Low-Strength Wastewater Treatment with Combined Granular Anaerobic and Suspended Aerobic Cultures in Upflow Sludge Blanket Reactors

Tuba Hande Ergüder¹ and Göksel Niyazi Demirer²

Abstract: Combined cultures were developed from anaerobic granular and suspended aerobic cultures in three upflow sludge blanket reactors aerated at 10 mL air/min 4 h/day (R2), every other day (R3), and 24 h/day (R4). The use of combined cultures was found to be advantageous compared to the anaerobic granules for the treatment of low-strength wastewaters. During municipal wastewater treatment at influent 5-day biochemical oxygen demand (BOD₅) concentration of 53–118 mg/L (hydraulic retention time: 0.75 day), combined cultures in R2, R3, and R4 exhibited average BOD₅ removal efficiencies of 52, 75, and 76%, respectively. The use of these cultures might be proposed as an alternative for municipal wastewater treatment due to their advantages such as achievement of required discharge standards, prevention of biomass loss/settleability problems unlike activated sludge systems and possible methanogenic activity, as well as high settling characteristics comparable to those of anaerobic granules.


CE Database subject headings: Aeration; Municipal wastes; Wastewater management; Reactors.

Introduction

Studies with combined anaerobic and aerobic cultures cultivated in one reactor (coupled reactor) have been carried out for the last 20 years and literature information is limited to a few studies (Beunink and Rehm 1988, 1990; Gerritse et al. 1990; Kurosawa and Tanaka 1990; Gerritse and Gottschal 1992; Zitomer and Shrout 1998; Yerushalmi et al. 1999; Gardin et al. 2001). The survival of anaerobic cultures under aerobic or microaerobic [dissolved oxygen (DO) concentration <1 mg/L, Yerushalmi et al. 2001] conditions is attributed to either intrinsic tolerance or formation of anaerobic niches (shielding effect) in these studies. Considering the location of the cultures in the reactor, studies can be classified as follows: (1) both anaerobic and aerobic species co-immobilized in natural polymers operating under aerobic and/or microaerobic conditions (Beunink and Rehm 1988, 1990; Kurosawa and Tanaka 1990; Meyerhoff et al. 1997); (2) granular anaerobic cultures or anaerobic cultures embedded into supporting media and suspended aerobic cultures placed in a reactor operating under aerobic/microaerobic conditions (Gerritse and Gottschal 1992; Gardin et al. 2001); (3) suspended anaerobic and aerobic cultures in the reactor operating under microaerobic/oxygen-limited conditions (Gerritse et al. 1990; Zitomer and Shrout 1998); and (4) mixed suspended anaerobic (or anoxic) and aerobic cultures in packed-bed bioreactors (Hutchins et al. 1992; Boopathi et al. 1998; Yerushalmi et al. 1999). In some of these studies with either free or co-immobilized cultures of anaerobes (or anoxic cultures) and aerobes, it is observed that DO concentrations display alternating values. Oxygen gradient results in alternating conditions from aerobic and/or microaerobic to anaerobic conditions either through the reactor content (as in packed-bed or slurry reactors) or from bulk liquid to the depths of the immobilized cocultures and thus leads to possible living conditions for different types of bacteria and makes this coexistence possible.

The advantages of using combined anaerobic and aerobic cultures in one reactor might be remarkable in treatment of several contaminants. Total mineralization of some polycyclic aromatic hydrocarbons and highly chlorinated solvents that require sequentially operated anaerobic and aerobic or anoxic reactors were achieved in one coupled reactor by combined anaerobic and aerobic cultures (Beunink and Rehm 1988, 1990; Gerritse and Gottshal 1992). The applications of coupled reactors can be extended for combined nitrification/denitrification, the treatment (decolorization and biodegradation) of azo dyes, and enhanced biological phosphorous removal. Besides, coupled reactors containing both anaerobic and aerobic cultures might be advantageous compared to the conventional anaerobic and aerobic treatment systems. They may achieve lower effluent biochemical oxygen demand (BOD) values than conventional anaerobic treatment processes and recover from organic shock loads more quickly (Zitomer and Shrout 1998). Aeration and oxygen-limited conditions resulted in decreased alkalinity requirement due to CO₂ stripping, increased chemical oxygen demand (COD) removal, pH recovery, and methane production compared to anaerobic systems (Zitomer and Shrout 1998; 2000). The large amount of excess sludge problem in the conventional activated sludge systems can also be ad-

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dressed in the coupled reactors via the existence of slowly growing anaerobic cultures, which means reduced cost for the handling, transport, and disposal of produced biosolids.

The activated sludge system surely is the most widely used biological process for the treatment of municipal wastewaters (Rittmann and McCarty 2001). However, combined anaerobic and aerobic cultures might have the possible advantages of each culture type and the treatment of municipal wastewaters with these cultures might be advantageous in terms of less aeration, lower biosolids, and additional methane production. Thus, it is worthwhile to investigate the treatment of low-strength wastewater with combined anaerobic and aerobic cultures (i.e., combined cultures). This study was conducted to develop combined cultures from a mixture of granular anaerobic and suspended aerobic cultures in upflow sludge blanket (USB) reactors for the treatment of low-strength wastewaters under alternating aerobic and/or microaerobic/anaerobic conditions. The term “combined cultures” is defined in this study as the mixture of anaerobic and aerobic cultures that could survive and operate under alternating aerobic and/or microaerobic/anaerobic conditions in the same reactor. It was also aimed to determine the optimum/feasible aeration protocol for the treatment of municipal wastewater with the cultures developed under the conditions mentioned.

Materials and Methods

Seed Cultures

Anaerobic granular sludge and aerobic activated sludge were used as seed cultures in the experiments. Anaerobic granular sludge and aerobic activated sludge were obtained from the upflow anaerobic sludge blanket (UASB) reactors of the Wastewater Treatment Plant of Tekel Pasabahce Liquor Factory located in Istanbul and the recycle line of the activated sludge units of the Greater Municipality of Ankara Central Wastewater Treatment Plant, respectively.

Basal Medium and Municipal Wastewater

Basal medium (BM) containing all the necessary micro- and macronutrients were used in the experiments. The BM constituents (Merck, Germany) and concentrations of each (given in parentheses as mg/L) are as follows: NH₄Cl (400), KCl (400), (NH₄)₂HPO₄ (80), CaCl₂ (80), CoCl₂.6H₂O (10), Na₂PO₄.12H₂O (10), KI (10), CuCl₂.2H₂O (0.5), ZnCl₂ (0.5), AlCl₃.6H₂O (0.5), Na₂MoO₄.2H₂O (0.5), H₂BO₃ (0.5), NiCl₂.6H₂O (0.5), Na₂WO₄.2H₂O (0.5), Na₂SeO₃.5H₂O (0.76), cysteine (10), NaHCO₃ (6,000), yeast extract (50), and resazurin (provided by Dr. Daniel H. Zitomer, Marquette University, Milwaukee) (1.04) as an oxidation-reduction indicator dye (Demirer and Speece 1998; Ferguson 1999). The municipal wastewater was obtained from the effluents of primary settling tanks of the Greater Municipality of Ankara Central Wastewater Treatment Plant. The characterization of the municipal wastewater is given in Table 1.

Experimental Procedure

Experiments were performed in four identical USB reactors constructed of cylindrical glass columns. Each reactor had an internal diameter of 44 mm, height of 670 mm, and an active volume of 0.79 L. Three of the reactors (test reactors; R2, R3, and R4) were seeded with a mixture of granular anaerobic sludge and suspended aerobic activated sludge (40:60 v/v) achieving volatile suspended solids (VSS) concentration of 11.08±0.79 g/L in each reactor. In order to be used as anaerobic control, one reactor (R1) was only seeded with anaerobic granular sludge. The amount of granular sludge in the anaerobic control reactor was the same as the granular sludge amount seeded to the test reactors, leading to a VSS concentration of 4.15±0.32 g/L.

Table 1. Characteristics of Municipal Wastewater

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Range</th>
<th>Average</th>
<th>Number of measurement</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCOD (mg/L)</td>
<td>155–244</td>
<td>188±30</td>
<td>7</td>
</tr>
<tr>
<td>BOD₅ (mg/L)</td>
<td>53–118</td>
<td>93±21</td>
<td>7</td>
</tr>
<tr>
<td>pH</td>
<td>7.3–7.8</td>
<td>7.5±0.2</td>
<td>10</td>
</tr>
<tr>
<td>Alkalinity (mg/L)</td>
<td>300–410</td>
<td>360±47</td>
<td>10</td>
</tr>
<tr>
<td>NH₄–N (mg/L)</td>
<td>12–22</td>
<td>17±3</td>
<td>3</td>
</tr>
<tr>
<td>PO₄–P (mg/L)</td>
<td>4–11</td>
<td>6±1</td>
<td>3</td>
</tr>
<tr>
<td>SS (mg/L)</td>
<td>17–76</td>
<td>45±27</td>
<td>10</td>
</tr>
<tr>
<td>VSS (mg/L)</td>
<td>17–62</td>
<td>36±18</td>
<td>10</td>
</tr>
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</table>

SCOD=soluble chemical oxygen demand; BOD₅=5-day biochemical oxygen demand; SS=suspended solids; VSS=volatile suspended solids.

Test reactors were aerated with a flow rate of 10 mL/min under different aeration periods to investigate the effect of aeration period and accordingly the DO concentration on combined culture development and low-strength wastewater treatment. One of the test reactors (R2) was aerated 4 h/day, while the other one (R3) was continuously aerated 1 day followed by noaeration period for the subsequent day. The remaining test reactor (R4) was continuously aerated throughout the operation period. The aeration was applied via porous air stones located at the bottom of the reactors. The anaerobic control reactor was not aerated. The reactors were operated in a temperature-controlled room at 35±2°C.

The reactors were operated in two phases (Phases 1 and 2) where low-strength wastewater treatment was studied in both. Synthetic wastewater was applied in Phase 1, while original municipal wastewater was used in Phase 2. In Phase 1, as carbon source glucose+acetic acid (HAc) (1:1 in terms of COD) dissolved in BM solution was continuously fed to all reactors by a peristaltic pump (MasterFlex L/S Standard Drive Pump, P-07521, Cole-Parmer, United States). In order to distribute the influent uniformly, glass beads (6 mm diameter) were placed at the inlet of the reactors. Low-strength wastewaters are characterized as those dilute industrial effluents with COD values less than 2,000 mg/L COD (Lettinga and Hulshoff Pol 1991) and as those municipal sewages with COD values less than 1,000 mg/L (Mergaert et al. 1992; Ndon and Dague 1994). Therefore, the COD value of the synthetic wastewater was initially set to 1,800–2,000 mg/L resembling the low-strength wastewater characteristics. The strength of the wastewater was gradually decreased to 550–600 mg COD/L, after at least 80% soluble COD (SCOD) reduction was achieved. The hydraulic retention time (HRT) of the reactors was initially set as 1.5 days. In Phase 2, to investigate the effect of original wastewater on treatment performance of the reactors, municipal wastewater was fed until the end of the experiment.

The sludge samples periodically withdrawn from all reactors were subjected to specific methanogenic activity (SMA) and specific oxygen uptake rate (SOUR) assays to investigate the combined culture development. Performances of the reactors were assessed by determining the flow rate on a daily basis and DO
concentration in the reactors, pH, volatile fatty acid (VFA), alkalinity, SCOD, 5-day BOD (BOD₅) (during Phase 2), suspended solid (SS), and VSS of the effluents on a weekly basis. At the end of the operational period, the reactor contents were analyzed for settling velocity and sludge volume index (SVI) tests.

**Analytical Methods**

Influent and 24 h composite effluent samples of reactors were analyzed for SCOD, BOD₅, SS, VSS and alkalinity, VFA contents, and pH. pH measurements were performed with a pH meter (Model 2906, Jenway Ltd., United Kingdom) and a pH probe (G-05992-55, Cole Parmer Instrument Co., United States). VFA (as HAc) and bicarbonate alkalinity were measured according to the procedure given by Anderson and Yang (1992). BOD₅ and SS/VSS contents were measured by following standard methods (5210 B and 2540 D, E) (APHA 1998). COD content was measured according to an EPA approved reactor digestion method (for a COD range of 0–1,500 mg/L) as given in *Hach Water Analysis Handbook* (1989). For COD analysis, Hach spectrophotometer (Model 45600-02, Cole Parmer Instrument Co., United States), vials and hand-made COD solutions were used. The calibration curve of the hand-made solution with respect to the *Hach Water Analysis Handbook* (1989) had R² of 0.9933 indicating the applicability of COD solution for the analyses. For SCOD analyses the samples were initially filtered through 0.45 μm sterilized filter paper (Millipore). DO concentration in the USB reactors was measured by inserting the DO probe through the sampling port which was located at midheight and specially designed for DO measurements. The sample withdrawn from this port was entrapped between the DO probe and the reactor, and thus not in contact with the atmosphere.

**Microbial Activity**

SOUR (mg DO/g VSS h) assay was performed following the standard methods (2710 B) (APHA 1998) by a DO meter (9071 Model, Jenway Ltd., United Kingdom). For SMA analyses, reactors of 110 mL were seeded with the sludge to be tested and BM was added (50 mL effective volume). After flushing with N₂/CO₂ (70/30) gas mixture for 3–4 min (to maintain anaerobic conditions), reactors were sealed with rubber septa. A certain amount of HAc was fed to the reactors to achieve COD concentration of 3,000 mg/L. Reactors were incubated in the temperature-controlled room at 35±2°C. Cumulative gas production was measured daily over 5–7 days and biogas methane (CH₄) content was determined daily by using KOH stock solution (Erguder and Demirer 2005). At the end of the assay defined with the cessation of the gas production, VSS contents of the reactors were measured. SMA was calculated as the initial, that is, maximum CH₄ produced per gram of VSS per hour (mL CH₄/g VSS h) (Zitomer and Shroot 1998).

**Physical Characterization**

Settling velocity tests (Eritzer and Wilderer 2001) were accomplished in a plexiglas cylinder (6 cm in diameter and 90 cm in height). Single granules were placed in the cylinder filled with water. Settling time was taken for the distance of 50 cm after the upper 30 cm of the water column where granules could reach their final settling velocity. SVI was measured according to Standard methods (APHA 1998).

**Statistical Analysis**

One-way analysis of variance (ANOVA) (at a significance level of 0.05) was performed to determine whether the cultures developed in the reactors or their performances are statistically different. ANOVA was applied to the calculated ratio values of SCOD removal efficiency per g VSS (of each USB reactor) by using a subprogram of Microsoft Office Software Excel 98.

**Results and Discussion**

**Performances of USB Reactors**

Experiments lasted 370 days. DO analyses performed periodically during aeration and no-aeration periods indicated that anaerobic conditions were achieved in R1 and in R2 and R3 during no-aeration periods (DO: 0.0 mg/L). The anaerobic conditions mentioned were also verified by the supernatant color of the reactors. Resazurin, which was fed with the influent, exhibits no color at an oxidation-reduction potential relative to a standard hydrogen electrode (Eh) below approximately −50 mV, but is pink under more oxidized conditions (Zitomer 1998). Under anaerobic conditions, the supernatants of R1, R2, and R3 were colorless most of the time. During aeration periods DO concentrations measured in R2 (4 h/day), R3 (24 h/day of a 2-day cycle), and R4 (24 h/day, continuously) ranged within 0.2–1.5, 0.6–2.3, and 0.8–5.1 mg/L, respectively. In other words, microaerobic/aerobic conditions prevailed in R4 most of the time, and in R2 and R3 during the aeration periods. The aerobic conditions were also verified by the pink color of the supernatants of the reactors mentioned. DO analyses indicated that via the varied aeration protocols, alternating cyclic anaerobic/aerobic and/or microaerobic conditions were achieved in R2 and R3, while microaerobic/aerobic conditions were obtained in R4. Continuous aeration of R4 resulted in a more aerated system compared to the other test reactors; however, investigating the effect of continuous aeration on combined culture development and corresponding treatment performances, and determining the optimum aeration protocol were already among the objectives of this study. Besides, it should be noted that anaerobic conditions would possibly prevail in R4 through the anaerobic granules, where oxygen could not diffuse (Kurosawa and Tanaka 1990; Lens et al. 1995) and the target (alternating microaerobic and/or aerobic/anaerobic) conditions might still be possible.

Throughout the operation period of 370 days, the influent pH, VFA, and alkalinity values ranged within 6.9–7.4, 200–360, and 1,300–1,950 mg/L, respectively. All reactors operated properly during this period in terms of effluent VFA and alkalinity contents. The assessment was made considering the optimum operational conditions given for more sensitive anaerobic cultures as 6.5–8.2, below 250 mg/L and 1,000–5,000 mg/L for pH, VFA, and alkalinity, respectively (Speece 1996). The effluent pH values were above the pH values given for optimum anaerobic conditions as 6.5–8.2 (Speece 1996). However, the reactor contents had pH values ranging from 7.25 to 7.49, which were well within the optimum pH values set for both anaerobic and aerobic treatment systems (Tchobanoglous and Burton 1991).

The performances of the reactors were also considered in terms of effluent VSS concentrations and removal efficiencies. The results of the effluent VSS analyses are illustrated in Fig. 1. Because the effluent VSS/SS ratios of the reactors were more or less the same through the study with average values of 0.9±0.2
Operational conditions and results of experiments

As seen in Fig. 1, throughout the operation period R1, R2, R3, and R4 displayed maximum effluent VSS concentrations of 90, 240, 260, and 460 mg/L, respectively. The expected increase in the wasted sludge amount with the increase in aeration period (4–24 h as in R2–R4, respectively) was attributed to the duration of the shear force applied. However, the sludge washout in all test reactors was not significant enough to negatively affect their performances which are discussed below.

It was also observed in Fig. 1 that effluent VSS concentrations of all test reactors displayed a decreasing trend with respect to time. This might be due to the gradually decreasing influent SCOD concentration (from 2,000 to 155 mg/L) leading to reduced new cell formation [Fig. 2(a)]. The sudden decrease in the effluent VSS concentrations (below 20 mg/L, Fig. 1) observed with the start of Phase 2 (municipal wastewater application) might be related to lower influent SCOD concentration. On the other hand, this sudden decrease might be explained by starvation-induced response of the cultures, since under starvation conditions bacteria become more hydrophobic which facilitates microbial adhesion and aggregation (Bossier and Verstraete 1996; Tay et al. 2001). It is likely that microorganisms are able to change their surface characteristics when they face starvation and aggregation is their strategy against starvation (Tay et al. 2001; Liu and Tay 2004). It was reported that cell surface hydrophobicity seemed to be insensitive to the changes in the organic concentrations in the range of 500–3,000 mg COD/L (Liu et al. 2003; Liu and Tay 2004). Therefore, starvation of the cultures might not be the case in Phase 1 where SCOD concentrations ranged within 500–2,000 mg/L [Fig. 2(a)]. However, the influent SCOD was as low as 155 mg/L in Phase 2. Therefore, the suspended cultures in the reactor content might have increased their surface hydrophobicity due to starvation and attached on to the granular cultures which might be an explanation of the sudden decrease in the effluent VSS concentrations of all reactors.

Phase 1 (Synthetic Wastewater Application)

Synthetic wastewater (glucose+HAcdissolved in BM solution) was fed to all reactors for 225 days. The SCOD value of the synthetic wastewater was initially set to 1,800–2,000 mg/L. HRTs of the reactors were initially set as 1.5 days and remained constant up to Day 147 [Fig. 2(a)]. As previously stated, the operational conditions such as influent SCOD concentrations and HRT values are given with the corresponding average SCOD removal efficiencies (and observed minimum-maximum values) of the reactors in Table 2.

As seen in Table 2, the influent SCOD concentration was decreased to 1,300–1,600 mg/L by Day 59. Until Day 104, the performances of all reactors were more or less the same in terms of average SCOD removal efficiencies ranging within 77–84%. Achieving removal efficiencies greater than 80%, the wastewater strength was decreased to a range of 600–850 mg SCOD/L on Day 105. However, with the decrease in the wastewater strength the average removal efficiencies of all reactors decreased to 56–60% (with minimum efficiencies of 38–52%, Table 2). Thus, on Day 147 HRT was decreased from 1.5 to 0.75 days to increase the space organic loading rate (SOLR) which resulted in increased average removal efficiencies up to 63–75% (with maximum values of 67–78%) [Days 147–181, Table 2, Fig. 2(b)]. HRT values were further decreased to 0.58 day and then to 0.33 day on Days 182 and 199, respectively. In accordance, influent SCOD was decreased to 400–650 mg/L (Days 182–225). However,
with the decrease of HRT from 0.75 day to 0.33 day, all reactors displayed decreasing (and fluctuating) performances in terms of SCOD removal [Days 182–225, Table 2 and Figs. 2(c and d)]. At a HRT of 0.33 day, R1, R2, R3, and R4 displayed SCOD removal efficiency ranges of 49–62, 28–51, 37–59, and 35–62%, respectively (Table 2).

The test reactors displayed significant removal efficiencies except for HRTs of 0.58 and 0.33 day (Table 2). This indicated that under optimum operational conditions (HRT: 0.75 day, SCOD: 650–750 mg/L) the cultures in the test reactors performed significant activities. In other words, under alternating cyclic anaerobic/aerobic and/or microaerobic conditions (R2 and R3) and microaerobic/aerobic conditions (R4), the cultures in the test reactors operated well. They also displayed significant SOUR and SMA activities, indicating their survival despite the conditions mentioned, which are discussed in the following parts. Therefore, it can be stated that combined cultures that could survive and operate were developed.

SCOD removal efficiency data in Table 2 might indicate that during Phase 1 almost similar removal efficiencies were obtained among the test and anaerobic control reactors. However, the sludge amount in the reactors should be considered while comparing the treatment performances. Therefore, the ratio of SCOD removal efficiency to VSS content was evaluated for each reactor [i.e., SCOD removal (%)/g VSS]. The calculated ratios of the test reactors were later normalized with the ratio of anaerobic control reactor in order to make a better removal efficiency comparison. The results are separately illustrated for Phases 1 and 2 in Figs. 3(a and b), respectively. As observed in Fig. 3(a), combined cultures developed in R2, R3, and R4 displayed better performances compared to anaerobic granules in R1 most of the time at low influent SCOD concentrations (even less than 800 mg/L). ANOVA performed for the ratio values (SCOD removal efficiency/g VSS) also indicated that combined cultures developed in all test reactors were not statistically different in terms of SCOD removal efficiency per g VSS. Combined cultures developed under varied aeration conditions displayed similar performances. In other words, aeration periods of 4 h/day (R2), every other day (R3), or continuously during 24 h (R4) did not change the removal efficiency of the combined cultures for low-strength wastewaters with SCOD strength of 400–2,000 mg/L. It can be concluded that the use of combined cultures developed under varied aeration conditions and from a mixture of anaerobic granular and suspended aerobic cultures was advantageous compared to the anaerobic granules for the treatment of synthetic wastewater with influent SCOD of 400–2,000 mg/L under the conditions studied [Fig. 3(a), Phase 1].

**Table 2. Operating Conditions and SCOD Removal Efficiencies of USB Reactors**

<table>
<thead>
<tr>
<th>Days</th>
<th>HRT(^b) (days)</th>
<th>Influent SCOD (mg/L)</th>
<th>Average SCOD removal efficiency(^a) (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>R1</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R3</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>R4</td>
</tr>
<tr>
<td>1–30</td>
<td>1.50</td>
<td>1,800–2,000</td>
<td>85±2 (84–89)</td>
</tr>
<tr>
<td>31–58</td>
<td>1.50</td>
<td>1,600–1,800</td>
<td>84±2 (78–88)</td>
</tr>
<tr>
<td>59–104</td>
<td>1.50</td>
<td>1,300–1,600</td>
<td>77±4 (72–83)</td>
</tr>
<tr>
<td>105–146</td>
<td>1.50</td>
<td>600–850</td>
<td>56±12 (40–69)</td>
</tr>
<tr>
<td>147–181</td>
<td>0.75</td>
<td>650–750</td>
<td>63±4 (59–67)</td>
</tr>
<tr>
<td>182–198</td>
<td>0.58</td>
<td>400–650</td>
<td>61±2</td>
</tr>
<tr>
<td>199–225</td>
<td>0.33</td>
<td>500–650</td>
<td>56±6 (49–62)</td>
</tr>
<tr>
<td>226–370</td>
<td>0.75</td>
<td>155–244</td>
<td>51±19 (22–77)</td>
</tr>
</tbody>
</table>

\(^a\)The ranges in parentheses indicate the minimum and maximum soluble chemical oxygen demand (SCOD) removal efficiencies achieved during the corresponding operational conditions.

\(^b\)HRT=hydraulic retention time.

**Fig. 3. Normalized SCOD removal efficiency/g VSS values of test reactors for comparison with anaerobic control reactor: (a) Phase 1; (b) Phase 2**
Phase 2 (Municipal Wastewater Application)

During Phase 2 (Days 226–370) municipal wastewater with influent SCOD concentrations ranging from 155 to 244 mg/L was fed to the reactors at a HRT of 0.75 day [Fig. 2(a)]. In fact, this range resembled the weak strength municipal wastewater rather than a typical municipal wastewater of medium strength (COD of 400 mg/L, Tchobanoglous and Burton 1991). At such low influent SCOD values, all the reactors displayed unstable SCOD removal performances. The SCOD removal efficiencies of R1, R2, R3, and R4 were fluctuating in the ranges of 22–77, 10–77, 21–88, and 17–73%, respectively (Table 2). A similar fluctuating trend was also observed for the normalized values of SCOD removal efficiencies per g VSS [Fig. 3(b)]. This fluctuation might be due to the fluctuating and greater COD/BOD$_5$ ratio values of the municipal wastewater studied (1.43–2.92) than the typical value of 1.14, which indicates low biodegradability (Tchobanoglous and Burton 1991). However, combined cultures (in R2, R3, and R4) still displayed better SCOD removal performances compared to anaerobic cultures (R1) for most of the time [Fig. 3(b)].

The performances of the reactors or cultures were also assessed in terms of their BOD$_5$ removal efficiencies (Table 3). For influent BOD$_5$ values of 53–118 mg/L, R1, R2, R3, and R4 displayed average BOD$_5$ removal efficiencies of 40, 52, 75, and 76%, respectively (Table 3). It was concluded as in Phase 1 that in treatment of municipal wastewater all the combined cultures developed under varied aeration conditions were more advantageous compared to the anaerobic granular cultures in terms of BOD$_5$ removal. Normalized BOD$_5$ ratio values (BOD$_5$ removal efficiency/g VSS) calculated for each reactor also indicated better performances of the combined cultures (in R2, R3, and R4) compared to anaerobic cultures (R1) (Fig. 4). Table 3 and Fig. 4 revealed that the combined cultures developed in R3 and R4, which were aerated every other day or continuously, respectively, displayed almost the same BOD$_5$ removal performances, which were greater than those in R2 (aerated 4 h/day). The same comment was also valid for the normalized BOD$_5$ removal efficiency/g VSS ratio values for most of the time (Fig. 4). Thus, it was concluded that aeration (by 10 mL/min) either every other day or continuously resulted in the development of combined cultures with similar performances. To develop combined cultures with high performances under the conditions studied, aeration applied every other day was sufficient rather than continuous aeration.

Despite the greater BOD$_5$ removal performances (75–76%) of combined cultures than those of anaerobic granules, efficiency values were comparably lower than those of conventional aerobic systems, which are reported as 85–95% for a typical activated sludge system treating domestic wastewater (Tchobanoglous and Burton 1991). However, the average effluent BOD$_5$ values of R2, R3, and R4 were 44, 22, and 21 mg/L, respectively, which are below the effluent discharge standards of Turkey set as 45 mg/L for 24 h composite samples of treated domestic wastewaters (Table 3) (Water Pollution Control Regulations 1988). Effluent BOD$_5$ values of R3 and R4 were also below the European Union effluent discharge standards set as 25 mg/L (EU 1991) and the effluent discharge standards of the United States set as 30 mg/L for a 30 day average and 45 mg/L for a 7 day average (Federal Register 1984) through the municipal wastewater application phase (Table 3).

### Physical Characteristics and Microbial Activity

#### Physical Characteristics

It was visually observed that the ratio of the brown cultures to black cultures were highest in R4 which was aerated continuously, while it was the lowest in R2 (4 h of aeration/day). The brown or yellow color was related to the aerobic cultures while the black cultures were highest in R4 which was aerated continuously. This fluctuation might be due to the fluctuating and greater COD/BOD$_5$ ratio values of the municipal wastewater studied (1.43–2.92) than the typical value of 1.14, which indicates low biodegradability (Tchobanoglous and Burton 1991). However, combined cultures (in R2, R3, and R4) still displayed better SCOD removal performances compared to anaerobic cultures (R1) for most of the time [Fig. 3(b)].

#### Microbial Activity

- **Fig. 4.** Normalized BOD$_5$ removal efficiency/g VSS values of test reactors for comparison with anaerobic control reactor.
fore attributed to the aerobic culture population escalating with increasing aeration period, but not to the phasing out of the anaerobic granules due to aerobic conditions and further recombination of aerobic suspended solids as Hu et al. (2005) mentioned because of the significant SMAs of the developed cultures and the physical characteristics comparable to granules observed through the experimental period (mentioned below). It was also observed that floc-like brown colored cultures (i.e., aerobes) were located around the granules but not distributed separately in the supernatant. The adhesion of the aerobic cultures and attachment to granules might be regarded as an effective strategy of cells against environmental stresses such as alternating DO conditions (Erguder and Demirer, Private Communication 2006) and starvation (Bossier and Verstrate 1996; Tay et al. 2001), the latter accomplished during municipal wastewater application.

The SVI and settling velocities of the combined cultures measured at the end of 370 days are given in Table 4. As seen in Table 4, the cultures in R2, R3, and R4 had average settling velocities of 27, 26, and 25 m/h, which are within the range of the values given for anaerobic granules as 20–50 m/h (Schmidt and Ahring 1996) and for aerobic granules as 22–77 m/h (Liu and Tay 2004; Hu et al. 2005). SVI values, namely, 54, 63, 66 mL/g for R2, R3, and R4 are greater than the SVI values of anaerobic granules given as 35–45 mL/g (Noyola and Moreno 1994) but very comparable to those of aerobic granules stated as 50–85 mL/g (Tay et al. 2001). Table 4 also indicated that seeding of the test reactors with both granular anaerobic and suspended aerobic cultures resulted in the increased SVI values and decreased average settling velocities of the anaerobic granules from 36 to 66 mL/g and from 34 to 25 m/h, respectively. This might be explained by the attachment of the aerobic cultures (like flocs) on the granules which increased in amount with the increasing aeration periods and resulted in the decreasing settling performances (Table 4, in the order of R2, R3, and R4). The other possible reason for decreasing SVIs might be the mechanical disintegration of the granular cultures into small sized granules, which is probably enhanced by the aeration period. The formation of small granules of varied sizes might explain the increasing settling velocity ranges and increasing standard deviations proportional to the increased aeration periods (Table 4). Similar increasing SVI values were also observed in a study where the anaerobic granules were exposed to increasing DO levels (from 0.5 to 8 mg/L) (Shen and Guiot 1996). However, they explained the impairment of the granule settleability with the negative influence of DO and in turn the float off the sludge bed and its washout, which was not the case in this study (Fig. 1).

### Microbial Activity

SMA and SOUR analyses were performed periodically for 370 days of the operation period to investigate the activities of the combined cultures and in turn their survival under alternating conditions (Table 5). Because the reactors were operated at varying influent SCOD, SOLR, and HRT values through the operation period [Table 2, Figs. 2(a and b)], SMA and SOUR results were only evaluated among the reactors independent of time. The cultures developed in each reactor were compared considering their SMA and SOUR activity ranges but not with respect to their trends.

As seen in Table 5, SMA values of the cultures in R1, R2, R3, and R4 were approximately in the range of 9–40, 18–62, 14–32, 11–35 mL CH4/g VSS h, respectively. The activities of the combined cultures in R3 and R4 were comparable to those of anaerobic granules in R1 and to the typical SMA of anaerobic granules reported as 10.5–44 mL CH4/g VSS h (Schmidt and Ahring 1996). SMA values of cultures in R2 were even greater than the values reported and those of anaerobic granules in R1. Limited aeration (4 h/day) might have provided a certain level of mixing and increased the mass transfer between the liquid (thus substrate) and granules (without disturbing the granular structure). This might have resulted in an increased layer of active anaerobic culture in the granules and thus greater SMA values than those of nonaerated control granules (R1). Nevertheless, it can be stated that all combined cultures displayed significant methanogenic activities despite the cyclic (R2 and R3) or continuous (R4) microaerobic/anaerobic conditions achieved during aeration periods that lasted for 1 year. The methanogens might have been located deep inside the granules, where the oxygen could not diffuse. Thus, after the oxygen diffusive layer of 200–400 μm (Kurosawa and Tanaka 1990; Lens et al. 1995) anaerobic cultures were supported inside to operate.

As seen in Table 5, combined cultures developed in R2, R3, and R4 had approximate SOUR activities of 7–28, 6–23 and 6–20 mg DO/g VSS h, respectively. These values were comparable and greater than that of activated sludge given as 10 mg DO/g VSS h (Lens et al. 1995). This indicated that combined cultures in all test reactors were constituted of aerobic cultures. Besides, the SOUR values resembling those of activated sludge rather than those of aerobic granules (29–90 mg DO/g VSS h, Tay et al. 2004) indicated that the aerobic cultures were not in the form of granules but most probably in the form of flocs (surrounding the granular anaerobes due to brown colored granules observed).

### Table 4. Physical Characteristics of Combined Cultures

<table>
<thead>
<tr>
<th>Reactor type</th>
<th>Aeration period (10 mL/min)</th>
<th>Settling velocity (m/h)</th>
<th>Sludge volume index (mL/g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>R1</td>
<td>—</td>
<td>26–45</td>
<td>34±6</td>
</tr>
<tr>
<td>R2</td>
<td>4 h/day</td>
<td>18–50</td>
<td>27±10</td>
</tr>
<tr>
<td>R3</td>
<td>In every other day</td>
<td>16–50</td>
<td>26±7</td>
</tr>
<tr>
<td>R4</td>
<td>Continuously</td>
<td>14–60</td>
<td>25±11</td>
</tr>
</tbody>
</table>

### Table 5. Results of SMA and SOUR Analyses

<table>
<thead>
<tr>
<th>Phases</th>
<th>Days</th>
<th>SMA (mL CH4/g VSS h)/SOUR (mg DO/g VSS h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1: (Synthetic WW)</td>
<td>46</td>
<td>40.0/24.3/6.8</td>
</tr>
<tr>
<td></td>
<td>98</td>
<td>17.0/18.5/7.9</td>
</tr>
<tr>
<td></td>
<td>155</td>
<td>20.9/26.0/7.5</td>
</tr>
<tr>
<td></td>
<td>219</td>
<td>29.6/49.8/21.5</td>
</tr>
<tr>
<td>2: (Municipal WW)</td>
<td>265</td>
<td>39.7/21.2/28.3</td>
</tr>
<tr>
<td></td>
<td>318</td>
<td>31.7/30.6/26.3</td>
</tr>
<tr>
<td></td>
<td>370</td>
<td>35.6/8.2/9.6</td>
</tr>
</tbody>
</table>

aCombined representation of the specific methanogenic activity (SMA) and the specific oxygen uptake rate (SOUR) data for R2, R3, R4 as SMA/SOUR. Standard deviations of SOUR values are in the range of 0.01–0.13.

bSOUR values of cultures in R1 were not measured.

cWW=wastewater.

dSMA values were measured on Day 327.
Despite of the short aeration period of 4 h, combined cultures developed in R2 displayed high and even a greater range of SOUR values (7–28 mg DO/g VSS h) than those of in R3 and R4. This might be explained by the increased oxygen uptake rates (OURs) of the cultures under environmental stress such as prolonged anaerobic and/or microaerobic conditions. The average OUR values of the cultures in R2, R3, and R4 were 0.2406, 0.1623, and 0.1646 mg DO/L min, respectively. Erguder and Demirer (2005) demonstrated a similar result where the combined cultures exposed to cyclic anaerobic to aerobic conditions had greater OUR values compared to those of combined cultures exposed to cyclic aerobic to microaerobic conditions (i.e., more oxygen load or prolonged oxygenation). Yerushalmi et al. (2002) stated that cultures might use an alternative metabolic pathway to more effectively use the oxygen under its decreasing availability, which was demonstrated by the decreased oxygen half saturation coefficients with the decreased initial DO concentrations from 1 to 0.05 mg/L.

Conclusions

Combined cultures were developed from anaerobic granular and suspended aerobic cultures in USB reactors. Developed cultures aerated during 1 year for 4 h/day (R2, cyclic anaerobic to microaerobic/aerobic conditions), every day (R3, cyclic anaerobic to microaerobic/aerobic conditions), and continuously (R4, aerobic/microaerobic conditions) survived under the conditions studied and were even well processed. SMA (11–62 mL CH4/g VSS h) and SOUR (6–28 mg DO/g VSS h) values of the combined cultures were comparable to those of anaerobic granules and activated sludge, respectively. Their average settling velocities (25–27 m/h) and SVI values (54–66 mL/g) were well within the range of the values given for granular cultures. Both microbial and physical characteristics of the combined cultures indicated the conservation of the granular structure of the anaerobic granules and most probably their entrapment by aerobic cultures.

The use of combined cultures was found to be advantageous compared to the anaerobic granules for the treatment of low-strength wastewaters. The aeration period did not affect the performances of the cultures during synthetic wastewater application (influent SCOD of 400–2,000 mg/L). During municipal wastewater treatment, combined cultures exhibited average BOD5 removal efficiencies of 52–76%, which were greater than those of anaerobic granules (40%). Among the cultures developed, combined cultures which were aerated every other day (i.e., exposed to alternating cyclic anaerobic to aerobic/microaerobic conditions) were selected as the most advantageous due to their higher removal efficiencies, slightly better settling characteristics, and lower oxygen requirement.

The use of combined cultures might be proposed as an alternative to the conventional activated sludge systems for municipal wastewater treatment due to the achievement of required effluent BOD5 discharge standards and similar effluent quality to those of activated sludge systems. The problems encountered in the activated sludge systems such as sludge bulking, high sludge production, and related handling problems were not experienced with the combined cultures. Besides, they had the advantages of advanced anaerobic treatment systems such as uninhibited activity and high settling characteristics as well as possible methanogenic activity comparable to those of anaerobic granules. The use of combined cultures under cyclic anaerobic to aerobic/microaerobic conditions might also be promising for the treatment of pollutants requiring sequential anaerobic and aerobic/anoxic conditions in the same reactor and should be further investigated.

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References


