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1                   **Microbial oxygen uptake in sludge as influenced by compost physical parameters**

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## 1 **Abstract**

2           The wide range of optimal values reported for the physical parameters of compost  
3 mixtures suggest that their interactive relationships should be investigated. The objective of this  
4 study was to examine the microbial O<sub>2</sub> uptake rate (OUR) in 16 sludge waste recipes, offering a  
5 range of moisture content (MC), waste/bulking agent (W/BA) ratio and BA particle size levels  
6 determined using a central composite experimental design. The 3 kg samples were maintained at  
7 a constant temperature and aeration rate for 28 days, during which a respirometer recorded O<sub>2</sub>  
8 uptake to provide a measure of microbial activity and biodegradability. The cumulative O<sub>2</sub>  
9 consumption after 14 and 28 days was found to be significantly influenced by MC, W/BA ratio,  
10 BA particle size and the interaction between MC and W/BA ratio ( $p < 0.05$ ). Using multivariate  
11 regression analysis, the experimental data was used to generate a model with good predictive  
12 ability for cumulative O<sub>2</sub> consumption after 28 days as a function of the significant physical  
13 variables ( $R^2 = 0.84$ ). The prediction of O<sub>2</sub> uptake by the model depended highly on the  
14 interaction between MC and W/BA ratio. A MC outside of the traditional 50-60 % (wet basis)  
15 range still resulted in a high level of microbial O<sub>2</sub> uptake as long as the W/BA ratio was adjusted  
16 to maintain a good O<sub>2</sub> exchange in the sample. The evolution of OUR in the samples was also  
17 investigated, uncovering strong associations between short and long-term respirometric indices,  
18 such as peak OUR and cumulative O<sub>2</sub> consumption ( $p < 0.005$ ). Combining peak OUR data with  
19 cumulative O<sub>2</sub> consumption after 14 days allowed for accurate predictions of cumulative O<sub>2</sub> after  
20 28 days of aeration ( $R^2 = 0.96$ ), implying that future studies need only run trials up to 14 days to  
21 evaluate the overall O<sub>2</sub> consumption or biodegradability of a sludge mixture.

22 **Keywords:** compost, biodegradability, respirometry, moisture content, bulking agent to waste  
23 ratio, particle size.

## 1 **1. Introduction**

2           The implementation of widespread composting initiatives is a vital challenge for our  
3 modern society. Increasing urbanization and industrialization has led to the production of large  
4 amounts of sludge from sewage and industrial wastewater treatment plants. In the US and EU,  
5 for example, over 7.5 million dry metric tons of sludge are produced annually from municipal  
6 sewage plants, while only about half is composted or used as landfill cover (EPA 2000; EEA,  
7 2001).

8           The lack of widespread sludge or biosolid composting initiatives is due, in large part, to  
9 the requirements of purchasing and handling costly bulking agent (BA) materials, and of  
10 undertaking extensive treatment periods of active biodegradation, followed by several months of  
11 maturation (Gupta and Garg, 2008). Selection of a waste recipe with optimal physical parameters  
12 can not only reduce the size of the operation and BA mass, but also create an environment  
13 conducive for high microbial activity. Increased microbial activity, consequently, translates into  
14 more extensive biodegradation at quicker rates in the active phase of composting (Haug, 1993).  
15 For sludge waste initially well-balanced in pH, carbon and nitrogen, the physical parameters of  
16 moisture content (MC), BA quantity and BA particle size are important in establishing the  
17 physical matrix of a mixture to be composted (Diaz et al., 2007). These parameters influence  
18 microbial activity directly or through their effect on mixture porosity and free-air-space (FAS),  
19 which in turn impact O<sub>2</sub> diffusion and sample compaction (Rynk, 1992; Liang et al., 2003;  
20 Agnew and Leonard, 2003).

21           The broad range of optimal values reported in compost literature for MC, waste/BA  
22 (W/BA) ratio and BA particle size may be indicative of an interaction effect between them,  
23 emphasizing the importance of the waste mixture as a whole. Indeed, Regan et al. (1973) in their

1 review of earlier publications reported a range of favourable MCs between 25-80 % on a wet  
2 basis, whereas more recent studies have shown an optimal range between 50 to 60 % (Tiquia et  
3 al., 1996). The 50-60 % range seems to be the standard, as higher levels can lead to early  
4 cooling or water-logging of the mixture (Golueke, 1977; Rynk, 1992). Moisture levels in the  
5 range of 80 %, however, have been successfully composted by Fernandes et al. (1994) using a  
6 BA of sphagnum peat moss with a high moisture-absorbing capacity, and Vallini and Pera  
7 (1989) using a BA of wood container residues and plastics with a high resistance to compaction.  
8 In fact, Fernandes et al. (1994) observed a lengthier compost operation with the use of straw in  
9 their sample instead of peat moss, as straw tends to collapse and block gas movement under high  
10 MC (Barrington et al., 2003). The density and matrix configurations of the waste mixture,  
11 determined by BA properties, may consequently be intimately connected with appropriate MC  
12 levels for a compost mixture. Using a waste/sawdust ratio of 1/5 (mass), Liao et al. (1993)  
13 successfully composted their mixture at a MC above 60 %, while Higgins et al. (1986) obtained  
14 similar positive effects with the use of lower W/BA ratios. For wood chips as BA, smaller  
15 particle sizes of 5 mm to 25 mm were shown to optimize compost performance (Raichura and  
16 McCartney, 2006), although material too finely divided can lead to structural collapses at high  
17 moisture (Das and Keener, 1997; Barrington et al., 2003). Richard et al. (2002) suggests the  
18 possibility of mitigating the effects of MC outside an optimal range by manipulation of substrate  
19 density and particle size. However, the interactive nature between MC and BA properties and  
20 their effect on microbial activity has yet to be clearly defined.

21         Respirometry is a useful tool in assessing microbial activity, as it is linked to the  
22 oxidation and thus biodegradation of waste hydrocarbons (Adani et al., 2001; Gea et al., 2004;  
23 Iannotti et al., 1994), in a metabolic process that consumes O<sub>2</sub> as the terminal electron acceptor

1 and produces CO<sub>2</sub>. For estimation of aerobic microbial biodegradation, the measurement of  
2 microbial O<sub>2</sub> uptake is more accurate, as anoxic zones can produce CO<sub>2</sub> and lead to  
3 misrepresentations of respirometry (Haug, 1993; Gomez et al., 2006). Early in aerobic  
4 biodegradation, O<sub>2</sub> uptake increases exponentially as microbial populations grow, then peaks  
5 when the availability of the most highly biodegradable substrates become limited, subsequently  
6 subsiding to a low and stable value as the remaining complex biodegradable matter is slowly  
7 solubilized and degraded (Tremier et al., 2005). Since the profile of the O<sub>2</sub> uptake rate (OUR)  
8 curve and the cumulative O<sub>2</sub> consumption provide a measure of microbial activity and overall  
9 biodegradability of the sample, finding associations between the two is useful.

10       Using mixtures of wood residues recovered from composting and wastewater sludge, the  
11 objectives of this study were therefore to (1) monitor O<sub>2</sub> uptake for different MCs, W/BA ratios  
12 and BA particle sizes under continuous aeration of biomass in order to establish their impact and  
13 the impact of their interactions on microbial activity; and (2) investigate the association between  
14 short and long-term measures of O<sub>2</sub> uptake to determine methods of predicting aeration  
15 requirements and biodegradability potential. To maximize the statistical strength of experimental  
16 data while minimizing the number of experimental trials needed, a central composite factor  
17 design was used to test key levels of the physical parameters. Amongst the large number of  
18 papers dealing with composting, only a limited number in recent years are based on an  
19 experimental design that enhances the reliability of results (Liang et al., 2003; Gea et al., 2007;  
20 Zambrano et al., 2007; Bueno et al., 2008a; Bueno et al., 2008b).

21

## 22 **2. Materials and Methods**

### 23 2.1 Experimental apparatus and substrate

1           The respirometric apparatus used in this study consisted of a 10 L cylindrical airtight  
2 reactor made of stainless steel (Figure 1). Inside the reactor, the substrate material was placed on  
3 a 3 mm mesh grid located 70 mm above the cell bottom forming a plenum receiving 65 L/h of air  
4 via a glass diffuser. To fully ventilate the entire sample, a continuous and ample airflow, as  
5 compared to composting, was supplied by re-circulating part of the exhaust air back into the  
6 cells. The entering and exhaust air stream was monitored for O<sub>2</sub> content using a paramagnetic O<sub>2</sub>  
7 gas analyser (MAIHAK Technology, Germany). Oxygen uptake measurements were made under  
8 continuous aeration of the biomass (dynamic method) rather than an intermittent aeration supply  
9 (static method) to minimize O<sub>2</sub> transfer limitations (Paletsky and Young 1995; Adani et al.,  
10 2001).

11           The apparatus controlled for other environmental factors influencing microbial activity.  
12 The temperature of the samples was held constant by placing each cell in a water-bath at 40 °C,  
13 which is optimal for mesophilic microbial activity (Tremier et al., 2005). The inlet air was  
14 preheated at 40 °C using a copper serpentine with a diameter of 10 mm, a thickness of 2 mm and  
15 a length of 2 m, submerged in the water-bath. The temperature of the samples was monitored by  
16 means of a Pt100 temperature probe (OMNI Instruments, UK) inserted in the centre of the cells.  
17 The inflowing air was saturated with moisture by bubbling through two water-filled glass bottles  
18 immersed in the water-bath. Moisture condensing in the exhaust air was collected in beakers  
19 above the water-bath to prevent both its return into the sample cell and its entrance into the gas  
20 analyzer.

21           The tested mixtures consisted of dewatered sludge obtained from an activated sludge  
22 reactor treating the wastewater of a slaughterhouse and, as BA, wood residues composed mainly  
23 of twigs and branches recycled from a composting operation. For consistency, a large sludge

1 sample was obtained prior to the trial, split into 10 kg sub-samples and stored at -20 °C until  
2 used. Table 1 describes the chemical properties of the experimental materials. The size of the  
3 respirometric apparatus developed was such that no pretreatment of the waste (e.g., grinding or  
4 suspension in aqueous solution) was required, thus creating a physical matrix representative of  
5 real sludge waste.

6

## 7 2.2 Experimental design and methodology

8 A central composite factor design method was utilized to measure the effect of a range in  
9 MC, W/BA ratio and BA particle size on the O<sub>2</sub> uptake of samples over a period of 28 days  
10 (Table 2). Sixteen (16) experiments were conducted using three independent variables, namely  
11 MC, W/BA ratio and BA particle size, where 8 of the experimental points corresponded to a  
12 three factor complete factorial design, 2 of the points to central values and six of the points to  
13 extreme values ( $\alpha=1.682$ ). Thus, five values were tested for each independent variable. To obtain  
14 the targeted (nominal) MCs of 20, 30, 45, 60 and 70 % on a wet basis, tap water was either added  
15 to the samples or they were dried at ambient air for 18 to 36 hours depending on the desired  
16 level. The sludge was manually mixed with BA to obtain W/BA ratios of 1/9.2, 1/7.9, 1/6, 1/4.1  
17 and 1/2.1, on a dry mass basis. Bulking agent particle sizes were obtained by sieving with rotary  
18 screens of 8 to 12.5 mm, 12.5 to 20 mm, 20 to 30 mm, 30 to 40 mm and > 40 mm. Samples with  
19 a wet mass between 2 and 4.7 kg and a volume of approximately 6 L were manually mixed and  
20 placed in the cell to prevent BA breakage and to minimize mixture compaction. All OURs were  
21 expressed on an initial dry mass basis of the trial samples. The 16 trials were performed in one  
22 single replicate and were randomly carried out in groups of six at any one time.



1           Once in the cell, all samples remained aerated until OURs reached a low and constant  
2 value, sometime between 12 and 22 days, depending on the activity of the sample. The cells  
3 were then opened and their contents weighed and manually mixed before being placed back into  
4 the cell. The cells were then re-aerated until OUR values diminished a second time, usually  
5 around 28 days. The manual mixing of the cells was performed to redistribute moisture and  
6 microorganisms and to restructure the sample (Berthe et al., 2007).

### 7 8 2.3. Chemical Analysis

9           The experimental materials, namely the sludge and BA, were analyzed for dry matter  
10 content (DM), organic matter (OM), total organic carbon (TOC), chemical oxygen demand  
11 (COD) and total Kjeldahl nitrogen (TKN) in triplicates, prior to the start of the experiment. Dry  
12 matter was measured by drying the wet samples at 80 °C to constant weight. Organic matter was  
13 measured by burning the dried ground samples at 550 °C (Afnor, 1985). Total organic carbon  
14 was obtained by oxidizing the dried ground samples to CO<sub>2</sub> and using infrared spectrometry to  
15 measure the emissions (SKALAR device, Breda, The Netherlands) (Afnor, 2001a). Chemical  
16 oxygen demand was determined by the dichromate oxidation of 50 mg of dried ground sample  
17 (Afnor, 2001b). Total Kjeldahl nitrogen was quantified on 50 mg of dried ground sample by  
18 digesting with 98 % sulphuric acid followed by steam distillation and titration (Afnor, 1995).

### 19 20 2.4 Statistical Analysis

21           Using the observed experimental respirometric data, univariate linear regression was first  
22 performed to delineate the marginal relationships of the measured MC, W/BA ratio and the  
23 average BA particle size, with each of cumulative O<sub>2</sub> consumption after 14 and 28 days, peak

1 OUR and time to peak OUR. Exploration of potential second-order associations was performed,  
2 although no quadratic relationship was uncovered. The potential impact of time at mixing upon  
3 cumulative O<sub>2</sub> consumption was also assessed and was found to be insignificant.

4 Multivariate linear regression was subsequently performed to decipher the associations  
5 between the physical parameters (including their interactions), and each of the experimentally  
6 recorded cumulative O<sub>2</sub> consumption, peak OUR, and time to peak OUR. The statistical software  
7 package Stata® (Statacorp LP, Texas, USA) was used to fit a linear regression model using a  
8 least-squares approach, in order to identify the relationship between the expected O<sub>2</sub> uptake (*Y*)  
9 and the physical variables. The regression model was based upon the equation:

$$10 \quad E(Y|x_1, x_2, x_3) = \beta_0 + \beta_1\chi_1 + \beta_2\chi_2 + \beta_3\chi_3 + \beta_{12}\chi_1\chi_2 + \beta_{13}\chi_1\chi_3 + \beta_{23}\chi_2\chi_3 + \beta_{123}\chi_1\chi_2\chi_3 \quad (1)$$

11 where  $\beta_0$  is the intercept;  $\beta_1, \beta_2, \beta_3$  are the main-effect coefficients;  $\beta_{12}, \beta_{13}, \beta_{23}, \beta_{123}$  are the  
12 interaction coefficients;  $\chi_1$ = MC, %;  $\chi_2$  = W/BA ratio, dimensionless;  $\chi_3$  = BA particle size, mm.  
13 The MC, W/BA ratio and BA particle size variables were centered at 45 %, 1/6 dimensionless,  
14 and 25 mm, respectively, to simplify the interpretation of the intercept and the interaction  
15 coefficients.

16 To determine associations between short and long-term indices of O<sub>2</sub> uptake, cumulative  
17 O<sub>2</sub> consumption at both 14 and 28 days were also separately regressed upon characteristics of the  
18 first OUR, such as peak OUR, average OUR in the 24 h period of highest respiration (DRI<sub>24h</sub>),  
19 time to peak OUR and total O<sub>2</sub> consumption up to the peak OUR. Trials 3, 6 and 7 were omitted  
20 in the analysis of cumulative O<sub>2</sub> consumption at 28 days, as these trials reached low and stable  
21 values before other trials and were stopped after 447 h (18 days), 596 h (24 days) and 639 h (26  
22 days), respectively.

1 Various diagnostic methods, such as regression residual summary measures, residual  
2 plots and added-variable plots, were used to evaluate the appropriateness of assumptions  
3 underlying linear regression. No overt departure from such assumptions was found.  
4

### 5 **3. Results and Discussion**

#### 6 3.1. Initial characterization of microbial oxygen uptake rate

7 Figure 2 illustrates the OUR profiles for trials 1, 5 and 13. In each trial, two peak OURs  
8 were observed, one within the first 14 days of aeration and the latter initiated after manual  
9 mixing of the samples once OUR had returned to low and stable values, between 12 and 22 days.  
10 Mixing of the samples served to redistribute moisture and microbial activity that may have  
11 accumulated in a certain region (Berthe et al., 2007) and consequently led to a smaller second O<sub>2</sub>  
12 uptake peak, as some remaining carbon was oxidized. For respirometric trials following similar  
13 time lines, Berthe et al. (2007) have shown that subsequent re-mixing produced smaller peaks in  
14 OUR. Thus, negligible biodegradable matter was assumed to remain after the second reduction  
15 in OURs. The second active stage of microbial activity generally subsided around 28 days.  
16

#### 17 3.2. Influence of physical parameters on cumulative O<sub>2</sub> consumption

18 The experimental cumulative O<sub>2</sub> consumption was calculated after both 14 and 28 days  
19 of aeration (Table 2), as these periods corresponded to an observed O<sub>2</sub> uptake peak and decline  
20 (Figure 1), and represent general active composting periods of high biodegradation for practical  
21 composting schemes (Haug, 1993). Regression analysis of cumulative O<sub>2</sub> consumption after 14  
22 and 28 days onto MC, W/BA ratio, BA particle size and their interactions generated a model

1 expressing mean cumulative O<sub>2</sub> consumption as a function of the significant physical parameters  
2 at centered values of 45 % MC, 1/6 W/BA ratio and a particle size of 25 mm:

$$3 \quad E(Y/x_1, x_2, x_3)_{14 \text{ days}} = 7120.48 + 92.88\chi_1 + 5852.52\chi_2 - 115.56\chi_3 - 1168.7\chi_1\chi_2 \quad (2)$$

$$4 \quad E(Y/x_1, x_2, x_3)_{28 \text{ days}} = 10566.46 + 119.62\chi_1 + 11043.23\chi_2 - 126.16\chi_3 - 1883.57\chi_1\chi_2 \quad (3)$$

5 where  $Y = \text{O}_2$  consumption, mmol/kg of DM;  $\chi_1 = (\text{MC} - 45)$ , %;  $\chi_2 = (\text{W/BA ratio} - 1/6)$ ,  
6 dimensionless and;  $\chi_3 = (\text{BA particle size} - 25)$ , mm.

7         The coefficients of determination ( $R^2$ ), providing a measure of the predictive ability of  
8 these regression models, were 0.85 and 0.84, at 14 and 28 days, respectively. The strong  
9 predictive ability of the models underlines the significant role played by each of MC, W/BA  
10 ratio, BA particle size and the interaction of MC and W/BA ratio in creating a physical  
11 environment conducive to microbial activity and organic matter oxidation.

12         The positive association between cumulative O<sub>2</sub> consumption and both MC and W/BA  
13 ratio indicates that the use of wetter recipes and less BA resulted in greater biodegradation after  
14 14 and 28 days. High MC is beneficial for microbial physiological activity, transport and  
15 hydrolysis (Rynk, 1992; Liang et al., 2003), while the use of a larger W/BA ratio increases  
16 carbon availability and favours microbe accessibility. The negative interaction between MC and  
17 W/BA ratio, however, altered the individual main effects of these parameters. The use of higher  
18 MCs coupled with larger W/BA ratios, can be expected to have caused a decrease in mixture  
19 porosity and FAS availability for O<sub>2</sub> diffusion, subsequently resulting in O<sub>2</sub> exchange limitations  
20 and reduced microbial O<sub>2</sub> uptake. This could explain why the model indicates that a low W/BA  
21 ratio performs better under high MC, generally yielding a larger cumulative O<sub>2</sub> consumption  
22 after 14 and 28 days. On average, greater O<sub>2</sub> consumption was also obtained with the use of

1 smaller BA particle sizes ( $p < 0.01$ ), reflecting the ability of microbes to maximize surface area  
2 attachment and access to carbon at such sizes (Raichura and McCartney, 2006).

3         Considering the acceptable accuracy of the models, cumulative O<sub>2</sub> consumption can be  
4 predicted for different physical parameter levels within the range of the experimental design.  
5 From equation (2), the intercept indicates that, on average, a total of approximately 7 mol O<sub>2</sub>/kg  
6 DM (766 m<sup>3</sup> of air/ton DM) is consumed after 14 days at the centred values for the physical  
7 variables. At 28 days, the O<sub>2</sub> consumption reaches approximately 10.5 mol O<sub>2</sub>/kg DM (1,150 m<sup>3</sup>  
8 of air/ton DM). At centered values for W/BA ratio and BA particle size, the models (Eqs. 2 and  
9 3) indicate that solely increasing MC in a sample from 45 to 60 %, for example, will cause  
10 cumulative O<sub>2</sub> consumption to increase by an average of 1.4 mol O<sub>2</sub>/kg DM (153 m<sup>3</sup> of air/ton  
11 DM) and 1.8 mol O<sub>2</sub>/kg DM (197 m<sup>3</sup> of air/ton DM) after 14 and 28 days of aeration,  
12 respectively. On the other hand, two samples at centered values for MC and BA particle size but  
13 differing in W/BA ratio by 15 %, will then, on average, experience cumulative O<sub>2</sub> consumptions  
14 that are 0.9 mol/kg DM (99 m<sup>3</sup> of air/ton DM) and 1.7 mol/kg DM (186 m<sup>3</sup> of air/ton DM)  
15 greater in the sample with a larger W/BA ratio, after 14 and 28 days, respectively. Finally, if MC  
16 and W/BA ratio remain constant, increasing the BA particle size by 10 mm results in a drop in  
17 cumulative O<sub>2</sub> consumption of 1.2 mol/kg DM (131 m<sup>3</sup> of air/ton DM) after 14 days and 1.3  
18 mol/kg DM (142 m<sup>3</sup> of air/ton DM), after 28 days. The models (Eqs. 2 and 3) only include main  
19 effects in the first-order and as such, the input of extreme values within the range of parameters  
20 tested yields maximum or minimum outputs.

21         The 28-day regression model (Eq. 3) was used to develop response curves of predicted  
22 cumulative O<sub>2</sub> consumption for different combinations of physical parameters within the limits  
23 tested (Figures 3-5). For a W/BA ratio of 1/6, higher MCs and/or smaller BA particle sizes lead

1 to increased cumulative O<sub>2</sub> consumption (Figure 3). Figure 4 illustrates that at a MC of 45 %,  
2 higher W/BA ratios and/or lower BA particle sizes yield larger cumulative O<sub>2</sub> consumption.

3 The strong influence of the MC and W/BA ratio interaction on microbial activity is  
4 illustrated in Figure 5. At a MC above 50 % and a W/BA ratio larger than 1/4.1, a further  
5 increase of moisture in the mixture is seen to reduce cumulative O<sub>2</sub> consumption. Tiquia et al.  
6 (1996) showed that a MC higher than 60 % had detrimental effects in their compost system.  
7 However, the model illustrates the possibility of increasing cumulative O<sub>2</sub> consumption for  
8 moisture levels up to 70 %, as long as W/BA ratios lower than 1/4.1 are used to maximize FAS  
9 and minimize compaction of the sludge mixture. Furthermore, despite past studies  
10 recommending the use of moisture levels above 40 % (Liang et al., 2003), the model shows good  
11 performance with moisture as low as 35 %, the lowest MC physically possible with a W/BA  
12 ratio of 1/2.8. Such low moisture still sustains microbial activity as long as the W/BA ratio  
13 remains high enough to provide sufficient wet mass and access to carbon. This seems to confirm  
14 that MC optimization should be done in consideration of compost mixture physical structure  
15 (Richard et al., 2002), incorporated in some studies as 75 % of the maximum water-holding  
16 capacity of the sample (Adani et al. 2001). The significant interdependence between MC and  
17 W/BA ratio partly explains the lack of consensus in past studies with respect to their optimal  
18 levels (Liao et al., 1993; Tiquia et al., 1996; Raichura and McCartney, 2006; Banegas et al.,  
19 2007).

20

### 21 3.3. Influence of physical parameters on peak oxygen uptake rates

22 For all respirometric trials, peak OUR occurred in the first active phase after 2 to 6 days  
23 of aeration and ranged from 23 mmol/h/kg DM to 103 mmol/h/kg DM (0.7 to 3.1 L/s/ton DM)

1 (Table 2). Regression analysis indicated that peak OUR had a significant positive association  
2 with MC ( $p < 0.01$ ) and a significant negative association with BA particle size ( $p < 0.005$ ).  
3 Although W/BA ratio had a strong main effect and interaction with MC on cumulative O<sub>2</sub>  
4 consumption, it did not significantly impact peak OUR at the onset of biodegradation. The  
5 influence of MC and BA particle size alone accounted for over 62 % of the variability seen in the  
6 peak OUR obtained in the trials ( $R^2 = 0.62$ ). The inclusion of the interaction between MC and  
7 particle size in the regression model alongside the main effects of MC and BA particle size ( $p =$   
8  $0.12$ ) increased the proportion of explained variability in peak OUR to 70 % ( $R^2 = 0.70$ ).

9       Samples with higher moisture and smaller BA particle sizes reached greater peak OURs,  
10 probably due to their direct role on microbial growth. A high MC in the sample provides ample  
11 water for physiological activities as well as transport of nutrients and the microorganisms  
12 themselves, while smaller BA particle sizes allow for increased surface area colonization and  
13 access to substrate for the microorganisms (Liang et al., 2003; Raichura and McCartney, 2006 ).

#### 15 3.4. Influence of physical parameters on time to peak O<sub>2</sub> uptake

16       The time elapsed to the first peak OUR was also considerably different between trials,  
17 ranging from 28 hours to over 137 hours (Table 2). The only physical factor significantly  
18 associated to time to peak O<sub>2</sub> uptake was MC, and this association was negative ( $p < 0.005$ ). As  
19 such, the use of higher moisture in the samples reduced the time needed to reach peak  
20 performance in the active phase, likely due to the strong deterministic role of water on microbial  
21 activity and nutrient transport. Although the influence of physical parameters on the kinetics of  
22 biodegradation needs further study, the results of our study suggest that MC may have an impact  
23 on the microbial growth rates or organic matter hydrolysis rates of an organic mixture. Indeed,

1 past studies have illustrated the effects of moisture on biodegradation kinetic models (Richard et  
2 al., 2002).

3

### 4 3.5. Predicting respirometric performance from different microbial O<sub>2</sub> uptake indices

5 Peak OUR and cumulative O<sub>2</sub> consumption are important respirometric measures for the  
6 design of compost aeration systems. They have also been used as indices for biological stability  
7 in composts, alongside DRI<sub>24 h</sub> (Adani et al. 2001; Barrena et al. 2009). Univariate and  
8 multivariate linear regression analyses were conducted to study the relationship between  
9 cumulative O<sub>2</sub> consumption at 28 days and cumulative O<sub>2</sub> consumption at 14 days, peak OUR,  
10 DRI<sub>24 h</sub> and time to peak OUR. The cumulative O<sub>2</sub> consumption after 14 days explained more  
11 than 89 % of the variability observed in cumulative O<sub>2</sub> consumption after 28 days (Table 3),  
12 illustrating the strong predictive power of O<sub>2</sub> uptake after just two weeks of aeration over the full  
13 biodegradability of the recipe material. Moreover, supplementing the above model with the peak  
14 OUR, time to peak OUR and cumulative O<sub>2</sub> consumption up to the peak further increased the  
15 ability of predicting cumulative O<sub>2</sub> consumption after 28 days ( $R^2 = 0.96$ ). This suggests that in  
16 future studies limiting the respirometric trials to two weeks would nonetheless allow for accurate  
17 predictions of cumulative O<sub>2</sub> consumption as a measure of the full biodegradation of sludge.

18 As expected, peak OUR and DRI<sub>24h</sub> were very strongly associated (Table 3). Moreover,  
19 peak OUR and DRI<sub>24 h</sub> had good predictive power for cumulative O<sub>2</sub> consumption at both 14 and  
20 28 days (Table 3). This suggests that the peak OUR and DRI<sub>24h</sub> achieved in the first few days of  
21 composting are good indicators of the aeration requirements and biodegradability potential of the  
22 waste, particularly after 14 days, once the main active phase and the majority of sludge  
23 biodegradation has taken place. Adding information pertaining to the time to peak OUR and the



1 cumulative O<sub>2</sub> consumption up to the peak did not improve the predictive ability of the  
2 regression model (data not shown), suggesting that the bulk of the information resides in the  
3 peak OUR itself.

4 Lastly, time to peak OUR and cumulative O<sub>2</sub> consumption after 14 days were found to be  
5 inversely associated (Table 3). Trials reaching their peak OUR sooner had larger cumulative O<sub>2</sub>  
6 consumption after 14 days reflecting an acceleration in active phase microbial biodegradation,  
7 since the bulk of O<sub>2</sub> uptake occurred around the peak. This indicates that a favourable physical  
8 compost recipe for practical compost schemes should attempt to reduce the time needed to reach  
9 peak OUR and thus stabilize the majority of its biodegradable matter.

10

#### 11 **4. Conclusion**

12 A study of the influence of moisture content (MC), waste to bulking agent (W/BA) ratio  
13 and BA particle size upon microbial O<sub>2</sub> uptake was successfully carried out using a central  
14 composite factor design that tested 16 different sludge waste compost recipes. Regression  
15 analysis indicated the presence of important interactions between sludge mixture physical  
16 parameters, namely MC and W/BA ratio. The interaction between these parameters had a strong  
17 impact upon cumulative O<sub>2</sub> consumption after 14 day and 28 days of biomass aeration, the latter  
18 endpoint reflecting the full biodegradability of the mixtures. Thus, focus should shift from  
19 optimizing individual physical parameter levels towards establishing optimal physical mixture  
20 recipes in an inclusive manner. Regression models predicting cumulative O<sub>2</sub> consumption as a  
21 function of the physical parameters emphasized the importance of the interaction between MC  
22 and W/BA ratio. They also served to illustrate the changes in cumulative O<sub>2</sub> consumption from  
23 alterations in MC, W/BA ratio and BA particle size in a sludge recipe.

1           Furthermore, strong associations were found between different short and long-term O<sub>2</sub>  
2 uptake indices. Short-term measures, such as peak OUR and DRI<sub>24h</sub>, can provide valuable  
3 information on microbial activity and serve as predictors of aeration requirements or  
4 biodegradability potential of a sludge-waste mixture reflected through cumulative O<sub>2</sub>  
5 consumption of the biomass.

6

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1 Table 1  
 2 Description of the experimental sludge and bulking agent

	Sewage sludge	Wood residues				
		8.5 - 12 (mm)	12.5 - 20 (mm)	20 – 30 (mm)	30 – 40 (mm)	>40 (mm)
Moisture content (%)	86.8	10.0	10.0	10.0	10.0	10.0
OM (% DM) <sub>(CV %)</sub>	83 <sub>(0.1)</sub>	91.0 <sub>(0.1)</sub>	92.1 <sub>(0.1)</sub>	93.9 <sub>(0.0)</sub>	93.1 <sub>(0.1)</sub>	89.3 <sub>(0.3)</sub>
COD (g/kg DM) <sub>(CV %)</sub>	1,331 <sub>(0.6)</sub>	1,356 <sub>(0.1)</sub>	1 362 <sub>(0.1)</sub>	1,370 <sub>(0.9)</sub>	1,531 <sub>(0.7)</sub>	1,369 <sub>(0.6)</sub>
TC (g/kg DM) <sub>(CV %)</sub>	484 <sub>(0.5)</sub>	514 <sub>(0.4)</sub>	508 <sub>(0.9)</sub>	510 <sub>(0.7)</sub>	517 <sub>(3.2)</sub>	486 <sub>(2.9)</sub>
TKN (g/kg DM) <sub>(CV %)</sub>	46.4 <sub>(1.5)</sub>	46.1 <sub>(0.8)</sub>	37.1 <sub>(1.0)</sub>	31.5 <sub>(0.5)</sub>	34.3 <sub>(0.5)</sub>	23.8 <sub>(0.9)</sub>

3 DM: dry matter basis; CV: coefficient of variation.

1 Table 2  
2 Experimental setup and respiration results

Trial number	Substrate physical recipes				COC <sub>14 days</sub> (mmol/kg DM)	COC <sub>28 days</sub> (mmol/kg DM)	Peak O <sub>2</sub> uptake rate (mmol/h/kg DM)	DRI <sub>24h</sub> <sup>†</sup> (mmol/h/kg DM)	Time to peak O <sub>2</sub> uptake rate (h)
	Nominal moisture content (%)	Measured moisture content (%)	Bulking agent particle size (mm)	Waste/bulking agent ratio (dry mass basis)					
1	45	41.5	20 – 30	1 / 9.2	5,216	7,170	42.9	40.9	56.5
2	60	61.6	12.5 – 20	1 / 7.9	9,404	13,277	50.1	48.8	60.3
3	45	52.5	8 – 12.5	1 / 6.0	11,227	11,796*	102.8	90.6	28.4
4	45	49.0	20 – 30	1 / 2.8	8,620	13,355	59.8	56.8	60.0
5	70	68.3	20 – 30	1 / 6.0	10,349	13,876	72.0	65.4	58.8
6	30	30.3	30 – 40	1 / 7.9	4,600	7,578*	23.1	21.5	95.2
7	30	35.6	12.5 – 20	1 / 4.1	9,267	13,735*	67.2	65.3	67.8
8	60	61.7	12.5 – 20	1 / 4.1	7,724	11,142	42.4	41.5	75.5
9	60	56.0	30 – 40	1 / 4.1	6,366	9,229	36.5	35	51.4
10	45	51.4	20 – 30	1 / 6.0	7,440	11,603	42.3	40.2	48.4
11	45	53.2	> 40	1 / 6.0	4,585	8,934	35.1	32.5	47.6
12	30	31.4	12.5 – 20	1 / 7.9	5,452	8,806	41.1	38.5	100.8
13	20	28.5	20 – 30	1 / 6.0	5,055	8,938	26.1	24.4	137.8
14	60	61.1	30 – 40	1 / 7.9	7,990	12,044	44.3	43.3	56.2
15	45	51.0	20 – 30	1 / 6.0	6,933	10,940	45.0	43.7	59.2
16	30	33.5	30 – 40	1 / 4.1	5,528	9,385	26.1	25.1	92.4

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4 COC<sub>14 days</sub>: cumulative O<sub>2</sub> consumption after 14 days; COC<sub>28 days</sub>: cumulative O<sub>2</sub> consumption after 28 days.

5 †DRI<sub>24h</sub>: Dynamic respiration index representing the average O<sub>2</sub> uptake rate during the 24 h period of highest microbial respiration.

6 \* Trials 3, 6 and 7 were stopped after 18, 24 and 26 days, respectively.

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Table 3  
Univariate linear regression models of different microbial O<sub>2</sub> uptake indices

	Cumulative O <sub>2</sub> Consumption after 336 hours (COC <sub>14 days</sub> )				Cumulative O <sub>2</sub> consumption after 672 hours (COC <sub>28 days</sub> )			
	Coefficient estimate	Standard error	<i>p</i> -value	Coefficient of determination (R <sup>2</sup> )	Coefficient estimate	Standard error	<i>p</i> -value	Coefficient of determination (R <sup>2</sup> )
COC <sub>14 days</sub>					1.1	0.11	0.000 <sup>***</sup>	0.89
Peak OUR	92.42	13.16	0.000 <sup>***</sup>	0.78	127.14	33.45	0.003 <sup>**</sup>	0.58
DRI <sub>24h</sub>	106.77	13.7	0.000 <sup>***</sup>	0.81	142.37	35.21	0.002 <sup>**</sup>	0.6
Time to peak OUR	-43.12	17.95	0.031 <sup>*</sup>	0.3	-26.62	22.84	0.27	0.11

6 \*\*\*, \*\*, \* Significant at p < 0.001, 0.005, 0.05, respectively.  
7 DRI<sub>24h</sub>: average O<sub>2</sub> uptake rate (OUR) during the 24 h period of highest microbial respiration.  
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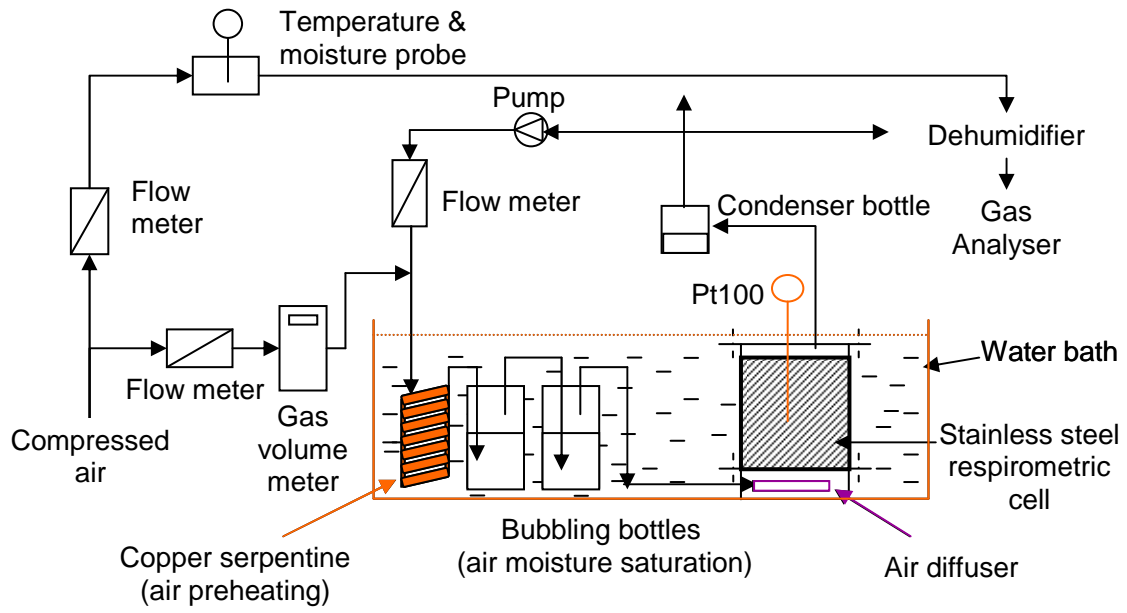
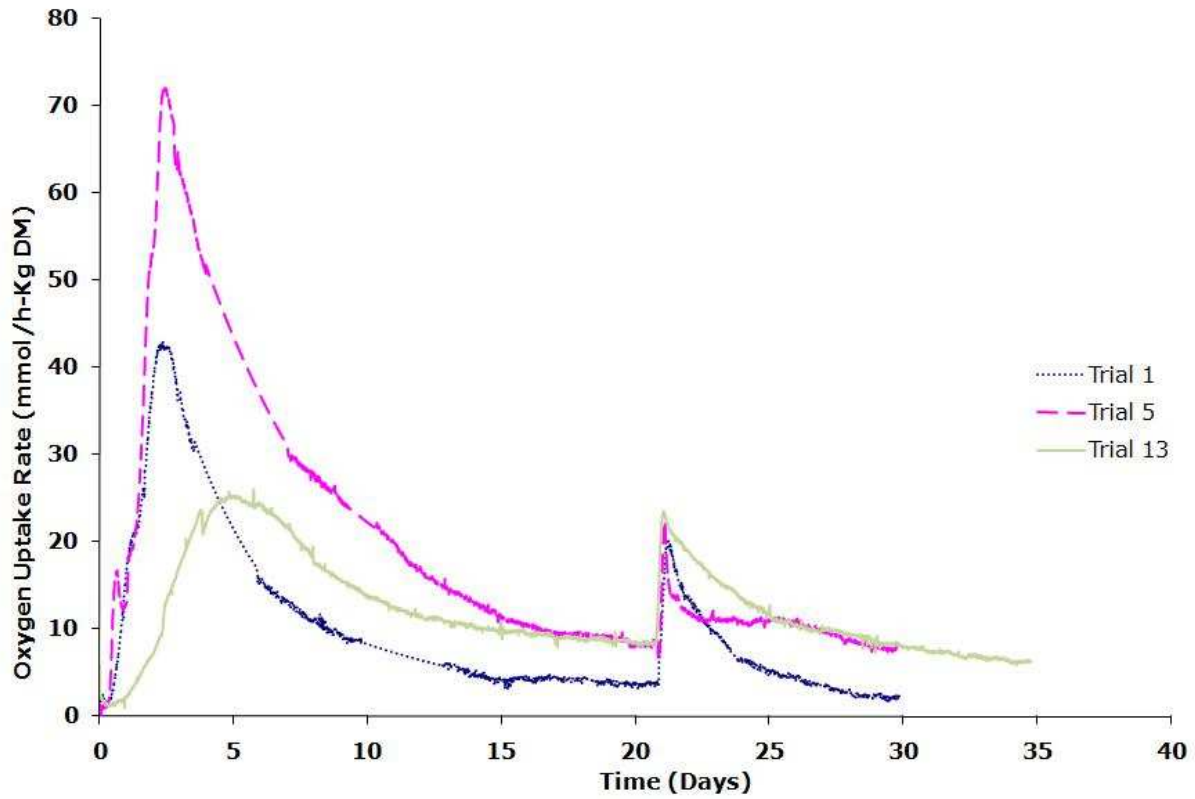
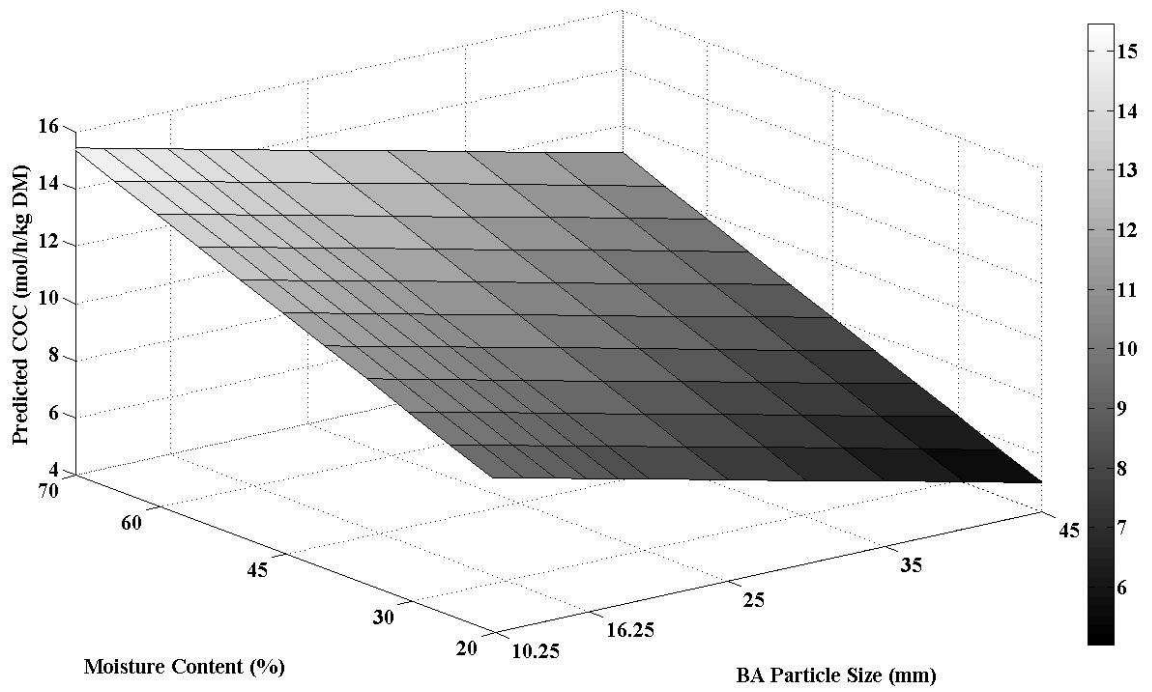


Fig. 1. Schematic diagram of respirometric apparatus adapted from Berthe et al. (2007).



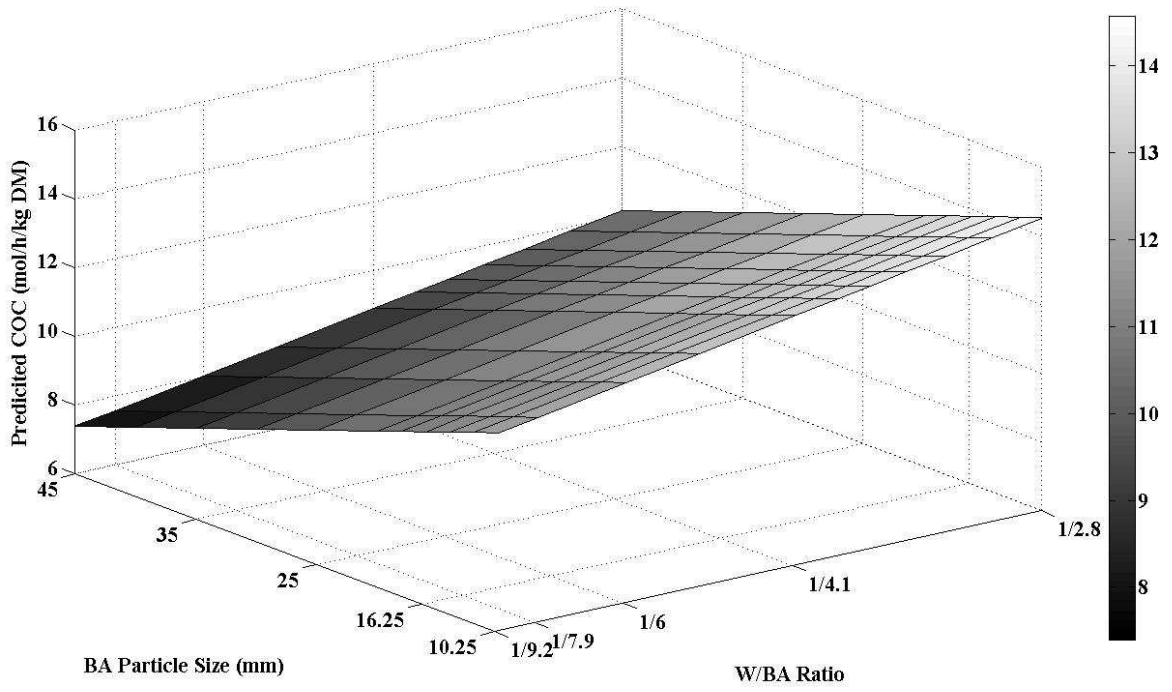
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 2 Fig. 2. Oxygen uptake rate (OUR) as a function of time for Trial 1: moisture content (MC) 41.5  
 3 %, waste/bulking agent (W/BA) ratio 1/9.2, BA particle size 20-30 mm; Trial 5: MC 68.3 %, W/BA ratio 1/6, BA particle size 20-30 mm;  
 4 Trial 13: MC 28.5 %, W/BA ratio 1/6, BA particle size 20-30 mm.  
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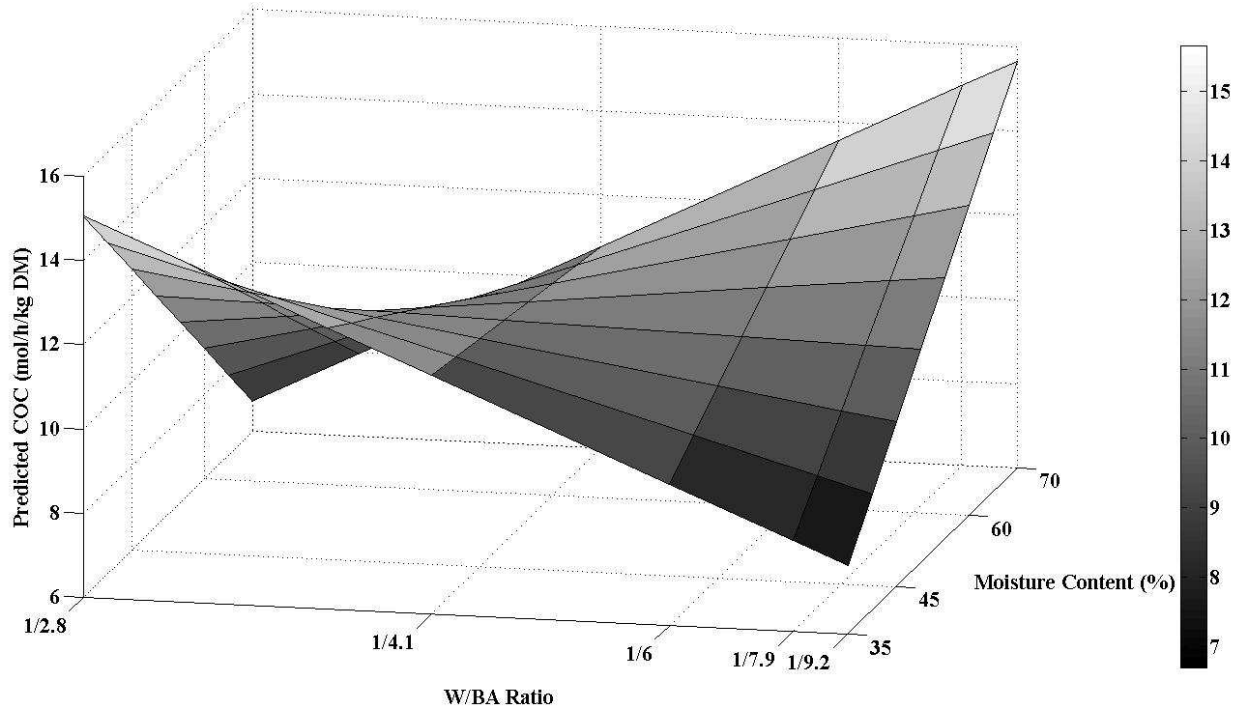
Fig. 3. The influence of moisture content and bulking agent (BA) particle size upon the predicted COC (cumulative O<sub>2</sub> consumption) at a constant waste/BA ratio of 1/6.

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Fig. 4. The influence of bulking agent (BA) particle size and waste/bulking agent (W/BA) ratio upon the predicted COC (cumulative O<sub>2</sub> consumption) at a constant moisture content of 45%.



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 2 Fig. 5. The influence of moisture content and waste/bulking agent (W/BA) ratio on the predicted  
 3 COC (cumulative O<sub>2</sub> consumption) at a constant BA particle size of 25 mm.