

Investigating the mechanism of sludge reduction in activated sludge with an anaerobic side-stream reactor

Dong-Hyun Chon, McNamara Rome, Hee-Sik Kim and Chul Park

ABSTRACT

To investigate the mechanism of sludge reduction in activated sludge (AS) with an anaerobic side-stream reactor (ASSR), four AS systems with different digestion schemes were operated in the laboratory. The four systems are: a) AS + ASSR; b) AS + aerobic digester; c) AS + anaerobic digester; and d) AS with no solids wastage. The average sludge yield of AS + ASSR from two phases was 0.14 mgVSS/mgCOD, which is 22–54% less than that from the three other systems. The accounting of biomass in AS + ASSR system revealed that 50% of sludge is degraded in ASSR while the other half is degraded in the aeration basin. Furthermore, both whole sludge and centrate from ASSR led to a significant oxygen uptake in AS, indicating the importance of aerobic biodegradation in AS + ASSR system. The extracellular polymeric substances (EPS) data showed that base-extractable EPS was much smaller for AS with ASSR than with no wastage. In contrast, cation exchange resin-EPS was similar for both systems. These results indicate that degradation of base-extractable EPS accounts for the lower sludge yield in AS + ASSR, and based on the literature this organic pool is believed to be aluminium and/or iron-bound EPS. The microbial profile data suggests that recirculation in AS + ASSR selects some unique microorganisms. Further research is warranted to study their role in sludge reduction.

Key words | activated sludge, digestion, EPS, side-stream reactor, sludge reduction, yield

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INTRODUCTION

The activated sludge process (ASP) is a primary method for the biological treatment of municipal and industrial wastewater. Despite its high organic matter removal efficiency, ASP generates large amount of excess sludge as a byproduct. The treatment of excess sludge can account for up to 60% of the operational costs of the facility, associated with conditioning, dewatering, disposal (Saby *et al.* 2003) and treatment of odor generated during the solids handling processes as well. A variety of chemical (*e.g.*, ozone and base treatment), physical (*e.g.*, sonication, mechanical shearing) or biological methods (*e.g.*, extended solids retention time (SRT)) have been developed and studied for sludge reduction. However, the performance of these processes has been highly variable in the field. Furthermore, these enhancement methods are usually costly and could also result in a poor sludge settling and an increased nitrogen concentration in the effluent (Low & Chase 1999).

The incorporation of an anaerobic side-stream reactor (ASSR) into the activated sludge system is a relatively new solids reduction process. The commercial sludge reduction method known as CannibalTM also incorporates an anoxic or anaerobic side-stream reactor into activated sludge, although some physical solids removal apparatuses are also included in this system (Johnson *et al.* 2008). The previous studies have demonstrated that operation of AS + ASSR-type system led to about 60% less solids generation than the conventional activated sludge system (Novak *et al.* 2007; Datta *et al.* 2009). Goel & Noguera (2006) also showed that the incorporation of ASSR into the enhanced biological phosphorous removal (EBPR) process led to sludge yield at 0.16 mg VSS/mg COD, which is about 63% lower than that of EBPR activated sludge alone (at SRT of 10 days). This yield is also 16–38% lower than that of the activated sludge with anaerobic digestion assuming that typical anaerobic digestion can lead to

40–55% volatile solids reduction in the digester. Note that all these yield values are for secondary activated sludge. For the mechanism of AS + ASSR, Novak *et al.* (2007) observed that much of organic matter, particularly protein, is solubilized in the anaerobic side-stream reactor and readily degraded in the main activated sludge reactor. The authors proposed that this solubilized organic pool is not typically degraded in the extended SRT system or aerobic digestion so that degradation of this material may be the key point of sludge reduction in activated sludge with an anaerobic side-stream treatment.

In spite of much interest in this new sludge reduction technique, the process is still at its early development and research stage. We believe that there have been no previous studies conducting a parallel comparison between activated sludge with ASSR and activated sludge with both anaerobic and aerobic digestions or extended SRT under controlled laboratory conditions. Consequently, the mechanism of this biological sludge reduction method is still not clearly understood. Moreover, previous studies only used synthetic wastewater as a feed and information regarding microbial community in this process remains unveiled. This lack of information has motivated us to conduct laboratory reactor study. In this study, several laboratory scale activated sludge systems were operated with different digestion schemes and their resultant sludge yield, oxygen uptake data, EPS, and microbial community profile were examined across the systems.

MATERIALS AND METHODS

Laboratory activated sludge systems

Activated sludge systems with four different digestion modes were investigated in this study. The overall schematics of these four systems are depicted in Figure 1. Briefly, four 5-litre laboratory-scale sequencing batch reactors (SBRs) were operated as activated sludge reactors with a settler. The R1 system incorporated an anaerobic side-stream tank reactor so that given amount of sludge, 10% biomass from SBR per day, was wasted to the anaerobic reactor and the treated sludge was recycled back to the main aerobic reactor. This ASSR was completely stirred tank reactor (CSTR) so SRT and HRT were same and were kept at 10 day. The R2 and R3 systems were operated as conventional activated sludge systems with aerobic and anaerobic digester as a sludge reduction method, respectively. Sludge from the digesters in R2 and R3 was wasted as a conventional treatment. The SRT of both aerobic and anaerobic digesters was also 10 day to compare sludge reduction efficiency from ASSR in R1. The SRT of two main aerobic SBRs in R2 and R3 was kept at 10 day. The R4 system was targeted to be an extended SRT system. There was no additional reactor for this system and no sludge was wasted from this system (except for intentional wastage of sludge for sampling) to evaluate the effect of a high SRT on sludge reduction.

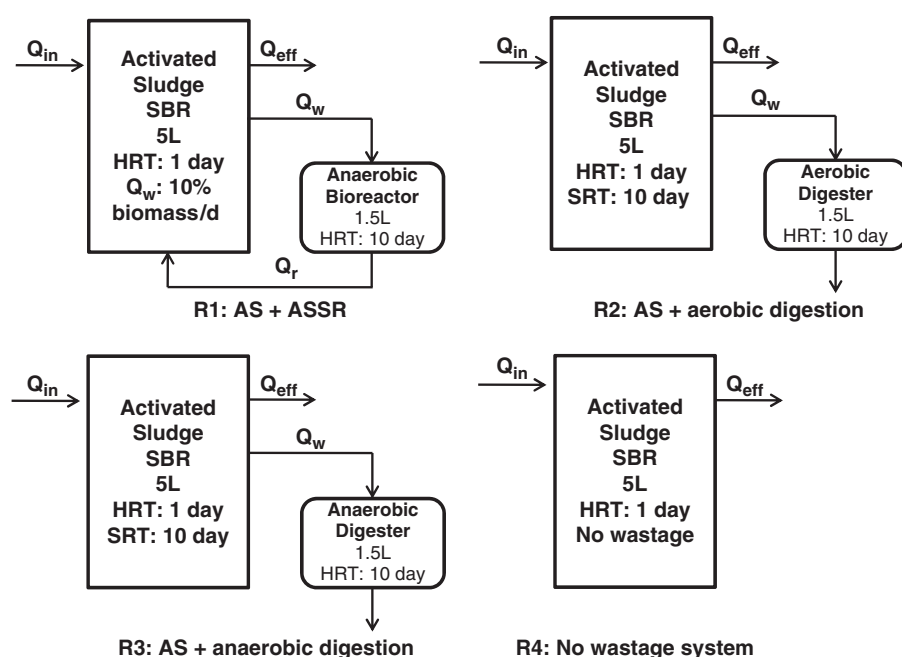


Figure 1 | Overall schematics of four laboratory activated sludge systems.

Total two operations were conducted for these laboratory reactor studies. Phase I and II were operated for 109 and 60 days, respectively. The seed sludge of phase I and II was obtained from the main aeration basin in Amherst Wastewater Treatment Plant (Amherst, MA) which uses a conventional activated sludge process. The feed was the combination of synthetic wastewater and primary effluent collected from the Amherst WWTP. The composition of synthetic wastewater followed the medium recipe shown in [Novak *et al.* \(2007\)](#).

Oxygen uptake rate

Four specific oxygen uptake rate (SOUR) tests were conducted in this study to further verify the sludge reduction mechanism in AS+ASSR system. They are: 1) 50 mL of whole sludge from ASSR+250 mL of activated sludge from R1; 2) 50 mL of centrate from ASSR+250 mL of activated sludge from R1; 3) 50 mL of R1 effluent+250 mL of activated sludge from R1; and 4) 50 mL of activated sludge from R1+250 mL of effluent from R1. The centrate of ASSR was obtained by centrifuging whole sludge of ASSR at 5,000 g for 5 minutes at ambient temperature. The oxygen uptake rate was measured using a dissolved oxygen meter (YSI Model 57, Yellow Springs, Ohio).

Extraction of EPS

The cation exchange resin (CER) and base extraction methods were adopted in this study following the procedures shown in [Park & Novak \(2007\)](#). Briefly, 200 mL of sludge suspension samples were initially centrifuged at 12,000 g for 15 min at 4°C. The designated extraction solution was then used to resuspend the resultant sludge pellet. For CER extraction procedure, the dose of CER (Dowex 50_8, Na⁺ form, 20–50 mesh) was adjusted to 60 g resin/g VS. The extraction solution was a low strength phosphate buffer saline (PBS) (2 mM KH₂PO₄, 6 mM Na₂HPO₄, and 10 mM NaCl). The extraction was performed at room temperature for 1 hr at 600 rpm in the extraction beaker which had four baffles within it. For base extraction, the sludge pellet was resuspended with 10 mM NaCl solution and the pH was adjusted to 10.5 using 1 N NaOH. Then the pH-adjusted sample was transferred to an extraction beaker and underwent an extraction at room temperature for 1 hr at 600 rpm in the presence of N₂ gas. After extraction, the suspensions were centrifuged at 12,000 g for 15 min at 4°C to obtain cell free extracts as indicated by [Nielsen & Keiding \(1998\)](#). The supernatant was then passed through a 1.5 µm filter to get a crude EPS extract.

Microbial community profile

The polymerase chain reaction (PCR) denaturing gradient gel electrophoresis (DGGE) was employed to investigate bacterial composition in different activated sludge systems. PCR was performed with the eubacterial 16S rRNA gene primer set (341f-GC and 786r), and PCR-amplified fragments were electrophoresed on an 10% polyacrylamide gel with a 40 to 60% urea-formamide gradient.

Analysis

Total solids (TS), total suspended solids (TSS), total volatile solids (VS), volatile suspended solids (VSS), and soluble chemical oxygen demand (COD) were measured according to *Standard Methods* (1995). The protein concentration in sludge and extracted EPS was determined by the [Frølund *et al.* \(1996\)](#) modification of the [Lowry *et al.* \(1951\)](#) method using bovine serum albumin as the standard. Polysaccharide was measured by the [Dubois *et al.* \(1956\)](#) method utilizing glucose as the standard.

RESULTS AND DISCUSSION

Sludge reduction in phase I

The reactors were operated for more than 100 days in phase I. A graphical method was used to determine the observed yield of sludge in each system. The Y-axis (gram VSS) designates “total mass” which includes the mass of sludge in the main activated sludge reactor, anaerobic side-stream reactor, and from cumulative solids wastage via effluent and intentional sampling. The X-axis (gram sCOD) is for “cumulative consumed soluble COD” in each system. The definition of yield is the amount of biomass generated per amount of substrate consumed so the slope of each linear regression line could be the observed yield of sludge for each system. As the data in [Figure 2](#) shows, the overall observed yields for R1 (AS+anaerobic side-stream reactor), R2 (AS+aerobic digestion), R3 (AS+anaerobic digestion), and R4 (no solids wastage) were found to be 0.11, 0.18, 0.24, and 0.14 mg VSS/mg COD, respectively. The observed yield in R1 was the lowest among the four activated sludge systems without showing any negative effects on sludge settling and effluent quality ([Figure 3](#)). While overall yield of R4 was also significantly lower than two conventional systems, settleability and effluent quality became deteriorated after 40 day of operation. Furthermore, several significant washout events

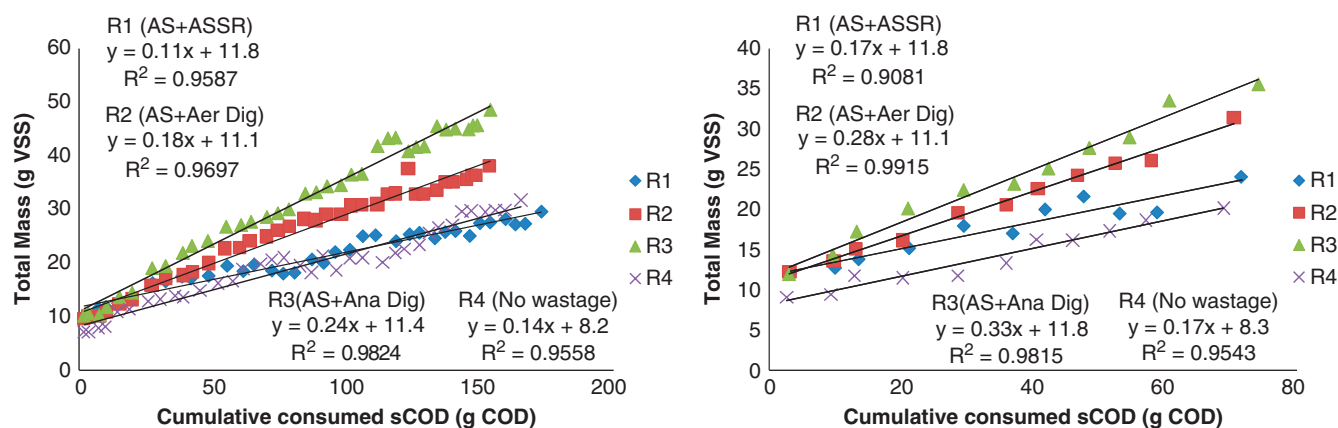


Figure 2 | The overall sludge yields in a) phase I and b) phase II. Subscribers to the online version of *Water Science and Technology* can access the colour version of this figure from <http://www.iwaponline.com/wst>.

occurred for R4 system (Figure 3), indicating that no sludge wastage method cannot be a proper choice for a conventional activated sludge system. Based on sludge yield values, the R1 system showed 39%, 54%, and 22% solids reduction compared to R2, R3, and R4, respectively. These results strongly indicate that the incorporation of anaerobic side-stream treatment into the activated sludge process is much more effective than conventional treatments (*i.e.*, anaerobic or aerobic digestion) with respect to sludge reduction.

Solids reduction in phase II

Phase II was operated for 60 days to verify the results obtained in phase I. The yields of sludge for R1, R2, R3, and R4 were 0.166, 0.276, 0.327, and 0.173 mg VSS/mg COD, respectively (Figure 2b). The yield in R1 was still the lowest among the four systems. R1 achieved 40%, 49%, and 4% solids reduction compared to R2, R3, and R4, respec-

tively. There were two accidental spills of sludge for R4 during the Phase II, which might account for lower sludge yield of extended SRT system in the second phase of operation. Except for that, sludge reduction trend was similar between Phase I and Phase II, supporting the outperformance of AS + ASSR over conventional sludge treatments.

In order to gain better insight of sludge reduction in R1 (AS + ASSR), solids reduction efficiency was directly determined using the solids data from influent and effluent of side-stream reactor or digesters and this value was compared to overall sludge yield obtained from graphical approach (Figure 2b). As Table 1 shows, observed yields of activated sludge in R2 and R3 were very close, 0.47 and 0.46 mg VSS/mg COD, respectively: this is expected because they were duplicate activated sludge having a different downstream digestion mode. The reduction of yield in the control systems (*i.e.*, 0.47 to 0.28 in R2; 0.46 to 0.32 in R3) was almost identical with the solids reduction efficiency for anaerobic or aerobic digesters. On the other hand, 34% of sludge reduction achieved in ASSR could not account for 64% reduction in sludge yield in R1 (*i.e.*, 0.46 to 0.17). This data strongly indicates that degradation mechanism in R1 is not just an anaerobic degradation of sludge in the side-stream

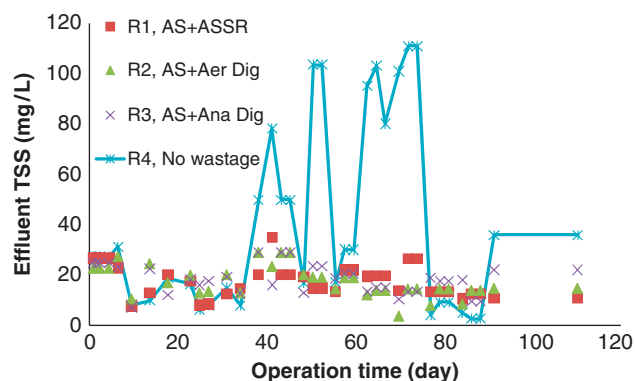


Figure 3 | Effluent TSS data from four different sludge reduction systems (Phase I). Subscribers to the online version of *Water Science and Technology* can access the colour version of this figure from <http://www.iwaponline.com/wst>.

Table 1 | Observed yield of sludge and solids reduction efficiency in three systems (phase II)

	R1	R2	R3
Yield of AS (mg VSS/mg COD)	–	0.47	0.46
System Overall Yield (mg VSS/mg COD)	0.17	0.28	0.32
Solids reduction by yield (%)	64	41	29
Solids reduction of ASSR or digesters (%)	34	41	30

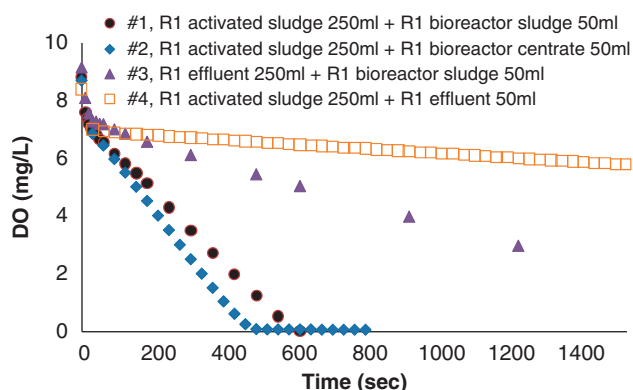


Figure 4 | Results of oxygen uptake tests (phase II). Subscribers to the online version of *Water Science and Technology* can access the colour version of this figure from <http://www.iwaponline.com/wst>.

reactor but also includes degradation of anaerobic sludge in the main aerobic basin.

Specific oxygen uptake rate tests

To further study the degradation mechanism in AS + ASSR, SOUR tests were conducted using various combinations of sludge content from the system of AS + ASSR. As the data in [Figure 4](#) shows, incubation of 50 mL of either whole sludge (test #1) or centrate (test #2) from ASSR with activated sludge biomass led to a rapid oxygen uptake. The incubation of whole sludge of ASSR with just effluent (test #3) showed much slower oxygen uptake. These results again suggest that one important reduction mechanism of AS + ASSR is that ASSR hydrolyzes sludge in anaerobic conditions and these hydrolyzed materials are efficiently degraded by activated sludge biomass in the aeration basin.

The decrease in dissolved oxygen in test #3 is still notable and a possible way to explain this data is that facultative

microorganisms from ASSR sludge adapt and start metabolizing solubilized materials under aerobic conditions. Little change in oxygen level in test #4 (250 mL of R1 activated sludge + 50 mL of effluent from R1) serves as a control for the SOUR tests.

Quantitative analysis of activated sludge EPS extracted by two methods

[Novak *et al.* \(2007\)](#) proposed that iron is reduced in an anaerobic side-stream reactor, releasing iron-associated organic matter into sludge solution. This released organic matter then returns to the main activated sludge reactor where it degrades under aerobic conditions. To test this hypothesis, extraction of EPS was performed using activated sludge from R1, R3, and R4. Base and CER extractions were employed to target and extract different cation-bound floc materials. [Park & Novak \(2007\)](#) reported that CER mainly removes divalent cation (Ca^{2+} and Mg^{2+}) from sludge and extracts divalent cation-associated EPS. On the other hand, base extraction (pH 10.5) hydrolyzes a significant amount of aluminium and iron, releasing aluminium and iron-bound floc materials. A later study by the same authors provided molecular evidence that proteins that are extracted by CER and base extractions are distinctively different indicating a different source of those materials ([Park *et al.* 2008](#)).

The concentrations of proteins and polysaccharides extracted from three different kinds of activated sludges are shown in [Figure 5](#). As the data shows, proteins and polysaccharides in activated sludge of R1 and R4 were much less than those from R3 (control activated sludge), suggesting that long solids retention time in R1 and R4 led to the degradation of EPS. This result is in accordance with the yield data seen

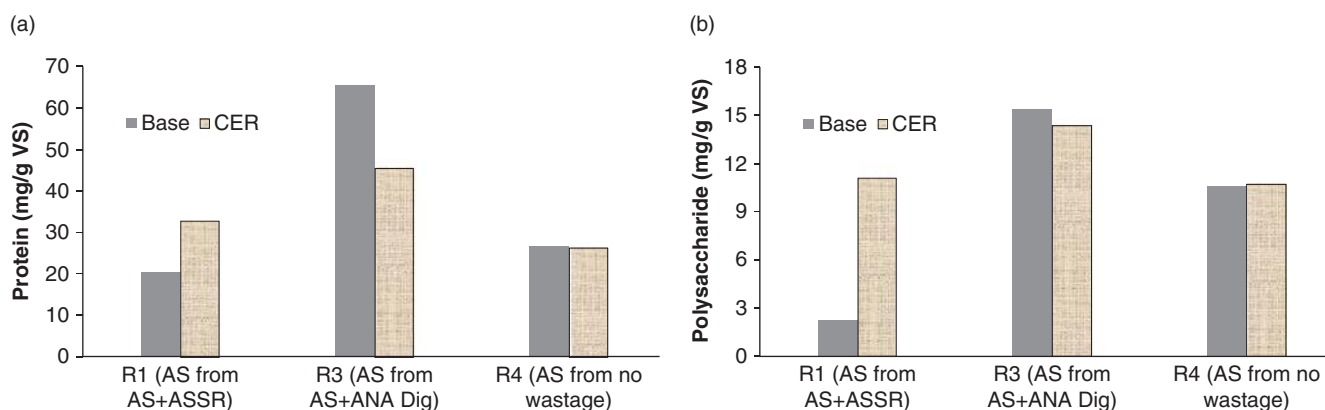


Figure 5 | The quantity of a) proteins and b) polysaccharides extracted by base and CER extraction methods. Sludge used for the extractions are activated sludge from R1, R3, and R4 systems. Subscribers to the online version of *Water Science and Technology* can access the colour version of this figure from <http://www.iwaponline.com/wst>.

throughout the study. The comparison between CER and base-extractable EPS data shows a very notable result. Base-extractable EPS of R1 was much lower than that of R4 and this trend was much more pronounced for polysaccharide-EPS. In contrast, the quantity of EPS for CER is similar for R1 and R4. These data suggest that the degradation of base-extractable materials, iron and/or aluminium-associated materials, accounts for the lower sludge yield in AS + ASSR system. In other words, these organic pools are not readily degraded in typical activated sludge systems even with long SRT but only degraded when both anaerobic and aerobic conditions are available.

Profile of bacterial community in different reactor systems

The current study also included the analysis of microbial community in various activated sludge systems. The results of PCR-DGGE data are shown in Figure 6. There were

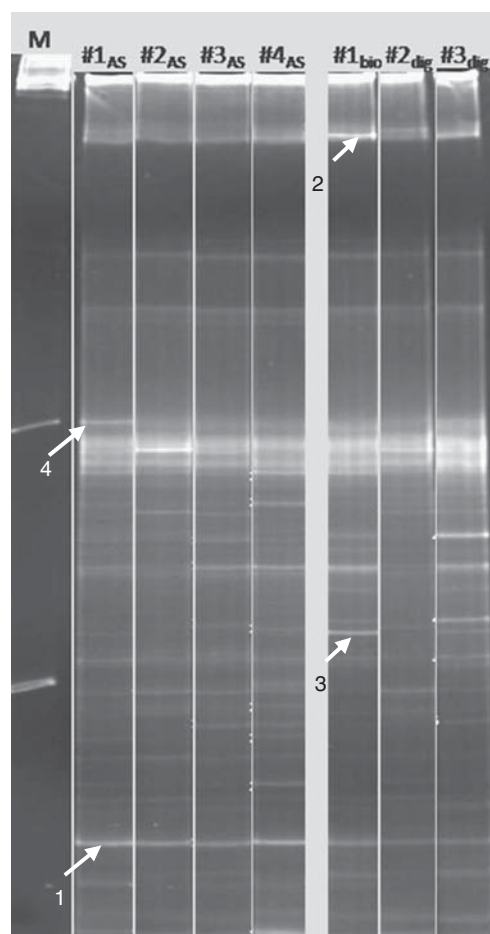


Figure 6 | The PCR-DGGE result for four activated sludges and three digested sludges in different digestion modes (phase I).

several common bacterial bands shown in all sludges investigated. One good example is band 1 which was shown in all DGGE lanes.

The systems that received a particular interest during microbial analysis was ASSR in R1 (#1_{bio} in Figure 6) and anaerobic digester in R 3 (#3_{dig} in Figure 6). There was high similarity for microbial composition between two anaerobic reactors, one with continuous recirculation and the other with permanent wasting. The results from Dendrogram analysis also revealed that there is about 75% similarity of microbial composition between the anaerobic side-stream reactor and the anaerobic digester (data not shown). Nevertheless, there were also some unique DGGE bands shown mainly in the anaerobic reactor from the R1 system. Good examples are band 2 and band 3 in the lane of #1_{bio}. These data suggest that there are some unique anaerobic microorganisms that can be enriched in a side-stream reactor with a continuous recirculation.

Interestingly, there was also some similarity between activated sludge from R1 (#1_{AS}) and R4 (#4_{AS}), indicating the selection of microorganisms which sustain under a long SRT conditions.

In spite of this observation, activated sludge from R1 showed less diverse microbial composition, which might be worth for a note. The DGGE data also shows that band 4 is also enriched in R1 activated sludge system. Future research is required to study the identification and role of these unique microorganisms in sludge reduction system.

CONCLUSIONS

Specific conclusions drawn from this study are shown below.

- The new solids reduction process that incorporates an anaerobic side-stream reactor was valid and much more effective than any other conventional methods.
- The solids production in the activated sludge with an anaerobic side-stream reactor was 40–55% less than the conventional systems with aerobic or anaerobic digesters at a 10 day SRT.
- For the system with anaerobic side-stream reactor, about 50% of sludge was digested in the side stream reactor while the other half was degraded in the aeration basin. This result strongly indicates that sludge reduction mechanism in R1 is combination of both anaerobic and aerobic digestion of activated sludge.
- Reduction of base-extractable EPS was much more pronounced for activated sludge with a side-stream than no

wastage system. On the other hand, reduction of CER-extractable EPS was similar for both systems. These results indicate that degradation of base-extractable EPS accounts for the lower sludge yield in the new sludge reduction system, and these organics are thought to be aluminum and/or iron-bound floc materials.

- There was high similarity of microbial composition between the anaerobic side-stream reactor and the anaerobic digester; however, there were unique microbial cells in the side-stream tank reactor as well.

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