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Enhanced Carbon Capture Biosorption through Process Manipulation

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Abstract

The feasibility of manipulating operating parameters, i.e. Food-to-microorganisms (F/M) ratio, SRT, and residual DO, to enhance biosorption performance was investigated. It was observed that lower F/M and longer SRT resulted in sludges which captured carbon mainly through carbon storage. Surface sorption, however, was the dominant mechanism for sludges grown under the higher DO condition. Generally, biosorption was optimal at pH 7. Sorption kinetic studies revealed that sludge cultivated under the low F/M ratio of 0.15 (Sludge S1) showed the best overall biosorption performance. Determination of calorific value revealed that Sludge S1 was able to capture energy as much as 0.9 kJ/g SS within 15 min contact time. About 66.3% of the overall biosorption capacity was attributed to carbon storage. Sludge S1 was able to accumulate organic substrate and stored this as polyhydroxyalkanoates (PHA). Culture-independent microbial community analysis through DGGE revealed the presence of strains capable of PHA-accumulation, e.g. *Rhodobacter* sp., and *Thauera* sp. While different dominating mechanisms resulted from different cultivation conditions, the best biosorption performance was significantly contributed by carbon storage activity.

1. Introduction

Current activated sludge-based operations in conventional wastewater treatment systems are considered economically and environmentally unsustainable due to the energy consumed (Sheik et al., 2014). The increasing concern over carbon footprints and global warming has led to more interest in energy reduction in wastewater treatment. The potential energy contained in raw municipal wastewater was reported to possibly exceed the energy requirements at a wastewater treatment facility (Shizas and Bagley, 2004).

Owing to its low cost and high efficiency, the usage of various types of biosorbent for the removal of toxic pollutants or for the recovery of valuable resources from wastewaters has been gaining momentum. Using sludge biomass as the sorbent, biosorption has been studied by numerous researchers but mainly focused on heavy metal and dye sorption (Park et al., 2010; Vijayaraghavan and Yun, 2008). Biosorption is generally accepted as a passive process (Chojnacka, 2010), which is affected by factors which include *pH*, *biosorbent (floc) size*, *biosorbent dosage*, *initial pollutant*

concentration, ionic strength, and temperature (Park, et al., 2010). The mechanisms involved in adsorptions of metal ions by biosorbent, as reviewed by Tsezos et al. (Tsezos et al., 2006), are passive and non-metabolic. As a generalization, the two possible major mechanisms involved are (i) ion exchange: cation-binding with ionisable functional; and (ii) complexation: complex formation of metal ions with organic molecules involving ligand centers in the organic species. Complexation may be electrostatic or covalent (Tsezos, et al., 2006).

In the context of energy recovery from wastewater influent, biosorption is described as the phenomenon where biomasses concentrate carbon substrates from the wastewater in a rapid manner (Guellil et al., 2001; Yu et al., 2009). This phenomenon is hereafter denoted as “*carbon capture biosorption*” (CCB). Only a few researchers have studied the CCB performance of activated sludge in terms of energy recovery. Both Guellil et al. (2001) and Yu et al. (Yu, et al., 2009) concluded that CCB in activated sludge involves only passive uptake (surface sorption). Whereas later study by Xiao et al. (Xiao et al., 2011) reported that CCB consisted of metabolically-mediated biological and physical-chemical components. This conclusion indicated surface sorption could be the first step but not necessarily the last step involved in CCB. Review of the literature would suggest possibility of enhancement of CCB performance by manipulating operating parameters, e.g. *Food-to-microorganisms (F/M)* ratio, Dissolved Oxygen (DO) level and Sludge Retention Time (SRT), as well as the consequent dominant mechanisms involved has yet to be investigated and reported.

Li et al. (2011) reported that, while low F/M ratio favors the growth of smaller granules, larger size granules appear in the system when high F/M ratio was applied during later stage of sludge granulation. It is envisaged that under low F/M ratio, a smaller floc size would facilitate uptake in low substrate environment. DO levels could also affect the growth pattern of the sludge (Takács and Fleit, 1995). Low DO environment leads to growth of filamentous bacteria, whereas high DO condition increases risk of fungi growth which would then compete with the bacterial species for the organic carbon (Li et al., 2010). Difference in the floc size and microbial community structure would result in different surface areas and this can have *crucial effect on CCB*. *With more available surface area, biomass tended to have better CCB capacity* (Kopac and Bozgeyik, 2010). Longer SRT leads to a denser and more compact floc structure, while in contrast, shorter SRT usually results in dispersed growth and fine flocs (Liao et al., 2006). On the other hand, Wang et al. (2006) suggested that SRT could affect the biomass’s *extracellular* polymeric substances (EPS) production. More EPS is produced at longer SRTs to improve floc aggregation. EPS acts like a glue-gel, entrapping particles. Ionic strength of the biomass, e.g. surface charge, could also affect CCB to certain extent. The

electron attraction or repulsion force between the sorbate (e.g. metal ion or negatively-charged colloids) and the biomass affects the CCB performance. In addition, Wilén et al. (2003) pointed out that the ionized group from the EPS of biomass could change the surface charge of the biomass.

The surface charge of microbial cells, EPS and sludge flocs originates from dissociation or protonation of carboxyl, phosphate and amino groups, and consequently depends on pH. Flocs and EPS of all activated sludge carry a net negative charge, mostly within the range of zeta potential -20 to -30 mV (H. Liu and Fang, 2002), due to the protonation of the anionic functional groups, such as carboxylic and phosphate. Zeta potential, which represents the potential drop in the diffuse double layer on the surface, is measured based on the electrophoretic mobility in an electric field. However, since sludge surface are inherently heterogeneous, whereas measurement of the electrophoretic mobility only yields an average value of the surface charge, local differences can be expected (Poortinga et al., 2002). Positively charged domains containing lysyl, histidyl and arginyl side chains, would result in localized positive charge on the cell surface (Tsezos, et al., 2006).

Polyhydroxyalkanoates (PHAs) is one of the most common carbon storage polymers among prokaryotic organisms, including species of both Bacteria and Archaea. PHAs are synthesized classically under unfavorable growth conditions due to imbalanced nutrient supply (Madigan et al., 2009). Such conditions result from an excess of carbon and energy-source coupled with a depletion of growth-essential substrates like nitrogen, phosphate, or dissolved oxygen (Koller et al., 2013). When conditions warrant PHAs are then broken down for biosynthesis or ATP production (Madigan, et al., 2009). Studies on carbon substrate uptake by PHA-accumulators, using acetate as the model substrate, showed that acetate uptake involved metabolically-mediated transport of acetate by the enzyme acetate permease, a symporter than transfer acetate together with proton. The efficiency of the enzyme relies on the membrane potential. Membrane potential is the difference in electric potential between the interior and the exterior of a cell (McMahon et al., 2010).

In this study, “*carbon capture biosorption*” (CCB) is defined as the phenomenon of carbon capture and concentration within the sludge floc. The CCB process could involve (i) surface sorption: passive uptake of organics through physical-chemical interactions; (ii) carbon storage: metabolically-mediated transport and accumulation of sorbed material or dissolved organic compounds within the cell; (iii) carbon entrapment: entrapment of larger particles in the open structure of the sludge floc, likely to be facilitated by the production of extracellular polymeric substances. *The application of CCB in the wastewater, and the possible ways to enhance CCB performance were investigated. The*

objectives of this study include (i) investigation on the effect of operation parameters, e.g. F/M ratio, DO level and SRT, on enhancing CCB performance; (ii) investigation on the effect of pH on biosorption performance; and (iii) elucidation of the mechanisms involved in CCB.

2. Materials and Methods

2.1 Sludge acclimation

Seed sludge was collected from a local water reclamation plant. Sludge acclimations were carried out through feast-famine cycle by employing a modified SBR with 6 stages: (i) 3 min fill (feast); (ii) 10 min contact (biosorption); (iii) 5 hrs react (famine); (iv) 40 min settling; (v) 6 min discharge; and (vi) 1 min idle. The total cycle time was 6 hrs, with a working volume of 3L, and a HRT of 12 hrs. Table 1 shows the six conditions for acclimating sludge. Conditions S1 and S2 were meant to investigate the effect of Food-to-microorganisms (F/M) ratio; conditions D1 and D2 to look into the effect of different Dissolved Oxygen (DO) level; whereas condition R1 and R2 look into the effect of different Sludge Retention Time (SRT). The reactors were operated within the pH range of pH6 to pH 8.

The bioreactors were fed with synthetic wastewater comprising 200 mg/L glucose, 358 mg/L Na-acetate.3H₂O, 200 mg/L starch (soluble), 125 mg/L urea, 100 mg/L NH₄Cl, 23.5 mg/L KH₂PO₄, resulting in a total COD of 600 mg /L. The mixture was supplemented with a trace elements solution (Smolders et al., 1994). Allylthiourea (ATU) was also added at 10 mg/L to inhibit nitrification. Bioreactor performance was monitored based on the residual COD over a SBR cycle and the final discharge quality. When the reactors reached pseudo steady state, sorption capacity of the sludge was determined as described in the following section.

2.2 Determination of Carbon Capture Biosorption Capacity

Mixed liquor (100 ml) was collected from the bioreactor at the end of the aerobic phase and allowed to settle for 30 min to achieve liquid-solids separation. 50 ml supernatant would then be decanted, and the remaining 50 ml mixed liquor transferred to a 100 ml Erlenmeyer flask. Synthetic wastewater (50 ml) was then quickly added into the flask to initiate the experiment (batch test). The duration of batch tests was 15 min as preliminary experiments revealed that carbon removal would reach steady-state within such period. Mixed liquor samples (3 ml each) would be withdrawn from the flask at the following time intervals: 10 sec, 2 min, 4 min, 6 min, 8 min, 10 min and 15 min. The samples were centrifuged at 10,000 rpm to separate the solids from the liquid. Residual COD in the supernatant was then determined. The effect of pH on biosorption performance would be investigated by conducting batch tests at pH 5, 6, 7, 8 and 9. All the batch tests were conducted in triplicates, under

organic loading of 0.1 g COD/g SS. The carbon capture capacity of the biomass was determined by the difference between initial COD and the residual COD at particular time intervals, expressed in mg COD/g SS, and denoted as the “overall capacity”.

2.3 Quantification of Individual Mechanisms

Inactivated sludge was used to obtain the baseline for surface sorption. The sludge was inactivated through sodium azide inhibition. Sodium azide was added at 0.2 g/g SS (Barbot et al., 2010) followed by 3 hours incubation. The sludges were then washed with standard phosphate buffer solution and immediately used for batch tests. Capacity of the inactivated sludge, determined through batch tests, was assumed to be attributed solely to surface sorption. On the other hand, since the capacity of the live sludge is attributed to both surface sorption and carbon storage activity, any difference in the capacity between live and inhibited sludge was assumed to be attributed to carbon storage activity.

2.4 Analytical Methods

Floc size was determined with a Mastersizer 2000 Particle Size Analyzer (Malvern Instruments). The *extracellular polymeric substances (EPS)* were extracted with the method reported in Liu et al. (2002), and were analyzed by investigating two major components, polysaccharides and proteins. The EPS polysaccharide fraction (EPSs) was determined with the phenol sulfuric acid assay. The EPS protein fraction (EPSp) was determined using the Pierce® BCA Protein Assay Kit (Thermo Scientific). Biomass calorific value was measured with a calorimeter (IKA C200). PHA contained in biomass was stained with the Sudan black B method and viewed with phase contrast microscopy. Biomass surface charge was measured in terms of its zeta potential with a Zetasizer Nano ZS (Malvern Instruments). COD was determined in accordance with the standard method [24].

2.5 Microbial Community Analysis by DGGE

The microbial communities in selected sludges were analyzed using Denatured Gradient Gel Electrophoresis (DGGE) with a denaturing gradient of 30–60% urea-formamide. The prominent bands were excised, and DNA was purified and cloned as reported by Lim et al. (2011). Insert-harboring clones were screened according to their restriction fragment length polymorphism (RFLP) patterns and selected for sequencing. The 16S-PCR amplicons were digested with Mbo I and Afa I (Rsa I) (Takara, Japan) to estimate the origins of the amplicons based on the electrophoresis patterns of the digested fragments. The sequences obtained in this study have been deposited in GenBank under accession numbers KM107810-KM107821.

3. Results and Discussion

3.1 Sludge Characteristics

Table 2 shows the sludge characteristics obtained under different operating conditions. All the sludges except Sludge D2 showed adequate settling ability, with SVIs ranging from 47 to 92 mL/g. Whereas Sludge D2 had a SVI value of 187, indicating the potential issue of sludge bulking in actual operation. Sludge S1 (low F/M ratio) produced the highest EPS at 150.7 mg/g VSS, followed by Sludge D1 (101.1 mg/g VSS) and S2 (98.5mg/g VSS). EPS content in Sludge R1, R2, and D2 were only in the range of 37.8 to 66.7 mg/g VSS, less than 50% of Sludge S1's content. However, the synthetic wastewater employed in this research study had mainly dissolved and colloid substrates. Given such circumstance, biomass EPS entrapment mechanism was anticipated to play a relatively less effective part in the overall CCB process in this instance.

As Sludge S1 was cultivated under substrate limiting condition (low F/M ratio), the smaller floc size might be due to the limited supply of nutrient. Smaller floc size resulted in higher surface area for substrate uptake/capture. Sludge S2, on the contrary, was cultivated under adequate supply of substrates and oxygen. Thus, Sludge S2 did not need to produce as much EPS to capture the substrates. For Sludge D1, oxygen was the limiting factor. But there were enough substrates in the wastewater and the SRT was long enough for the floc to aggregate. Therefore, Sludge D1 showed a relatively larger particle size of 245.0 μm . The floc size of Sludge R1 (d_{50} , 119.1 μm) was only half of Sludge R2. With an SRT twice as long as Sludge R1's, Sludge R2 was able to aggregate and formed larger flocs, and produced more EPS (57.99 mg/g VSS) compared to Sludge R1.

Sludge S1 and D2 had relatively smaller mean particle diameters (d_{50}) of 96.2 μm and 96.5 μm , resulting in higher specific surface areas potentially being available for surface sorption. On the contrary, Sludge D1 and R2 showed comparatively larger d_{50} of 245.0 μm and 242.6 μm , putting them at a disadvantage for surface sorption. The surface charge for the acclimated sludges were generally similar, ranging from -21.5 mV to -26.1 mV, more negatively-charged than baseline Sludge D2 (-13.6 mV). As the colloids in the feed were also negatively-charged, the repulsive force between the colloidal fraction and the sludge surface would be lesser in Sludge D2. A hypothesis is made here that by factoring in the specific surface area and the sludge surface charge, it is envisaged that Sludge D2 would show the highest surface sorption capacity among the acclimated sludges.

3.2 Carbon Capture Biosorption Capacity

The condition in bioreactor D2 was chosen to simulate the condition in a conventional activated sludge process (Metcalf et al., 1991). Thus, Sludge D2 can be regarded as a baseline for the other acclimated sludges discussed in this study. Batch tests revealed that Sludge S1 had the highest overall capacity among the six acclimated sludges. Optimal pHs in terms of overall capacity were pH 7 (57.2 mg COD/g SS) and pH 8 (59.8 mg COD/g SS). On the contrary, baseline case Sludge D2 showed the lowest overall capacity (23.1 to 29.1 mg COD/g SS) under all pHs. At pH 5, all the sludges up-took the least organic substrate from the wastewater. Figure 1 shows the effect of pH on the overall capacity and the individual mechanisms.

Comparison of batch tests results between live and inactivated sludge revealed that the overall CCB is not only attributable to the surface sorption mechanism, but also to carbon storage activity (hereafter abbreviated as “storage activity”). Among the six acclimated sludges, baseline Sludge D2 delivered the highest surface sorption capacity (Figure 1b), ranging from 18.3 mg COD/g SS to 22.5 mg COD/g SS. This observation was consistent with the hypothesis in Section 3.1. As shown in Table 2, the larger surface area of Sludge D2 (0.138 m²/g), led to better surface sorption (Kopac and Bozgeyik, 2010). Although the surface area of Sludge S1 and D2, as well as S2 and R1 were of similar order, only baseline Sludge D2 was more substantially lower negatively-charged (zeta potential, -13.6 mV). Zeta potential measurement revealed that the colloids in the synthetic wastewater were mild negatively-charged, in the range of -3.0 to -5.0 mV. Thus, there was less repulsive force between the colloidal fraction and the surface of Sludge D2, resulting in a better surface sorption performance. Nevertheless, despite the higher surface sorption capacity observed in baseline Sludge D2, its lower storage capacity disadvantaged it with respect to overall CCB performance.

It was observed that storage activity was sensitive to extreme pHs (Figure 1c). Sludge S1 showed the highest storage capacity at 35.3 to 37.6 mg COD/g SS under optimal pH (pH 7 and 8), compared to about 5.0 mg COD/g SS observed in baseline Sludge D2 under the same pHs. The storage capacity of Sludge S1 decreased significantly - by at least 30% at pH 5, 6 & 9. In fact, Chong et al. (1997) reported that extreme pH condition could affect bioactivity of the bacteria. One possible explanation could be that when under extreme pH conditions, bacteria would be under stress and shut down the communication channels to the outside environment temporarily. Therefore some of its activities (e.g. carbon storage) could be inhibited. Another possible reason could be the change in membrane potential under low pH. In neutral and near- neutral pH, acetate uptake is energized primarily by the membrane potential. In the case of GAO (glycogen-accumulating organisms), one group of PHA-

accumulator, acetate permease-mediated symport is energized by the membrane potential generated through the fumarate reductase system (consuming NADH, and producing NAD^+), as well as ATP synthase (McMahon, et al., 2010). Low pH leads to cytoplasmic acidification in microorganisms, and causes disruption of membrane potential and enzymatic reactions (Jeong et al., 2008). In order to restore to neutral or near-neutral pH, the fumarate reductase system has to work harder and consequently more NAD^+ is being generated. High amounts of NAD^+ inhibit the enzymes involved in PHA synthesis, e.g. 3-ketohiolase and acetoacetyl-CoA reductase (Koller, et al., 2013), resulted in low storage activity. Whereas under higher pH (pH 9), the excess hydroxide ions cause the acetate uptake to be more difficult due to lack of proton to establish enough membrane potential. Consequently, the storage activity decreases significantly compared to optimal pH 7 and 8 (Figure 1c).

At pH 7 (Figure 2) and pH 8, about 60% of Sludge S1's overall capacity was attributed to carbon storage. Sludge S1 was cultivated not only through the feast-famine cycle but also under low F/M ratio of 0.15 and long SRT of 30 days, which had preferentially enriched for a microbial community with storage activity, e.g. PHA-accumulators. Sudan black B staining confirmed storage of PHA within the microbial cells of Sludge S1 after the biosorption experiment. The increase in inclusion bodies stained black compared with the baseline (data not shown) indicated the accumulation of carbon in the form of PHA. The mean microbial life-time in the reactor would be determined by the SRT, and this has impact on the microbial population make-up. Studies done by Van Aalst Leeuwen et al. (Leeuwen et al., 1997) reported that faster growing organisms show less storage activity. This explained the lower storage activity observed among five of the sludges (Sludge S2, D1, D2, R1, and R2), which were cultivated under higher loading, and shorter SRT. On the contrary, results obtained by Chua et al. (2003) suggested that shorter SRT may select microbial community with higher storage capacity than that selected under longer SRT.

Among the six acclimated sludges, Sludge S1 was able to capture the most energy in terms of calorific value, e.g. 0.86 kJ/g at pH 7 and 0.91 kJ/g at pH 8, 15 min contact time. Whereas baseline Sludge D2 showed the lowest increase in calorific value (0.41 kJ/g to 0.47 kJ/g) regardless of pH condition (Figure 3). This trend is consistent with the batch tests result which showed that Sludge S1 had the highest overall biosorption capacity, and baseline Sludge D2 had the lowest overall biosorption capacity (Figure 1a). The overall energy captured [kJ/g SS] correlated well with the overall biosorption capacity [mg COD/g SS] and the storage capacity determined through batch tests, with Pearson coefficient of 0.918 and 0.933 respectively (Figure 4a). However, the overall energy

captured correlated poorly with the surface sorption capacity (Pearson coefficient -0.254, Figure 4b). In addition, the overall capacity correlated well with the storage capacity (Pearson coefficient 0.938, Figure 5a) but correlated poorly with surface sorption capacity (Figure 5b). This suggested carbon storage could be the more stable mechanism, rather than surface sorption, for capturing energy from wastewater containing significant amounts of soluble COD.

3.3 Correlation of Operating Parameters with Sludge Properties and Capacity

3.3.1 Sludge Retention Time

Table 3 summarizes the Pearson correlation coefficients between process operating parameters with sludge properties and capacities. SRT correlated positively with total EPS produced (Pearson coefficient 0.940). Sludge grown under longer SRT produced more amounts of total EPS, which is consistent what was reported by Wang et al. (2006). When higher amount of total EPS was produced, the EPS was able to entrap and sorb the colloids in the synthetic wastewater used, resulted in higher surface sorption (Pearson coefficient 0.568) and carbon storage (Pearson coefficient 0.682). The concurrent positive effect of higher total EPS on both of the mechanisms also produced a higher overall capacity (Pearson coefficient 0.755). In contrast, SRT only correlated weakly with SVI, floc size (d_{50}) and surface charge (zeta potential), with Pearson coefficient -0.202, -0.380, -0.129, respectively.

3.3.2 Surface Charge and Floc Size

Kara et al (2008) reported that as the floc surface became more negatively charged, the floc became more fragile. This trend is expected as higher negativity of the surface would cause higher electrostatic repulsion between the floc components. On the other hand, if zeta-potential higher than $\pm 30\text{mV}$, long term stability is typical observed and flocculation will be less (Mehta et al., 2011). Besides, the authors' experiences were also consistent with this information, in which acclimated sludge with surface charge of -30mV had difficulty to form flocs or flocculation only happened intermittently (data not shown). If the zeta potential of the acclimated sludges is more than $\pm 30\text{mV}$, the floc size would then be significantly affected by zeta-potential. Since the zeta-potential of the acclimated sludges reported in this study are in the range of -13.6 mV to -26.1 mV . The effect of zeta-potential on the floc size was minimal. In fact, the floc size (d_{50}) correlated weakly (Pearson coefficient -0.114, Table 3) with zeta potential.

3.3.3 Dissolved Oxygen

Within the range of DO level in this study, higher DO resulted in sludge with smaller floc size, higher SVI, and less negatively charged (Pearson coefficient -0.636, 0.919, and 0.779, respectively, Table 3). As reported by Li et al. (2010), higher DO increases risk of fungi growth, resulted in floc structure that was less dense (higher SVI). A less dense floc structure with a smaller floc size provided more surface area per unit volume. Whereas a less negative surface charge lessened the electric double layer repulsion force between sorbent-sorbate. These resulted in increased surface sorption (Figure 6). In contrast, there was weak correlation between the floc size with the carbon storage capacity (Pearson coefficient 0.028).

The results also showed that surface charge correlated well with carbon storage activity. A more negatively-charged surface correlated with a higher carbon storage activity (Figure S1a & b, Supplementary Material). The exact underlying reason(s) for these observations is not really clear. One possible explanation could be that a more negative surface charge caused steric hindrance between the floc structures, opening up more area for diffusion of the carbon substrate into pores and became more accessible to carbon uptake transporters located on the cell surface. As a result, the carbon storage capacity increased as zeta potential became more negative. In contrast, the surface sorption decreased marginally as the surface negativity increased (Figure S1).

It was also observed that while surface sorption increased marginally with SVI, carbon storage capacity correlated negatively with SVI (Figure S2, Supplementary Material). Tang et al.(2002) reported that floc settling behavior is dependent on floc size, effective density and porosity. The porosity of flocs can act to reduce drag by allowing advection of the suspending medium through the floc structure (Bushell et al., 2002). Therefore a lower SVI would imply better compactibility, and possibly lower degree of porosity. Since higher SVI implies relatively higher porosity, more surfaces per unit volume were available for surface sorption. In fact, under pH 7, the surface sorption capacity increased marginally with the SVI (Figure S2). On the other hand, it was observed that denser flocs (lower SVI) and possible lower porosity did not hinder the carbon storage capacity. Oshiki et al (2010) commented that due to the PHA granules in the cells, PHA-accumulating cells are also denser. A lower SVI suggested denser flocs and the possible enrichment of PHA-accumulators.

3.4 Microbial Community Analysis by DGGE

Sludge S1 and S2 were selected for microbial community analysis as Sludge S1's overall biosorption performance was the best among the acclimated sludges. Whereas the overall biosorption

performance of Sludge S2, was at similar level with most of the remaining acclimated sludge. Microbial community analysis of Sludge S1 and S2 through DGGE revealed the DNA sequences related to species capable of PHA-accumulation, e.g. *Rhodobacter* sp., and *Thauera* sp. (Chodak, 2008; Oshiki et al., 2008), were detected in all of the prominent bands of Sludge S1. In addition, sequence related to *Nitrospira* sp., *Microbacterium* sp., *Flavobacterium* sp., and phylum Bacteroidetes were also recovered from Sludge S1 (Table 4). In previous studies by Majone et al. (2006), *Thauera* sp. and *Flavobacterium* sp. were detected in the acclimated activated sludge for PHA production, under loading 0.24 mg BOD₅/(mg SS.day) with 10 days sludge age. The PHA storage was 20x faster and PHA content was about 4x higher than the original activated sludge (Majone, et al., 2006). Oshiki et al. (2008) reported that *Thauera* sp. were among the PHA-accumulators detected in full scale activated sludge processes showing PHA-accumulating ability. However, Liu et al. (2001) reported that no cellular PHA inclusions were detected in the *Cytophaga-Flavobacterium* cluster of *Cytophaga-Flexibacter-Bacteroides* (CFB) phylum. Besides, no PHA-accumulation activity was reported for *Nitrospira* sp. and *Microbacterium* sp. Thus, the high storage capacity (35.3 mg COD/g SS at pH 7) observed in Sludge S1, which was at least 59.7% higher than the remaining acclimated sludges, could very likely be attributed to the presence of *Thauera* sp., and *Rhodobacter* sp.

Whereas among the species detected in the prominent bands of Sludge S2, no DNA sequence related to PHA-accumulators were recovered. DNA sequences closely-related to *Chryseobacterium* sp., *Flavobacterium* sp., *Brevundimonas* sp. were detected. Both *Chryseobacterium* sp. and *Flavobacterium* sp. belong to the CFB phylum. Although previous studies did report that at least three types of filaments that occurred in activated sludge belong to the CFB phylum (Kämpfer and Wagner, 2003), and two out of five filamentous strains isolated by Seviour et al. (1997) were identified as *Chryseobacterium*-like organisms belonging to the CFB phylum - there was no definitive indication that the two strains detected in the DGGE were actually filaments. One strain of *Brevundimonas vesicularis* isolated from paper machine slime deposit was able to produce polysaccharide (Rättö et al., 2005), and one strain was able to synthesis PHA using acid-hydrolyzed sawdust as carbon source (Silva et al., 2007). The sequence closely-related to *Brevundimonas* sp. which was detected in Sludge S2 could indicate another possible PHA-accumulator in. However, there have been no reports in the literature on ability of the *Brevundimonas* sp. strain to either produce slime (EPS) or accumulate PHA from municipal wastewater.

3.5 Manipulation of main mechanisms involved in carbon entrapment

The preceding data and discussion would suggest it is possible to improve biosorption performance by selecting a suitable set of operating conditions which favored the most relevant mechanisms. For example, EPS entrapment mechanism should be emphasized when carbon in the wastewater contained much particulate. The treatment plant could then apply low F/M (e.g. condition S1) ratio or low DO (e.g. condition D1) to promote EPS production to entrap the particulate COD. Conversely, if soluble COD dominated, carbon storage would be important. The plant could divert part of the incoming influent to a side stream reactor operated under conditions which favored enrichment of population capable of carbon storage, e.g. low F/M ratio, longer SRT, neutral pH. The cultivated sludge could then be dosed into the contact tank to capture carbon through carbon storage, to supplement the overall capacity. In the event the major component of carbon in the wastewater was colloidal, high DO level could be applied to alter the microbial population which entrap carbon predominantly through surface sorption.

3.6 General Discussion and Potential Future Investigation

As both of the surfaces of the sorbents (sludge) and sorbate (colloids) in this study are negatively charged, it would be expected that the negatively-charged colloids would experience double layer repulsion when approaching the sludge surface. However, batch tests showed positive interactions between the sorbents and the sorbate. In the case of sludge, which predominant population is bacteria, the surface are inherently heterogeneous (Poortinga, et al., 2002). Whereas zeta potential measurement only yields an average value of the surface charge, while local differences can be expected at the molecular level (Poortinga, et al., 2002). The positive interactions between the colloids with the sludge surface would arise from positively charged domains on the cell surface structure. When the colloids come closer the cell surface, Van de Waals attractive force could further facilitate the capture of colloid, overcoming the double layer steric force from negative charged region. Nevertheless, fundamental studies on the removal mechanisms of anionic colloids present in wastewater have yet to be reported. In addition, it is not exactly clear why the carbon storage capacity increased as the surface charge negativity increased (Section 3.3.3). Further investigations are still required to elucidate the capture/uptake mechanism(s) of negatively charged colloids in the CCB, e.g. the functional groups on the cell surface at the binding site for the sorbent-sorbate positive interactions, as well as how the carbon uptake transporters involved in carbon storage operate despite more negatively charged surface.

4. Conclusions

This study showed that biosorption was not solely a physical-chemical process. With respect to carbon capture, biosorption process also involved carbon storage. A number of mechanisms could be present in the biosorption process. By manipulating operating conditions, sludge could be prepared such that particular mechanisms could be enhanced. Where such manipulation had been successful, biosorption performance was enhanced by about 120% in the best case compared to the baseline.

Nomenclature

ATP	Adenosine triphosphate
ATU	Allylthiourea
SBR	Sequencing batch reactor
CCB	Carbon capture biosorption
CFB	<i>Cytophaga-Flexibacter-Bacteroides</i>
COD	Chemical oxygen demand
DGGE	Denaturing gradient gel electrophoresis
DO	Dissolved oxygen
EPS	Extracellular polymeric substances
F/M ratio	Food-to-microorganisms ratio
HRT	Hydraulic retention time
NAD ⁺	Nicotinamide adenine dinucleotide (oxidized form)
NADH	Nicotinamide adenine dinucleotide (reduced form)
PHA	Polyhydroxyalkanoate
SRT	Sludge retention time
SS	Total suspended solid
VSS	Volatile suspended solid

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Figure 7. DGGE profiles of the bacterial communities in Sludge S1 and S2

Table 1. Sludge acclimation conditions

Parameters	S1	S2	D1	D2	R1	R2
F/M ratio	0.15	0.45	0.3	0.3	0.3	0.3
DO (mg/L)	2.5 - 3.0	2.5 - 3.0	0.5 - 1.0	5.0 - 6.0	2.5 - 3.0	2.5 - 3.0
SRT (day)	30	12	12	12	3	6

Note: bold type face indicates parameters that were different from the baseline condition D2.

Table 2. Key properties of the biomass acclimated under different conditions

Parameters	Acclimated Sludge					
	S1	S2	D1	D2	R1	R2
MLSS (g/L)	3.13	3.18	3.17	3.03	2.68	2.87
MLVSS (g/L)	2.96	3.06	3.02	2.76	2.51	2.65
Particle size, d_{50} (μm)	96.2	123.5	245.0	96.5	119.1	242.6
Specific surface area (m^2/g)	0.140	0.085	0.032	0.138	0.089	0.045
EPS (mg/g VSS)	150.7	98.5	101.1	66.7	37.8	58.0
Zeta-potential (mV)	-25.6	-25.2	-24.2	-13.6	-26.1	-21.5
SVI (mL/g)	57	89	47	187	92	68

Table 3. Pearson correlation coefficients (r) between process operation parameters with sludge properties and capacity, at pH 7, 15 min contact time

r	Surface sorption capacity ^c , mg COD/g SS	Carbon storage capacity ^c , mg COD/g SS	Overall capacity ^c , mg COD/g SS	Energy captured ^d , kJ/g SS	F/M	DO	SRT	SVI	Total EPS	d ₅₀
F/M^a, day⁻¹	-0.179	-0.606	-0.614	-0.458	-	-	-	-	-	-
DO^a, ppm	0.620	-0.403	-0.281	-0.425	0.000	-	-	-	-	-
SRT^a, day	0.711	0.680	0.777	0.505	-0.607	-0.005	-	-	-	-
SVI^b, mL/g	0.474	-0.712	-0.603	-0.745	0.200	0.919	-0.202	-	-	-
Total EPS^b, mg/g SS	0.568	0.682	0.755	0.535	-0.411	-0.273	0.940	-0.408	-	-
d₅₀^b, μm	-0.697	0.028	-0.094	0.160	0.122	-0.636	-0.380	-0.535	-0.172	-
Zeta-potential^b, mV	0.399	-0.587	-0.495	-0.597	0.027	0.779	-0.129	0.841^e	-0.301	-0.114

a: Process operating parameters. b: Sludge properties. c: Sludge capacities. d: Sludge capacities in terms of energy captured.

e: Not reported in main text as the correlation between these two parameters is not conclusive when viewed on an x-y plot.

Bold type face: $0.8 < r < 1.0$; *Italic type face:* $0.6 < r < 0.8$

Table 4. Sequence and phylogenetic affiliation of the bacteria based on bands in the bacterial community DGGE gel of Sludge S1 and S2

Band ^a	Phylogenetic relationship	Accession no.	Similarity (%)
	Closest relative		
S1-1(I) ^b	<i>Rhodobacter blasticus</i> strain ATCC 33485	NR043735	99
S1-1(II)	<i>Nitrospira</i> sp. clone 5	HE601935	99
S1-2(I)	<i>Microbacterium</i> sp. MRS-1	KF774194	98
S1-2(II)	Bacteroidetes bacterium clone 5.17m5	JN679190	99
S1-2(III) ^c	<i>Flavobacterium lindanitolerans</i> strain IP10	NR044208	99
S1-2(IV) ^b	<i>Thauera</i> sp. 'CJSOPY1 (T-IV)'	EF205258	98
S1-3(I) ^b	<i>Thauera</i> sp. TS4	EU073070	99
S1-3(II) ^b	<i>Rhodobacter</i> sp. clone DWIIA07	HQ711905	99
S2-1 ^c	<i>Chryseobacterium</i> sp. MH19	EU182848	100
S2-2(I) ^c	<i>Flavobacterium lindanitolerans</i> strain IP10	NR044208	99
S2-2(II) ^c	<i>Flavobacterium lindanitolerans</i> strain IP10	NR044208	100
S2-3 (I)	<i>Brevundimonas</i> sp. LC516	JQ014587	100

^aThe letter and number combination designates the test sample; the number after the dash corresponds to the band; the Roman numerals distinguish different species within the same band

^bPHA-accumulator

^cPossible filamentous bacteria

Supplementary Material

Figure S1. Correlation between zeta-potential with surface sorption and carbon storage capacity, for (a) Sludge S2, D1, D2, R1 (surface sorption or two mechanism equally dominant); (b) Sludge S1, R2 (carbon storage dominant)

Figure S2. Relation between Sludge volume index (SVI) with surface sorption and carbon storage capacity, at pH 7, 15 min contact time