Anaerobic -aerobic system for beverage effluent treatment: Performance evaluation and microbial community dynamics

Vinay Kumar Tyagi[a, b], Jianbo Liu[c], Leong Soon Poh[a], Wun Jern Ng[a*]

[a] Environmental Bio-innovations Group (EBiG), School of Civil & Environmental Engineering, Nanyang Technological University, 60 Nanyang Avenue, Singapore 637551

[b] Department of Civil Engineering, Indian Institute of Technology Roorkee, Roorkee-247667, India

[c] Advanced Environmental Biotechnology Center (AEBC), Nanyang Environment and Water Research Institute (NEWRI), Nanyang Technological University, 1 Cleantech Loop, CleanTech One, #06-08, Singapore 637141

***Corresponding author:**

Prof. Wun Jern NG

Environmental Bio-innovations Group (EBiG),

School of Civil and Environmental Engineering,

Nanyang Technological University

N1-01a-29, 50 Nanyang Avenue, Singapore 639798

E-mail addresses: WJNG@ntu.edu.sg

Tel: (65) 6790-5266

Abstract

A novel 'anaerobic filter' with a pulsing bio-cord bed, the anaerobic pulsed bed filter (APBF), was integrated with the aerobic sequencing batch reactor (aeSBR) to treat effluent from a beverage manufacturer. The APBF reactor achieved 61% COD and 70% BOD removal at 8h HRT and 5.45 kg COD/m^3.d OLR. The combined APBF-aeSBR system achieved COD, BOD, TSS and TN removals of 97%, 98%, 80% and 82%, respectively. Notwithstanding the APBF's immobilized biomass, it was suspected the range of loadings could cause changes in the microbial community. 16S rRNA high throughput sequencing

analysis of the immobilized biomass showed Euryarchaeota (7.60%-44.42%), Proteobacteria (4.65%-37.98%), Bacteroidetes (9.72%-27.13%), Firmicutes (8.39%-22.69%) and Synergistetes (0.73%-13.20%) were the dominant phyla. The diversity and density of bacteria and archaea keep decreased with the increased OLR from 2.15 (HRT= 48h) to 5.45 (HRT= 8 h) kg COD/m^3.d.

Keywords: Wastewater treatment; Water reclamation; APBF, aeSBR; Beverage industry

Abbreviation

APBF	Anaerobic pulsed bed filter
BOD	Bio-chemical oxygen demand
CIP	Cleaning in place
COD	Chemical oxygen demand
COP	Clean-out-of-place
CSD	Carbonated soft drink
DAP	Di-ammonium phosphate
HRT	Hydraulic retention time
MLSS	Mixed liquor suspended solids
OLR	Organic loading rate
OTUs	Operational taxonomic units
RCL	Retort caninng line
aeSBR	Aerobic sequencing batch reactor
SRT	Solids retention time
TN	Total nitrogen
TS	Total solids
TSS	Total suspended solids

VS	Volatile solids
VSS	Volatile suspended solids
WWTP	Wastewater treatment plant

1. Introduction

The combination of increasing water demand and water scarcity, stringent government regulations, and increasing public pressure on sustainable usage of water, has given impetus to consideration of more effective water management strategies. This is so in the water intensive food and beverage industry (Alkayaa and Demirer, 2015). The industry typically uses voluminous amounts of fresh water in the production processes such as boiling, cooking, cleaning, fermenting, washing, evaporating, pasteurizing and sterilizing, of which almost half is discharged as effluent. The effluent could then contain high concentrations of cleaning agents, organics , suspended solids (TSS), total nitrogen (TN), elevated chemical oxygen demand (COD) and biochemical oxygen demand (BOD), and have substantial pH variations (Amuda and Amoo, 2007; Isla et al., 2013; Oktay et al., 2007; Ait Hsine et al., 2005). If a "closed circuit", comprising wastewater treatment and water reclamation and reuse can be arranged, then this would serve to reduce the water demand and environmental impact (Tong and Elimelech, 2016).

Biological treatment processes are typically more economical for removal of organic compounds in wastewaters. Anaerobic treatment offers a two-fold advantage – removing organic pollutants without need for oxygen while producing biogas which can be a potential source of energy (Khan et al., 2011). Since the 1970's, (high-rate) anaerobic treatment has been increasingly applied to industrial effluents and this included numerous full-scale upflow anaerobic filters. Organic loading rates of up to 10 kg COD/m^3.day have been applied satisfactorily (van Lier et al., 2015). A key requirement of anaerobic systems is requirement

79 for relative stability in wastewater properties including the hydraulic and organic loads.
80 Aerobic treatment is then typically deployed to polish anaerobically treated effluent to meet
81 effluent discharge requirements, and for purposes of water reclamation and reuse (Khan et
82 al., 2011). In an integrated anaerobic–aerobic system, the intention then is to have the
83 anaerobic reactor remove a large portion of the organic content with as small a reactor
84 volume as is viable. Then, in an aerobic post-treatment step, the residual pollutants are
85 removed to meet the discharge limits and possibly the water reclamation system's feed water
86 quality requirements. Often, such an integrated biological treatment system would strive to
87 achieve positive energy balance, reduced sludge generation and smaller space requirements
88 (Simate et al., 2011).
89 A novel anaerobic pulsed bed filter (APBF) filled with bio-cord carrier was proposed
90 for the treatment of beverage industry wastewater. The anaerobic microorganisms attached to
91 a support medium which can be "expanded" at predetermined intervals. This 'pulsing"
92 action would reduce incidence of bed clogging while enhancing capacity of the system to
93 accommodate fluctuations in hydraulic loads. The residual organics in APBF effluent will be
94 treated in the aerobic sequencing batch bioreactor (aeSBR). Since production at the beverage
95 manufacturer can be on a campaign basis, it is necessary to consider a system which can be
96 "adjusted" to respond to the consequent wastewater changes – hence the aeSBR. The aeSBR
97 was selected also because it can better suppress bulking sludge which can result from sugars
98 in the wastewater.
99 This study was intended to demonstrate, at pilot-scale, beverage wastewater treatment
100 with the APBF-aeSBR system can provide stable treated effluent suitable as feed to a water
101 reclamation system. With a successful demonstration, scale-up to a full-scale plant can then
102 be undertaken. The full-scale effluent treatment and water reclamation system is anticipated
103 to recover water sufficient for 35% of the total daily water consumption (sufficient for

104 cooling and boiler water make-up). An added interest is the microbial community in the
105 APBF as it accommodated the various hydraulic and organic loadings imposed on it.
106
107 **2. Material and methods**
108 2.1. Wastewater, seed sludge and sample collection
109 Discharges from the "retort canning line" (RCL) and "carbonated soft drink" (CSD)
110 production line at the factory were collected so that their respective variations might be
111 determined and hence impact on equilization requirements. This wastewater streams had
112 varying concentrations of organic compounds such as sugars, polysaccharides and soymilk
113 proteins and natural flavouring materials.
114 For treatment plant wastewater feed characterization, grab samples were collected
115 from the equalization tank inlet and outlet for analysis twice a week over 9 months (February
116 2016-October 2016). To evaluate pilot plant performance, 24 hours composite samples were
117 collected at the plant's inlet (before two stage pH-adjustment), APBF outlet, and aeSBR
118 outlet. The samples were collected for analysis thrice a week over the plant's 5 months
119 operation period. All samples were transferred to the laboratory on ice.
120 The APBF seed inoculum was collected from an anaerobic digester treating sewage
121 sludge (primary and waste activated sludge) at a local wastewater treatment plant (WWTP).
122 The aeSBR seed inoculum was obtained from the sludge return line of the secondary
123 sedimentation tanks at same WWTP. The physico-chemical characteristics of the APBF seed
124 sludge were: pH 7.10 ± 0.2, total solids (TS) = 14.50 ± 0.15 g/L, volatile solids (VS) = 10.70
125 g/L ± 0.12 g/L, TSS = 13.60 ± 0.20 g/L, volatile suspended solids (VSS) =10.10 ± 0.20 g/L.
126 The characteristics of aeSBR seed sludge were as follows: : pH 6.50 ± 0.2, total solids (TS) =
127 3.25 ± 0.15 g/L, volatile solids (VS) = 2.68 g/L ± 0.20 g/L, TSS = 3.05 ± 0.12 g/L, volatile
128 suspended solids (VSS) =2.58 ± 0.16 g/L.

2.2. Design and operation of the pilot plant

Characterization of the various wastewater streams at the factory confirmed need for accommodation of the occasional large variations in influent hydraulic loads and properties such as pH, COD and TSS. A two-stage pH correction system was implemented before the biological treatment stage to reduce the scale of pH variation which if uncorrected could inhibit the bioprocesses.

The main components of the pilot plant are as follows: (a) Two-stage pH adjustment unit where pH was adjusted to 6.5- 8.5 using sodium hydroxide (NaOH) and hydrochloric acid (HCl); (b) Nutrient and alkalinity dosing system before feeding to the APBF. Di-ammonium phosphate (DAP) and urea were added to provide sufficient N and P in the ratio of COD:N:P = 250:5:1. Sodium bicarbonate was added as buffer to maintain the alkalinity around 2500 mg/L; and two biological treatment stages comprising (c) the APBF followed by (d) the aeSBR. The APBF was fed in the upflow mode through the flow distributor at its base. The reactor had dimensions of 700 mm × 700 mm × 2300 mm (LxDxH) with an effective volume of 1 m^3 and was mounted with fiber polypropylene microbial support medium (bio-cord) at a packing ratio of 60% (v/v), in terms of bulk volume. The bio-cord has a specific surface area of 32 m^2/m^3 and specific weight of 3.7 kg/m^3. An intermittently operated recirculation pump generated the hydraulic pulse which resulted in the shear used to control biofilm thickness. The APBF was seeded with 0.3 m^3 of anaerobic sludge (i.e. 30% of the reactor volume). The aeSBR was operated cyclically with the following phases – aerobic feed, aeration, settle and discharge. The reactor was 1300 mm in diameter, 1360 mm high and had volume of 1.8 m^3. The solids retention time (SRT) was maintained at about 15 days with mixed liquor suspended solids (MLSS) concentration of 3000±350 mg/L and volatile suspended solids

concentration of 2610 ±230 mg/L. The coupled APBF and aeSBR was operated for 150 days. Plant operation was automated with a programmable logic controller (PLC).

2.3. Pilot plant operation

The APBF was started with 48 hours hydraulic retention time (HRT). This was then reduced in steps to 24 h, 16 h, 12 h and 8 h following satisfactory performance at each HRT. The corresponding aeSBR HRTs were 42 h, 24 h, 20 h, 17 h and 12 h. Table 1 summarizes the pilot system operating conditions over the entire study period.

Table 1. Pilot system operating conditions.

2.4. Analytical methods

pH, BOD_5, COD, TSS, alkalinity, oil and grease, total dissolved solids (TDS), ammonia nitrogen (NH_4^+-N), sulfate (SO_4), phosphate (PO_4-P), Total Nitrogen (TN), total hardness, and total and dissolved silica were determined in accordance with Standard Methods (APHA, 2012). For VFA analysis, the filtered sample (0.9 mL) was acidified with 0.1 mL formic acid (10%, V/V). The acidified sample was chromatographed using gas chromatograh (Agilent, USA), mounted with a DB-FFAP column (Phenomenex, USA) and a flame ionization detector (FID).

APBF sludge morphology was observed using a field-emission scanning electron microscope (JEOL 6340) equipped with an energy dispersive X-ray spectrometer (EDS) (JOEL 4340) operated at 5 kv (Sun et al., 2018).

2.5. DNA extraction and microbial community analysis

The biomass samples (0.5 mL) were collected from the APBF bio-cords in 2 mL microcentrifuge tubes, and centrifuged for 30 s at 10000 rpm. The supernatant was decanted and, the pellet (sludge) was collected and rinsed twice by 1 mL phosphate buffer (PBS 1X). Before DNA extraction, the sludge samples has 5X dilution to achieve around 10^{10} cells per mL cell concentration. The MagNA Pure Compact nucleic acid isolation kit (Roche, Germany) was used to extract the DNA from the samples. The DNA was preserved at -20 °C prior to analysis. The V4 regions of bacteria and archaea 16S rDNA genes were amplified using the Primer 515F(5'- GTGCCAGCMGCCGCGGTAA-3') and 806R (5'-GGACTACNNGGGTATCTAAT -3') (Yan et al. 2017). Illumina MiSeq platform (Macrogen Inc., Korea) was used to perform the sequencing. To define the operational taxonomic units (OTUs), clustering was sequences at 0.03 distance level within 97% similarity.

The BLASTN was used for taxonomical classification of OTUs based on the data obtained from NCBI, RDPII and Green Genes, (www.ncbi.nlm.nih.gov,http://rdp.cme.msu.edu) (Yan et al. 2017).

Fig 1. Flow schematic and photographs of the pilot scale system.

3. Results and discussion

3.1. Wastewater characterization

Table 2 summarizes the physico-chemical characteristics of the wastewater samples collected at different locations of the factory.

Table 2. Physico-chemical characteristics of raw wastewater.

The CSD and RCL effluents varied substantially in terms of pH, organics (COD, BOD, TSS), and nutrients (N and P) as these result from campaign manufacturing. Cleaning (cleaning in place, CIP and cleaning out of place, COP) also caused large swings in the wastewater characteristics. Use of cleaning chemicals like nitric acid, phosphoric acid, caustic soda, hydrogen peroxide, sodium hypochlorite, acetic acid, and sulphuric acid resulted in large pH variations. Alkalinity in the incoming wastewater (avg. 163 mg $CaCO_3$/L) was low and expected to provide insufficient pH buffer at the APBF when subjected to high organic loads. Sodium bicarbonate was dosed to increase alkalinity (avg. 2500 mg/L). Sharp increases in TSS and COD values could have resulted from product rejects, which were then discharged with the wastewater. The existing equalization tank could not mitigate such variations sufficiently with its retention time of only 3 hours. The wastewater did, however, largely have BOD:COD ratios of about 0.58 which suggested good biodegradability. TN and phosphate in wastewater fluctuated and was dependent on the product manufactured. Soya based production resulted in higher TN and phosphate concentrations. However, carbonated soft drinks production resulted in low nutrients concentration and would have resulted in nutrient deficiency (Henze et al., 1997) if not corrected. The wastewater COD:N:P ratio was adjusted by supplementing nitrogen (urea) and phosphorous (DAP).

3.2. Performance evaluation of pilot system

3.2.1 pH

Although the feed wastewater had pH range of 4.50 - 12.20, the two-stage pH adjustment system maintained APBF influent pH at 7.0 - 8.5 (Figure 1). APBF effluent pH was then 6.40 -7.63 (avg. 7.10). On several occasions, pH in the APBF drops below 6.4. This was due to surges in organics concentration (4 - 5 times higher influent COD versus average of 2500

mg/L). aeSBR effluent pH ranged from 8.17 to 8.60 (avg. 8.45). Aeration in the aeSBR had stripped CO_2 from the wastewater hence increasing the pH. The sodium bicarbonate buffer added to the APBF feed would have generated CO_2. additional to the anaerobic process. Increasing the size of the equalization tank (to at least one working shift i.e. 12 h) should help reduce the pH and organic load variations and hence reduce need for alkalinity supplementation. Intermittent recirculation at the APBF was noted to have helped with maintaining a more stable pH.

Fig 1. pH profile at different stages of the pilot system operation i.e. Raw combined wastewater (Raw WW), and effluent of APBF and aeSBR .

3.2.2. Organics removal

Over the five HRTs, APBF organic loading rates (OLR) had ranged from 0.83 to 8.67 kg COD/ m^3.d and on the aeSBR from 0.25 to 2.36 kg COD/ m^3.d (Figure 2). The average OLR applied to the APBF and aeSBR over the period had increased from 2.15 to 5.45 kg COD/m^3·d and 0.56 to 1.53 kg COD/m^3·d, respectively. At the shortest HRT, the APBF (8 h) and aeSBR (12 h) had OLR ranging from 2.65 to 7.60 kg COD/ m^3.d (avg. 5.45 kg COD/ m^3.d) and 0.25 to 2.36 kg COD/ m^3.d (avg. 1.53 kg COD/ m^3.d), respectively. The APBF was able to cope with the large fluctuations in OLR within a stage and as OLR increased from stage to stage.

Fig 2. Profile of daily organic loading rates on the APBF and aeSBR across different APBF HRTs.

Influent COD varied significantly - from 585 to 9085 mg/L with an average of 3200 mg/L (Figure 3a). Occasional discharge of product rejects resulted in the 2 to 3 fold increases

in COD values over the average COD. While sharp surges in organics loading could typically upset the anaerobic processes, and lead to poorer performance (Sheldon and Erdogan, 2016), the APBF could accommodate the organic load surges. Over the study period, the APBF removed up to 80% COD, while the integrated system removed up to 99% (Figure 3b). At the shortest HRT, COD removal was still 61% by the APBF and 97% by the integrated APBF-aeSBR system. The average treated effluent COD of 40 mg/L was below the target value of 75 mg/L, which was set for feed into the UF/RO water reclamation system.

Figure 3c shows efficiency of the APBF and combined APBF-aeSBR system against the OLR applied. Over the whole study period, the APBF and combined system's COD removal were always above 52% and 92%, respectively. Average COD removal rates of 1.68, 1.95, 3.10, 3.59 and 3.20 kg /m^3.d were achieved by the APBF at 2.15, 2.75, 4.30, 4.89 and 5.45 kg COD/m^3.d OLR, respectively. At low OLR (2.15 kg COD/m^3.d), average COD removals by the APBF and combined system were 80% and 98.3% respectively. As the OLR increased to 2.75, 4.30 and 4.89 kgCOD/m^3.d, COD removal by the APBF and APBF-aeSBR had remained relatively stable i.e. about 75%, and between 96% and 99%, respectively. However, when the OLR had increased to 5.45 kgCOD/m^3.d, APBF COD removal decreased to 61%, although the combined system's performance remained at 97%.

Fig 3. (a) tCOD profile at various treatment stages of the pilot system (b) tCOD removal by the APBF and the integrated APBF-seSBR system (c) Effect of OLR on COD removal by APBF and APBF-SBR.

The wastewater BOD ranged from 420 to 3270 mg/L (avg. 1560 mg/L). At the shortest HRT of 8h, APBF BOD removal was 70% and APBF-aeSBR was 98%, with final effluent BOD of 15 mg/L. The wastewater TOC ranged from 270 to 3350 mg/L (avg. 955

mg/L) over the study period. At 8h HRT, the APBF-aeSBR system removed 98% TOC with effluent TOC of 11 mg/L (avg.). The influent TSS concentration ranged from 50 to 1000 mg/L (avg 225 mg/L). High influent TSS was observed when there was soya based production. At 8h HRT, 80% TSS removal with final effluent TSS of 30 mg/L was observed.

3.2.3 Nutrients removal

Nutrients removal would be monitored when there was soya based production. Total nitrogen in the raw wastewater fluctuated from 1.9 mg/L to 85 mg/L (avg. 22 mg/L) (Figure 4). Carbonated soft drinks production, resulted in low TN (≈ 5 mg/L) and N supplementation would be required . A similar trend was observed for phosphate. Therefore, urea and DAP were supplemented in correlation with the production schedule at the beverage plant. The influent TN concentration ranged from 35 to 65 mg/L and was 9 to 23 mg/L after the aeSBR. TN removal rates at the five HRTs tested ranged from 60–82 %. At the shortest aeSBR HRT (15 h), TN removal was 82% with effluent TN concentration of 9 mg/L (avg.). A progressive improvement in TN removal was observed as aeSBR HRT in SBR was decreased from 60 to 15 h. The improvement of TN removal was likely due to acclimatization of Nitrifying and Denitrifying bacteria to the cyclic program of the aeSBR.

Ammonia nitrogen was largely not present or at low concentrations in the raw wastewater (Avg. 1.8 mg/L, range 0.0- 21.0 mg/L). The ammonia nitrogen in APBF effluent ranged from 0.0 mg/L to 70.0 mg/L (Avg. 27.0 mg/L). Apart from the soya rich wastewater, DAP addition as a nutrient supplement also contributed to ammonia nitrogen. The aeSBR effluent ammonia nitrogen concentration of 3.2, 9.0, 0.68, and 0.04 mg/L with corresponding ammonia nitrogen removal of 72%, 70%, 97% and 99.8% was observed at 42 h, 24 h, 17 h and 12 h HRT respectively. At 12h HRT, effluent ammonia nitrogen concentration was 0.12 mg/L with corresponding ammonia nitrogen removal of 99.4%.

Fig. 4. Total nitrogen removal profile in pilot system.

3.2.4. Effect of OLR on TVFA profile

Figure 5 shows total VFA (TVFA) had remained below 400 mg/L up to 5 kg COD/m^3d OLR. TVFA increased from 430 to 1100 mg/L when OLR increased beyond 5 kg COD/m^3d. COD removal efficiency had declined from 73 to 61%, when the OLR of the APBF increased from 4.89 to 5.45 kg COD/m^3d and HRT decreased from 12 h to 8 h. To reduce the VFAs concentrations and neutralize pH, intermittent recirculation of APBF mixed liquor was performed. This helped stabilize pH throughout the reactor and reduced VFA concentrations.

Fig. 5. VFA concertation and OLR applied to the APBF.

3.3. Biomass growth in the APBF

The morphology of biofilm developed on the support medium (bio-cord) in the APBF was investigated by scanning electron microscopy. The surface of the virgin bio-cord fibers was rough, and spiral shaped with sharp edges. A large number of cocci and bacilli, possibly *Methanospirillum* and *Methanobacterium*-like and archaea, were pre-dominant on the surface of the bio-cord, and were heavily aggregated due to the presence of extracellular polymeric substance (EPS). While it was difficult to draw any quantitative conclusions from the SEM micrographs, the impression was that very dense microbial colonization on the surface of bio-cord fibers resulting in a thick biofilm.

3.4. Microbial community dynamics

For the APBF-SBR system, the performances in terms of pH, COD removal and nutrient removal were stable in the study period. In contrast, the APBF showed varying effluent in

courses of increasing organic loading rates and variant influence properties. The variant performance should be a result of dynamic microbial community change in response to the operation conditions. On the other hand, obvious attached film on bio-cord in APBF was observed as showed in section 3.4, the dynamic microbial profile was not clear. In this section, the microbial community analysis based on next generation sequencing was conducted to further understand the variant performance of APBF.

3.4.1. Diversity and abundance

The number of OTU gradually decreased from 891 at HRT=48h to 610 at HRT=8h. The Chao1 index showed the same trend as OTU, but the change in the Shannon and Simpson had not correlated well with the HRT change. The Good's coverage was around 99.65% in all samples, indicating that almost all bacterial species in the samples were detected. In general, the number and diversity of OTU decreased with the shortening of HRT, indicating that some slower growing bacteria were washed out and this would have affected the APBF's performance.

3.4.2. Comparison of microbial community structure

From the anaerobic digester system, the seed sludge was sampled as control while sludge samples from 3 different reactor segments under 5 different operational HRTs were taken as well. For the 16 sludge samples, genomic DNA was extracted and v4 region in 16s rRNA gene was sequenced by next generation sequencing as described in Section 2.4. Figure 6 presents the bacterial structure of sludges samples by the relative abundance of OTUs at different taxonomic levels. Compared with seed sludge, sludge samples from different reactor segments under different OLR showed dynamic change and distinctive distribution pattern.

At the phyla level, 14 prokaryotic phyla were higher than 1% in all sequenced samples with the larger proportions being *Euryarchaeota* (account for 7.60%-44.42%), *Proteobacteria* (account for 4.65%-37.98%), *Bacteroidetes* (account for 9.72%-27.13%), *Firmicutes* (account for 8.39%-22.69%) and *Synergistetes* (account for 0.73%-13.20%) as shown in Figure 6a. . *Euryarchaeota* was the only archaea phylum present and microbes in this phylum plays a crucial role in the process of methanogenesis. With the APBF operation progressed, the abundance of *Euryarchaeota* gradually increased but showed a decrease by 12.63% at an OLR of 5.45 kg COD/m^3.d (avg.), with an average abundance of 10.73, 20.14, 37.62, 35.14 and 30.70% at average OLR (HRT) of 2.15 (48 h), 2.75 (24 h), 4.30 (16 h), 4.89 (12 h) and 5.45 (8 h) kg COD/m^3.d, respectively. Besides, *Euryarchaeota* abundance showed a large difference at different reactor segments at OLR (avg.) of 2.75, 4.30, and 4.89 kg COD/m^3.d. For example, when OLR= 4.89 kg COD/m^3.d, the *Euryarchaeota* abundance were 26.50, 34.66 and 44.26% at the top, middle and bottom of reactors, respectively. Following *Euryarchaeota*, the proportion of phyla *Proteobacteria* showed a trend of decreasing first and then increasing, with an average abundance of 28.86, 12.72, 6.37, 21.03 and 22.94% at OLR= 2.15, 2.75, 4.30, 4.89 and 5.45 kg COD/m^3.d, respectively. *Proteobacteria* is widely found in various biological processes and contributes to the organic pollutants removal. *Bacteroidetes* were also dominating in APBF system, while the proportion was decreased (from an abundance of 21.30% at OLR=2.15 kg COD/m^3.d to 12.22% at OLR= 5.45 kg COD/m^3.d) along with the HRT reduced. For *Firmicutes* the proportion remained at a relatively high level with minor change along with OLR changes. The maximum proportion of 20.83% appeared at OLR=4.30 kg COD/m^3.d, followed by 12.69, 16.34, 11.63 and 16.16% at OLR=2.15, 2.75, 4.89 and 5.45 kg COD/m^3.d, respectively. Microbes in *Firmicutes* are usually anaerobes involved in in hydrolysis and acidification under anaerobic condition. Similar to *Firmicutes*, inconspicuous changes were

found for *Synergistetes* between different samples with an average proportion of 8.50 ± 2.34% in all process. In contrast, the abundance of *Chloroflexi* declined from 9.08% in OLR=2.15 kg COD/m^3.d (HRT= 48 h) to 0.61% in OLR=5.45 kg COD/m^3.d (HRT= 8h), indicating the gradual wash-out at the shortened HRT. The unclassified phylum accounted for about 10%, which is common in reported results (Yan et al., 2017**)**. The rest phylum contributed to the remaining proportion which was always below 20%.

A deeper classification is the basis for study of the function of microbial populations, which can then allow better understanding and explanation the relationship between operational factors and microorganisms in biological treatment processes. Figure 6b shows the heat map made of genera abundance higher than 1 % from detected 352 prokaryotic genera. As for archaea, the abundance of the 6 archaea genera, *Methanobacterium*, *Methanothrix*, *Methanospirillum*, *Unclassified-Euryachaeota*, *Unclassified-Methanoregulaceae* and *Methanomassiliicoccus* were all relatively low at OLR = 2.15 kg COD/m^3.d. Only the average abundance of the genus *Methanolinea* reached 5.26% and this genus belongs to hydrogenotrophic methanogenic archaea capable of utilizing H_2/CO_2 and formate for growth. The dominant of this genus implied a high methane yield. When the OLR was halved from 2.15 kg COD/m^3.d, the proportions of *Methanobacterium* and *Methanothrix* increased to 6.70% and 10.42%, respectively, and became dominant archaea within the system. As the OLR of the system continued to increase, these two genera archaea had maintained a high proportion. Both of them have been confirmed to be able to utilize H_2/CO_2 and sometimes formate and alcohols as substrates for growth and methane production (Yan et al., 2017). But genus *Methanolinea* proportion declined to around 0.51% with OLR increased. The *Methanospirillum* abundance had been stable at 2.56 ± 1.10%, indicating that it had no obvious response to OLR or HRT.

As for bacteria, there are 19, 12 and 7 genus bacteria belonging to *Proteobacteria*, *Bacteroidetes* and *Firmicutes*, respectively. Except some unclassified genera, no genus was widely present under various OLR conditions. However, under specific OLR, some individual genus did have higher abundance than neighboring OLR and other bacterial genera, indicating competitive advantages in the specific scenario. In detail, *Acinetobacter* only performed a high proportion at OLR=2.15 kg COD/m^3.d (account for 5.16-28.17%) and decreased to low abundance at increased OLR. In contrast, OLR of 4.89 kg COD/m^3.d (HRT= 12 h) favored the growth of genus *Vampirovibrio* and the abundance showed increasing along the flow direction with the abundance at the top, middle, and bottom of the reactor was 13.81, 13.59, and 7.85%, respectively. But its specific functions were not clear yet. As the most common genus of *Firmicutes*, *Streptococcus* has the highest proportion at OLR-= 4.30 kg COD/m^3.d, with an average abundance of 6.90, while the abundances under other conditions are 0.28, 3.45, 1.27, and 3.87%. *Streptococcus* has the capability of metabolizing a wide variety of carbohydrates such as glucose, fructose, mannose, cellobiose, lactose, trehalose, maltose and galactitol and producing VFAs (Lee et al., 2008). Same with *Streptococcus*, carbohydrates were also metabolized into VFAs by genus *Cloacibacillus*, belonging to phylum *Synergistetes*, an average abundance of 5.68, 10.70, 5.06, 5.21 and 4.96% were obtained at OLR=2.15, 2.75, 4.30, 4.89, and 5.45 kg COD/m^3.d, respectively. Unclassified-*Bacteroidetes* involved in the hydrolytic and acidogenic step, and it showed a relatively high abundance under all conditions.

Fig. 6 Relative abundance percentages of OTUs at (a) taxonomic phylum level and (b) heat map of genera for sludge samples from anaerobic digester system. Taxa represented occurrence at >1% frequency in at least one sample.

4. Conclusion

- APBF reactor achieved 61% COD and 70% BOD removal at OLR of 5.45 kg COD/m^3.d and HRT of 8h.
- Integrated APBF-aeSBR system achieved 97% COD, 98% BOD, 80% TSS and 82% TN removal.
- High throughput sequencing analysis showed the presence of fourteen phyla bacteria in APBF, which accounted around 98% of total.
- The diversity and density of bacteria and archaea (OTUs) keep decreased with the increased OLR from 2.15 (HRT= 48h) to 5.45 (HRT= 8 h) kg COD/m^3.d.
- Integrated APBF-aeSBR system can be scaled up and optimized to deploy as an effective and promising solution for beverage industrial wastewater treatment.

Acknowledgement

This research was supported by the Singapore National Research Foundation under its Incentive for Research and Innovation Scheme (IRIS) and administered by the Environment and Water Industry Program Office (EWI) of the PUB. The authors would like to thank our industrial associate, Yeo Hiap Seng Limited (YHS) and Mr. Weldon Huang, for collaboration and kind support during the entire study period. We are also thankful to Dr. Chaozhi Pan for reviewing the manuscript and his constructive comments.

Appendix A. Supplementary data

Supplementary data (SEM micrograph of APBF sludge; OTU and alpha diversity of all anaerobic biomass samples) are provided in supplementary file.

References

1. Ahring, B.K., Angelidaki, I., 1995. Volatile fatty acids as indicators of process imbalance in anaerobic digestion. Appl. Microbiol. Biotechnol. 43, 559–565.
2. Alkaya, E., Demirer, G.N., 2015. Water recycling and reuse in soft drink/beverage industry: A casestudy for sustainable industrial water management in Turkey. Resour. Conser. Recycl. 104, 172–180.
3. Amuda, O.S., Amoo, I.A., 2007. Coagulation/flocculation process and sludge conditioning in beverage industrial wastewater treatment. J. Hazard. Mater. 141, 778–783.
4. APHA, 2005. Standard Methods for the Examination of Water and Wastewater, 22nd ed. American Public Health Association, Washington DC.
5. Ait Hsine, E., Benhammou, A., Pons, M., 2005. Water resources management in soft drink industry-water use and wastewater generation. Environ. Technol. 26, 1309–1316.
6. Banu, J.R., Kaliappan, S., Beck, D., 2006. Treatment of Sago Wastewater Using Hybrid Anaerobic Reactor. Water Qual. Res. J. Canada 41 (1), 56–62.
7. Franco, A., Gresia, G., Roca, E., Rozzi, A., Lema, J.M., 2002. Influence of pulsation on start-up of UASB reactors. Wat. Sci. Technol. 45 (10), 163–168.
8. Henze, M., Harremes, P., Cour Jansen J.La, Arvin, E., 1997. Wastewater treatment - biological and chemical processes, 2nd ed., Springer, Berlin, Heidelberg, New York.
9. Isla, M.A., Comelli, R.N., Seluy, L.G., 2013. Wastewater from the soft drinks industry as a source for bioethanol production. Bioresour. Technol. 136, 140–147.
10. Jiang, J., Zhang, Y., Li, K., Wang, Q, Gong, C., Li, M., 2013. Volatile fatty acids production from food waste: Effects of pH, temperature, and organic loading rate. Bioresour. Technol. 143, 525–530.

11. Khan, A.A., Gaur, R.Z., Tyagi, V.K., Khursheed, A., Lew, B., Mehrotra, I., Kazmi, A.A. 2011. Sustainable options of post treatment of UASB effluent treating sewage: a review. Resour. Conserv. Recycl. 55 (12), 1232–1251.
12. Lee, C., Kim, J., Shin, S.G., Hwang, S., 2008. Monitoring bacterial and archaeal community shifts in a mesophilic anaerobic batch reactor treating a high-strength organic wastewater. FEMS Microbiol Ecol. 65, 544–554 .
13. Lim, S.J., Kim, B.J., Jeong, C.M., Choi, J.D.R., Ahn, Y.H., Chang, H.N., 2008. Anaerobic organic acid production of food waste in once-a-day feeding and drawing-off bioreactor. Bioresour. Technol. 99 (16), 7866–7874.
14. Oktay, S., Iskender, G., Germirli Babuna, F., Kutluay, G., Orhon, D., 2007. Improving the wastewater management for a beverage industry with in-plant control. Desalination 211, 138–143.
15. Sheldon, M.S., Erdogan, I.G., 2016. Multi-stage EGSB/MBR treatment of soft drink industry wastewater. Chem. Eng. J. 285, 368–377.
16. Simate, G.S., Cluett, J., Iyuke, S.E., Musapatika, E.T., Ndlovu, S., Walubita, L.F., Alvarez, A.E., 2011. The treatment of brewery wastewater for reuse: State of the art. Desalination, 273, 235–247.
17. Sun, F., Xiao, K.K., Zhu, W., Withanage, N., Zhou Y., 2018. Enhanced sludge solubilization and dewaterability by synergistic effects of nitrite and freezing. Water Res. 130, 208-214.
18. Tong, T., Elimelech, M., 2016. The global rise of zero liquid discharge for wastewater management: drivers, technologies, and future directions. Environ. Sci. Technol. 50 (13), 6846-6855.

19. van Lier, J.B., van der Zee, F.P., Frijters, C.T.M.J., Ersahin, M.E. (2015). Celebrating 40 years anaerobic sludge bed reactors for industrial wastewater treatment. Rev. Environ. Sci. Biotechnol. 14, 681–702.
20. van Haandel, A. C., Lettinga, G., 1994. Anaerobic Sewage Treatment. A Practical Guide for Regions with a Hot Climate. John Wiley and Sons Ltd, Chichester.
21. Yan, W., Shen, N., Xiao, Y., Chen, Y., Sun, F., Tyagi, V.K., Zhou, Y., 2017. The role of conductive materials in the start-up period of thermophilic anaerobic system. Bioresour. Technol. 239, 336-344.

505 **Captions for Tables**

506

507 **Table 1.** Pilot system operating conditions

508 **Table 2.** Physico-chemical characteristics of raw wastewater

509

510

511 **Table 1.**

Phase	Duration, Weeks	APBF			SBR		
		HRT, h	**Flow rate,** m^3/d	**OLR,** kg $COD/m^3.d$	**Flow rate,** m^3/d	**HRT,** h	**OLR,** kg $COD/m^3.d$
I	**2**	48	0.5	0.83- 4.54 (Avg 2.15)	0.25	42	0.36- 092 (Avg. 0.56)
II	**3**	24	1.0	1.35- 4.20 (Avg 2.75)	0.5	24	0.33- 1.64 (Avg. 0.80)
III	**3**	16	1.5	1.92- 6.05 (Avg. 4.30)	1.125	20	0.36- 1.36 (Avg. 0.94)
IV	**4**	12	2.0	2.86- 8.67 (Avg. 4.89)	1.5	17	0.59-1.36 (Avg. 0.93)
V	**4**	8	3.0	2.65- 7.60 (Avg. 5.45)	2.0	12	0.25-2.36 (Avg. 1.53)

512

513

514 **Table 2.**

Parameters		RCL	CSD	After Equalization tank
pH	Avg.	6.72	6.71	6.76
	Min.	2.55	3.20	3.94
	Max.	10.49	10.19	11.80
Alkalinity (mg $CaCO_3$/L)	Avg.	250	130	163
	Min.	0	0	0
	Max.	1834	834	1966
Total Hardness(mg/L as $CaCO_3$)	Avg.	169	55	NA
	Min.	95	53	NA
	Max.	308	56	NA
TDS (mg/L)	Avg.	924	313	416
	Min.	93	2	32
	Max.	11600	1652	4747
Oil and Grease (mg/L)	Avg.	49	25	23
	Min.	11	11	0
	Max.	271	118	145
TSS (mg/L)	Avg.	630	80	325
	Min.	1	1	4
	Max.	10500	236	1820
COD (mg/L)	Avg.	2380	9683	2675
	Min.	111	250	150
	Max.	22240	99420	8538
BOD (mg/L)	Avg.	NA	NA	1576
	Min.	NA	NA	139
	Max.	NA	NA	3796
Total Nitrogen (mg/L)	Avg.	53	29	44
	Min.	0	0	0
	Max.	827	250	170
NH_4-N	Avg.	1.3	ND	4.0

(mg/L)	Min.	0.3	0.0	0.1
	Max.	3.0	0.0	44
PO_4 (mg/L)	Avg.	46	35	34
	Min.	6	6	0
	Max.	287	246	192
SO_4 (mg/L)	Avg.	NA	NA	79
	Min.	NA	NA	60
	Max.	NA	NA	119
Dissolved Silica (as SiO_2)	Avg.	6.61	6.59	NA
	Min.	5.45	6.40	NA
	Max.	7.43	6.80	NA
Total Silica (as SiO_2)	Avg.	6.78	6.63	NA
	Min.	5.79	6.85	NA
	Max.	7.48	6.45	NA

Avg.= Average; Min.= Minimum; Max.= Maximum; ND: not detected; NA: not available

Number of samples, n= 23 for RCL; n= 37 for CSD; n= 96 for “After” equalization tank

Captions for Figures

Fig 1. pH profile at different stages of the pilot system operation i.e. Raw combined wastewater (Raw WW), and effluent of APBF and aeSBR.

Fig 2. Profile of daily organic loading rates on the APBF and aeSBR across different APBF HRTs.

Fig 3. (a) tCOD profile at various treatment stages of the pilot system (b) tCOD removal by the APBF and the integrated APBF-seSBR system. (c)**.** Effect of OLR on COD removal by APBF and APBF-SBR.

Fig 4. Total nitrogen removal profile in pilot system.

Fig. 5. VFA concertation and OLR applied to the APBF.

Fig. 6. Relative abundance percentages of OTUs at (a) taxonomic phylum level and (b) heat map of genera for sludge samples collected from different levels of APBF. Taxa represented occurrence at >1% frequency in at least one sample.

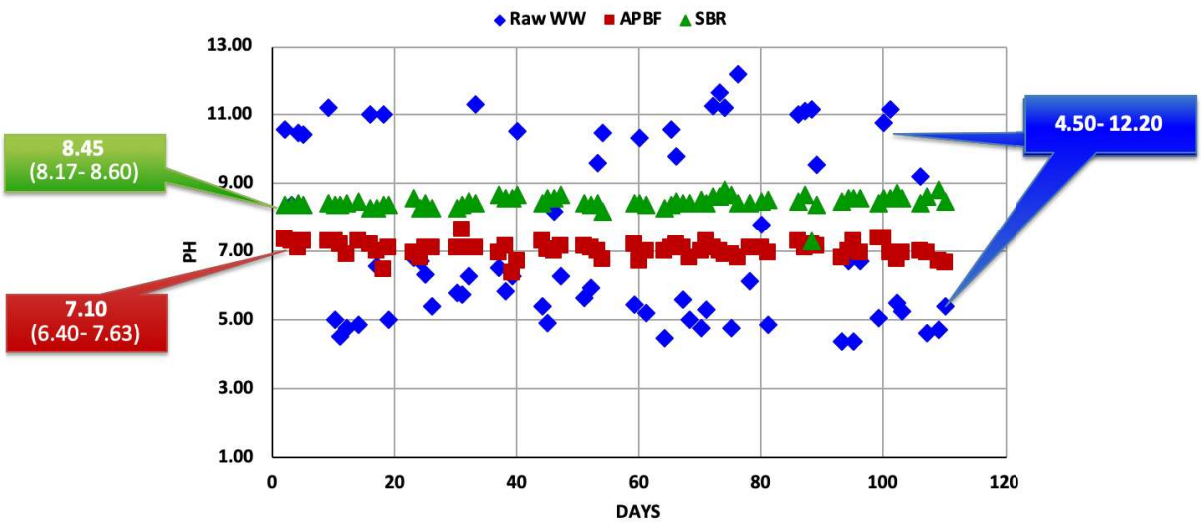

Raw WW
APBF
SBR
13.00
11.00
9.00
7.00
5.00
3.00
1.00
PH
0
20
40
60
80
100
120
DAYS
8.45
(8.17- 8.60)
7.10
(6.40- 7.63)
4.50- 12.20

Fig 1.

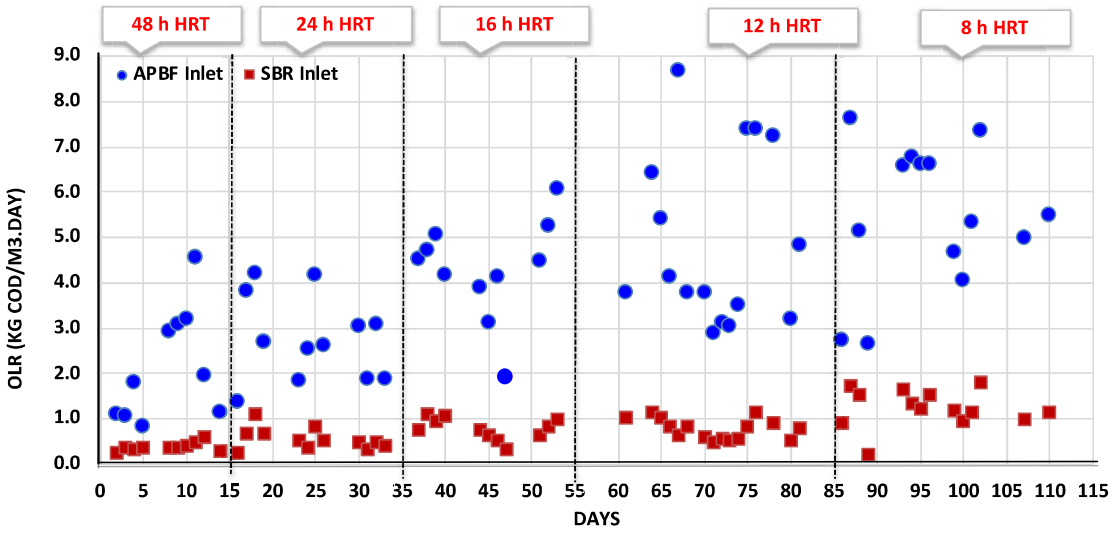
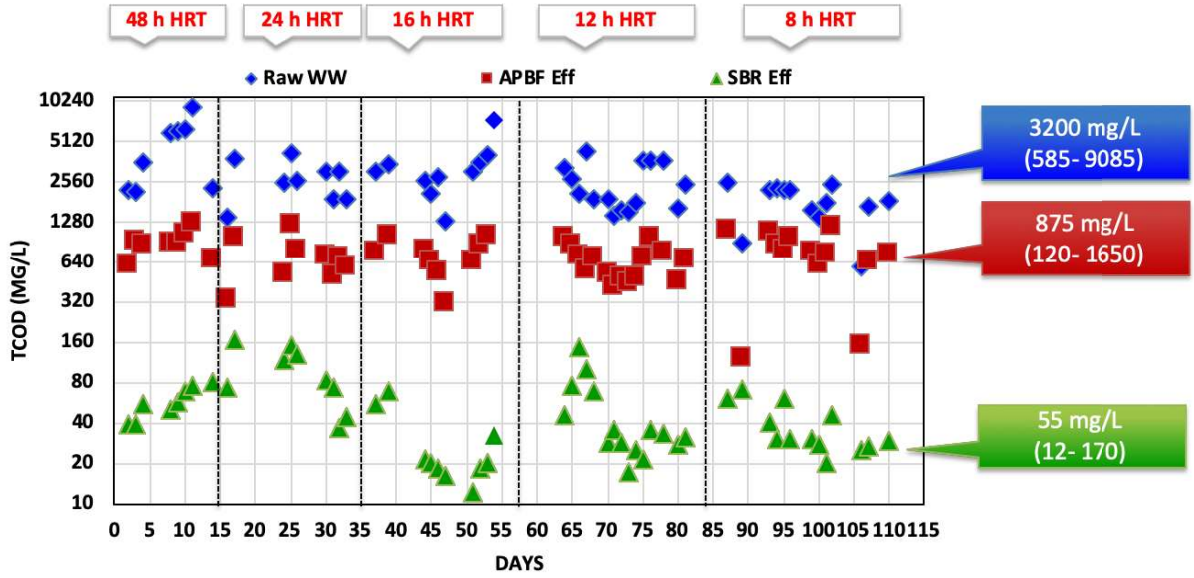
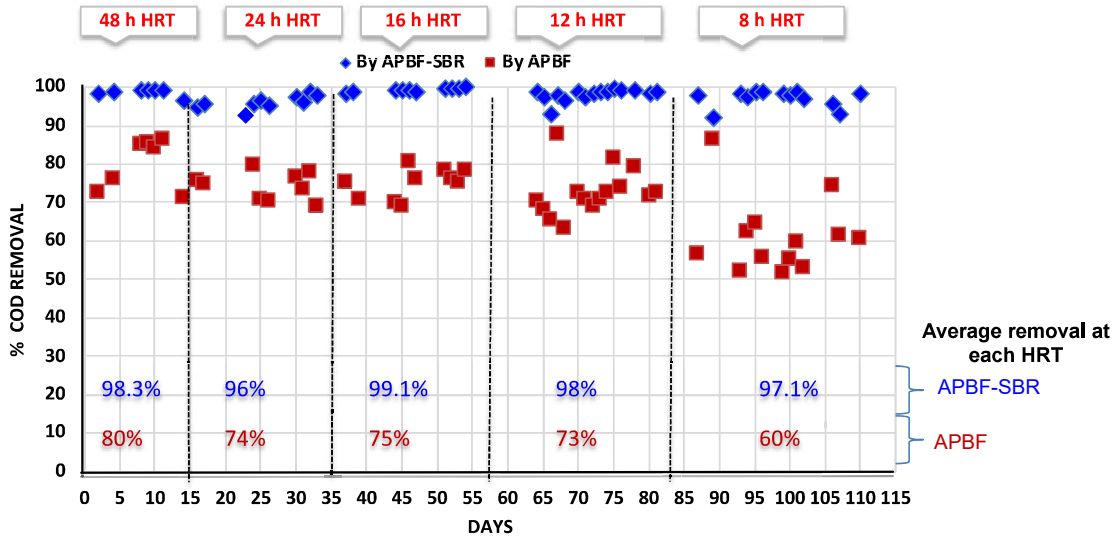

48 h HRT
24 h HRT
16 h HRT
12 h HRT
8 h HRT
APBF Inlet
SBR Inlet
OLR (KG COD/M3.DAY)
DAYS
9.0
8.0
7.0
6.0
5.0
4.0
3.0
2.0
1.0
0.0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115

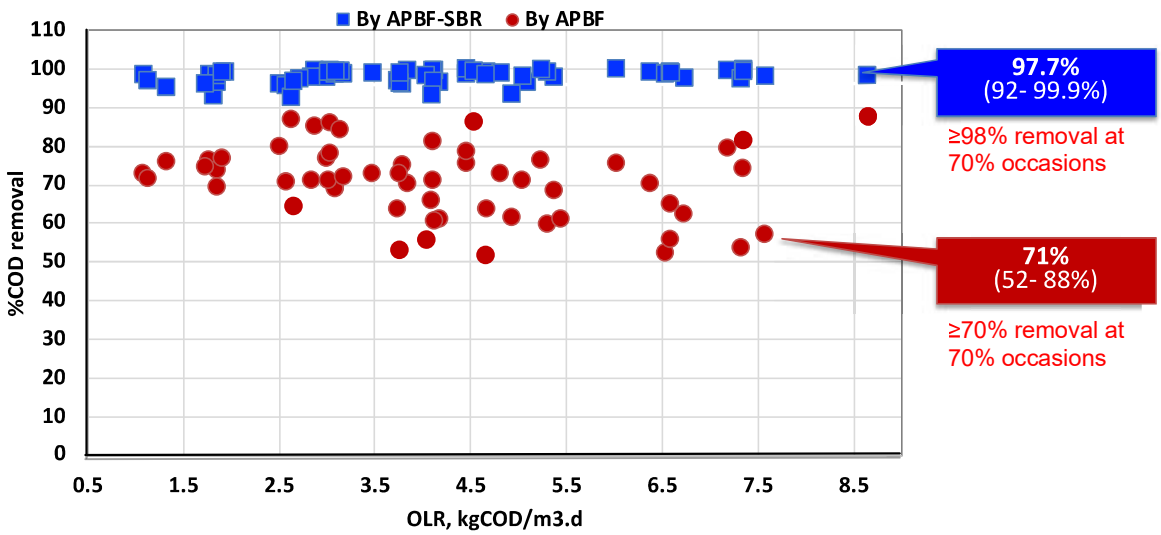
Fig 2.


48 h HRT
24 h HRT
16 h HRT
12 h HRT
8 h HRT
Raw WW
APBF Eff
SBR Eff
TCOD (MG/L)
10240
5120
2560
1280
640
320
160
80
40
20
10
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115
DAYS
3200 mg/L
(585- 9085)
875 mg/L
(120- 1650)
55 mg/L
(12- 170)

(a)


48 h HRT
24 h HRT
16 h HRT
12 h HRT
8 h HRT
By APBF-SBR
By APBF
% COD REMOVAL
100
90
80
70
60
50
40
30
20
10
0
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110 115
DAYS
98.3%
96%
99.1%
98%
97.1%
80%
74%
75%
73%
60%
Average removal at each HRT
APBF-SBR
APBF

(b)


By APBF-SBR
By APBF
%COD removal
OLR, kgCOD/m3.d
97.7%
(92- 99.9%)
≥98% removal at
70% occasions
71%
(52- 88%)
≥70% removal at
70% occasions

(c)

Fig 3.

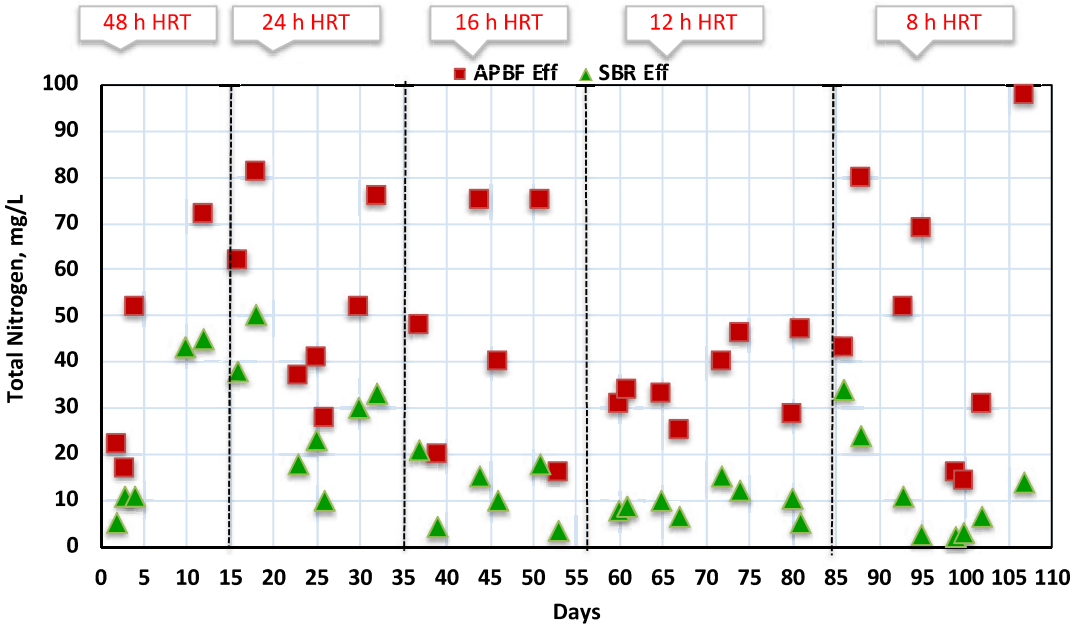

48 h HRT
24 h HRT
16 h HRT
12 h HRT
8 h HRT
APBF Eff
SBR Eff
Total Nitrogen, mg/L
Days
0
10
20
30
40
50
60
70
80
90
100
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80 85 90 95 100 105 110

Fig 4.

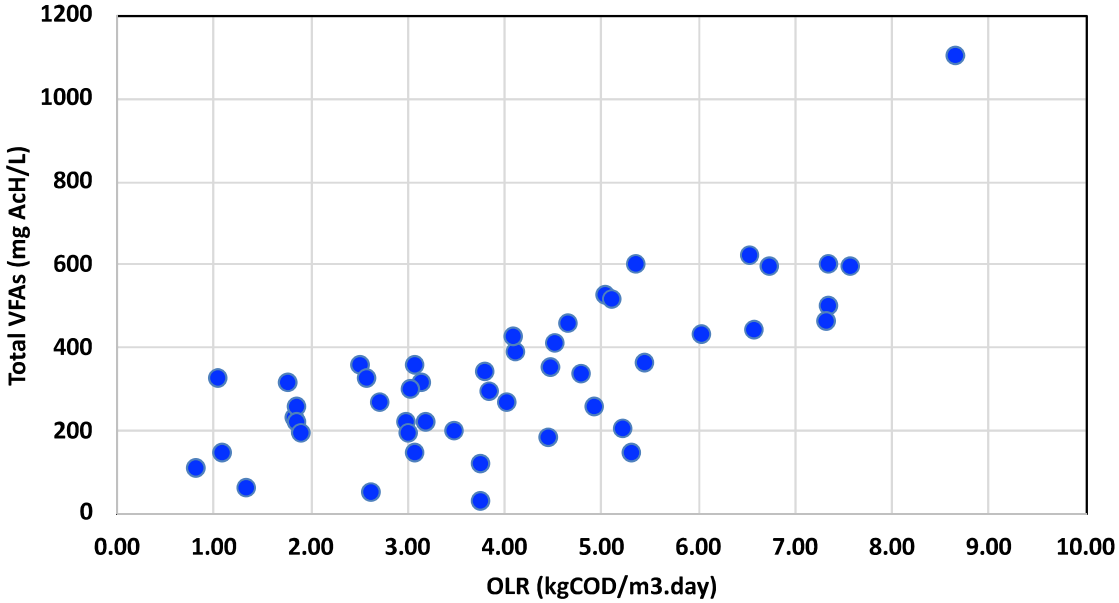

1200
1000
800
600
400
200
0
Total VFAs (mg AcH/L)
0.00
1.00
2.00
3.00
4.00
5.00
6.00
7.00
8.00
9.00
10.00
OLR (kgCOD/m3.day)

Fig. 5.

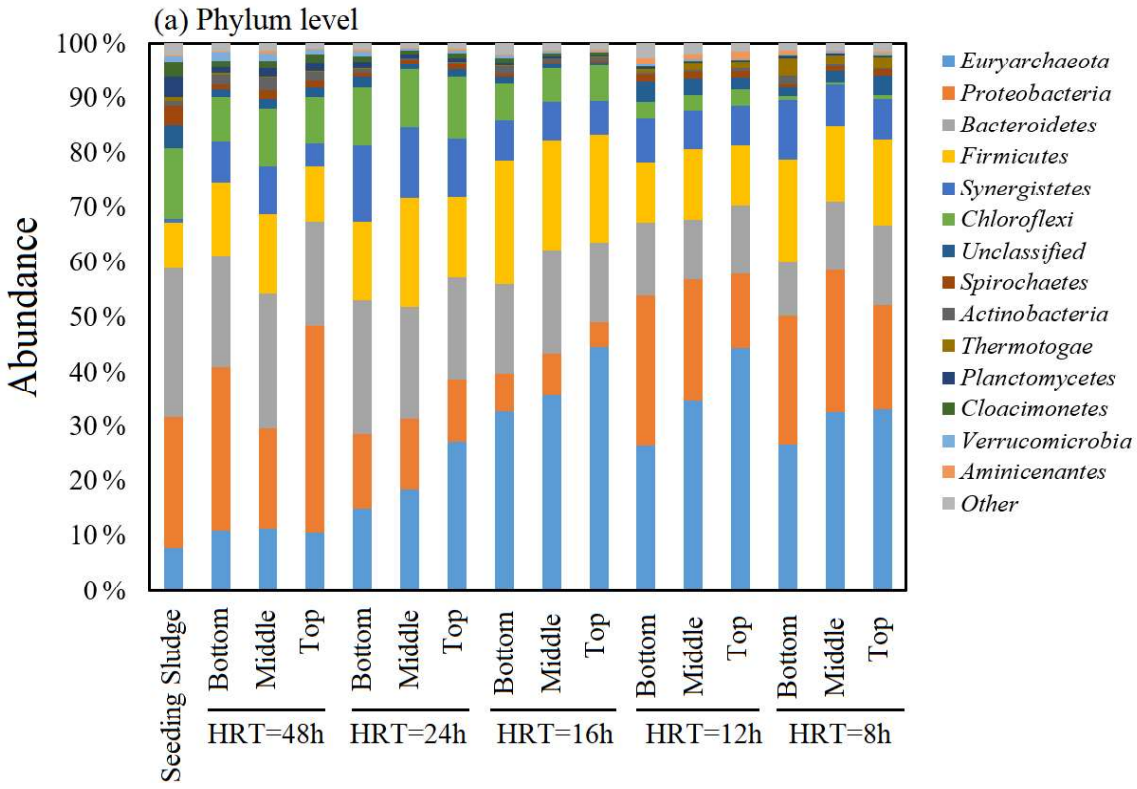

(a) Phylum level
Abundance
100 %
90 %
80 %
70 %
60 %
50 %
40 %
30 %
20 %
10 %
0 %
Seeding Sludge
Bottom
Middle
Top
Bottom
Middle
Top
Bottom
Middle
Top
Bottom
Middle
Top
Bottom
Middle
Top
HRT=48h
HRT=24h
HRT=16h
HRT=12h
HRT=8h
Euryarchaeota
Proteobacteria
Bacteroidetes
Firmicutes
Synergistetes
Chloroflexi
Unclassified
Spirochaetes
Actinobacteria
Thermotogae
Planctomycetes
Cloacimonetes
Verrucomicrobia
Aminicenantes
Other

Fig 6 (a)

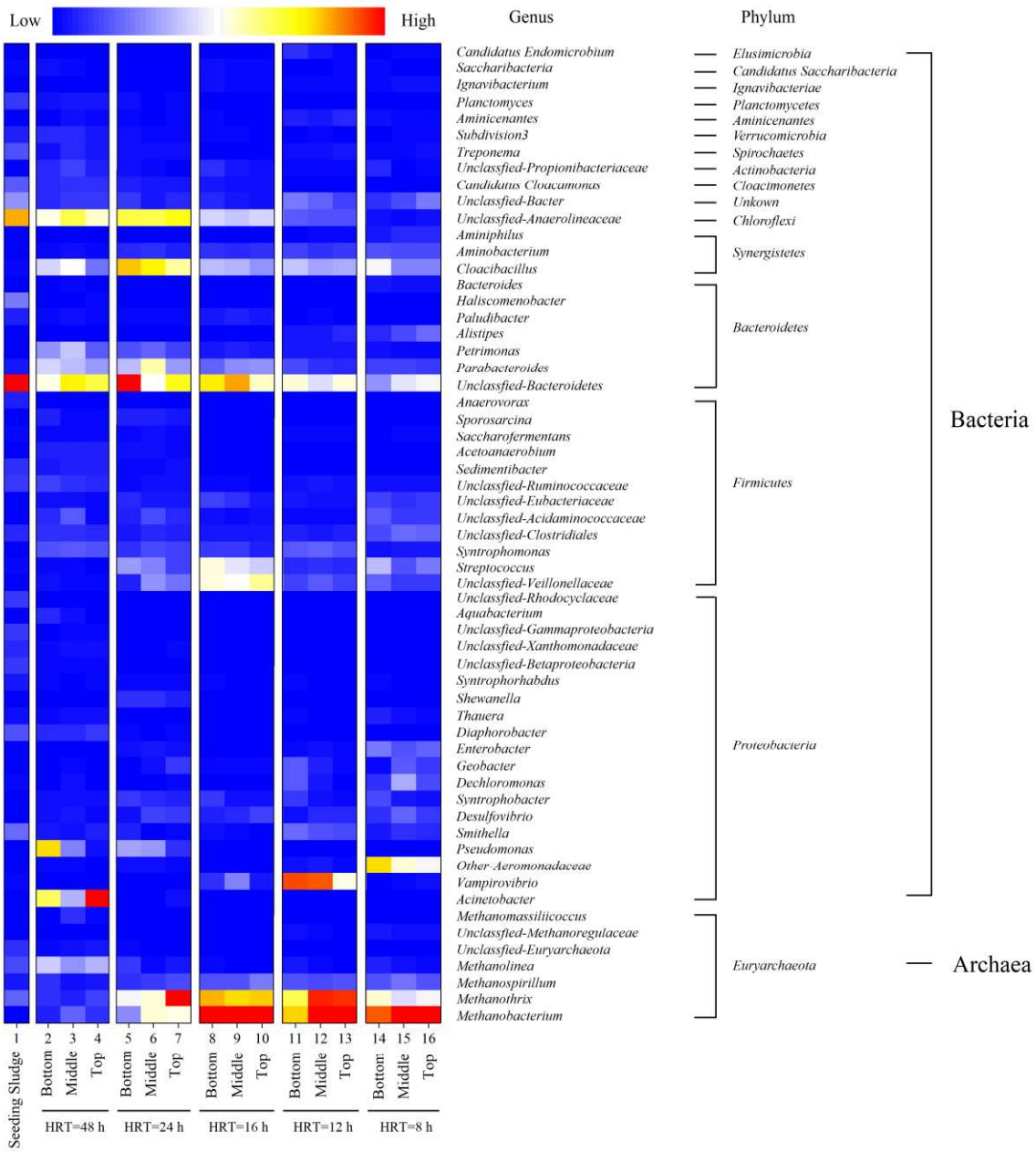
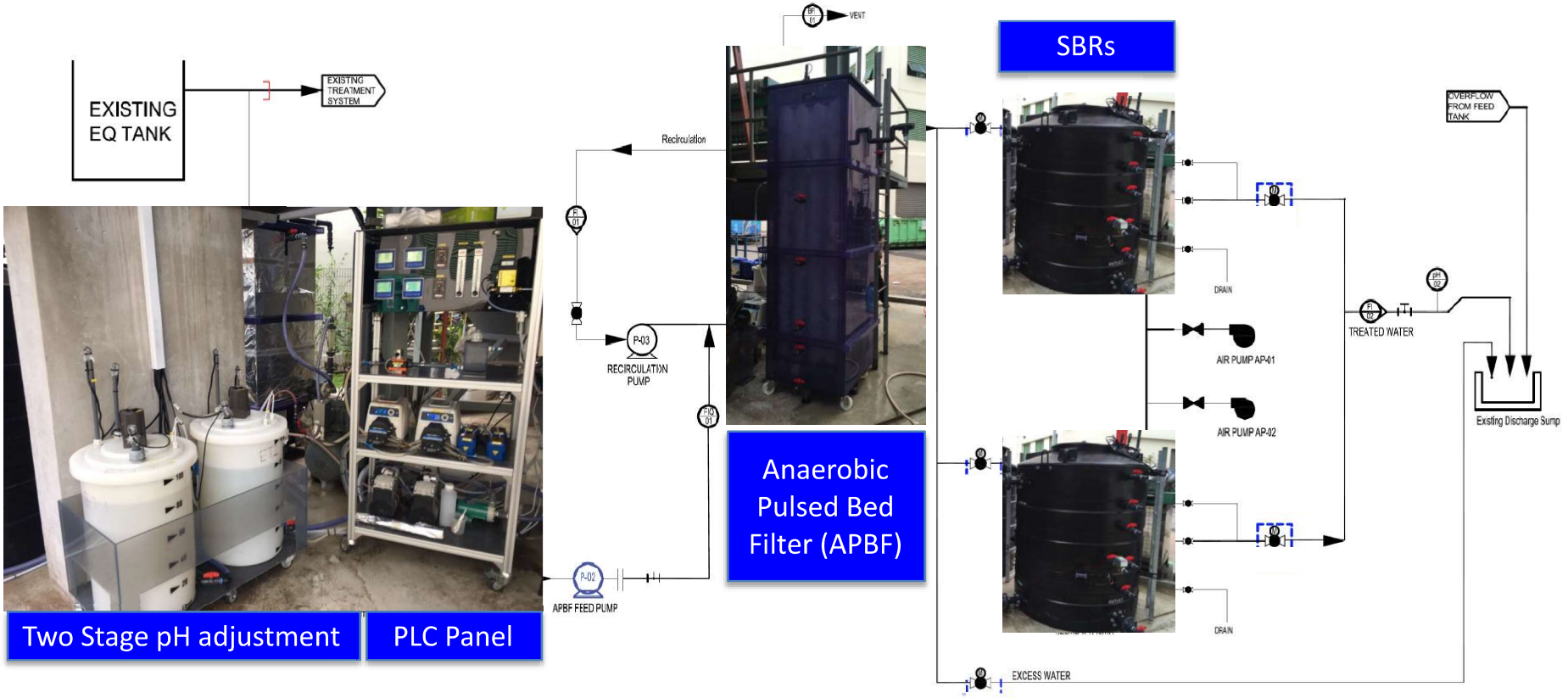

Low
High
Genus
Phylum
Candidatus Endomicrobium
Saccharibacteria
Ignavibacterium
Planctomyces
Aminicenantes
Subdivision3
Treponema
Unclassfied-Propionibacteriaceae
Candidatus Cloacamonas
Unclassfied-Bacter
Unclassfied-Anaerolineaceae
Aminiphilus
Aminobacterium
Cloacibacillus
Bacteroides
Haliscomenobacter
Paludibacter
Alistipes
Petrimonas
Parabacteroides
Unclassfied-Bacteroidetes
Anaerovorax
Sporosarcina
Saccharofermentans
Acetoanaerobium
Sedimentibacter
Unclassfied-Ruminococcaceae
Unclassfied-Eubacteriaceae
Unclassfied-Acidaminococcaceae
Unclassfied-Clostridiales
Syntrophomonas
Streptococcus
Unclassfied-Veillonellaceae
Unclassfied-Rhodocyclaceae
Aquabacterium
Unclassfied-Gammaproteobacteria
Unclassfied-Xanthomonadaceae
Unclassfied-Betaproteobacteria
Syntrophorhabdus
Shewanella
Thauera
Diaphorobacter
Enterobacter
Geobacter
Dechloromonas
Syntrophobacter
Desulfovibrio
Smithella
Pseudomonas
Other_Aeromonadaceae
Vampirovibrio
Acinetobacter
Methanomassiliicoccus
Unclassfied-Methanoregulaceae
Unclassfied-Euryarchaeota
Methanolinea
Methanospirillum
Methanothrix
Methanobacterium
Elusimicrobia
Candidatus Saccharibacteria
Ignavibacteriae
Planctomycetes
Aminicenantes
Verrucomicrobia
Spirochaetes
Actinobacteria
Cloacimonetes
Unkown
Chloroflexi
Synergistetes
Bacteroidetes
Firmicutes
Proteobacteria
Euryarchaeota
Bacteria
Archaea
1
2 3 4
5 6 7
8 9 10
11 12 13
14 15 16
Seeding Sludge
Bottom
Middle
Top
HRT=48 h
HRT=24 h
HRT=16 h
HRT=12 h
HRT=8 h

Fig 6 (b)

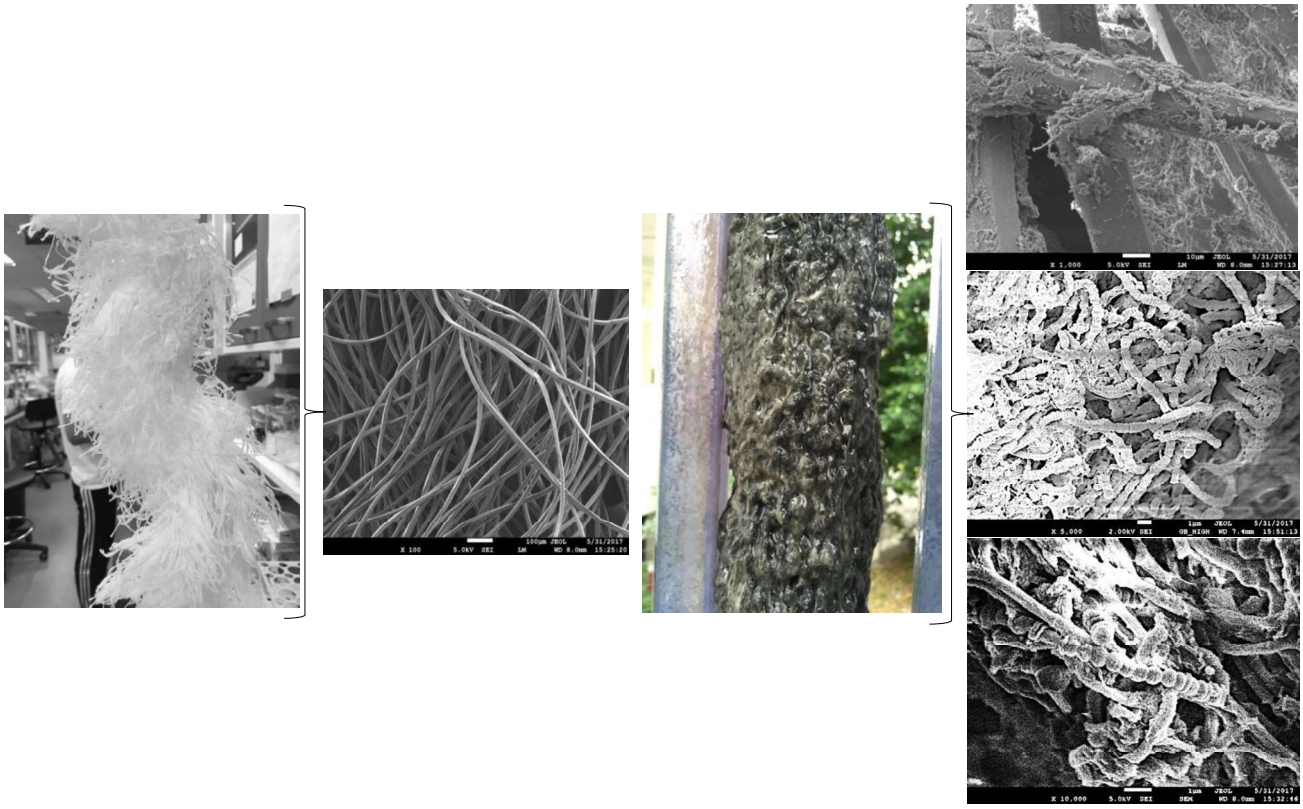
Fig. 6

Graphical abstract

A novel APBF reactor integrated with SBR



Appendix A. Supplementary data



(a) (b)

Figure S1. SEM micrograph of APBF support medium (a) virgin medium (b) mature medium.

Table S1. Index of α diversity and OTU lists

HRTs, h	Sample Type	OTUs	Chao1	Shannon	Simpson	Good's Coverage
	Seed Sludge	1005	1106.44	7.31	0.98	99.59%
48	B	890	1107.18	6.18	0.96	99.64%
48	M	894	1068.28	6.49	0.97	99.59%
48	T	906	1062.51	5.93	0.95	99.63%
24	B	840	1119.03	5.86	0.96	99.57%
24	M	713	969.28	5.60	0.95	99.60%
24	T	802	1035.33	5.76	0.96	99.70%
16	B	748	947.20	5.26	0.94	99.65%
16	M	649	840.15	5.00	0.93	99.72%
16	T	630	833.60	4.62	0.89	99.72%
12	B	718	866.08	5.76	0.95	99.71%
12	M	668	866.89	5.45	0.93	99.76%
12	T	646	827.25	5.08	0.90	99.77%
8	B	651	782.10	5.52	0.95	99.74%
8	M	564	699.00	5.42	0.94	99.78%
8	T	589	745.67	5.46	0.93	99.79%

B: Reactor bottom, M: Reactor middle, T: Reactor top