

**BIOMASS SELECTION FOR OPTIMAL ANAEROBIC TREATMENT OF
OLIVE MILL WASTEWATER**

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Abstract

This research was conducted to identify the most efficient biomass out of five different types of biomass sources for anaerobic treatment of Olive Mill Wastewater (OMW). This study was first focused on examining the selected biomass in anaerobic batch systems with sodium acetate solutions (control study). Then, the different types of biomass were tested with raw OMW (water-diluted) and with pretreated OMW by coagulation-flocculation using Poly Aluminum Chloride (PACl) combined with hydrated lime ($\text{Ca}(\text{OH})_2$). Two types of biomass from wastewater treatment systems of a citrus juice producing company “PriGat” and from a citric acid manufacturing factory “Gadot”, were found to be the most efficient sources of microorganisms to anaerobically treat both sodium acetate solution and OMW. Both types of biomass were examined under different concentration ranges ($1\text{-}40\text{ g l}^{-1}$) of OMW in order to detect the maximal COD tolerance for the microorganisms. The results show that 70-85% of COD removal was reached using Gadot biomass after 8-10 days when the initial concentration of OMW was up to 5 g l^{-1} , while similar removal efficiency was achieved using OMW of initial COD concentration of 10 g l^{-1} in 2-4 days of contact time with the PriGat biomass. The physico-chemical pretreatment of OMW was found to enhance the anaerobic activity for the treatment of OMW with initial concentration of 20 g l^{-1} using PriGat biomass. This finding is attributed to reducing the concentrations of polyphenols and other toxicants originally present in OMW upon the applied pretreatment process.

Keywords: Olive mill wastewater; Anaerobic treatment; Batch system; Biomass; Polyphenols

INTRODUCTION

Olive mill wastewater (OMW) generated by the olive oil extraction process is the main waste product of this industry. Approximately, 1.8×10^6 tons of olive oil is produced annually worldwide with the majority (98%) of it being produced in the Mediterranean basin [1]. It is reported that OMW resulting from the production process surpasses 30 million m^3 per year [2] in the Mediterranean region. Treatment of OMW is a serious problem mainly due to its high organic COD concentration, and because of its resistance to biodegradation due to its high content of inhibitors for biomass growth, e.g. phenolic compounds [3]. In addition, OMW typically contains polysaccharides, lipids, proteins, and a number of monocyclic and polymeric aromatic molecules [4] that might exhibit inhibition effects towards some specific anaerobic microorganism populations.

The OMW is a significant source of environmental pollution in the Mediterranean countries [5, 6]. In general and for economic reasons, OMW is often disposed into evaporation ponds and left to dry throughout the summer season [7, 8]. OMW has a negative impact on the regional environment due to its severe toxicity to microorganisms in domestic wastewater treatment plants, its strong and unpleasant odor after anaerobic digestion, and also due to its potential threat to surface and groundwater sources.

The seasonal production and high organic load of OMW makes anaerobic treatment a very reasonable treatment option for this type of aqueous waste [9]. Within the different anaerobic treatment systems studied so far, up-flow anaerobic biomass blanket reactor

(UASB) is considered as an advanced model of bioreactors to treat agro-industrial wastewaters characterized with a high organic load. Previous research work shows that a very high efficiency of COD removal has been achieved using UASB reactors with an influent organic loading rate of $8 \text{ g COD l}^{-1} \text{ d}^{-1}$ [10]. Most research studies in this field report that the major problems of UASB system are the long-term start-up period to obtain maximum performance, in addition to the instability of the biological activity as a result of washing-out a significant part of the biomass from the reactor or fluctuations on organic load. Also, other problems are encountered such as the high toxicity of phenolic compounds, tannins, and adjusting the pH in the medium of the reactor [11] when dealing with wastewaters generated from the agro-industry.

Suitable inoculum (biomass) source is highly important for both shortening the start-up period for the reactor and overcoming the low biodegradability of toxic compounds typically present in OMW. Therefore, the main goal of this study is to examine the most suitable biomass to be used for OMW in anaerobic treatment systems, mainly UASB reactors.

MATERIALS AND METHODS

Biomass

Five different types of biomass were collected from five different sources. These included a UASB reactor for treating domestic wastewater located in Sakhnin, a town in the Galilee area, a municipal wastewater treatment plant at Haifa (HWTP), a UASB reactor at the industrial wastewater treatment plant at Gadot, Haifa (a citric acid

manufacturing factory), a cesspool for evaporation of OMW in the Sakhnin region, and a UASB reactor for treating wastewater from the citrus juice industry at the PriGat factory in Israel.

OMW Characterization

OMW for this study was obtained from different olive mills in the Galilee area and was refrigerated at 4°C. The parameters COD, BOD, TSS, VSS, pH, and alkalinity of the collected OMW samples were determined according to the “Standard Methods for the Examination of Water and Wastewater, 20th Edition 1998”. The total polyphenols in OMW were determined according to the Folin-Cioacalteau method (see Kachouri & Hamdi 2003 [12]). Table 1 shows the typical characteristic parameters of OMW from the region. All chemicals of analytical grade were purchased from Sigma.

Biomass Characterization

The main characteristics of the different types of biomass are given in Table 2. The differences in concentrations of mixed liquor suspended solids (MLSS) and mixed liquor volatile suspended solids (MLVSS) for the tested OMW samples were due to the solid contents of the original biomass collected from the specific source. Biomass dry weight for the OMW samples was checked by taking an amount of mixed liquor suspended biomass, which was centrifuged at 5000 rpm for 10 minutes. The two phases obtained after centrifugation, were separated by decantation and then a fixed amount of the

accumulated biomass was weighted and considered as wet base biomass. The obtained wet biomass amount was heated in an oven at 110°C for 24 hours, and the resulting weight was considered as the dry weight of the biomass.

The relatively low MLVSS/MLSS (0.227) of Gadot biomass was due to the presence of an inert carrier material (such as sand and silt), where the high MLVSS/MLSS (0.7260) ratio of the biomass from PriGat was due to the high organic content of the distillery wastewater that was absorbed or mixed with the biomass.

Anaerobic Batch Experiments

Anaerobic batch experiments were carried out in 1 l Erlenmeyer flasks connected with tubing to gas measuring tubes of 500 ml in volume. All gas collection tubes were calibrated daily to determine the volume of gas accumulated. For each biomass source, either different initial concentration ranges of sodium acetate solutions, or OMW with COD concentrations in the range of 993-35,500 mg l⁻¹, were added. Similar volumes of standard solutions containing trace elements and yeast extract were added to all of the anaerobic batch experiments in addition to a source of carbon (either sodium acetate or OMW). Sucrose solutions (5% of total COD, 700 ml in each flask) were added to two flasks containing OMW, where the first had a COD of 1 g l⁻¹ and the second had a COD of 20 g l⁻¹, in order to examine the effect of easy biodegradable compounds on the total efficiency of biodegradation. Each flask was supplemented with 50 g of biomass from a different source and 0.4 g urea as a source of nitrogen. The medium in each flask was adjusted to pH = 7.0 and kept constant at this value by appropriate addition of NaOH or

HCl solutions each of 0.1 M. The flasks were immersed in a shaker bath at temperature 37°C and shaken at 50 rpm. Samples were taken daily for COD and pH tests. The COD was determined by taking 5 ml of the flask content, centrifuged at 5000 rpm for 10 minutes, then tested according to the “Standard Methods for the Examination of Water and Wastewater, 20th Edition 1998”. The gas collection tubes were monitored to determine the volume of the accumulated gas.

Physico-chemical Pretreatment of OMW

Coagulation-flocculation experiments were performed in batch reactors, equipped with controllable speed mixers and sampling ports at different vertical locations. A typical physico-chemical procedure was started by adding 5 g l⁻¹ of polyaluminium chloride (PACl) into 1 l of raw OMW and then mixing the solution at 150 rpm for 5 minutes. Calcium hydroxide (lime, 40 g) was then added to the OMW solution and mixing was resumed at 30 rpm for 30 min. After the flocculation step, the solution was filtered through a Whatman GFA filter paper. The filtrate, the so called “pretreated OMW”, was used in the anaerobic batch system in order to examine the effect of this type of physico-chemical pretreatment on the availability of OMW for the different biomass sources. The characteristics of OMW before and after the applied physico-chemical treatment are presented in Table 3.

RESULTS AND DISCUSSION

Biomass Activity with Sodium Acetate

In a preliminary work, the anaerobic activity of the five different sources of biomass was evaluated using a solution of sodium acetate as a sole carbon source. This model system was used to figure out the extent of the anaerobic activity for the five different biomass samples collected from the various sources.

Figure 1a shows the biological activity for the five different types of biomass as COD biodegradation (removal) rate of sodium acetate solution. It is clear that the two biomasses from PriGat and Gadot UASB reactors revealed the best biodegradation rate of acetate (in terms of decrease in COD concentration versus time). It can also be seen in Figure 1a that the PriGat biomass shows higher removal kinetic and extent than the Gadot biomass, where more than 90% of COD removal was achieved during the first 2-3 days. The results presented in Figure 1a also show that 8-9 days were required at least to reach the maximum COD removal efficiency of 80% using the biomass from Gadot. The PriGat biomass shows better performance in terms of both organic matter biodegradation rate and eventual extent of biodegraded organic matter.

Figure 1b illustrates the rate of biogas production from the anaerobic mediums containing sodium acetate solution and using the five different types of biomass. The rate and the extent of biogas production were consistent with the rate of sodium acetate removal (COD Removal) presented in Figure 1a, where the biomass of PriGat and Gadot showed the best performance.

Biomass Activity with OMW

In order to confirm the results obtained for the control study as presented in Figures 1a and 1b two sets, each of five batch anaerobic systems containing water-diluted OMW with two various initial COD concentrations, each were inoculated with one of the five different biomass sources. Figure 2 shows the rate of the biological activity using the five different sources of biomass with OMW at initial COD concentrations of 1 g l^{-1} (Figure 2a) and 20 g l^{-1} (Figure 2b). Figures 2a and 2b show obviously that the biomass from PriGat has the highest anaerobic biological activity in the treatment of OMW at both low and high concentration ranges. Similar to the control study with acetate, a maximum COD removal of 80% at the low concentration range (1 g l^{-1}) was reached after 2-3 days of contact time with PriGat biomass, while 10 days were required to reach the same removal level using the biomass from Gadot. This result confirms that the PriGat biomass comprised the most appropriate populations of anaerobic microorganisms for the treatment of OMW compared to other types of selected biomass.

Effect of Concentration Range of OMW on the Biomass Activity

The biomass of Gadot and PriGat were selected for our further study in order to test the effect of a broader COD concentration range on the biological activity of OMW biodegradation under anaerobic conditions. Figure 3 presents the results for the effect of initial COD concentrations of OMW on the biological activity of biomass from PriGat. It

can be seen from the results that the initial COD concentration of 5 g l^{-1} does not affect the rate of biodegradation of OMW as well as having no effect on the extent of the ultimate removal percentage (80%) obtained after 4 days. However, the results in Figure 3 show that starting with initial COD concentrations of OMW equal or higher than 20 g l^{-1} caused a major inhibition effect on the anaerobic biodegradation rate of OMW as well as on the final extent of COD removal. It can also be seen in Figure 3 that the initial COD concentration of 10 g l^{-1} was observed to be such an “intermediate” concentration level, where the biodegradation rate was slow in the first three days, but the ultimate degraded organic materials present in OMW was similar to the extent obtained when using lower initial concentration of COD for OMW. Figure 3 also shows that 73-85% of the COD content in the low range ($1\text{-}10 \text{ g l}^{-1}$) was removed within 4-5 days of contact time compared to about 40% COD removal obtained when the initial COD concentration of 20 g l^{-1} was used with the PriGat biomass. The results also show that only 25-28% of the COD concentration was removed when the initial COD concentration was of 40 g l^{-1} . These results indicate that the equivalent concentration of the refractory compounds (assumed to be polyphenols) in OMW of 20 g l^{-1} or more as COD has significantly inhibited the biomass anaerobic activity.

A similar phenomenon to that presented in Figure 3 has been observed with the biomass from Gadot, but with different kinetics behavior (Figure 4). Also, the effect on the extent in terms of final removal at both low and high concentration ranges was similar to that obtained when the biomass from PriGat was used. The rate of OMW biodegradation using the biomass of PriGat is much higher than that obtained using Gadot biomass, where more than 9-10 days (Figure 4) were required to achieve the same

removal percentage obtained in about 5 days of contact time using the biomass of PriGat (Figure 3).

Effect of Physico-chemical Pretreatment of OMW on the PriGat Biomass Activity

The physico-chemical pretreatment of raw OMW decreases significantly the concentrations of COD, BOD and polyphenols as presented in Tables 3 and 4. Given the latter parameter as the main anaerobic degradation inhibiting factor, this pretreatment is supposed to positively affect the biodegradation of OMW using PriGat biomass which exhibited the optimal activity at up to 10 g l^{-1} of initial COD concentration without physico-chemical pretreatment of OMW. The biodegradation rate of different COD concentrations of OMW after physico-chemical treatment is shown in Figure 5.

Compared with the results presented in Figure 3 it is clear here that the physico-chemical pretreatment of OMW resulted in enhancing the anaerobic biodegradation rate for the organic materials in OMW with initial COD concentration of 20 g l^{-1} . On the other hand, there was no observation of any inhibition effect on the COD removal rate of pretreated OMW with higher initial concentrations (30 and 40 g l^{-1}). However, slow down in the biodegradation rate for the organic materials present on OMW at initial COD concentrations is not linear with the equivalent concentration of polyphenols as can be seen in Figure 5. For example, the biodegradation rate of the organic materials at initial COD concentration of 30 g l^{-1} , after the physico-chemical treatment of OMW, was expected to be higher than the biodegradation rate obtained when using non-pretreated

OMW at initial COD concentration of 10 g l^{-1} with a similar concentration level of polyphenols in both mediums (Table 3). This result means that at initial COD concentrations higher than 30 g l^{-1} there are other factors excluding polyphenols that might affect the anaerobic biodegradation rate for the organic materials present in OMW. To the best of our knowledge, this phenomenon is reported for the first time, and therefore, a further investigation study to interpret this result is under progress.

CONCLUSIONS

Two types of biomass, collected from the wastewater treatment systems of a citrus juice producing company “PriGat” and of citric acid manufacturing factory “Gadot” both located in Israel, were found to be the most efficient sources of microorganisms to anaerobically treat both sodium acetate solution and OMW. The results show that 70-85% of COD removal was reached using Gadot biomass after 8-10 days when the initial COD concentration of OMW was up to 5 g l^{-1} , while similar removal efficiency was achieved using OMW of initial COD concentration of 10 g l^{-1} in 2-4 days of contact time with the PriGat biomass.

The physico-chemical pretreatment of OMW was found to enhance the anaerobic biodegradation rate for OMW with initial concentration of 20 g l^{-1} using PriGat biomass. A removal efficiency of 80% was observed when OMW was first physico-chemically pretreated, while only 40% of removal efficiency was reached using water-diluted OMW with the same initial COD and biomass concentrations. This finding is attributed to the high removal efficiency of polyphenols and other toxicants by the proposed pretreatment process for OMW.

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REFERENCES

1. Benitez F.J., Beltran-Heredia, J., Torregrosa, J., and Acero, J.L., Aerobic degradation of olive mill wastewater. *Appl. Microbiol. Biotechnol.*, **47**, 185-188 (1997a).
2. Baccari M., Bonemazzi F., Majone M., and Riccardi C. Interaction between Acidogenesis and Methanogenesis in the Anaerobic Treatment of Olive Oil Mill Effluents. *Wat. Res.*, **30**, 1, 183-189 (1996).
3. Ramos-Comenzana A., Monteolica-Sanchez M and Lopez MJ, Bioremediation of alpechin. *J. Biodeter. Biodegr.*, **35**: 249-268 (1995).
4. Ethaliotis C., Papadopoulou K., Kotsou M., Mari I., Balis C., Adaptation and population dynamics of *Azotobacter vinelandii* during aerobic biological treatment of olive mill wastewater. *FEMS Microbiol. Ecol.*, **30**, 301-311 (1999).
5. Basheer S., Sabbah I., and Marzook T., Olive mill wastewater treatment in the Galilee Region. Annual Report, Israeli Ministry of Agriculture, (1999).

6. Borja, R., Martin, M., Maestro, R., Alba j., Fiestas, J.A., Enhancement of the anaerobic digestion of olive mill wastewater by the removal of phenolic inhibitors. *Process Biochem.*, **27**, 231-237 (1992).
7. Benitez F.J., Beltran-Heredia, J., Torregrosa, J., Acero, J.L., and Cercas V., Chemical pretreatment by ozone of wastewater from olive oil mills. *Technol. Environ. Chem.*, **60**, 97-109 (1997b).
8. Boari, G., Brunetti, A., Passino,R., Rozzi, A. Anaerobic digestion of olive mill wastewater. *Agri. Wastes*, **10**, 161-175 (1984).
9. Erguder T.H., Guven E., and Demirer, Anaerobic treatment of olive mill wastes in batch reactors. *Process Biochem.*, **36**, 243-248 (2000).
10. Sabbah I., Marzook T., and Basheer S., The effect of pretreatment on anaerobic activity of olive mill wastewater using batch and continuous systems. *Process Biochem.*, **39**, 1947-1951 (2004).
11. Hamdi, M., Anaerobic digestion of olive mill wastewater. *Process Biochem.*, **31**, 105-110 (1995).
12. Kachouri F., and Hamdi, M., 2004, Enhancement of polyphenols in olive oil by contact with fermented olive mill wastewater by *Lactobacillus plantarum*. *Process Biochem.*, **39**, 841-845 (2004).

Table 1: Characteristics of olive mill wastewater used for the biomass selection study
(batch systems).

Parameter	Value
pH	5.0
Total alkalinity as CaCO ₃	1.172 g l ⁻¹
Total polyphenols	6.8 g l ⁻¹
COD(total)	163.5 g l ⁻¹
COD (soluble)	131.5 g l ⁻¹
BOD	27.5 g l ⁻¹
TSS	86.84 g l ⁻¹
VSS	0.503 g l ⁻¹
N-NH ₄ ⁺	0.11 g l ⁻¹

Table 2: Characteristics of five different biomass sources tested in this study.

Biomass type	MLSS (g l⁻¹)	MLVSS (g l⁻¹)	MLVSS/MLSS	Wet Weight (g)	Dry Weight (g)	% Dry/Wet weight
Sakhnin UASB	102.4	69.5	0.678	50.0	7.7	15.4
Haifa (HWTP)	23.3	16.25	0.697	43.6	4.2	9.6
Gadot UASB	411.4	93.6	0.227	49.8	20.0	40.2
OMW- evaporation pond	48.7	35.4	0.726	50.0	5.1	10.2
PriGat UASB	74.05	53.4	0.722	50.0	6.5	13

Table 3: The physico-chemical pretreatment effect on the OMW characteristics.

Parameter	Raw OMW	After pretreatment using PACl and Ca(OH) ₂ followed by filtration
pH	4.9	12.3
Conductivity ($\mu\text{mho cm}^{-1}$)	10,200	24,200
TS (g l^{-1})	89.54	59.24
TSS (g l^{-1})	36.89	ND
VSS (g l^{-1})	35.12	ND
COD Total (g l^{-1})	184.7	72.62
COD Soluble (g l^{-1})	97.95	72.62
BOD Total (g l^{-1})	72.5	37.2
BOD Soluble (g l^{-1})	35.0	31.2
P as PO ₄ (mg l^{-1})	1242	58.3
N-total (mg l^{-1})	1,522	237.7
Oil Total (g l^{-1})	16.7	0.065
Oil Soluble (g l^{-1})	1.08	0.064
Polyphenols (g l^{-1})	5.84	0.843

Table 4: The COD concentrations for samples of water-diluted OMW and their content of polyphenols before and after pretreatment with PACl followed with treatment with calcium hydroxide solution (lime).

OMW initial COD concentration (g l ⁻¹)	Polyphenols conc. before pretreatment (mg l ⁻¹)	Polyphenols conc. after pretreatment (mg l ⁻¹)
5	150	60
10	300	120
20	634	230
30	950	350
40	1,270	460

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- Figure 1b: Kinetics of biogas production (ml) using five different types of biomass in batch system. The anaerobic biodegradation conditions: See Figure 1a.
- Figure 2: Kinetics of COD (mg l^{-1}) removal of OMW presented as COD measured at different time intervals (COD_t) per the initial COD concentration (COD_i). The anaerobic biodegradation conditions: Two sets of five OMW solutions (1l) of COD_i of 1 g l^{-1} (graph a) and of 20 g l^{-1} (graph b) were inoculated each with 50 g of biomass from the five different sources. The solutions were shaken in a shaking bath incubator at 50 rpm, temperature of 37°C and pH=7.
- Figure 3: The effect of initial COD concentration of OMW on the kinetic activity of biomass from PriGat. The activity profile is presented as COD measured at different time intervals (COD_t) per the initial COD concentration (COD_i). The anaerobic biodegradation conditions: OMW solutions (1 l) of various initial COD concentrations contained in 1 l flasks were inoculated each with 50 g of biomass from PriGat. The flasks were shaken in a shaking bath incubator at 50 rpm, temperature of 37°C , and pH 7.
- Figure 4: The effect of initial COD concentration of OMW on the kinetic activity of biomass from Gadot. The activity profile is presented as COD measured at different time intervals (COD_t) per the initial COD concentration (COD_i). The anaerobic biodegradation conditions: OMW solutions (1 l) of various initial COD concentrations contained in 1 l flasks were inoculated each with 50 g of biomass from Gadot. The flasks were shaken in a shaking bath incubator at 50 rpm, temperature of 37°C , and pH 7.
- Figure 5: The effect of initial COD concentration of pretreated OMW on the kinetic activity of biomass from PriGat. The activity profile is presented as COD measured at different time intervals (COD_t) per the initial COD concentration (COD_i). The anaerobic biodegradation conditions: Five flasks of 1 liter containing OMW solutions (1 l) of various initial COD concentrations which were pretreated as described in the Materials and Methods section, were inoculated each with 50 g of biomass from PriGat. The flasks were shaken in a shaking bath incubator at 50 rpm, temperature of 37°C , and pH 7.

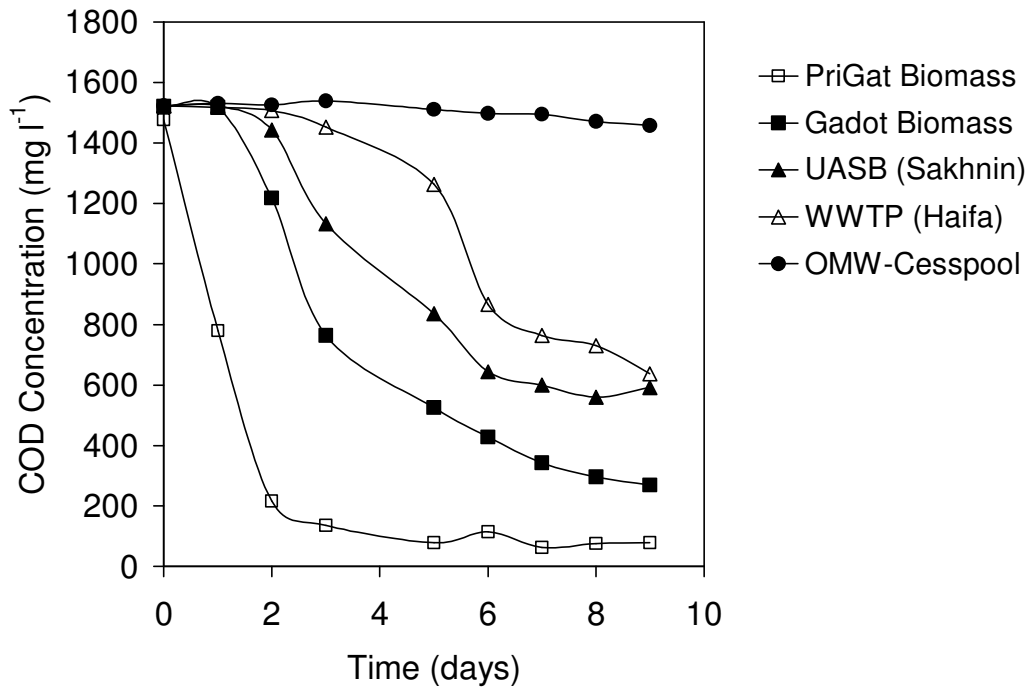


Fig 1a

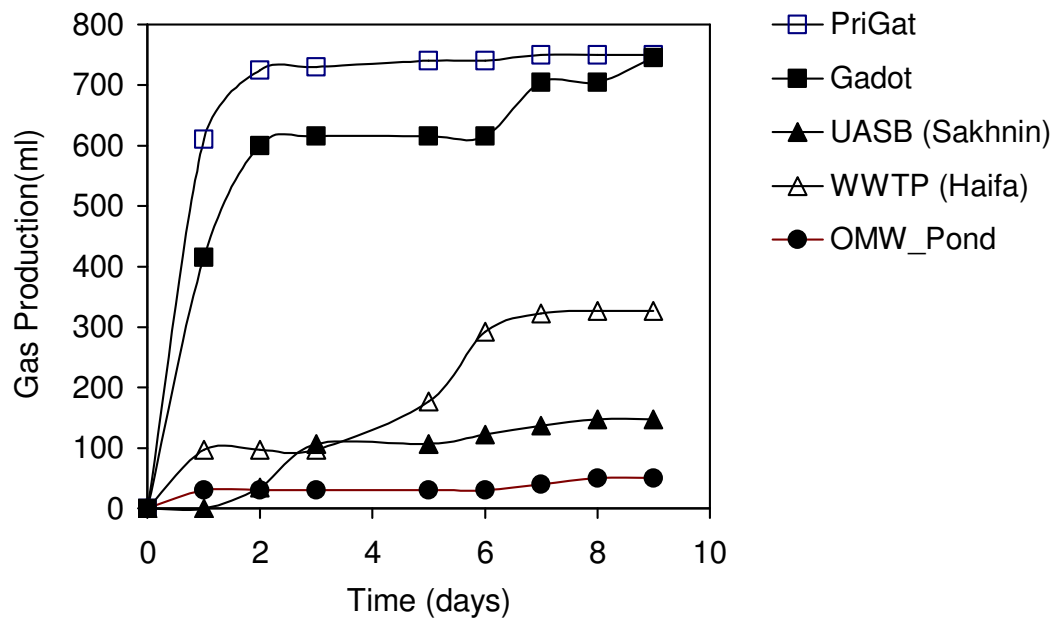


Fig 1b

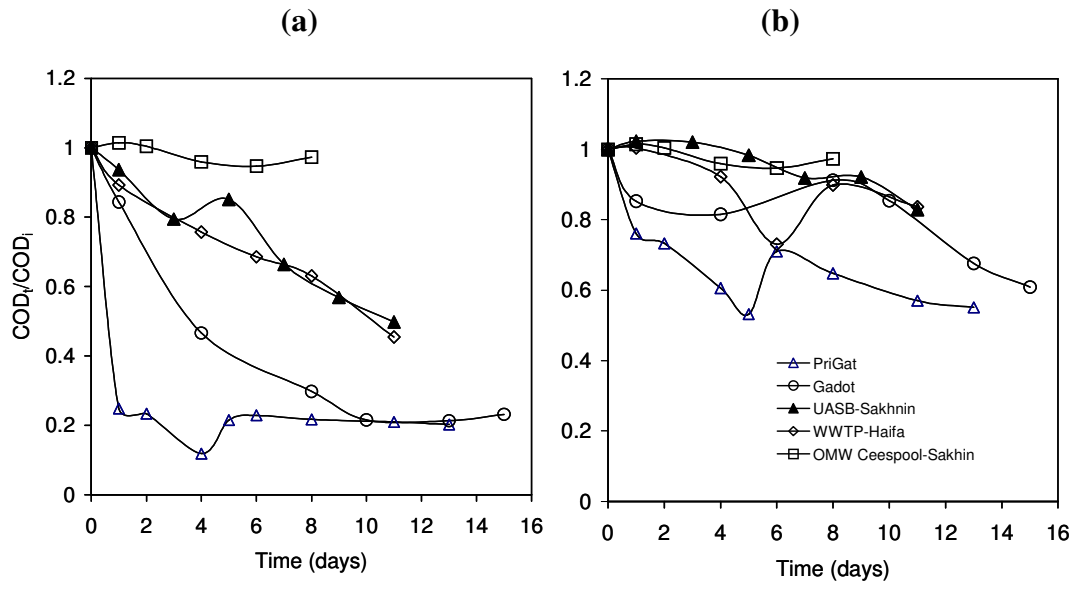


Fig 2

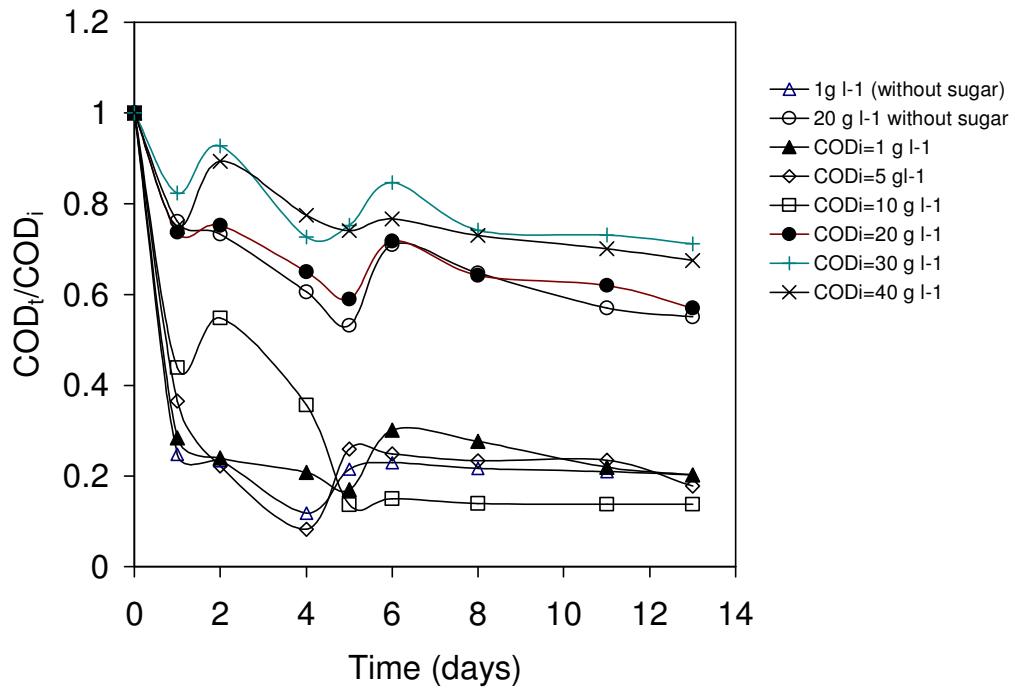


Fig 3

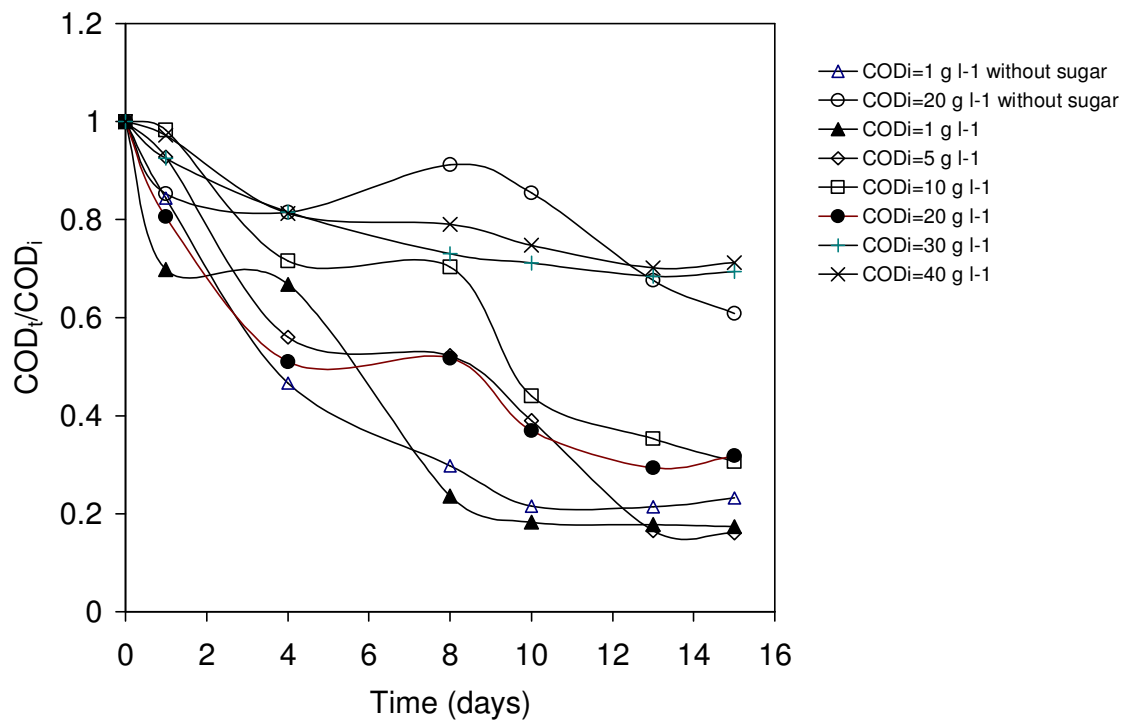


Fig 4

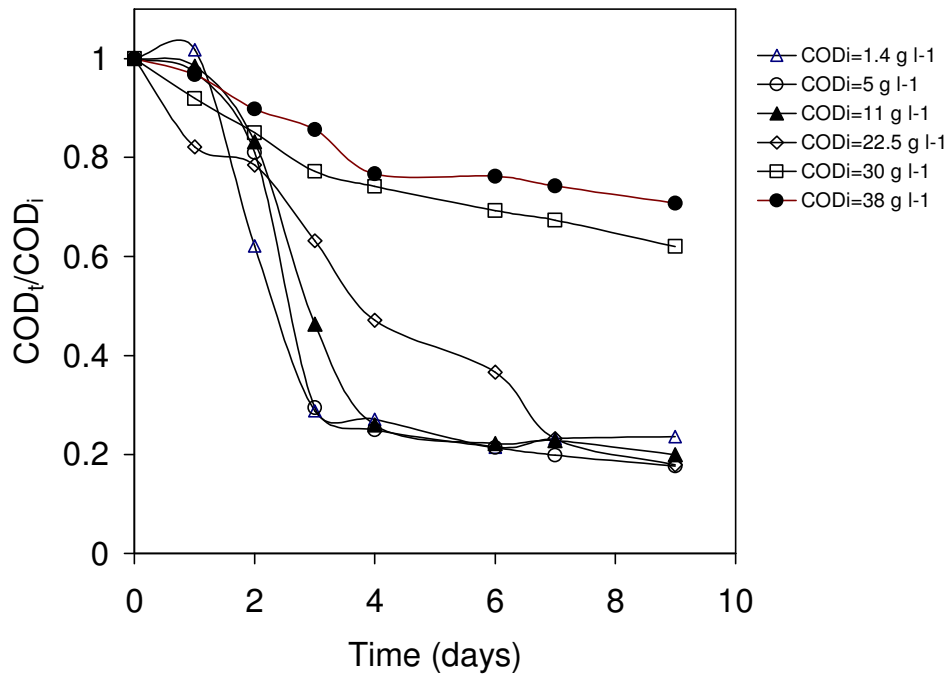


Fig 5