Effect of dissolved oxygen and temperature on macromolecular composition and PHB storage of activated sludge

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Abstract

The macromolecular composition of activated sludge (lipids, intracellular proteins and intracellular polysaccharides) was studied together with its capacity to store macromolecules such as polyhydroxybutyrate (PHB) in a conventional activated sludge system fed with synthetic sewage water at an organic load rate of 1.0 kg COD/(m^3·d), varying the dissolved oxygen (DO) and temperature. Six DO concentrations (0.8, 1.0, 1.5, 2.0, 2.5 and 8 mg/L) were studied at 20°C with a sludge retention time (SRT) of 6 days. In addition, four temperatures (10ºC, 15ºC, 20ºC and 30ºC) were assessed at constant DO (2 mg/L) with 2 days SRT in a second experimental run. The highest lipid content in the activated sludge was 95.6 mg/g VSS, obtained at 30°C, 2 mg/L of DO and a SRT of 2 days. The highest content of intracellular proteins in the activated sludge was 87.8 mg/g VSS, obtained at 20°C, 8 mg/L of DO and a SRT of 6 days. The highest content of intracellular polysaccharides in the activated sludge was 76.6 mg/g VSS, which was achieved at 20°C, a SRT of 6 days and a wide range of DO. The activated sludge PHB storage was very low for all the conditions studied.

Keywords: Activated sludge, dissolved oxygen, macromolecular composition, PHB, temperature.

Introduction

A large amount of excess waste activated sludge (WAS) from urban wastewater treatment plants is generated daily worldwide.[1, 2] In aerobic treatment systems 0.5 to 0.6 kg of mixed liquor volatile suspended solids (MLVSS) per kg of biological oxygen demand (BOD_5) removed is produced. Not only that, the treatment and disposal of the excess WAS from sewage sludge originating from wastewater treatment plants accounts for as much as 60% of the total cost of wastewater treatment.[3]

48 At worldwide level, the common practices for the management of sewage sludge include: codisposal
49 landfilling, land application and thermal processing. In Chile, sludges are arranged in monofills or
50 codisposal landfills, with limited application to soils due to environmental restrictions. In order to
51 stabilize sludge, the major sewage water treatment plants in most countries digest it anaerobically,
52 producing biogas, which is sometimes used as energy. However, for different reasons the exploitation
53 of biogas for energy purposes is limited. Therefore, this way of recovering part of the investment made
54 in constructing the plant is not widely used in some countries such as Chile. Therefore, it is advisable
55 to look for other alternatives for the economic valorisation of the sludge generated in wastewater
56 treatment plants by activated sludge systems.
57 On the other hand, secondary sludge typically consists of valuable organic substances such as nucleic
58 acids, enzymes, proteins, and polysaccharides,[4, 5] and between 66-81% of total solids are volatile
59 solids (VS). Proteins, carbohydrates and lipids were in the ranges 0.34-0.47 g equivalent bovine serum
60 albumin/g VS, 0.17-0.30 g equivalent glucose/g VS and 0.00-0.09 g/g VS, respectively.[6]
61 Huynh et al.[7] discovered that the predominant fatty acids of sludge oil extracted from activated sludge
62 are palmitic acid (19-27%), palmitoleic acid (15-20%), and octadecenoic isomers (20-33%). These
63 same researchers observed that the amount of neutral lipids from activated sludge is 7.87% of its dry
64 weight, using for this quantification subcritical water treatment instead of the traditional systems
65 regularly used.
66 It is well known that under transient conditions the growth of the heterotrophic biomass becomes
67 imbalanced because there is a faster adaptation to the changing environment. Two physiological
68 adaptations can occur: an increase in the growth rate and/or substrate storage. The presence of
69 biodegradable storage compounds such as polyhydroxyalkanoate (PHA) and glycogen in activated
70 sludge has been repeatedly reported.[6, 8-10] The forms of PHA in activated sludge are mainly
71 polyhydroxybutyrate (PHB) and polyhydroxyvalerate (PHV).[11]

Many studies have been carried out on activated sludge to produce PHA, most of them using feast-famine regimes or nutrient pulses.[12] It has been observed that its production depends on operational parameters such as the type of carbon source, C/N ratio, organic load, dissolved oxygen (DO) concentration, pH, sludge retention time (SRT), temperature, magnetic field intensity, and nutrient deficiencies such as nitrogen and phosphorus.[13-15]

There is currently great interest worldwide to produce various commercially usable macromolecules from the WAS from sewage treatment plants.[13, 15] With this in mind, research has been carried out to study different alternatives, such as: PHA production as an alternative to the usual petroleum-derived plastics;[9] the production of oils, chars or gases by means of pyrolysis;[16-18] the production of proteins[19] and biodiesel production from either the lipids present in sludge[7] or the lipids stored by yeasts growing on pre-treatment sewage sludge.[20]

As most of the large sewage water treatment plants have activated sludge systems where the heterotrophic biomass grows in transient states, the objective of this study was to evaluate in a laboratory-scale activated sludge system the effect of two of the most important above-mentioned variables such as DO and temperature on the macromolecular composition (intracellular carbohydrates, intracellular proteins and lipids) and on the PHB stored by the activated sludge. The influence of the mentioned variables on COD removal efficiency was also assessed.

Materials and methods

Experimental set up

Two laboratory-scale activated sludge systems (ASS) were used. Each one is composed of an aerobic reactor and a secondary clarifier. The useful volumes of the tank reactors and settlers were 2.56 and

96 2.18 L, respectively. The reactors had a cylinder-conical geometry. An air pump with variable flow-
97 rate was used to control the DO in the systems. The air inlet was located at the bottom of the tanks.
98 The DO in the reactors was measured with a DO electrode. A cooler-heating blanket connected to a
99 thermostatic bath controlled the temperature of each reactor.
100 Sludge from a conventional full-scale activated sludge wastewater treatment plant was used as
101 concentrated mixed liquor for the laboratory-scale reactors (AAS). The main characteristics of this
102 inoculum were: pH, 7.25; total suspended solids (TSS): 4900 mg/L; and volatile suspended solids
103 (VSS), 3900 mg/L. The influent of the AAS was a synthetic wastewater with a chemical oxygen
104 demand (COD) concentration of approximately 600 mg/L. It was prepared with starch (400 mg/L),
105 sunflower oil (35 mL/L), ovoalbumin (40 mg/L), urea (26 mg/L), KH_2PO_4 (10.5 mg/L), $CaCl_2 \cdot 2H_2O$
106 (44 mg/L), $MgSO_4 \cdot 7H_2O$ (0.98 mg/L), KCl (42.5 mg/L), $NaHCO_3$ (17.5 mg/L), yeast extract (125
107 mg/L) and a trace element solution (2 mL/L). The composition of the trace element solution was based
108 on $FeCl_3 \cdot 4H_2O$ (1000 mg/L), $CoCl_2 \cdot 6H_2O$ (1000 mg/L), $MnCl \cdot 4H_2O$ (250 mg/L), $CuCl_2 \cdot 2H_2O$ (15
109 mg/L), H_3BO_3 (25 mg/L), $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ (45 mg/L), $NaSeO_3 \cdot H_2O$ (50 mg/L), $NiCl_2 \cdot 6H_2O$ (25
110 mg/L), EDTA (500 mg/L), and HCl 36% (0.5 mL/L). The COD concentration of the synthetic
111 wastewater can be considered as intermediate according to the literature, where values in the range of
112 350-1000 mg/L are reported.[21]
113
114 ***Operational conditions***
115 The ASS was operated in continuous mode at a constant organic load rate (OLR) of 1.0 kg COD/($m^3 \cdot d$)
116 and a hydraulic retention time (HRT) of 14.4 h for all conditions studied. Two different operating
117 strategies were evaluated: (1) operation of ASS at ambient temperature (20°C) with the DO varying
118 from 0.8, 1.5, 2, 2.5 to 8 mg/L, with a SRT of 6 days; (2) operation of ASS at a constant DO of 2
119 mg/L, varying the operational temperature from 10ºC, 15ºC, 20ºC to 30°C, with a SRT of 2 days. A

recirculation ratio (recirculation flow/raw wastewater) of 0.5 was applied. All experiments were carried out in duplicate reactors and the final results averaged.

Analytical methods

Influent and effluent COD, total suspended solids (TSS) and volatile suspended solids (VSS) within the reactor were measured according to Standard Methods.[22] Lipids, intracellular proteins, intracellular polysaccharides and PHB content of the sludge were measured when a steady-state was reached. Steady-state was assumed when the percentage of COD removal was kept about 90% for more than 10 days. To measure the intracellular proteins and the intracellular polysaccharides, the cells were lysed by means of treatment for 30 min in boiling sodium hydroxide (2M), then cooled on ice and neutralized with 2M hydrochloric acid.[23] Finally, the samples were subjected to vortex and centrifuged, taking the supernatant for the analysis of non-cellular organic matter. The lipid, protein and polysaccharide content in the sludge was measured by Soxhlet,[24] the Lowry modified method[25] and the Dubois method,[26] respectively. The biomass PHB content was obtained by HPLC using a Biorad HPX-87H column with a DAD L-7450A detector, prior to extracting with potassium hydroxide.[23]

Results and discussion

Effect of dissolved oxygen concentration

The ASS operated at 1 kg COD/(m^3·d) OLR, 20°C, a SRT of 6 days and DO of between 0.8 and 8.0 mg/L, keeping an average TSS of 3 g/L in the reactor, of which VSS was about 78%. Average COD removal efficiencies reached 94% for most cases studied, as can be observed in Figure 1.

144 COD removal efficiencies of 83% and 57% were achieved in the aerobic treatment of diluted palm oil
145 mill effluent (POME) (COD = 1000 mg/L) in AAS operating at 36h and 24h HRTs respectively, and at
146 DO concentrations in the range of 1.8-2.2 mg/L.[27]
147 Lipids, intracellular protein and intracellular polysaccharide contents of the active sludge at different
148 DOs are presented in Figure 2. As can be observed, high variations in lipid and intracellular protein
149 contents were obtained. However, the intracellular polysaccharides remained steady at a value of
150 around 76.6 mg/g VSS.
151 The highest lipid and intracellular protein contents in the activated sludge were found at 8 mg/L of DO
152 (57.8 and 87.8 mg/g VSS, respectively). The maximum lipid value obtained corresponded to 67% of
153 the maximum value reported by Mottet et al.[6] The maximum intracellular protein and intracellular
154 polysaccharide values obtained from activated sludge corresponded to 56% and 47% of the values
155 reported by Frølund et al.,[28] respectively, for sludge where the extracellular polymeric substances
156 have been subtracted. The values obtained in the present work were considered adequate, taking into
157 account that they did not consider the proteins and carbohydrates contained in the cell membranes and
158 walls.
159 In the present work sludge lipid content positively correlated with DO through the following equation:
160 $L = 24.17 \ln(DO) + 8.59$ (1)
161 where L is the lipid content expressed in mg/g VSS and DO is the dissolved oxygen concentration
162 expressed in mg/L, the regression coefficient being 0.983. An increase in the sludge intracellular
163 protein content was also observed with increased DO, but no correlation among the values obtained
164 was observed.
165 The PHB content of the sludge obtained at the different DOs studied (Figure 3) varied between 0.44
166 and 2.28 mg/g VSS, very low values compared with those obtained in transient systems.[29] A positive

effect on the PHB intracellular storage by the biomass was not observed with limited oxygen as was also previously reported by Padian et al.[30]

By contrast, another previous study focussing on PHB production by activated sludge in a two-stage process revealed that the rate of substrate uptake, as well as the yield and content of PHB increased with an increase in DO concentration.[12] The studies reported by Qu and Liu[12] also showed that an enhanced F/M ratio favored PHB accumulation.

Effect of temperature

The ASS operated at 1 kg COD/(m^3·d) OLR, a SRT of 2 days, DO of 2 mg/L and temperatures ranging between 10ºC and 30°C, keeping an average reactor TSS concentration of about 1.7 g/L, of which the VSS were approximately 80%. For all the cases studied, high COD removal values were achieved, with average values of 90% at temperatures of 20ºC and 30°C, as can be seen in Figure 4.

It was previously reported [21] that 40% COD removal efficiencies were achieved in activated sludge systems treating synthetic wastewater (1000 ± 20 mg/L) and real domestic wastewater at 10ºC. At higher temperatures (25-30ºC) the efficiency of the reactor was 80%.[21] In the same way, Song et al.[31] reported that aerobic granular sludge was cultivated in sequencing batch airlift reactors (SBAR) at 25ºC, 30ºC and 35ºC. These above-mentioned results also showed that 30ºC was optimum for mature granule cultivation, where the granules had a more compact structure, better settling ability and higher bioactivity with COD removal efficiency of 97% and a total phosporus removal rate of 75%.[31]

Activated sludge reactors operating at temperatures in the range of 20-30ºC were also used for treating saline wastewaters generated by marine-product industries. COD removal efficiencies of 88% were achieved at an OLR of 1 g COD/(L·d) when the system is inoculated with NaCl-acclimated culture.[32]

Finally, and also for comparative purposes, the influence of the temperature on aerobic treatment was

studied in a sequencing batch reactor over 40 weeks in mesophilic (35ºC) operation.[33] An average COD removal percentage of 75% was achieved in this case.

The lipids, intracellular protein and intracellular polysaccharide contents of the biomass were strongly affected by temperature (Fig. 5). It was observed that at the highest temperature, the contents of these macromolecules increased, obtaining 95.6 mg/g VSS of lipids, 36.0 mg/g VSS of intracellular proteins, and 74.3 mg/g VSS of intracellular polysaccharides at 30ºC.

Only the lipid content (L, in mg/g VSS) of the activated sludge could be properly correlated to temperature (T in °C) through the following equation (regression coefficient: 0.994):

$$L = 3.90*T-23.2 \quad (2)$$

Mottet et al.[6] analysed the lipid content of sludge from activated sludge systems with capacities higher than 62,000 equivalent inhabitant and their results showed that when decreasing the sludge age both in mesophilic and thermophilic systems, the lipid content increased. However, it was not possible to evaluate the effect of the operating temperature on lipid content and these authors [6] could not find a correlation between these two parameters for a fixed sludge age.

The lipid content of the sludge increased as DO and temperature increased, and the highest values were obtained at a temperature of 30ºC and a DO of 2 mg/L. However, from the results obtained it was not possible to evaluate the interaction of both parameters.

It was not possible to detect PHB at any temperature, and this suggests that in conventional activated sludge systems there is no selection or acclimatizing pressure for the enrichment of microorganisms that can accumulate this type of polymer.

Conclusion

The macromolecular composition of sludge can vary according to the operational conditions (such as DO and temperature) of the ASS. The lipid content of activated sludge increased when either DO or temperature was increased. On the other hand, the highest activated sludge intracellular polysaccharide and protein contents were obtained at a temperature of 20ºC and a SRT of 6 days. The influence of DO on the polysaccharide content was not significant, while the maximum protein content was obtained at a DO of 8 mg/L. Temperatures of 20 ºC and 30 ºC and DO values between 2.0-2.5 led to achieve COD removal efficiencies of about 90%. In ASS at the above-mentioned operational conditions, the sludge generated had very low PHB contents.

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FIGURE CAPTIONS

Figure 1. Average COD removal (%) as a function of DO (the dotted vertical lines indicate a change in conditions).

Figure 2. Lipid, intracellular protein and intracellular polysaccharide contents of the biomass obtained from ASS operating at 1.0 kg COD/(m^3·d) OLR , 20°C, SRT of 6 days for the different DO conditions tested (the standard deviations of the plotted values were less than 5% in all cases).

Figure 3. PHB contents of the biomass obtained from ASS operating at 1.0 kg COD/(m^3·d) OLR, 20ºC and SRT of 6 days for the different DO conditions tested (the standard deviations of the plotted values were less than 5% in all cases).

Figure 4. Average COD removal as a function of temperature (■: influent COD; ♦: effluent COD; ▲: COD removal efficiency) (the dotted vertical lines indicate a change in conditions).

Figure 5. Lipid, intracellular protein and intracellular polysaccharide contents of the biomass obtained from ASS operating at 1.0 kg COD/(m^3·d) OLR, 2 mg/L of DO and SRT of 2 days for the different temperatures assayed (the standard deviations of the plotted values were less than 5% in all cases).

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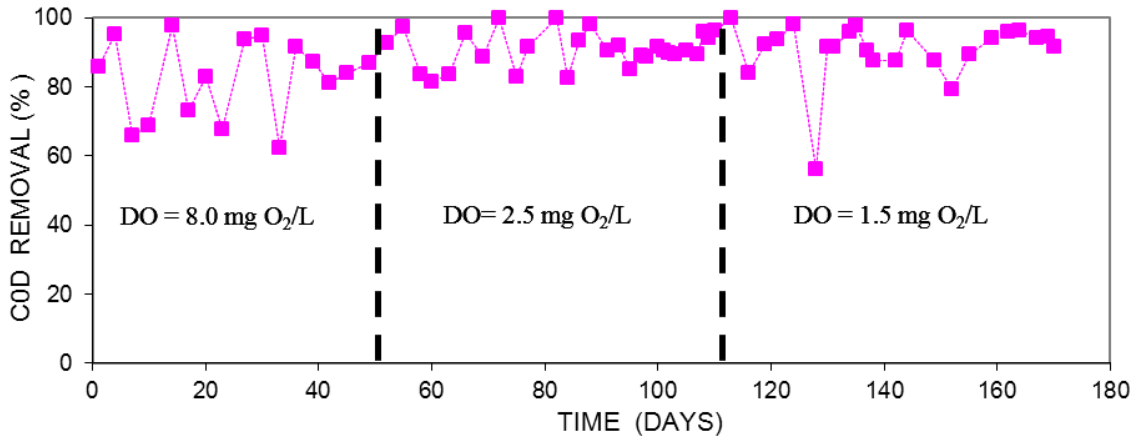
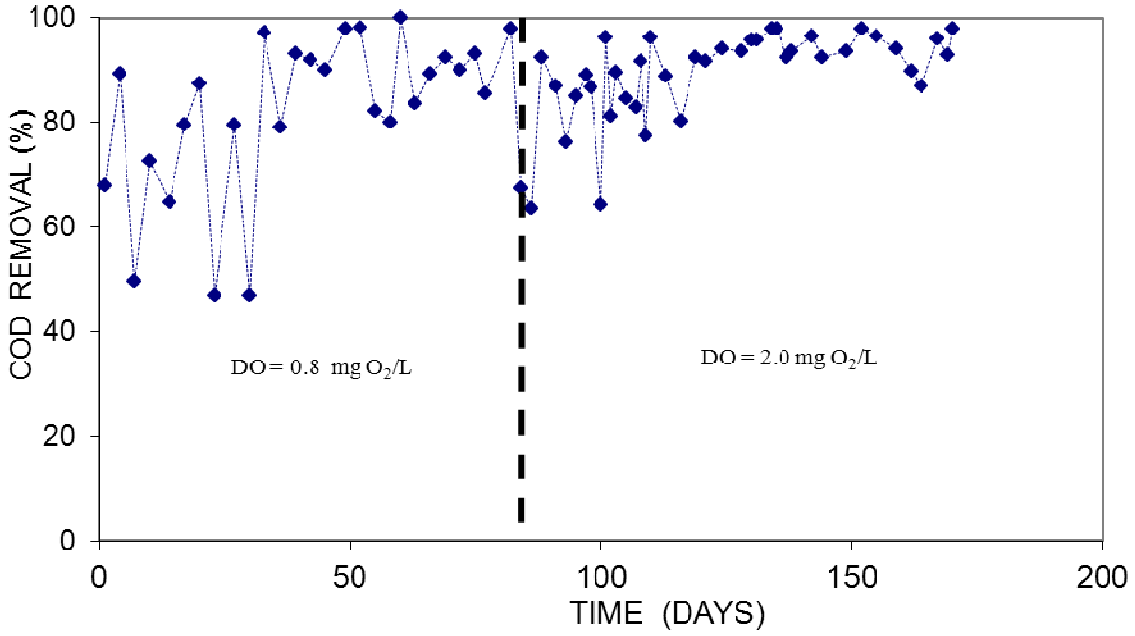


357



358

359 **Fig. 1**

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362

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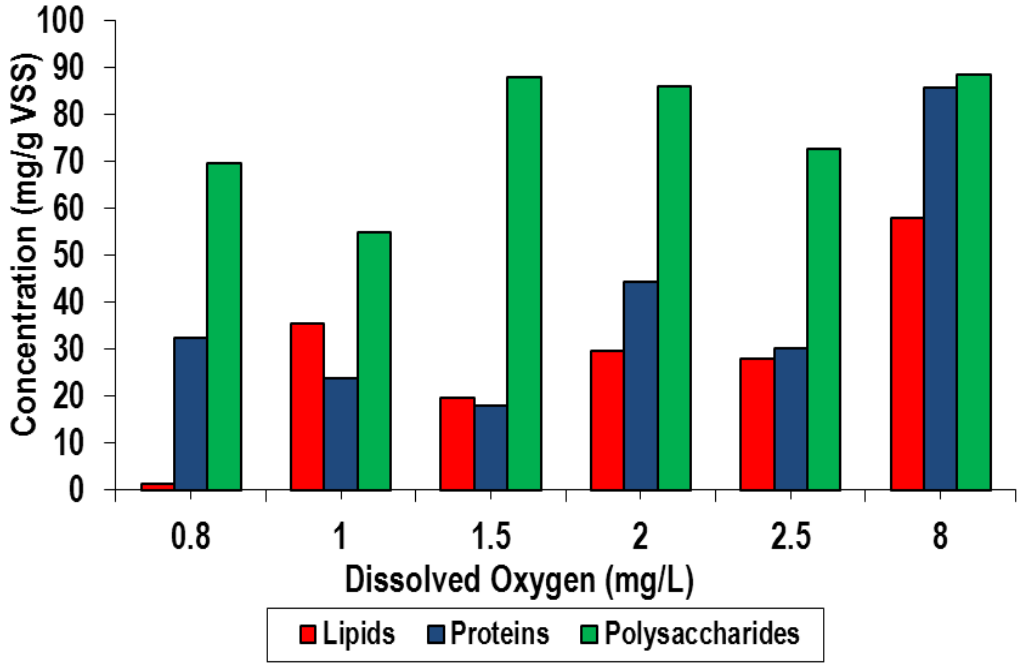

Concentration (mg/g VSS)
100
90
80
70
60
50
40
30
20
10
0
0.8
1
1.5
2
2.5
8
Dissolved Oxygen (mg/L)
Lipids
Proteins
Polysaccharides

Fig. 2

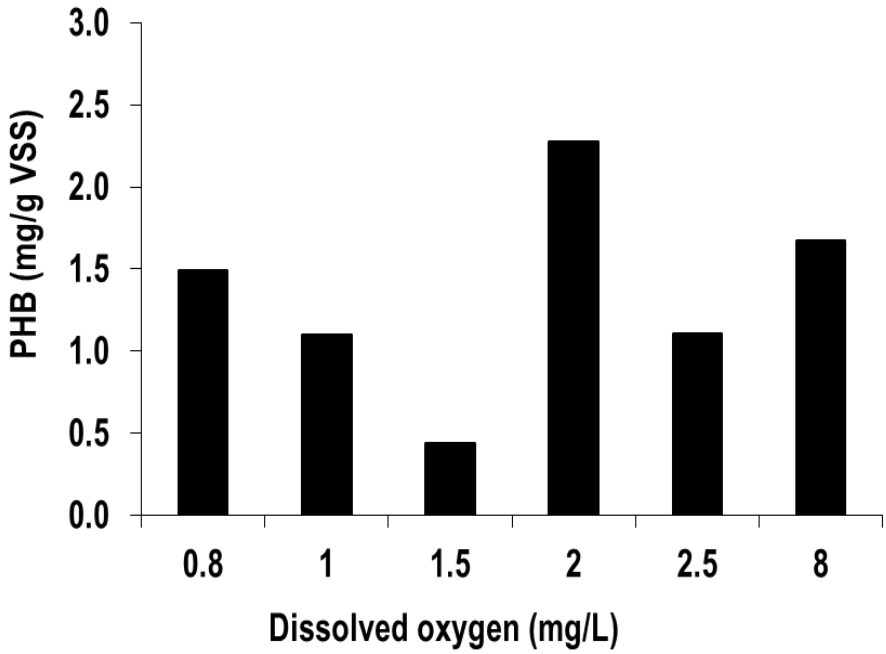

3.0
2.5
2.0
1.5
1.0
0.5
0.0
PHB (mg/g VSS)
0.8
1
1.5
2
2.5
8
Dissolved oxygen (mg/L)

Fig. 3

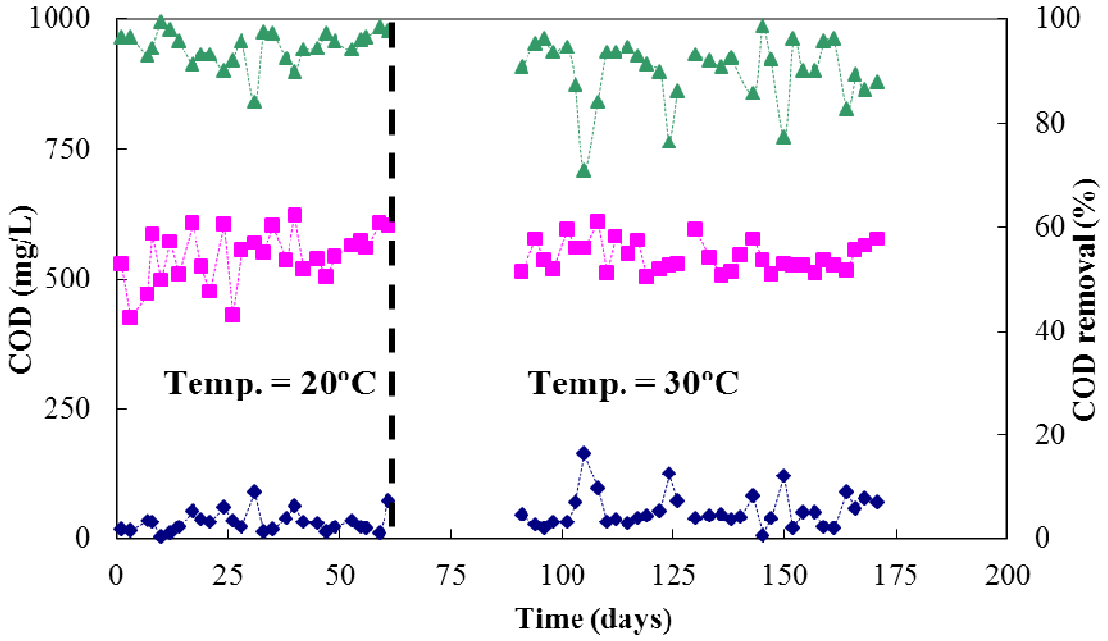
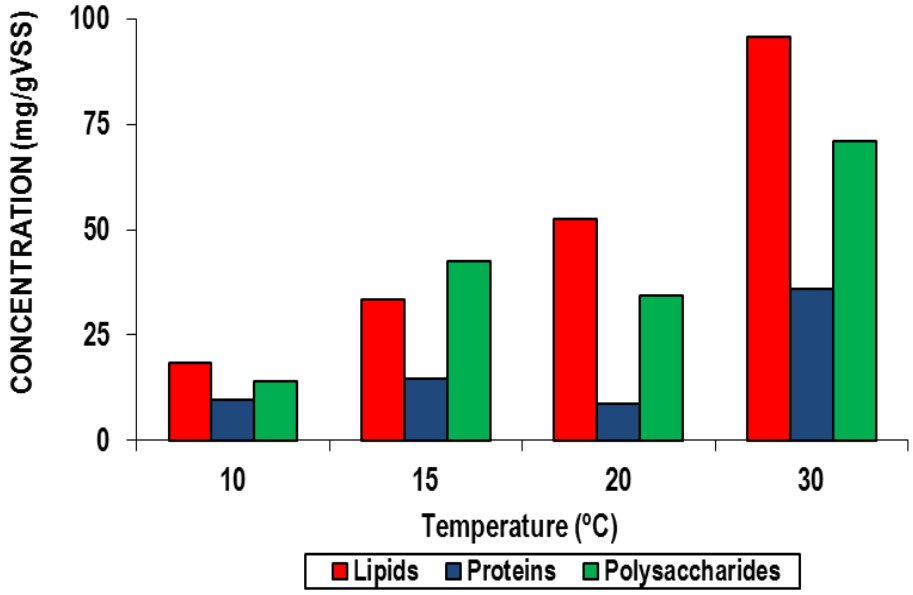

1000
750
500
250
0
COD (mg/L)
100
80
60
40
20
0
COD removal (%)
Temp. = 20°C
Temp. = 30°C
0
25
50
75
100
125
150
175
200
Time (days)

Fig. 4

409



410

411 **Fig. 5**

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