) and (B) depict the changes in TOC, total N (nitrogen), and total P (phosphorous) removal efficiencies with time in R-1 and R-2, respectively. TOC removal rate was continuously increased in both the reactors with the progress of experiment but in R-1, the removal rates were much higher than R-2. TOC removal rate in R-1 on day 13 was 70%, while R-2 showed 49% to that of 43% on day 1. After this, a slow tendency in TOC removal rates was found. At the close of experiment, R-1 and R-2 showed 84% and 62% removal efficiencies, respectively. The high removal rate in R-1 can be attributed to its augmented MLSS concentrations which correspond to higher microbial metabolic activities in SBR type of reactor. As the F/M ratio was higher in the beginning of aeration period in R-1 due to supply of whole food at once, the microbial activities were amplified and produced a lot of microbial products like EPS as mentioned previously, and also significant increase in its MLSS concentrations took place. These high MLSS and EPS contents in R-1 showed that much of the influent organic carbon was utilized by micro-organisms to synthesize organic polymers, therefore its low values in the effluent were found. During the one cycle of SBR, TOC removal rates were also measured and it was noted that almost 80% of TOC removal occurred during the first hour of the aeration period while 18% TOC removal took place in the next two hours. No substantial removal rates were seen after that in the aeration mode. In the case of CFSTR, not much changes in microbial activities took place due to similar operating procedure, comparatively low values of EPS and MLSS were found; as a result, the removal rates were too lower than SBR. During the process, biomass also produced soluble microbial products (SMP) like loosely bound EPS (results not shown) by consuming organic material which is released due to cell lysis. These were also higher in SBR than CFSTR, further indicating the improved TOC removal in SBR system.

Total N and P in the effluent samples were monitored to determine their removal efficiencies by granulated activated sludge. Results showed that the removal rates gradually increased and reached the optimum values at the end. Maximum nitrogen removal efficiency of R-1 was 71% on day 35, while that of R-2 was 83% on the same day. Phosphorous removal efficiencies were found to be 56 and 64% at the completion of experiment in R-1 and R-2, respectively, which were quite less than the nitrogen removal rates. These results showed that N and P were much efficiently removed in R-2 than R-1. Different removal rates in these systems may be due to different nature of micro-organisms produced in them. It has been reported by many researchers that, by changing the operating variables like feed regime, aeration period, and settling time of bioreactors profoundly, change in the diversity of microbial communities, but no variations among the individual reactors had been

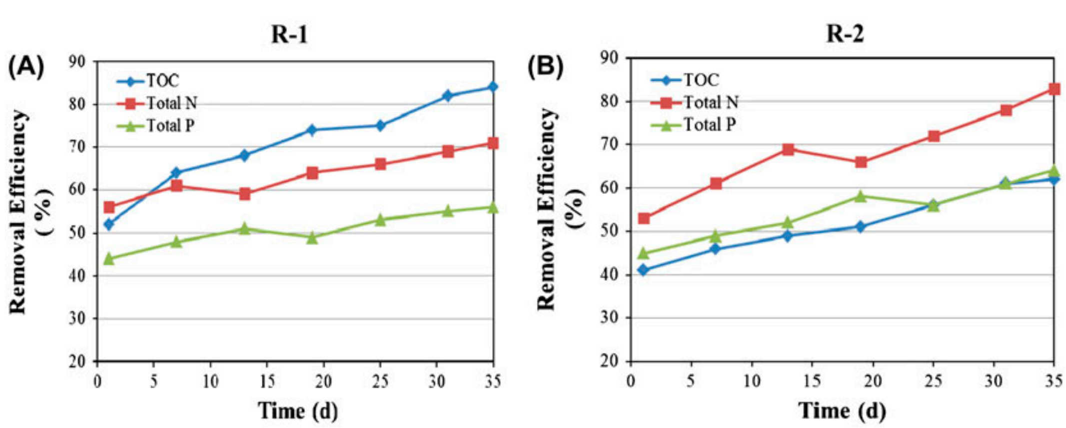

Fig. 4. The removal efficiencies (%) of TOC, total N, and total P in (A) R-1 & (B) R-2 with time (d).

noted [20,21]. High removal rates of nitrogen and phosphorous in CFSTR showed that it may generate more autotrophic microbes, while SBR created more heterotrophic microbes. Some researchers found that high substrate carbon favors the growth of heterotrophic microbes [19]. As discussed earlier, that in SBR, the substrate loading rate was very high in the beginning, so more chances were there to form heterotrophic organisms than CFSTR. Heterotrophic organisms normally produce very compact and stable granules as these grow inside the granules, while autotrophs are not capable for imparting stability to granules [22]. This may be the reason that granules of R-1 were more compact and larger in size as compared to R-2.

The difference of N and P removal efficiencies between two reactors was due to the changes in their particle size. Particle size analysis showed that sludge in R-1 was much bigger in size than R-2. The small granules of R-2 showed a clear advantage over the large granules of R-1 in the uptake of these nutrients. In comparison to larger granules, the smaller granules had more surface area and were very efficient for the ammonia and phosphate degradation. Protein is concentrated on the inner, while polysaccharides on the outer surface of aerobic granules [23]. The mechanism of nitrogen removal can be considered as electrophilic–nucleophilic reaction between protein and nitrogen of ammonia or nitrate etc. As the protein is positively charged, it behaved like electrophile while the nitrogen atom of ammonia, nitrate, or nitrite is nucleophilic in nature due to the presence of a lone pair of electrons and negative charges, respectively. Due to the smaller size of granules in R-2, the protein (electrophile) was more in contact with N (nucleophile) atom of its above-mentioned chemical species, and higher electrostatic attractions were found. Due to these electrophilic and nucleophilic attractions, the removal of nitrogen by sludge particles was enhanced and consequently increased its removal efficiency. In the case of R-1, the sludge particles were much larger and exposure of proteins to nitrogen was not very prominent, so the removal rates were low, even though it had higher protein contents than R-2.

4. Conclusions

The results of the present study showed that SBR system enhanced the granulation speed and produced large size sludge particles as compared to CFSTR which had granule size almost half to that of SBR. Therefore, the settleability of sludges in the SBR was significantly improved than CFSTR. TOC removal rates of SBR were very high due to high conversion of influent carbon into EPS and other microbial products. However, for nitrogen and phosphorous removal, the CFSTR system was better due to the generation of small size particles. The small particles of CFFSTR had high surface area and also more contact with boundary EPS components, particularly proteins, thus increased in the electrophilic–nucleophilic interactions occurred which helped to raise the removal rates.

Abbreviations

TOC	—	total organic carbon
SBR	—	sequencing batch reactor
CFSTR	—	continuous flow stirred tank reactor
EPS	—	extracellular polymeric substances
F/M	—	food/micro-organism
MLSS	—	mixed liquor suspended solids
COD	—	chemical oxygen demand
CER	—	cation exchange resin
SVI_{30}	—	sludge volume index
SMP	—	soluble microbial products

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