Comparison of Oxygen Transfer Parameters from Four Testing Methods in Three Activated Sludge Processes

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In this investigation, the mass transfer of oxygen was determined using four different testing methods in three activated sludge processes, as per the guidelines established by the American Society of Civil Engineers (ASCE 1997). The testing methods applied included the steady-state oxygen uptake rate (OUR), the non-steady-state changing power level (CPL), the non-steady-state hydrogen peroxide addition (HPA) and the off-gas methods. The analysis indicated that steady-state OUR and off-gas methods resulted in comparable estimates of oxygen transfer parameters, with somewhat higher variations observed in the data from the off-gas method. The application of HPA and CPL methods produced variable results under the same process conditions and these testing methods affected the process. Based on the comparative evaluation conducted in these controlled experiments, the validity of HPA and CPL tests to measure the oxygen transfer under process conditions is questionable. Overall, the off-gas method appears to be superior, as it does not require steady-state process conditions. However, under suitable conditions the steady-state OUR method may be an economical option to study oxygen transfer under process conditions.

Key words: activated sludge process, hydrogen peroxide, oxygen transfer testing, oxygen uptake rate

Introduction

Lewis and Whitman (1924), Higbie (1935) and Danckwerts (1951) initially explained the mass transfer of oxygen in clean water. The basic parameters that determine the mass transfer process are: (i) the amount of oxygen gradient, (ii) thickness of the liquid film, (iii) area and time of contact between oxygen and water, and (iv) the mixing conditions in the reactor. The impacts of physical variables such as temperature, pressure, reactor-geometry, mixing, surface tension and viscosity on oxygen transfer in clean water are well documented (Bewtra and Nichols 1964; U.S. EPA 1979; Eckenfelder 1980; WPCF and ASCE 1988; Gillot et al. 2000; Pincince 1999; Mueller et al. 2002). The influence of suspended and dissolved solids on clean water oxygen transfer depends on the nature of solids, and their physical and chemical interaction with the dissolved oxygen molecule (Wilhelm et al. 1977; Casey and Karmo 1974; Leu et al. 1998). An increase in dissolved inorganic salts tends to reduce oxygen solubility due to association of these salts with water molecules and decrease in molecular attraction between water and oxygen molecules. Ultimately, the net effect depends on the type and concentration of inorganic salts in water (Stack 1979). Similarly, the effect of a known chemical or surfactant addition to clean water depends on the properties (solubility, ionization, hydrophobic or hydrophilic character) and quantity of added chemical (Gurrol and Nekouinaini 1985; Backman et al. 1987a,b). The general role of surfactants in decreasing clean water oxygen mass transfer is well known (Davis and Acrivos 1966; Mancy and Barlage 1968; Eckenfelder 1980; Backman et al. 1987a,b; Wagner and Popel 1996; Rottorp 1998; Lin et al. 1998; Gillot et al. 2000). In the case of diffused aeration, diffuser-related variables including the orifice diameter, bubble diameter, material of construction, depth of submergence, airflow rate per diffuser, layout, diffuser density, wetting property and fouling nature influence clean water oxygen transfer (Bowers 1955; Eckenfelder 1959; Barnhart 1969; Mavinic and Bewtra 1974; Motarjemi and Jameson 1978; Huibregtse et al. 1983; Boyle and Redmond 1983; U.S. EPA 1989a,b; Geary and Rice 1991; Ashley et al. 1991, 1992; Kim and Boyle 1993; Fujie et al. 1997; Mueller et al. 2002). Based on a large number of studies a clean water oxygen-transfer standard was established to measure the oxygenation capacity of aeration devices (ASCE 1992). Thus, the mass transfer of oxygen is well understood in clean water; however, this is not the case under activated sludge process conditions.

The major differences between the clean and process water systems are: (i) the presence of much higher concentrations of soluble and particulate, organic and inorganic contaminants of influent origin, as well as those produced in the biochemical reactions, (ii) the heterogeneous, complex nature of biological flocs, and (iii)
actively respiring microorganisms associated with the biological flocs under the process conditions. The influence of physical, chemical and biological reactor conditions on \( \alpha \) (the ratio of mass transfer coefficient under process conditions to clean water) and \( \text{OTE}_f \) (oxygen transfer efficiency) is not clearly known. Several studies reported on oxygen transfer parameters under process conditions (Boyle and Campbell 1984; Hwang and Stensstrom 1985; Boyle et al. 1989; Mines and Sherrard 1987; Mueller and Boyle 1988; Mueller and Stensel 1990; Reiber and Stensel 1985). However, limited data are available on the influence of process-related variables (influent characteristics, solids retention time [SRT], hydraulic retention time [HRT], biomass recirculation rates, process configuration, biochemical reaction rates, etc.) on oxygen transfer, as stated in the aeration manuals (U.S. EPA 1989a,b). Unfortunately, the situation has not improved since the publication of these documents. In particular, little information exists on \( \alpha \) and \( \text{OTE}_f \) in biological nutrient removal (BNR) processes. Fisher (1996) and Fisher and Boyle (1999) indicated insignificant influence of upstream anaerobic and anoxic zones on \( \alpha \) and \( \text{OTE}_f \) in a BNR process. However, this is contrary to the other observations (Randall et al. 1992a,b). Even in the case of conventional activated sludge systems, a survey of full-scale systems showed that low SRT, non-nitrifying systems had lower \( \alpha \) and \( \text{OTE}_f \) (Groves et al. 1992). It is important to further investigate the fundamental role of biochemical reactions (carbon oxidation, nitrification, denitrification and enhanced phosphorus removal) on \( \text{OTE}_f \) under controlled conditions, given the high costs associated with energy utilized for aeration.

Therefore, the overall goal of this research was to study the impact of biochemical reactions on oxygen transfer under controlled process conditions in three different, laboratory-scale, activated sludge processes, namely, the completely mixed activated sludge (CMAS), the modified Ludzack-Ettinger (MLE) and the enhanced biological phosphorus removal - University of Cape Town (UCT) process, by varying the solids retention time (SRT) and the influent carbon, nitrogen and phosphorus concentrations (Mahendraker 2003). As part of this investigation, four different methods of measuring the oxygen transfer under process conditions, as per the ASCE guidelines (ASCE 1997) were employed. They were the steady-state oxygen uptake rate (OUR) method, the non-steady-state changing power level (CPL) method, the non-steady-state hydrogen peroxide addition (HPA) method and the off-gas method. In addition to measuring the oxygen transfer coefficient, a careful monitoring of the process performance was carried out throughout the study. The objective of this particular manuscript is to present a comparative evaluation of oxygen transfer coefficient data obtained from different methods and to comment on the methods of testing.

### Materials and Methods

#### Activated Sludge Processes

The CMAS, MLE and UCT processes investigated are shown in a simplified manner in Fig. 1. In these processes, the aerobic, anoxic and aerobic reactor volumes were 16, 8.55 and 3.85 L, respectively, whereas the clarifier volume was equal to 5.25 L. The influent, return activated sludge (RAS) and internal biomass recirculation rates were constant at 3 L/h. Each of the reactors had a mixer operating at 60 rpm and the clarifier had an internal scraper rotating at 4 rpm to dislodge the attached biomass. The entire setup was located in a temperature-controlled room set at 20 ± 0.5°C. The aerobic reactor was fitted with a fine-pore ceramic diffuser (Model Number AS2, Aquatic Ecosystems, Fla.) made from heat-bonded silica with a maximum pore size of 140 microns, producing air bubbles of 1- to 3-mm radius in clean water. Air was supplied via filter, pressure regulator, pressure gauges and high-precision flow metre (accuracy ±2%, Labcor catalogue #P-32044-18), as shown in Fig. 2A. The liquid and diffuser submergence depths were equal to 525 and 465 mm, respectively.

The synthetic wastewater used in these experiments was made from glucose (245 to 320 mg/L), yeast extract (45 mg/L), peptone (45 mg/L), sodium acetate (175 to
350 mg/L), ammonium chloride (60 to 155 mg/L), potassium phosphate monobasic (20 to 50 mg/L), calcium chloride (31.50 mg/L), potassium chloride (22.50 mg/L), manganous sulfate (0.09 mg/L), ferrous sulfate (1.0 mg/L) and zinc sulfate (0.45 mg/L) in tap water. All compounds were reagent grade, except potassium chloride and sodium bicarbonate, which were commercial grade and may have had a slightly higher amount of other compounds (as contaminants) than the reagent-grade chemicals. The required influent carbon (COD), nitrogen (TKN) and phosphorus (TP) were adjusted by the amount of sodium acetate (or glucose), ammonium chloride and potassium phosphate monobasic, respectively, added during the feed preparation. Many factors affect the mass transfer of oxygen under process conditions (U.S. EPA 1989a,b). Therefore, a number of variables were maintained uniform to reduce the experimental noise. They included: (i) reactor geometry, (ii) mechanical mixing intensity in all the reactors, (iii) aerobic reactor DO concentration (2.5 ± 0.5 mg/L), (iv) temperature, (v) air supply pressure and quality (dry air supplied at 1 atm pressure and 20ºC), (vi) RAS and internal biomass recirculation rate(s) (3.0 L/min), (vii) feed flow rate (3.0 L/min), (viii) nominal HRT in the aerobic reactor, (ix) concentrations of inorganic metals and (x) diffuser design. For each testing period, lasting for a maximum of 15 days, a new diffuser with known clean water oxygen transfer parameters was employed to minimize diffuser fouling affecting the oxygen transfer measurements.

Daily grab samples were collected from the feed tank, aerobic, anoxic and anaerobic reactors and effluent for analysis. The parameters analyzed included mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS), chemical oxygen demand (COD), total organic carbon (TOC), ammonia-nitrogen (NH₃-N), nitrite plus nitrate nitrogen species (NO₂⁻-N, NO₃⁻-N), orthophosphate (PO₄-P), total phosphorus (TP), total Kjeldahl nitrogen (TKN), conductivity and sludge volume index (SVI) following the Standard Methods (APHA et al. 1992). In addition, dissolved oxygen (DO), oxidation-reduction potential (ORP) and pH in the aerobic reactor were continuously monitored and recorded by an on-line data acquisition system. The airflow rate (Qₘᵣ) was set in such a way as to maintain a DO concentration between 2.5 ± 0.5 mg/L in the aerobic reactor, at all times to ensure a common oxygen gradient. A barometer constantly monitored the atmospheric humidity and pressure in the temperature-controlled experimental room. The solids retention time (SRT) was controlled by the daily measurement of suspended solids in all the reactors, clarifier underflow and effluent, and wasting appropriate solids from the system.

**Oxygen Transfer Testing Methods under Process Conditions**

**Steady-state OUR test.** Under the steady-state process conditions, when the reactor DO concentration is constant, the oxygen uptake rate (also denoted as R = OUR) is equal to the oxygen transfer rate (OTR) assuming no other oxygen-consuming reaction is simultaneously occurring. The basis of this test is a mass balance of DO across a completely mixed aerobic reactor operating under steady-state conditions, which results in equation 1, as described in the guidelines (ASCE 1997):

\[
\frac{dC}{dt} = \frac{1}{\theta_T} (C_1 - C) + K_L a_f (C_{sf} - C) - R
\]

where \(\theta_T\) is the true hydraulic retention time of the aerobic reactor \((h) = V_R/Q\), where \(V_R\) is aerobic reactor volume \([L]\) and \(Q\) is total influent flow to the aerobic reactor \([L/h]\)], \(C_1\) is DO entering the aerobic reactor \((mg/L)\), \(C\) is DO in the aerobic reactor at any time \(t\) \((mg/L)\), \(K_L a_f\) is volumetric mass transfer coefficient \((L/min)\), \(C_{sf}\) is DO saturated concentration as time approaches infinity \((mg/L)\), and \(R\) is microbial oxygen uptake rate \((OUR) \ (mg \ O_2/L/h)\). Under steady-state conditions, \(dC/dt = 0\) and the oxygen transfer rate \((OTR)_f = R \ (g/d)\).

In these investigations, since the aerobic reactor received feed or mixed liquor that was devoid of DO, \(C_1 = 0\). Rewriting equation 1, one gets equation 2:
where, $C_R$ is the steady-state DO concentration in the aerobic reactor (mg/L). The $\theta_T$ values in the CMAS, MLE and UCT processes were 2.67, 1.78 and 1.78 h, respectively. The conditions to be satisfied for the duration of the steady-state test are (ASCE 1997): (i) a constant respiration rate, (ii) constant influent flow and characteristics, (iii) constant influent DO, (iv) constant recycle flow rates, (v) constant biomass concentration, and (vi) reactor DO greater than 1 mg/L. The temperature correction for $K_{Laf}$ and $\alpha$ were determined using equations 3, 4 and 5, respectively:

$$K_{Laf} = \left[ \frac{R + \frac{C_R}{\theta_T}}{C_{sf} - C_R} \right]$$

(2)

$$\alpha = \frac{K_{Laf(\text{processwater})}}{K_{Laf(\text{cleanwater})}} = \frac{K_{Laf}}{K_f}$$

(3)

$$\% OTE_f = \frac{OTR_f}{M_{O_2}} \times 100$$

(4)

$$\frac{C_R - C}{C_R - C_0} = \exp - \left( K \times t \right)$$

(5)

where, $K = (K_{f,\alpha} + 1/\theta_T)$ and $K_{f,\alpha} = (K-1/\theta_T)$. The DO concentration versus time data were subjected to non-linear, least square approximation regression using the statistical software SYSTAT 9 (SPSS Inc. 1999) to estimate $K$ and $C_0$. The estimated value of $K_{f,\alpha}$ was corrected to 20°C and $\alpha$ value was calculated. Then, the oxygen transfer rate under field condition ($OTR_f$) was determined using equation 7.

$$OTR_f = \frac{\alpha(SOTR)(T_{20} - T)}{C_{20} - \beta \times C_{20} - C_R}$$

(6)

where $\beta$ is the ratio of saturated DO concentration in process water to that in clean water under similar conditions of temperature and partial pressure ($\beta = 0.99$), SOTR is the standard oxygen transfer rate and it was obtained from the clean water test data of the diffuser (g/h), and $C_{20}$ is the clean water DO saturated concentration at 20°C, 1 atmospheric pressure and 100% relative humidity (9.08 mg/L).

Non-steady-state tests. The two non-steady-state tests applied in this study were: (i) the hydrogen peroxide addition (HPA) method and (ii) the changing power level (CPL) technique. These tests involved measuring DO concentration over time in the reactor, after elevating it by the external addition of $H_2O_2$ or by increasing aeration from the steady-state normal operating conditions and analyzing the data according to equation 6 (ASCE 1997):

$$\frac{C_R - C}{C_R - C_0} = \exp - \left( K \times t \right)$$

(7)

Non-steady-state hydrogen peroxide addition (HPA) method. In this test, $H_2O_2$ was used as a source of instantaneous oxygen to the mixed liquor, while the process was operating under uniform conditions at constant air supply. Because of the slug addition of oxygen, the DO concentration increases rapidly and eventually the excess oxygen is desorbed returning the process to the steady-state DO concentration ($C_0$). The test creates non-steady-state conditions as far as the DO concentration in the liquid phase is concerned. The DO concentration decrease with time was analyzed as per the procedure given in the previous section. The major advantages of this method are: (i) reactor hydrodynamic conditions remain unchanged, (ii) it is easy to execute and (iii) the low cost of testing. Theoretically, 2.125 mg $H_2O_2$ is required to generate one mg $O_2$ and a catalyst like the $Fe^{2+}$ will be required for the Fenton reaction, which is usually present under the process conditions. Initial tests indicated that an addition of 1 mL of 30% concentration of $H_2O_2$ resulted in DO increase from 2.5 mg/L to 9 to 10 mg/L in the reactor under process conditions. Based on these results, in the UCT 10-day SRT first and second runs, 1 mL (2 tests per day) of $H_2O_2$ was added and in the UCT 15-day SRT first run, 0.75 mL (1 test per day) of $H_2O_2$ was added. In addition, a trial $H_2O_2$ test was conducted a day before starting the tests to verify the DO increase in the reactor.

Non-steady-state changing power level (CPL) method. In these tests, airflow rate was increased from the process set point to a higher level for 5 to 7 min, and then returned to the normal operating level. This produced an increase of up to 3 mg/L of DO above the steady-state concentration. Eventually, at the end of the
test the DO concentration returned to the steady-state concentration (Cₘ) and the data obtained were analyzed in the same manner as described before. The advantages of this method are: (i) no addition of chemical and (ii) existing equipment may be sufficient to carry out the test. However, the test rapidly changes the hydrodynamic characteristics in the reactor.

**Off-gas method.** The OTEf was determined from the molar ratios of the inlet and outlet gas fractions as described by Redmon et al. (1983) and ASCE (1997).

\[
\% \text{OTE}_{\text{f}} = \left[ \frac{MR_i - MR_e}{MR_i} \right] \times 100
\]  

(8)

Where MRᵢ and MRₑ are the molar ratios of the inlet and outlet oxygen to inert gas fractions, respectively, defined as follows:

\[
MR_i = \frac{MF_i}{(1 - MF_i - MF_{CO2\text{inf}} - MF_{wv\text{inf}})}
\]  

(9)

\[
MR_e = \frac{MF_e}{(1 - MF_e - MF_{CO2\text{eff}} - MF_{wveff})}
\]  

(10)

where, MFᵢ and MFₑ are molar fraction of oxygen in the inlet and outlet gases, MF₀₂ᵢ and MF₀₂ₑ are molar fraction of carbon dioxide in the inlet and outlet gases, MFᵢwv and MFₑwveff are molar fraction of water vapour in the inlet and outlet gases. In this work, MFᵢwv = 0 (dry influent air). The assumptions to apply this method are: (i) inert gases and nitrogen are neither produced nor consumed in the process, (ii) process conditions are uniform at the sampling location, (iii) DO concentration in the liquid is constant and (iv) the surface aeration is negligible compared to diffused aeration. Using the OTEᵢ value obtained from equation 8, the OTRᵢ (g O₂/d) was calculated.

\[
\text{OTR}_{\text{i}} = \frac{(\text{OTE}_{\text{i}}/100)^*Q_{\text{air}}^*\rho_{\text{oxygen}}}{(\text{Q}_{\text{air}})^*\rho_{\text{oxygen}})*24*60}
\]  

(11)

where Qᵢ is the airflow rate (mL/min), MF₀₂ᵢ and ρᵢ are the mass fraction of oxygen in air (0.232), and ρᵢ is the density of air (0.0012 g/mL). The arrangement for off-gas collection shown in Fig. 2B consisted of: (i) 65-mm inverted plastic funnel immersed to 10 mm in the liquid, (ii) 350-mL gas collection bulb with a gas-tight septum and (iii) associated tubing. The funnel diameter at the liquid-gas interface was approximately 50 mm. The total surface area of the reactor and that occupied by the funnel were 31,400 and 2000 mm², respectively, with the funnel occupying 6.4% of the total area. The volume of the gas collection system including the funnel and tubing was close to 500 mL. Considering the maximum applied airflow rate of 1338 mL/min (in the UCT 15-day SRT low nitrogen and phosphorus run) the minimum average residence time of gases in the collection system based on a proportional flow through the funnel was equal to 5.9 min. This time was much less than 15 min of interval allowed between two successive sample collections. The gas samples were collected in a 1-mL gas-tight syringe (Hamilton Model 1001) and analyzed immediately in a Fisher Hamilton Gas Partitioner (Model 29) connected to a Spectraphysics SP 4290 integrator. On each of the days, at least six off-gas samples were analyzed. Furthermore, the funnel position changed to a new location after each sample collection, so that on average 40% of the total area was covered, which was more than the minimum 2% test surface area specified in the guidelines (ASCE 1997). In addition, 2 to 3 influent air samples were analyzed to verify the influent air quality on each test day.

Since temperature (20 ± 0.5°C), total pressure (1 atm or 760 mm Hg) and sample volume (1 mL) were constant, the number of moles of each gas within the sample was directly proportional to the volume of the gas in the sample. Further, the number of moles of a gas in a given volume is the molar concentration of that gas. In addition, the pressure exerted by a gas is directly proportional to its molar concentration, at constant temperature, volume and total pressure. Thus, in this work, the molar fraction was equal to the partial pressure fraction of the gas. The gas analyzer output data were in percentage CO₂, N₂, and O₂ v/v for total of 1 mL sample, at one atmospheric pressure and 20 ± 0.5°C. These data were converted to mole fractions by dividing values by 100 to get the partial pressure fractions of the gases. In this study, water vapour pressure was present only in the off-gases; when a gas is collected over water, only a part of the total pressure is exerted by the gas itself and some of the pressure is exerted by the water vapour. The actual pressure on the gases is the total outside pressure minus the water vapour pressure, at that particular temperature. Therefore, in the calculation of volume collected over water, the partial pressure of water vapour was subtracted from the total pressure under which the gas was collected, to obtain the true pressure on the gases. The output data from the gas analyzer did not include the effect of water vapour present in the off-gas samples. Therefore, the effect of water vapour partial pressure at the process temperature was corrected. The gas analyzer readings were reduced by the fraction equal to the (760-water vapour partial pressure in mm Hg at the process temperature)/(760), Based on the procedure explained above, OTEᵢ and OTRᵢ values were determined.

**Results and Discussion**

Figure 3 presents the aerobic reactor dissolved oxygen dynamics in HPA, CPL and OUR tests. Figure 3A shows a rapid DO increase when hydrogen peroxide was added to the reactor. This confirmed the presence of completely mixed conditions in the reactor, supporting an earlier
finding of a dye tracer study, conducted before bringing the reactor into service (Mahendraker 2003). Figure 3B shows a sluggish increase in DO in a non-steady-state CPL test, contrary to the expectations. A lag time of approximately five minutes existed before an increase of up to 3 mg/L of DO could be observed in the tests, indicating that a rapid increase in airflow rate did not quickly increase reactor DO under the process conditions. It was possible that the sudden increase in turbulence deflocculated and increased oxygen utilization by the biomass, as mentioned by Markl et al. (1991), delaying the rise in residual DO in the CPL test. However, in the non-linear regression analysis, only the desorption portion of the curve, starting from the highest DO concentration and ending with the concentration close to that existing prior to the test was considered, and the lack of rapid DO increase by itself was not a factor in the estimation of $K_{LAV}$ and $C_0$. Figure 3C shows an example when three OUR tests were performed in a single day. This figure also shows the DO concentration in the aerobic reactor while conducting the OUR tests. It is clear from Fig. 3C that the process was operating under steady-state conditions and a withdrawal of 350 mL mixed liquor (out of the total 16 L) did not affect the aerobic reactor DO concentration.

Table 1 presents the results of the steady-state OUR and the non-steady-state HPA tests conducted in the UCT process operating at 10 and 15 days of SRT. In the first and second experiments, on a test day, OUR followed by HPA tests were conducted in duplicate, whereas, in the third experiment duplicate OUR tests followed by a single HPA test were conducted each day. In the fourth and fifth sets of experiments, only OUR tests (2–3 tests per day) were conducted. The duration between the end of the first experiment and the start of the second experiment was 10 days (one SRT) and in all other cases it was 30 days or more. The variations in oxygen transfer parameters determined by the HPA method were between -9.3 to +3.9% of the values obtained from the OUR method, which compare well with data in literature (ASCE 1997; Mueller and Boyle 1988; Capela et al. 2004). In this study, a remarkable consistency between the OUR and HPA test results can be seen. Furthermore, considering the small size of reactor, high values of oxygen transfer parameters were a surprise in the first three experiments. It is difficult to compare oxygen transfer parameters from different studies because of the number of associated variables. However, $\alpha$ values arrived at in the first three experiments were above the averages reported in the literature with real wastewaters (Redmon 1998; Boyle et al. 1994). For example, the mean $\alpha$ ranged from 0.39 to 0.51 in a municipal wastewater pilot-scale BNR process using fine-pore diffusers in the study conducted by Fischer (1996) and Fischer and Boyle (1999). At one of the activated sludge plants (site C), Redmon et al. (1983) found 54% higher OTEf in a HPA test compared to the off-gas test and the reasons for the variation were unclear from this report.

In addition to the surprisingly high oxygen transfer parameters, the H$_2$O$_2$ addition resulted in rapid increases in ORP and pH and such changes potentially alter the metabolic functioning of microorganisms (Mahendraker et al. 2002). This test also led to a loss in process efficiency in terms of COD, ammonia and phosphorus removal by the process (Mahendraker et al. 2002, In press). On some of the test days, more than 100% COD and nitrogen mass balances were determined (Mahendraker 2003), which indicates that the hydrogen peroxide and its reaction products caused release of carbon and nitrogen from cell or biomass components (Cabiscol et al. 2000). In particular, one of the reaction products,
hydroxyl radical (OH•), is the most potent oxidizing radical with no known antioxidant enzymes (Halliwell 1984; Brock et al. 1994). The high rate of respiration was likely due to the microbial need to repair damaged components and oxidize what is not possible to repair. In essence, oxidative stress conditions increased the maintenance energy and respiration rate (Russell and Cook 1995). The capacity of a bacterial culture to neutralize the oxidative conditions depends on the availability of antioxidant enzymes such as superoxide dismutase, catalase, peroxidase or glutathione (Brock et al. 1994). Under normal circumstances, bacterial cells regulate the production of H₂O₂ and other reactive oxygen species generated in the aerobic respiration process (Flecha and Demple 1995, 1997). However, the external addition of H₂O₂ seemed to have upset the control mechanism by creating additional demand for the antioxidant enzymes. A detailed account of the impacts of the HPA test on the activated sludge process was presented earlier (Mahendraker et al. 2002, In press).

The same-day application of OUR and HPA tests affected the process performance (Experiments 1, 2 and 3), therefore, it was concluded that the oxygen transfer parameters derived under these conditions were suspect, even though the data were similar between OUR and HPA methods. The data obtained with the application of only the OUR method under similar influent and process operating conditions in Experiments 4 and 5 showed that the results from the OUR method in the earlier period when both the HPA and OUR methods were employed on the same day, in Experiments 1, 2 and 3 were affected by the addition of hydrogen peroxide. Mines and Sherrard (1987) reported enhanced oxygen transfer rates utilizing a BOD type of test, which was similar to the current study. Subsequently, Mueller and Stensel (1990) demonstrated that a BOD type of test overestimated OUR and oxygen transfer parameters under oxygen-limited and high organic load conditions. Mueller and Stensel (1990) observed correct estimations of oxygen transfer rates in the absence of oxygen limitation and when low residual substrate concentration was present in the BOD-type test. In this study, the loading and SRT were common between the periods when: (i) OUR and HPA tests were applied. Therefore, the reason for the discrepancy in the results appears to be the action of hydrogen peroxide and the resultant reactive oxygen species. Furthermore, low F/M ratios (at 10- and 15-day SRTs = 0.24 and 0.21 g COD applied/g MLSS, respectively) and soluble substrate concentrations (15 to 30 mg COD/L) existed in these experiments, unlike the experimental conditions that were existing in the study conducted by Mines and Sherrard (1987). There was limited biodegradable substrate available in the OUR tests in the present study.

<table>
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<tr>
<th>Experiment number</th>
<th>Process description</th>
<th>Q_{afr} (mL min^-1)</th>
<th>No. of test days</th>
<th>Method</th>
<th>K_{1afr} (h^-1)</th>
<th>α</th>
<th>OTR_f (g d^-1)</th>
<th>OTE_f (%)</th>
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<tr>
<td>1</td>
<td>UCT 10-day SRT</td>
<td>854</td>
<td>7</td>
<td>OUR</td>
<td>6.28 ± 0.47</td>
<td>0.86 ± 0.06</td>
<td>14.37 ± 1.07</td>
<td>4.23 ± 0.31</td>
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<td></td>
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<td></td>
<td>HPA</td>
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<td>4.04 ± 0.24</td>
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<td>-9.3</td>
<td>-4.7</td>
<td>-4.5</td>
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<tr>
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<td>UCT 10-day SRT</td>
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<td>OUR</td>
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<td>5.10 ± 0.23</td>
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<td>OUR</td>
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<td>14.37 ± 1.34</td>
<td>4.40 ± 0.58</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% Mean variation</td>
<td>3.9</td>
<td>3.5</td>
<td>-3.6</td>
<td>0.2</td>
</tr>
<tr>
<td>4</td>
<td>UCT 15-day SRT</td>
<td>854</td>
<td>13</td>
<td>OUR</td>
<td>4.12 ± 0.26</td>
<td>0.60 ± 0.04</td>
<td>9.94 ± 0.81</td>
<td>2.93 ± 0.24</td>
</tr>
<tr>
<td></td>
<td>Run #2</td>
<td></td>
<td></td>
<td>HPA</td>
<td>————</td>
<td>————</td>
<td>————</td>
<td>————</td>
</tr>
<tr>
<td>5</td>
<td>UCT 10-day SRT</td>
<td>854</td>
<td>5</td>
<td>OUR</td>
<td>3.82 ± 0.16</td>
<td>0.55 ± 0.02</td>
<td>9.04 ± 0.61</td>
<td>2.66 ± 0.18</td>
</tr>
<tr>
<td></td>
<td>Run #3</td>
<td>1096</td>
<td>6</td>
<td>OUR</td>
<td>3.7 ± 0.32</td>
<td>0.42 ± 0.04</td>
<td>8.94 ± 0.84</td>
<td>2.05 ± 0.19</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>HPA</td>
<td>————</td>
<td>————</td>
<td>————</td>
<td>————</td>
</tr>
</tbody>
</table>

Mean variation is with respect to OUR data = 100*(Mean HPA - Mean OUR)/Mean OUR.

The mean of data ± standard deviation is shown.
Furthermore, most of the residual COD present was probably from the soluble microbial products (SMP) rather than original acetate and glucose substrates.

Table 2 provides comparative data between the steady-state OUR and the non-steady-state CPL methods under identical process conditions. In this case, the CPL test was temporally separated from the OUR tests. After completion of the fourth experiment (Table 1), the operation of the process was continued to perform the CPL tests for six days in a row. It was found that the oxygen transfer parameters from the CPL tests were close to double the values obtained in the steady-state OUR tests (Table 2). As explained earlier in the Materials and Methods section, in the CPL test, the air supply was increased suddenly to measure 2 to 3 mg/L of DO increase from the steady-state value and then brought back to the normal operating level. Therefore, the results of this test suffer the consequences of an abrupt change in the hydraulic and mixing conditions within the reactor. The swift increase in airflow created highly turbulent conditions in the reactor.

The effects of sudden change in turbulence on the biological floc depend on floc characteristics, including floc strength that keeps the flocs together (Droppo 2004; Liao et al. 2002; Mikkelsen and Nielsen 2001). According to the general flocculation theory, the steady-state size distribution of flocs in a reactor for the given process operating condition and mixing intensity is a net result of competition between agglomeration (flocculation) and break-up (deflocculation) of particles (Thomas et al. 1999; Biggs and Lant 2000; Spicer and Pratsinis 1996; Bouyer et al. 2004; Han et al. 2003). The primary particles (a few microns in size) form the basis of the fractal (self-similar) structure of larger parent flocs (Thomas et al. 1999; Spicer and Pratsinis 1996). When flocs are subjected to short periods of increase in turbulence, the floc distribution is governed by floc disruption and the floc size distribution reaches a temporary unstable equilibrium between flocculation and break-up, which is the limit at which the break-up mechanism fails to produce additional fragmentation (Akers et al. 1987). Under a step-change increase in turbulent conditions, flocs having weak links easily break up and this process may not necessarily involve only large flocs. The exposure to turbulent conditions results in stronger and denser flocs with reduced average floc size, as most of the weak links are removed in the process (Akers et al. 1987; Spicer and Pratsinis 1996). Thus, sudden increase in turbulence increases the shear and reduces the overall floc size, shifting the entire size distribution into smaller sizes (Govoreanu et al. 2003). This effect is prominent when the linkage between primary particles is weak (Spicer and Pratsinis 1996).

The CPL test would have caused deflocculation of weak flocs and reduction in the average size of flocs, resulting in higher oxygen transfer parameters. The confirmation of the floc break-up hypothesis was indirectly confirmed by the increase in SVI as the application of the CPL test continued. The average SVI during the steady-state OUR test period was less than 50 mL/g, which reached 125 mL/g with simultaneous increase in the clarifier sludge blanket height from 50 to 225 mm, at which point it was decided to stop the CPL test to avoid solids wash-out from the system. Interestingly, Capela et al. (2004) reported that in reaeration tests with aeration switched off (while mixing mechanically), the KLaf values decreased by as much as 43% (when compared to the data from the off-gas method). It seems, Capela et al. (2004) produced a temporary reduction in deflocculation and an increase in flocculation in the reaeration tests by stopping aeration to the reactor, which would have resulted in higher floc size and reduced oxygen transfer parameters as per the flocculation theory (Han et al. 2003; Bouyer et al. 2004). In effect, the data obtained by Capela et al. (2004) were opposite to what was found in the present study because of the reverse treatment during the non-steady-state tests, but the overall results in both the studies were in conformity with the flocculation theory. As explained earlier, an increase up to 100% in KLaf compared to the steady-state OUR value and increase in SVI were observed in the present study, when aeration was increased and then decreased to the normal operating level in the CPL tests (Mahendraker et al. 2005). Observations made in the current and Capela et al. (2004) studies indicate the importance of hydrodynamic conditions and the sensitivity of the

TABLE 2. Comparative oxygen transfer data in UCT 15-day SRT runs—OUR and CPL methodsa,b, c

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Process description</th>
<th>Qafr (mL min⁻¹)</th>
<th>No. of test days</th>
<th>Method</th>
<th>KLa (h⁻¹)</th>
<th>α</th>
<th>OTRf (g d⁻¹)</th>
<th>OTEf (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>UCT 15-day SRT</td>
<td>854</td>
<td>13</td>
<td>OUR</td>
<td>4.12 ± 0.26</td>
<td>0.60 ± 0.04</td>
<td>9.94 ± 0.81</td>
<td>2.93 ± 0.54</td>
</tr>
<tr>
<td>6</td>
<td>Run #2</td>
<td>6</td>
<td>15</td>
<td>CPL</td>
<td>8.13 ± 0.60</td>
<td>1.04 ± 0.15</td>
<td>19.85 ± 1.99</td>
<td>6.01 ± 0.54</td>
</tr>
</tbody>
</table>

aThe mean of data ± standard deviation is shown.
bMean variation is with respect to OUR data = 100*(Mean CPL - Mean OUR)/Mean OUR.
cOn each test day, duplicate tests were conducted and the data for the day is the mean of these tests.

The effects of sudden change in turbulence on the biological floc depend on floc characteristics, including floc strength that keeps the flocs together (Droppo 2004; Liao et al. 2002; Mikkelsen and Nielsen 2001). According to the general flocculation theory, the steady-state size distribution of flocs in a reactor for the given process operating condition and mixing intensity is a net result of competition between agglomeration (flocculation) and break-up (deflocculation) of particles (Thomas et al. 1999; Biggs and Lant 2000; Spicer and Pratsinis 1996; Bouyer et al. 2004; Han et al. 2003). The primary particles (a few microns in size) form the basis of the fractal (self-similar) structure of larger parent flocs (Thomas et al. 1999; Spicer and Pratsinis 1996). When flocs are subjected to short periods of increase in turbulence, the floc distribution is governed by floc disruption and the floc size distribution reaches a temporary unstable equilibrium between flocculation and break-up, which is the limit at which the break-up mechanism fails to produce additional fragmentation (Akers et al. 1987). Under a step-change increase in turbulent conditions, flocs having weak links easily break up and this process may not necessarily involve only large flocs. The exposure to turbulent conditions results in stronger and denser flocs with reduced average floc size, as most of the weak links are removed in the process (Akers et al. 1987; Spicer and Pratsinis 1996). Thus, sudden increase in turbulence increases the shear and reduces the overall floc size, shifting the entire size distribution into smaller sizes (Govoreanu et al. 2003). This effect is prominent when the linkage between primary particles is weak (Spicer and Pratsinis 1996).

The CPL test would have caused deflocculation of weak flocs and reduction in the average size of flocs, resulting in higher oxygen transfer parameters. The confirmation of the floc break-up hypothesis was indirectly confirmed by the increase in SVI as the application of the CPL test continued. The average SVI during the steady-state OUR test period was less than 50 mL/g, which reached 125 mL/g with simultaneous increase in the clarifier sludge blanket height from 50 to 225 mm, at which point it was decided to stop the CPL test to avoid solids wash-out from the system. Interestingly, Capela et al. (2004) reported that in reaeration tests with aeration switched off (while mixing mechanically), the KLaf values decreased by as much as 43% (when compared to the data from the off-gas method). It seems, Capela et al. (2004) produced a temporary reduction in deflocculation and an increase in flocculation in the reaeration tests by stopping aeration to the reactor, which would have resulted in higher floc size and reduced oxygen transfer parameters as per the flocculation theory (Han et al. 2003; Bouyer et al. 2004). In effect, the data obtained by Capela et al. (2004) were opposite to what was found in the present study because of the reverse treatment during the non-steady-state tests, but the overall results in both the studies were in conformity with the flocculation theory. As explained earlier, an increase up to 100% in KLaf compared to the steady-state OUR value and increase in SVI were observed in the present study, when aeration was increased and then decreased to the normal operating level in the CPL tests (Mahendraker et al. 2005). Observations made in the current and Capela et al. (2004) studies indicate the importance of hydrodynamic conditions and the sensitivity of the
mass transfer coefficient to increase or decrease in turbulence in the reactor. It seems, changes to floc structure occur during the reaeration tests and monitoring these changes can lead to an improved understanding of mass transfer parameters. Given the discrepancy observed in the mass transfer coefficient obtained using the non-steady-state H$_2$O$_2$ method and the CPL or reaeration method, there is a need to further investigate the validity of these testing methods by closely monitoring the floc-related parameters in addition to the process performance under controlled conditions. As such, the HPA and CPL tests negatively affected the process in this investigation. Therefore, the oxygen transfer parameters obtained from these tests are suspect.

Table 3 presents the oxygen transfer parameter data obtained from steady-state OUR and off-gas methods in four different experiments and two activated sludge processes (UCT and MLE). Here, in the off-gas analysis only OTE$_f$ and OTR$_f$ were calculated. As shown in Table 3, the OTE$_f$ and OTR$_f$ data from these two methods were comparable, although variations (shown by the standard deviation) were higher in the data from the off-gas method compared to that from the steady-state OUR method, possibly due to errors in sampling and injection steps of the analysis. One of the reasons for higher variations in the data from the off-gas method may be the low transfer efficiencies measured in this research, particularly in the ninth and tenth experiments in the MLE process, where the OTE$_f$ was less than 2% (Table 3). Chiesa et al. (1990) reported that when the OTE$_f$ measured decreased from 10 to 3%, the coefficient of variation increased from 3 to 10% suggesting an exponential error distribution pattern in the off-gas analysis. Babcock and Stenstrom (1993) reported on the precision and accuracy of the off-gas testing method and stated that as the measured OTE$_f$ decreases the percent error of the measurement tends to increase. They estimated ±10% accuracy in determining the OTE$_f$ using the off-gas method, based on data from the literature. Redmon et al. (1983) observed a similar range of oxygen-transfer parameters using the steady-state OUR and off-gas methods in full-scale activated sludge plant studies. More recently, Krause et al. (2003) found a comparable oxygen transfer rate using OUR and off-gas methods in a full-scale membrane reactor.

In the CMAS process experiments only the steady-state OUR test was implemented and Table 4 presents the summary of oxygen transfer parameters obtained at two different SRTs and four different airflow rates. The major difference among the CMAS experiments was the nitrification rate. In the eleventh experiment the nitrification rate was equal to 1.34 g NH$_3$-N/d, whereas, in the twelfth and thirteenth experiments the rate was higher at 2 to 2.2 g NH$_3$-N/d. In the twelfth experiment, two different airflow rates (2311 and 3475 mL/min) were required to maintain the reactor DO within 2.5 ± 0.5 mg/L and the

<table>
<thead>
<tr>
<th>Experiment number</th>
<th>Process description</th>
<th>$Q_{af}$ (mL min$^{-1}$)</th>
<th>No. of test days</th>
<th>Method</th>
<th>$K_l a_f$ (h$^{-1}$)</th>
<th>$\alpha$</th>
<th>OTR$_f$ (g d$^{-1}$)</th>
<th>OTE$_f$ (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>UCT 15-day SRT</td>
<td>1320</td>
<td>11</td>
<td>OUR</td>
<td>5.84 ± 0.17</td>
<td>0.61 ± 0.02</td>
<td>13.82 ± 0.86</td>
<td>2.63 ± 0.16</td>
</tr>
<tr>
<td></td>
<td>Low nitrogen &amp;</td>
<td>1320</td>
<td>16</td>
<td>Off-gas</td>
<td>—</td>
<td>—</td>
<td>13.86 ± 1.50</td>
<td>2.64 ± 0.54</td>
</tr>
<tr>
<td></td>
<td>phosphorus run</td>
<td></td>
<td></td>
<td>% Mean</td>
<td>variation</td>
<td>0.3</td>
<td>—</td>
<td>0.4</td>
</tr>
<tr>
<td>8</td>
<td>MLE 10-day SRT Run</td>
<td>1320</td>
<td>8</td>
<td>OUR</td>
<td>4.54 ± 0.16</td>
<td>0.46 ± 0.02</td>
<td>10.89 ± 0.29</td>
<td>2.08 ± 0.05</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1320</td>
<td>15</td>
<td>Off-gas</td>
<td>—</td>
<td>—</td>
<td>10.71 ± 1.30</td>
<td>2.06 ± 0.20</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% Mean</td>
<td>variation</td>
<td>1.7</td>
<td>—</td>
<td>-1.0</td>
</tr>
<tr>
<td>9</td>
<td>MLE 15-day SRT Run</td>
<td>1540</td>
<td>6</td>
<td>OUR</td>
<td>3.96 ± 0.24</td>
<td>0.41 ± 0.03</td>
<td>9.04 ± 0.78</td>
<td>1.45 ± 0.12</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1540</td>
<td>8</td>
<td>Off-gas</td>
<td>—</td>
<td>—</td>
<td>10.50 ± 1.63</td>
<td>1.74 ± 0.24</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% Mean</td>
<td>variation</td>
<td>16.2</td>
<td>—</td>
<td>20.0</td>
</tr>
<tr>
<td>10</td>
<td>MLE 10-day SRT</td>
<td>1320</td>
<td>7</td>
<td>OUR</td>
<td>3.20 ± 0.15</td>
<td>0.39 ± 0.02</td>
<td>7.80 ± 0.38</td>
<td>1.49 ± 0.07</td>
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<td></td>
<td>High nitrogen run</td>
<td>1320</td>
<td>6</td>
<td>Off-gas</td>
<td>—</td>
<td>—</td>
<td>9.50 ± 1.23</td>
<td>1.81 ± 0.23</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% Mean</td>
<td>variation</td>
<td>21.8</td>
<td>—</td>
<td>22.7</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1540</td>
<td>8</td>
<td>OUR</td>
<td>3.54 ± 0.23</td>
<td>0.35 ± 0.02</td>
<td>8.64 ± 0.83</td>
<td>1.42 ± 0.13</td>
</tr>
<tr>
<td></td>
<td></td>
<td>1540</td>
<td>9</td>
<td>Off-gas</td>
<td>—</td>
<td>—</td>
<td>9.91 ± 0.65</td>
<td>1.62 ± 0.11</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>% Mean</td>
<td>variation</td>
<td>14.7</td>
<td>—</td>
<td>14.1</td>
</tr>
</tbody>
</table>

*On each test day, duplicate OUR tests were conducted and the data for the day is the mean of these tests.

*On each test day, a minimum of 6 off-gas tests were conducted and the data for the day is the mean of these tests.

*Mean variation is with respect to OUR data = 100*(Mean CPL-Mean OUR)/Mean OUR.

*The mean of data ± standard deviation is shown.
higher airflow produced the lowest transfer efficiency of 0.88% among the experiments. A critical comparison of the steady-state OUR data in Table 4 with those in Tables 1 to 3 indicates higher variations in the oxygen transfer parameters in the CMAS process, compared to the UCT and MLE processes. This may be due to the fact that all of the biochemical reactions were concentrated in a single reactor and the total amount of solids was low in the CMAS process. It can also be seen from the total data presented in this study that the variations in the estimated parameters using the steady-state OUR method decreased as the process was changed from CMAS to MLE to UCT. The relatively high respiration rates resulted in increased variations in oxygen transfer parameters in the CMAS process, which was similar to the observation made by Krause et al. (2003).

This study was conducted using laboratory-scale reactors, and size of bioreactors influences the measured oxygen transfer parameters. However, application of four methods on a single system operating under identical process conditions while using the same aeration device (without fouling in the case of diffused aeration) is expected to produce the same quality of information that was observed in the present investigation. The key is to closely maintain and monitor the process parameters before, during and after the oxygen transfer tests to understand the test impacts, particularly when the intrusive HPA and CPL methods are employed to determine the oxygen transfer parameters under the actual process conditions.

Conclusions

The following conclusions can be drawn based on this study:

1. The non-steady-state HPA and CPL tests produced variable results, and these tests affected the process. Therefore, the oxygen transfer data from these tests are suspect.
2. The steady-state OUR and off-gas methods produced comparative oxygen transfer parameters.
3. In this study, the variations in oxygen transfer parameters determined from the steady-state OUR method increased, as the configuration changed from CMAS to MLE to UCT processes.

The steady-state OUR and off-gas methods seem to be suitable techniques for measuring the oxygen transfer under process conditions. The advantages of the off-gas method are: (i) wastewater treatment processes need not be operating under steady-state conditions, and (ii) mixed liquor is not directly involved in the test. The disadvantages of the off-gas method are: (i) the need for specialized instrumentation and equipment, (ii) the method can be applied only for subsurface diffused aeration systems, (iii) the necessity to collect off-gas samples from a large surface area and (iv) relatively high cost of testing. The major limitation in applying the OUR technique is the requirement of a steady-state process condition, which is particularly difficult to achieve in full-scale systems. Sometimes, even under a steady-state condition, it is possible to observe spatial OUR variations within the bioreactor due to non-ideal reactor conditions, complicating the application of the steady-state OUR method. However, where feasible, the OUR technique may be an economical procedure to study oxygen transfer under the process conditions.

Acknowledgements

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