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Biosorption for Carbon Capture on Acclimated Sludge

– Process Kinetics and Microbial Community

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Abstract: This study investigated the biosorption process kinetics and the associated microbial community. Seed sludge from the aeration tank of a wastewater treatment plant in Singapore was acclimated with synthetic wastewater formulated to contain colloidal (ca. 40%) and dissolved COD (Chemical Oxygen Demand). The COD removal kinetics and the individual mechanisms involved were determined by subjecting the acclimated sludge to increasing organic loadings (0.1, 0.5, 1.0, and 2.5 g COD per g suspended solid) of synthetic wastewater. Under pH 7, sorption capacity of the acclimated sludge increased with organic loading. Comparison between live and azide-inactivated sludge revealed that under organic loading of 1.0 g COD/g SS, a level similar to a typical contact tank for carbon capture, at least 74% of the biosorption capacity was contributed by carbon storage. Kinetics data suggested that carbon storage was the predominant mechanism in the first 20 to 30 min of the carbon capture biosorption process. The removal kinetics of dissolved COD can be represented by a pseudo-second-order model and intraparticle diffusion model. These suggested the rate-limiting steps could include chemisorption and intraparticle diffusion. On the other hand, colloid COD removal can be described as a first order process with respect to initial organic loading. Taxa capable of carbon-storage which include *Chloroflexi*, *Thiobacillus* sp., *Xanthobacter* sp., *Mycobacterium* sp., and *Nakamurella* sp., were uniquely detected in the acclimated sludge.

Keywords: Activated sludge, Biosorption, Biosorption kinetics, Carbon capture, Carbon storage, Energy recovery

Nomenclature

A_p	Intraparticle diffusion constant
ATP	Adenosine triphosphate
ATU	Allylthourea
CCB	Carbon capture biosorption
C	Dissolved COD at t contact time, mg COD/L
C_e	Dissolved COD at equilibrium, mg COD/L
C_0	Initial dissolved COD, mg COD/L
C_{OL}	Organic loading
COD	Chemical oxygen demand
COD _c	Colloidal COD fraction
COD _s	Dissolved COD fraction
COD _T	Total COD fraction
DGGE	Denatured gradient gel electrophoresis
DO	Dissolved oxygen
EPS	Extracellular polymeric substance
EPS _p /EPS _s	Ratio of protein to polysaccharide fraction in EPS
F/M ratio	Food-to-Microorganisms ratio
k_1	Rate constant of pseudo-first-order process, min ⁻¹
k_2	Rate constant of pseudo-second-order process, g/mg.min
k_{ca}	Rate constant of colloidal COD removal with respect to organic loading, mg COD/g COD.L.min
k_f	Rate constant of film mass transfer, min ⁻¹
k_p	Rate constant of intraparticle diffusion, g/g.min ^{0.5}
MLSS	Mixed liquor suspended solids
OTU	Operational taxonomic unit
PCR	Polymerase chain reaction
PHA	Polyhydroxyalkanoate
$Q_{C, t}$	Amount of colloidal COD sorbed at t min contact time per unit weight of sorbent, mg/g
Q_{se}	Amount of dissolved COD sorbed at equilibrium per unit weight of sorbent, mg/g
$Q_{S, t}$	Amount of dissolved COD sorbed at t min contact time per unit weight of sorbent, mg/g
$Q_{T, t}$	Overall capacity, amount of total COD sorbed at t min contact time per unit weight of sorbent, mg/g
R_C	Specific colloidal COD removal rate
R_S	Specific dissolved COD removal rate
R_T	Specific total COD removal rate
SBR	Sequencing batch reactor
SRT	Sludge retention time
SS	Suspended solids
t	Contact time t , min
TAG	Triacylglycerols
TSS	Total suspended solids
WWTP	Wastewater treatment plant

1. Introduction

Conventional activated sludge-based technologies used in wastewater treatment rely on bio-oxidation through aeration for pollutant removal, and are thus energy intensive. Lately, there has been a paradigm shift on the role of wastewater treatment plants (WWTPs), from pollutants removal to resource recovery. Moreover, global warming concerns call for WWTPs which are more environmentally sustainable. Due to such concerns, the AB-process has been received considerable attention. The AB-process is a two-stage approach with pre-treatment in an extremely high loaded biosorption stage (A-stage) for carbon capture, followed with a treatment in a low loaded biological stage (B-stage) ensuring removal of dissolved organics and ammonia. The organic carbon captured is then recovered as energy from the solids train containing A- and B-stage sludge through biogas generation in anaerobic digesters [1]. Through this approach, energy can therefore be recovered from the wastewater influent to improve the energy self-sufficiency of treatment plants.

Researchers had attempted to demonstrate the feasibility of carbon capture (energy) from raw municipal wastewater using biomass, i.e. activated sludge, anaerobic sludge or acclimated sludge [2-8]. Earlier studies had concluded the process involved solely physical-chemical processes [2, 3], which is consistent with the common definition of a metabolically-passive “biosorption” process [9]. Xiao et al. had, however, reported active sludge tended to give better biosorption than inactive sludge. It was then concluded biosorption is a combination of metabolically-mediated biological and physical-chemical processes [10]. Lim et al. [5] reported the biological component involved in carbon capture by activated sludge can be attributed mainly to carbon storage. Carbon storage can occur in the form of poly-hydroxyalkanoates (PHA) [11] and triacylglycerols (TAG) [12]. In addition, bacteria in activated sludge can also form other storage products such as glycogen [13]. Therefore, insofar as carbon capture is concerned, usage of the term “*biosorption*” alone can cause confusion as the phenomenon can go beyond physical-chemical process(es). To better describe, without ambiguity, the phenomenon where biomass capture, concentrate and retain carbon within the sludge flocs, Lim et al. [5] had proposed the term “*carbon capture biosorption*” (hereafter abbreviated as “CCB”). CCB could involve (i) surface sorption: metabolically-passive uptake of organics; (ii) carbon storage: metabolically-mediated uptake of organics and accumulated within the cell; and (iii) carbon entrapment: entrapment of larger particles in the open structure of the sludge floc, facilitated by extracellular polymeric substances (EPS) [5].

It has been reported removal of the colloidal fraction reaches equilibrium in about 10-20 min, whereas removal of the dissolved fraction reaches equilibrium in 40-45 min [2, 3, 5, 6]. However, there have been few reports on the predominant mechanism(s) and possible limiting steps involved, if any, throughout the CCB process. To the authors' knowledge, despite various adsorption kinetic models

having been used to describe the adsorption of metal ions, few such attempts have been reported for CCB [7]. Guellil [2] reported CCB of total COD obeyed the first order kinetic model. Whereas a more recent study conducted by Modin et al [7] reported that the sorption of TSS and dissolved TOC occurred by a near-instantaneous sorption event followed by a slower process that obeyed first order kinetics. Wei and Hong [14] demonstrated the overall biosorption process for organic pollutants was well described by the pseudo-second-order model, although the kinetic data were for specific pollutants and the organic loading applied was not reported. There are obviously gaps in the understanding of CCB capacity and removal kinetics of COD components at loadings higher than 0.4 g COD/g SS. Typical organic loadings observed in the contact tank of the AB-process was 0.18 to 0.63 g COD/g SS [1], but previous studies on CCB had been conducted at organic loadings of 0.1 to 0.33 g COD/g SS [2, 3, 5, 6]. There is also still a relative lack of information on the microbial communities involved in CCB except for the earlier attempt by Lim et al [5] using DGGE.

In this study, the CCB capacity of seed sludge was enhanced through feast-famine cycle acclimation, under low Food-to-Microorganisms (F/M) ratio. The investigation had attempted to elucidate the predominant mechanism(s) involved in CCB under increasing organic loadings. The COD removal kinetics and the individual mechanisms involved were determined by subjecting the acclimated sludge to increasing organic loadings (0.1, 0.5, 1.0, and 2.5 g COD/g SS) of synthetic wastewater. Dynamics of the removal of COD fractions during CCB were analysed using pseudo-first-order and pseudo-second-order kinetic models. Microbial community analyses, with next-generation sequencing technique, were also attempted to identify the groups associated with higher CCB capacity.

2. Materials and Methods

2.1 Sludge Acclimation

Seed sludge was obtained from the aeration tank of a local WWTP in Singapore. Sludge acclimation was carried out at 30°C, pH 6.8-7.3, in a Sequencing Batch Reactor (SBR) with a cycle time of 6 hours comprising 6 stages: (i) filling, 5min; (ii) contact, 10 min; (iii) sludge discharge, 2 min; (iv) aeration, 290 min, (v) settling, 38 min; and (vi) decantation, 15 min. Working volume was 6 L. The mixed liquor suspended solids (MLSS) of the seed sludge inoculated was about 3 g/L. The reactor was operated under F/M 0.1, volumetric exchange ratio of 50%, DO 2.0-2.2 ppm, and 13 days SRT (sludge retention time). The synthetic wastewater (SW1) was formulated to simulate effluent from the Primary Settling Tank which had 42% dissolved COD, 54% colloidal COD and 4% particulates COD. The SW1 was formulated with sucrose, 50 mg/L; sodium acetate trihydrate, 179 mg/L; starch (cooked), 150 mg/L; urea, 62.5 mg/L; yeast extract, 1 mg/L; bacto peptone, 1 mg/L; ammonium chloride, 180 mg/L; and monopotassium phosphate, 23.5 mg/L. The SW1 was prepared using tap water and cooked before use. The total COD of cooked SW1 was then 300 mg COD /L with about 40%

in colloidal form and the balance dissolved. The mixture was supplemented with a trace elements solution [15]. ATU was added to inhibit nitrification. The sludge acclimated with SW1 is hereafter designated as “Sludge A1”.

2.2 Sludge Characterizations

Sludge surface charge was determined in terms of zeta-potential by using the Zetasizer Nano ZS (Malvern Instruments). The particle size distribution of the sludge was determined using the Laser Diffraction Particle Size Analyzer (SALD-3101, Shimadzu, Japan). The EPS was extracted from the sludge with the formaldehyde-NaOH method [16]. The EPS polysaccharide fraction (EPSs) was determined with the phenol-sulfuric acid assay [17] while the protein fraction (EPSp) was determined with the DC Protein Assay (Bio-Rad).

2.3 Carbon Capture Biosorption Capacity and Kinetics

Biosorption capacity of the acclimated sludge was determined with batch tests at pH 7. Mixed liquor (300 ml) was collected from the bioreactor at the end of aeration phase and settled for 30 min. The supernatant was then decanted. The 2x concentrated sludge (150 ml) was transferred into conical flask. SW1 (150 ml) was then added into the flask rapidly to start the batch test. Experiments were conducted at 30°C, with continuous N₂ purge to maintain anaerobic conditions. The acclimated sludge was subjected to organic loadings 0.1, 0.5, 1.0, 2.5 g COD/g SS by manipulating the strength of SW1 from the composition indicated in the preceding section. COD was determined in accordance with Standard Methods [18]. Mixed liquor sampled from the flask was centrifuged at low speed (100 xg) for liquid-solids separation, before the supernatant was filtered through grade 392 filter paper (5-8 µm retention size, Sartorius Stedim) to obtain the non-settleable (colloidal and dissolved) fraction, also defined as total fraction (COD_T) in this study. Overall biosorption capacity (hereafter denoted as “overall capacity”) was determined by the difference between initial COD_T and the residual COD_T at particular time point, expressed in mg COD/g SS. The colloidal COD (COD_C) was defined as the fraction that passes through grade 392 filter paper, but retained by 0.45 µm filter. Whereas the fraction that passed through 0.45 µm filter was defined as the dissolved COD (COD_D). Baseline sludge (hereafter designated as “Sludge K”) from the same WWTP was subjected to SW1 under organic loadings 0.1 and 0.5 g COD/g SS, at pH 7.

Biosorption capacity attributed to carbon storage activity was determined following sodium azide inactivation. Briefly, sodium azide was added to the concentrated biomass and incubated for 30 min before introducing the SW1, under loadings 0.1, 0.5, 1.0 g COD/g SS, at pH 7. The mixed liquor contained a final concentration of 0.2% (w/v) sodium azide. While the overall capacity of live sludge was assumed to be attributable to surface sorption and carbon storage, capacity of the inactivated

sludge was assumed to be contributed only by surface sorption. Hence, the difference in the CCB capacity between live and inactive sludge was attributed to carbon storage.

2.4 Community Analysis through Amplicon Sequencing

Genomic DNA extracted using FastDNATM SPIN Kit for soil (MP Biomedicals). Universal primers targeting the bacterial 16S rRNA gene, 27F (AGAGTTTGATCCTGGCTCAG, [19] and 534R (ATTACCGCGGCTGCTGG, [20] were used. The primer pair amplifies a DNA fragment of ca. 500 bp of the 16S rRNA gene (Variable V1 to V3 region). PCR amplification was performed using 1X Platinum® High fidelity buffer, 400 pM dNTP, 1.5 mM MgSO₄, 2mU Platinum® Taq DNA Polymerase High Fidelity, 5 µM barcoded V1-V3 adaptor mix, and 10 ng template DNA. PCR conditions were 95°C, for 2 min, 30 cycles of (95 °C, for 20 sec, 56°C for 30 sec, 72°C for 60 sec) and a final step of elongation at 72°C for 5 min. PCR products were purified using Agencourt AmpureXP (Beckman Coulter) with a ratio of 1.8 bead solution/PCR solution. The concentration of DNA was determined using the QuantIT HS kit (Life Technologies). Barcoded amplicons were pooled in equimolar amounts and paired-end sequenced (2*300 bp) on the Illumina MiSEQ. Paired end reads were merged using FLASH and the sequences were clustered into OTUs (97%) and classified using QIIME V. 1.8 [21] with the GreenGenes database (13_5) as the reference database. Data analysis was performed by using R (v.3.1.1; <http://www.r-project.org/>). The OTUs unique to either one of the sludges (indicator species) were identified using the indicpecies package (ver.1.7.2).

The raw data of bacterial 16S rRNA-encoding gene sequence reads was deposited in the Sequence Read Archive (SRA) of NCBI (Bioproject: PRJNA261264). The sample identifications for microcosms in the sludges are: baseline Sludge K, SAMN03070058, and acclimated Sludge A1, SAMN03070059. Sequences associated with the indicator species unique to Sludge A1, and Sludge K were deposited into GenBank, with the accession numbers KM580373 to KM580412.

3. Results and Discussion

3.1 Sludge Properties and Capacity

Sludge A1 had a larger floc size (d_{50}) of 441 μm , resulting in a smaller surface area per unit volume compared to baseline Sludge K (Table 1). The total EPS produced in Sludge A1 was 93.3 mg/g SS, about 74% of the amount in Sludge K (126.0 mg/gSS). In addition, the EPS_p/EPS_s ratio in Sludge A1 was 2.06, lower than that of Sludge K's (3.00). On the other hand, Sludge A1 showed higher surface negativity (zeta potential = -19.6 mV) compared to Sludge K (-16.8 mV). Lower EPS_p/EPS_s ratio implied lower protein content and/or higher polysaccharide content in the EPS produced.

Flocs of activated sludge carry net negative charge, due to the protonation of the anionic groups, e.g. carboxylic and phosphate groups. On the contrary, the lysyl, histidyl and arginyl side chains of the amino groups on the cell surface would result in localized positive charge [22]. The carboxyl groups in polysaccharide (EPS_s) contributed to the negative charge. Thus the lower EPS_p/EPS_s in Sludge A1 corresponded with its higher surface negativity and vice versa for Sludge K (Table 1). Wang et al. had also reported that the surface properties correlated to EPS_p/EPS_s ratio rather than the individual EPS components. The EPS_p/EPS_s ratio positively correlated with hydrophobicity, and negatively correlated with surface negativity [23]. Nevertheless the effect of hydrophobicity was not evident herein.

Preliminary batch tests subjecting Sludge A1 to SW1 suggested that at pH 7, COD removal by Sludge A1 plateaued after 60 min contact time (Figure S1, Supplementary Material). Consequently, the batch tests were conducted for 60 min. Table 1 shows the sludge properties and overall biosorption capacity (hereafter denoted as “overall capacity”) of Sludge A1 and baseline Sludge K. When subjected to synthetic wastewater (SW1), Sludge A1 demonstrated higher overall capacity than baseline Sludge K in terms of COD_T removal regardless of the organic loading. Under the higher loading of 0.5 g COD/g SS, Sludge A1's overall capacity in 60 min contact time ($Q_{T, 60}$) was 242.3 mg COD/g SS, approximately 90-fold higher than that of Sludge K (2.74 mg COD/g SS).

Figure 1a shows that the overall capacity of Sludge A1 correlated positively with the loading when subjected to loading between 0.1 and 1.0 g COD/g SS. Although under loading 2.5 g COD/g SS Sludge A1 demonstrated overall capacity of 266.9 mg COD_T/g SS, predominantly attributed to COD_C uptake, CODs uptake rate was highly variable. The standard deviation for CODs uptake under loading 2.5 g COD/g SS was then unacceptable (Figure S2, Supplementary Material). Hence, the data presented hereafter are those obtained under loadings 0.1, 0.5 and 1.0 g COD/g SS. The overall capacity in 60 min (Q_{T60}) increased from 89.7 mg COD/g SS to 242.3 mg COD/g SS, when the loading increased from 0.1 to 0.5 g COD/g SS. Q_{T60} plateaued at this level even though the loading

was further increased. 52.6% to 67.7% of the material sorbed was contributed by the capture of CODs under loading 0.1, 0.5 and 1.0 g COD/g SS (Figure 1a).

The surface sorption capacity was determined using inactivated sludge. The addition of sodium azide blocked ATP synthesis inside the cell, inactivating the biomass's respiratory process [24]. Since carbon storage is a metabolically-mediated process, the capacity of the inactivated sludge was then assumed to be attributable only to surface sorption. Therefore, the difference between the biosorption capacity of live and inactive biomass could be attributed to carbon storage. It was observed carbon storage capacity (hereafter denoted as "storage capacity") correlated positively with the organic loading. Figure 1b shows the storage capacity increased from 45.1 mg COD/g SS to 227.5 mg COD/g SS as the loading increased from 0.1 to 1.0 g COD/g SS. In contrast, the portion contributed by surface sorption remained at the level of 40 to 62 mg COD/g SS.

It was demonstrated that through a feast-famine cycle with low F/M ratio, the sludge could be acclimated to respond to 1.0 g COD/ g SS loading with a maximum biosorption capacity of around 250 mg COD/g SS.

3.2 Biosorption Kinetics

3.2.1 Trend of removal

Figure 2a, b, and c show that throughout the batch tests, the CODs specific removal rates (R_S) were in a decreasing trend. R_S decreased substantially as CODs decreased to the equilibrium concentration (C_e), at which R_S became zero or close to zero (Figure 2d, e, and f). Similar trend was also observed for the COD_T specific removal rates (R_T). In contrast, the specific colloid removal rates (R_C) remained stable throughout the same period of time, independent of the sorbate concentration at t time. In 30 to 40 min contact time, R_S gradually decreased to the extent of lower than R_C , whereas R_T decreased to the level similar to R_C . This suggested that CODs removal was the predominant mechanism in the initial 20 to 30 min. However, once the biomass's capacity towards CODs was saturated, colloid removal became the predominant mechanism in the later part of CCB. This observation is consistent with previous reports that CCB for CODs reached equilibrium in about 40 to 45 min contact time [2, 3]. As for COD_c, in contrast to observations reported previously [2, 3], the removal of COD_c only reached equilibrium following 50 min contact when Sludge A1 was subjected to loading of 0.1 g COD/g SS but was not observed under higher loading. This could be because at higher loading, the absolute amount of COD_c present in the synthetic wastewater was in excess. Although under the higher loading of 1.0 g COD/g SS, the CCB capacity was about 250 mg COD/g SS, the equilibrium CODs was 180 mg COD/L. These values suggested the necessity for multiple stages CCB when faced with high initial loadings, in order to achieve higher carbon capture in absolute amounts.

3.2.2 Colloidal COD Fraction

Figure 2d, e, and f shows the relationships between specific removal rates of COD total, colloidal, and dissolved fractions with respect to the same COD fraction at time t , under organic loadings 0.1, 0.5, 1.0 g COD/g SS. R_C was independent of COD_c, suggesting a zero order relation:

$$R_C = k_C \quad \text{Equation 1}$$

k_C = colloid removal kinetic constant

However, the colloid removal kinetic constant (k_C) correlated linearly with the organic loading (Figure S3, Supplementary Material), resulting in a first order relationship with respect to organic loading (C_{OL}). Thus, specific colloidal removal rate can be expressed as:

$$R_C = k_C = k_{c\alpha} \cdot C_{OL} \quad \text{Equation 2}$$

where

$$k_{c\alpha} = 2.816 \text{ mg COD}/(\text{g COD} \cdot \text{L} \cdot \text{min})$$

C_{OL} = Organic loading, g COD/g SS

Positive linear correlation between the organic loading and the specific colloids removal rate ($R^2=0.911$, Figure S3) suggested that at higher organic loading the interaction between the colloids and the sludge surface increased, resulting in higher colloids removal. However, under higher loading, the biomass surface was rapidly saturated with colloids, causing unstable sorbent-sorbate interaction. Desorption of COD_c was observed from 40 min onward. Residual CODs also increased after 30 min and had started to fluctuate (Figure S4, Supplementary Material).

3.2.3 Dissolved COD Fraction

Pseudo-first-order [25] and pseudo-second-order [26] models were used for fitting the biosorption kinetics data for CODs. The pseudo-first-order (Equation 3) and the pseudo-second-order (Equation 4) processes can be written as follows

$$\ln(Q_{se} - Q_{s,t}) = \ln Q_{se} - k_1 t \quad \text{Equation 3}$$

$$\frac{t}{Q_{s,t}} = \frac{1}{k_2 \cdot Q_{se}^2} + \frac{t}{Q_{se}} \quad \text{Equation 4}$$

Where Q_{se} is the amount of CODs sorbed at equilibrium per unit weight of sorbent, mg/g; $Q_{s,t}$ is the amount of CODs sorbed at t contact time, mg/g; k_1 (min^{-1}) and k_2 (g/mg min) are the rate constants of pseudo-first-order and pseudo-second-order process, respectively. The first-order kinetic process has been used for reversible reaction with an equilibrium being established between liquid and solid phase. Whereas the pseudo-second-order model is based on the assumption that the rate limiting step may be a chemisorption involving valency forces through sharing or exchange of electrons between adsorbent and adsorbate. The pseudo-second-order model has been applied successfully to the adsorption of metal ions, dyes, herbicides, oils and organic substances from aqueous solutions [27]. The experimental and calculated Q_{se} values, k_1 , k_2 and regression coefficient (R^2) values obtained are shown in Table 2. The R^2 values of the pseudo-second-order model were better than those of the pseudo-first-order model. In addition, there is close agreement between experimental and calculated for $Q_{s,t}$ values for the pseudo-second-order model (Figure 3a). This suggests the possibility that chemisorption force could be one of the rate limiting steps for CODs uptake before the CODs was transported into the cell for accumulation.

In order to identify any other limiting steps from a mechanistic viewpoint, the experimental data was also evaluated with the film mass transfer model (Equation 5) and intraparticle diffusion model (Equation 6) [14].

$$-k_f t = 2.303 \log \frac{C}{C_0} \quad \text{Equation 5}$$

$$Q_{s,t} = k_p t^{1/2} + A_p \quad \text{Equation 6}$$

Where C is CODs at t contact time (mg/L). C_0 is initial CODs (mg/L). $Q_{s,t}$ is the amount of CODs sorbed at t time, mg/g. k_f is the film mass transfer rate constant (min^{-1}), k_p is the intraparticle diffusion rate constant ($\text{g/g.min}^{0.5}$). A_p is the intercept of the plot $Q_{s,t}$ versus $t^{1/2}$ (mg/g), which is directly proportional to the boundary layer thickness. If the regression of $Q_{s,t}$ versus $t^{1/2}$ passes through the origin, then intraparticle diffusion is the sole rate-limiting step [28]. R^2 values show that generally the experimental data fitted the intraparticle diffusion model better (Table 2). A_p increased along with higher loading suggested boundary layer effect. Intraparticle diffusion rate constant k_p increased with higher loading suggesting higher intraparticle diffusion rate due to driving force by a higher concentration gradient present [29]. While overall experimental data for loading 0.1 g COD/g SS followed the intraparticle diffusion model ($R^2=0.9399$), two linear portions were observed for loadings 0.5 and 1.0 g COD/g SS (Figure 3b). This suggested two-stage diffusion of the COD into the sludge floc. In addition, the deviation of the line from the origin for the plot $Q_{s,t}$ versus $t^{1/2}$ suggested that intraparticle diffusion was not the only rate limiting step under loadings 0.1, 0.5 and 1.0 g COD/g SS.

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The biosorption kinetics data showed the possible rate limiting steps in CCB of CODs could include chemisorption and intraparticle diffusion.

3.5 Microbial Community Analysis

Figure 5 shows that half of the OTUs recovered from Sludge A1 were closely-related to the phylum *Chloroflexi*, with a relative abundance of 55%. In contrast, only 7% of the OTUs detected in Sludge K were closely-related to *Chloroflexi*. Phase contrast micrograph revealed that Sludge A1 flocs were relatively more compact with filamentous bacteria extending out from the main body of the floc (Figure S6, Supplementary Material). As Sludge A1 was cultivated under low F/M ratio, at low substrate concentrations, filaments would gain advantage by extending outside the floc to access substrate. On the other hand, for the case of Sludge K, at higher substrate concentrations the filaments could remain inside the floc without suffering disadvantage because the substrate was not limiting inside the floc [30]. In fact, the relative abundances of *Chloroflexi* in Sludge A1 (55%), and Sludge K (7%) correlated positively with the floc size (d_{50}) which were 441 μm , and 115 μm , respectively (Table 1). The more abundant filamentous bacteria (*Chloroflexi* and others) in Sludge A1 formed the skeleton of the floc for more floc-forming bacteria to come together and form bigger flocs. In comparison, Sludge K flocs were looser with few filaments extending out from the floc. Kragelund et al reported that the surface of *Chloroflexi* to be hydrophilic (surface negativity) compared to other filamentous bacteria present in activated sludge [31]. Thus, the higher relative abundance of *Chloroflexi* (55%) in Sludge A1 could have also resulted in a higher surface negativity (zeta-potential= -19.8 mV), and a lower EPS_{sp}/EPS_s ratio.

Analysis using the indicpecies package in R (v.3.1.1) revealed that about 74% of the total microbial population in Sludge A1 were unique, whereas only about 32% of the total microbial population in Sludge K were unique. Phylogenetic relationships between the indicator species are presented in Figure 5 and the closest relatives are summarised in Table 3. Among the microbial population uniquely detected in Sludge A1, about 66% were closely-related to *Chloroflexi*. Polysaccharide degradation and PHA storage capability among filamentous *Chloroflexi* in activated sludge samples have been observed by ecophysiology study conducted by Kragelund et al [31]. Other taxa with carbon storage ability were also among the OTUs unique to Sludge A1, these include *Thiobacillus* sp., *Xanthobacter* sp. (PHA storage), and members of *Actinobacteria*: *Mycobacterium* sp. and *Nakamurella* sp (TAG storage). Ability to store carbon in the form of TAG is widespread among *Actinobacteria* [12]. Besides, annotation of *Mycobacterium canettii* genome, a member of *Actinobacteria*, revealed a putative gene for beta-ketothiolase (accession no. G0TN44), the enzyme involved in the initial stage of PHA-synthesis. PHA synthase had been reported in *Nakamurella multipartite* (Genbank accession no.C8X953). In total, about 79% of the OTUs uniquely detected in Sludge A1, but not in Sludge K, were closely-related to taxa with potential of carbon storage. On the contrary, only about 55% of the OTUs unique to Sludge K were closely-related to taxa with potential carbon storage ability: eg. *Chloroflexi*, *Accumulibacter* sp., *Dechloromonas* sp., *Nitrobacter* sp. [32],

and *Lysobacter* sp. [33]. Besides, two OTUs (K-OTU11259 & K-OTU882) were closely-related to sequences recovered from PHA storage community (accession no. AF204243 & AB515764). Since baseline Sludge K was collected from the aeration tank of a full scale plant in which carbon source was always present, the enrichment of carbon storage communities was not anticipated.

3.6 General Discussion

When subjected to SW1 under loadings 0.1 and 0.5 g COD/g SS, the overall capacity of Sludge A1 was approximately 90-fold higher than the baseline Sludge K in terms of COD_T removal (Table 1). In terms of COD fractions, much of the COD sorbed by Sludge K was from the colloidal fraction. Under loading 0.1 g COD/g SS, 6.44 and 8.98 mg COD/g SS of colloidal fraction were sorbed by Sludge K in 30 min and 60 min contact time, respectively (Table S1, Supplementary Material). These values were only about 20% of the colloidal fraction sorbed by Sludge A1 under the same condition. The COD_C sorbed by Sludge K improved significantly when the loading was increased to 0.5 g COD/g SS, and was then 22.3 mg COD/g SS - comparable with Sludge A1 at 30 min contact time (28.8 mg COD/g SS, Table S1). The smaller floc size (115 µm) in Sludge K, would have resulted in a larger surface area per unit volume but this had apparently not provided Sludge K significant advantage in sorbing COD_C. A possible reason for this could be Sludge A1's floc structure, which had an abundance of filamentous bacteria extending from the main body of the floc (Figure S6, Supplementary Material). These filamentous bacteria could have provided a larger effective surface area for sorbing COD_C. There was minimal contribution from CODs removal towards the biosorption capacity in Sludge K, suggesting insignificant carbon storage activity. In addition, desorption of the CODs into the mixed liquor was observed in Sludge K at loading 0.5 g COD/g SS, leading to negative sorption capacity recorded - at about -20 mg COD/g SS (Table S1). The release of CODs could also be caused by the hydrolysis of COD_C sorbed by Sludge K at higher loading. In contrast, Sludge A1 biosorption capacity contributed by CODs removal was 37.1 to 164.0 mg COD/g SS, significantly higher than Sludge K under all conditions.

The biosorption capacities in terms of COD_T or the various COD fractions showed limited correlation with total EPS content and surface charge. Sludge K had the highest total EPS (126.0 mg/g SS) but the lowest removal of organics. Also, the small difference in surface negativity did not show good correlation with the difference in biosorption capacities between Sludge A1 and Sludge K.

As the synthetic wastewater used in this study was formulated to contain colloidal (ca. 40%) and soluble COD, it was envisaged that carbon entrapment by the EPS would play a less important role in carbon capture. It was reported that significant carbon storage capacity, in the form of PHA, was especially observed in activated sludge cultivated in feast-famine condition, with alternating high and

low organic loading [34]. As both the surface of *Chloroflexi* and starch were hydrophilic [31], capture of COD_c increased with organic loading (Figure 1). In addition, the filamentous *Chloroflexi* in Sludge A1 provided more surface to sorb the COD_c (starch), which was partially degraded into simpler form (CODs) and diffused into the floc and captured by other groups through carbon storage. However, the kinetics data showed that in Sludge A1 intraparticle diffusion could still be one of the rate-limiting steps under all loadings (Section 3.2.3). The PHA storage capability of *Chloroflexi* (relative abundance 55% in Sludge A1) could then have also contributed to the higher overall capacity in Sludge A1 compared to baseline Sludge K.

For Sludge A1, the overall capacity ($Q_{T,30}$ and $Q_{T,60}$) was highly correlated with the carbon storage capacity regardless of contact time (Pearson coefficient = 0.996 and 0.992, Table S2). This suggested that carbon storage could be the more dominant mechanism. In contrast, Dionisi et al [35] reported anoxic batch tests results had shown that as organic loading increased, the carbon storage capability of mixed culture decreased. The high correlation between the biosorption capacity in terms of CODs ($Q_{S,30}$ and $Q_{S,60}$) and the carbon storage capacity (Pearson coefficient: 0.969 and 0.900, Table S2) suggested the possibility that CODs was captured through carbon storage. On the other hand, while it was envisaged that COD_c was captured through surface sorption, the removal of COD_c (e.g. 126.5 mg COD/g SS under loading 1.0 g COD/g SS, Figure 1a) was higher than the portion attributed to surface sorption (e.g. 39.4 mg COD/g SS, Figure 1b) under the same loading. Besides, the COD_c biosorption capacity ($Q_{C,30}$ and $Q_{C,60}$) showed low negative correlation with the surface sorption capacity (Pearson coefficient = -0.491 and -0.285, Table S2). Thus, it was clear that surface sorption acted as the first step but not necessarily the final step of carbon capture biosorption in Sludge A1. In addition, the starch used in SW1 to simulate colloidal COD could be degraded easily. This suggested that for easily-degradable COD_c, surface sorption was a preliminary step preceding hydrolysis of COD_c [2], before part of the sorbed COD_c was transported into the cell for carbon storage.

As carbon storage is a non-equilibrium process [9], carbon capture through this mechanism is more stable. On the contrary, desorption can easily occur for materials sorbed through surface sorption. In a recent study by Huda et al [36], it was reported methane production by anaerobic digestion using excess sludge as the substrate could be improved by using sludge in which PHA was accumulated. However, it is reckoned that one potential limitation of using acclimated biomass for energy recovery is its slower growth rate due to the low F/M and feast-famine conditions. The biomass yields of the acclimated Sludge A1 was in the range of 0.14-0.2 g biomass/g COD, which was only 50% that of aerobic heterotrophs at 0.36 g biomass/g COD [37]. Another factor to be taken into consideration for the process economics would be the carbon mineralization during the acclimation process. In real application, the acclimation can be carried out in a side stream and the acclimated sludge is then

dosed into the contact tank for carbon capture. Another alternative would be to operate the activated sludge-based treatment facilities at WWTP to encourage carbon storage, with at least partial-enrichment. In addition, while easily-degradable starch was used to simulate COD_c in this study, future studies on not-easily-degraded colloid systems could provide more insights on whether carbon storage would be the subsequent step following surface sorption of such colloid systems in CCB process.

5. Conclusions

By acclimating sludge under low F/M ratio through feast-famine cycle, groups of microorganisms with carbon storage ability were selectively enriched. The acclimated sludge showed an enhanced biosorption performance when subjected to organic loading as high as 1.0 g COD/g SS. CODs removal was the more predominant mechanism in the first 20 to 30 min of CCB process, regardless of the loading applied. Therefore for acclimated sludge with enhanced CCB capacity towards CODs, the HRT of the contact tank is to be within 30 min in order to maximize CCB through carbon storage. The possible rate limiting steps in CCB of CODs could include chemisorption and/or intraparticle diffusion.

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Figure 1.

Figure 2.

Figure 3.

Figure 4.

Figure 5a.

Figure 5b.

Table 1.

Parameters	Sludge A1	Sludge K ^a
Particle size, d ₅₀ (µm)	441	115
Zeta-potential (mV) @ pH7	-19.6	-16.8
Total EPS (mg/g SS)	93.3±9.7	126.0±26.4
EPS _p /EPS _s ^b	2.06	3.00
<i>Biosorption performance towards SW1</i>		
Overall capacity ^c (mg COD _T /g SS)		
O.L. 0.1, Q _{T,30} ^f	74.3 ± 19.5	7.33 ^d
Q _{T,60} ^e	89.7 ± 18.3	11.8 ^d
O.L. 0.5, Q _{T,30} ^h	156.8 ± 37.6	-2.74 ^d
Q _{T,60} ^g	242.3 ± 84.2	2.75 ^d

(a): baseline sludge, sludge from aeration tank of local water reclamation plant;

(b): EPSs, polysaccharide; EPS_p, protein;

(c) subjected to synthetic wastewater (SW1) under organic loading (O.L.) 0.1 and 0.5 g COD/g SS, and reported in terms of 30 and 60 min contact time (Q_{T,30} and Q_{T,60}, respectively); (d) n = 1;

F/M equivalent: (e) 2.4 g COD/(g SS.day); (f) 4.8 g COD/(g SS.day);

(g) 12 g COD/(g SS.day); (h) 24 g COD/(g SS.day).

Table 2.

Organic loading	$Q_{se, exp}$	Pseudo first-order kinetic			Pseudo second-order kinetic			Film mass transfer ^a		Intraparticle diffusion		
		k_1	$Q_{se, cal}$	R^2	k_2	$Q_{se, cal}$	R^2	k_f	R^2	k_p	A_p	R^2
0.1	50.42	0.0327	42.25	0.9334	7.81×10^{-4}	60.24	0.9541	0.0053	0.9431	4.77	6.33	0.9399
0.5	120.61	0.0228	75.27	0.8840	5.29×10^{-4}	133.33	0.9637	0.0015	0.8135	14.33 ^b	10.15 ^b	0.9203 ^b
1.0	183.33	0.0273	129.27	0.8261	2.78×10^{-4}	204.81	0.9617	0.0009	0.7062	21.41 ^b	15.41 ^b	0.8952 ^b

Unit: organic loading (g COD/g SS); $Q_{se, exp}$, $Q_{se, cal}$ (mg COD/g SS). (a) determined by $-\log(C/C_0)$ versus t plot (Figure S5, Supplementary Material); (b) determined from the initial linear portion of $Q_{s,t}$ versus $t^{0.5}$ plot.

Table 3.

OTU ^a	Abundance (reads)	Phylum	Phylogenetic relationship	Accession no.	Similarity (%)
			Closest relative		
A1-OTU2441 ^b	4375	<i>Chloroflexi</i>	Uncultured <i>Caldilinea</i> sp. clone CFX153	HQ262529	96
A1-OTU2888 ^b	3994	<i>Chloroflexi</i>	Uncultured bacterium clone PAE-EN23_17	KC238414	100
A1-OTU2437 ^b	409	<i>Chloroflexi</i>	Uncultured bacterium clone ncd557a11c1	HM278533	93
A1-OTU3357 ^b	640	<i>Chloroflexi</i>	Uncultured <i>Chloroflexi</i> bacterium clone TDNP_Wbc97	FJ517062	98
A1-OTU9001 ^b	1250	<i>Chloroflexi</i>	Uncultured <i>Caldilinea</i> sp. clone CFX153	HQ262529	99
A1-OTU9513 ^b	617	<i>Chloroflexi</i>	Uncultured <i>Anaerolineae</i> bacterium clone RLBP1855	KC449700	97
A1-OTU5357 ^b	446	<i>Chloroflexi</i>	Uncultured bacterium clone mesophilic_alkaline-76	GU455191	99
A1-OTU7945	1229	<i>Acidobacteria</i>	Uncultured <i>Acidobacteria</i> bacterium clone FII-TR131	JQ579926	97
A1-OTU2435	235	<i>Acidobacteria</i>	Uncultured bacterium clone Exudates_32_non-tox-D12	HQ856368	100
A1-OTU1981 ^b	78	<i>Actinobacteria</i>	<i>Nakamurella multipartita</i> DSM 44233	CP001737	98
A1-OTU8998 ^b	670	<i>Actinobacteria</i>	<i>Mycobacterium</i> sp. DSM 3803	AY147261	99
A1-OTU2895	432	<i>Nitrospira</i>	<i>Nitrospira</i> sp. enrichment culture clone 1	KJ598609	98
A1-OTU10415	461	<i>Firmicutes</i>	<i>Lactococcus</i> sp. YM05001	EU689103	100
A1-OTU8995	301	TM 7	Uncultured candidate division TM7 bacterium clone S5B_99	JQ433793	98
A1-OTU2439 ^b	299	<i>α-Proteobacteria</i>	Uncultured <i>Rhodomicrobium</i> sp. partial 16S rRNA gene, clone CL2.C35	FM175375	100
A1-OTU10411 ^b	688	<i>α-Proteobacteria</i>	Uncultured <i>Xanthobacter</i> sp. clone MFC63G08	FJ823934	99
A1-OTU2887 ^b	317	<i>β-Proteobacteria</i>	Uncultured bacterium clone SludgeG_bottom_39	AB516185	98
A1-OTU3351 ^b	261	<i>β-Proteobacteria</i>	Uncultured <i>Thiobacillus</i> sp. clone ENR06	FJ536941	99
A1-OTU9056	334	<i>β-Proteobacteria</i>	Uncultured bacterium clone B152	HQ640614	99
A1-OUT3354	397	<i>γ-Proteobacteria</i>	<i>Dokdonella kunshanensis</i> strain DC-3	JQ341904	98
A1-OTU9516	267	<i>γ-Proteobacteria</i>	Uncultured bacterium clone 1505b PN-3	JQ766317	98
Total abundance (reads)	17700				

Note (a): operational taxonomic unit; (b): potential carbon storage ability

Table 3. (cont.)

OTU ^a	Abundance (reads)	Phylum	Phylogenetic relationship	Accession no.	Similarity (%)
			Closest relative		
K-OTU12069 ^b	375	<i>Chloroflexi</i>	Uncultured <i>Chloroflexi</i> bacterium clone HUY-H05	AB638624	98
K-OTU8094 ^b	1128	<i>Chloroflexi</i>	Uncultured bacterium clone MBB1_495	JQ985237	96
K-OTU3766	286	<i>Acidobacteria</i>	Uncultured <i>Acidobacteria</i> bacterium clone M07A002	AB604792	99
K-OTU2382	246	<i>Acidobacteria</i>	<i>Geothrix fermentans</i> strain HradG1	HF559181	99
K-OTU11042	222	<i>Acidobacteria</i>	Uncultured bacterium clone EDBAC04G09	JF709383	98
K-OTU3416	531	α - <i>Proteobacteria</i>	Uncultured bacterium clone MD01d3_6748	JQ384677	99
K-OTU6493 ^b	291	α - <i>Proteobacteria</i>	<i>Nitrobacter</i> sp. 219	AM286375	100
K-OTU1767 ^b	521	β - <i>Proteobacteria</i>	Uncultured Candidatus <i>Accumulibacter</i> sp. clone 5.17h7	JN679162	99
K-OTU5544 ^b	330	β - <i>Proteobacteria</i>	<i>Dechloromonas</i> sp. JJ	AY032611	99
K-OTU11259 ^b	231	β - <i>Proteobacteria</i>	Uncultured beta proteobacterium GC24	AF204243	100
K-OTU3251	432	β - <i>Proteobacteria</i>	Uncultured <i>Rhodocyclaceae</i> bacterium clone 3.29h30	JN679082	99
K-OTU8824 ^b	246	β - <i>Proteobacteria</i>	Uncultured bacterium clone SludgeF_bottom_92	AB515764	100
K-OTU100778 ^b	838	γ - <i>Proteobacteria</i>	Uncultured bacterium clone GD 1-106	KC551584	100
K-OTU1686 ^b	224	γ - <i>Proteobacteria</i>	<i>Lysobacter</i> sp. CC-Bw-6	KC820654	100
K-OTU1588	458	<i>Bacteroidetes</i>	Uncultured bacterium clone a-100	JX040393	99
K-OTU2052	306	<i>Bacteroidetes</i>	Uncultured <i>Bacteroidetes</i> bacterium	CU923678	97
K-OTU11766	290	<i>Bacteroidetes</i>	Uncultured <i>Bacteroidetes</i> bacterium clone XH12	JQ861365	97
K-OTU11129	475	<i>Bacteroidetes</i>	Uncultured bacterium clone N1_4_272	JQ116086	100
K-OTU3421	238	<i>Nitrospira</i>	Candidatus <i>Nitrospira defluvii</i> clone B14	GQ249372	100
Total abundance (reads)	7668				

Note (a): operational taxonomic unit; (b): potential carbon storage ability

Supplementary Material

Figure S1. Evolution of residual COD total fraction, under organic loadings (O.L.) 0.1, 0.5, 1.0, and 2.5 g COD/g SS, pH 7

Figure S2. Biosorption capacity of Sludge A1, in terms of dissolved COD, under organic loadings 0.1, 0.5, 1.0, and 2.5 g COD/g SS, pH 7, 60 min contact time.

Figure S3. Effect of organic loading towards the specific colloids removal rate of live Sludge A1.

Figure S4. Evolution of residual COD total, colloidal and dissolved fractions, under organic loading 2.5 g COD/g SS, pH 7

Figure S5. Variation of $-\log(C/C_0)$ with contact time t . O.L.: organic loading

Figure S6. Phase contrast micrographs of (a) Sludge A1; (b) Sludge K. Scale bars represent 100 μm

Table S1a. Comparison of biosorption capacities (mg COD/g SS) of Sludge A1 and baseline Sludge K, in terms of total (Q_T), colloidal (Q_C) and dissolved (Q_S) fractions, under loading 0.1 and 0.5 g COD/g SS in 30 min contact time

Organic loading, g COD/g SS	Sludge A1 ^a			Sludge K ^b		
	$Q_{T, 30}$	$Q_{S, 30}$	$Q_{C, 30}$	$Q_{T, 30}$	$Q_{S, 30}$	$Q_{C, 30}$
0.1 ^c	74.3 ±19.5	37.1 ±10.9	37.2 ±13.0	7.33	0.88	6.44
0.5 ^d	156.8 ±37.6	128.0 ±53.2	28.8 ±16.8	-2.74	-25.0	22.3

Note: (a) n = 3; (b) n = 1;

F/M equivalent: (c) 4.8 g COD/(g SS.day); (d) 24 g COD/(g SS.day)

Table S1b. Comparison of biosorption capacities (mg COD/g SS) of Sludge A1 and baseline Sludge K, in terms of total (Q_T), colloidal (Q_C) and dissolved (Q_S) fractions, under loading 0.1 and 0.5 g COD/g SS in 60 min contact time

Organic loading, g COD/g SS	Sludge A1 ^a			Sludge K ^b		
	$Q_{T, 60}$	$Q_{S, 60}$	$Q_{C, 60}$	$Q_{T, 60}$	$Q_{S, 60}$	$Q_{C, 60}$
0.1 ^c	89.7 ±18.3	49.7 ±4.6	40.0 ±14.6	11.8	2.82	8.98
0.5 ^d	242.3 ±84.2	164.0 ±85.9	78.3 ±1.7	2.74	-21.1	23.8

Note: (a) n = 3; (b) n = 1;

F/M equivalent: (c) 2.4 g COD/(g SS.day); (d) 12 g COD/(g SS.day)

Table S2. Pearson correlation coefficient^a (r) between biosorption capacities, in terms of total (Q_T), colloidal (Q_C) and dissolved (Q_S) fractions, versus surface sorption and carbon storage capacity in 30 and 60 min contact time

r	$Q_{C, t}$	$Q_{S, t}$	Surface sorption capacity, t	Carbon storage capacity, t
$Q_{T, t=30min}$	0.159	0.987	-0.938	0.996
$Q_{T, t=60min}$	0.895	0.948	0.172	0.992
$Q_{C, t=30min}$	-	-0.003	-0.491	0.246
$Q_{C, t=60min}$	-	0.706	-0.285	0.944
$Q_{S, t=30min}$	-	-	-0.870	0.969
$Q_{S, t=60min}$	-	-	0.478	0.900

Note (a): r was calculated base on results obtained under organic loadings 0.1, 0.5, and 1.0 g COD/g SS.