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Minimizing discrepancies in oxygen demand-based biodegradability (ODB) results using Taguchi method

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ABSTRACT

Oxygen demand-based biodegradability (ODB) suffers from a "discrepancy in the results" problem. Therefore, the objectives of this paper were to (1) determine the impacts of standards pH, initial substrate concentration, initial seed volume, and temperature on the accuracy of the ODB results; (2) identify the effect of increasing the test temperature to the mesophilic range; and (3) optimize the ODB conditions. The results showed, under standard environmental conditions, five-day, 20°C, and pH of 6.5 up to 8.5, the average ODB was 46 with 21% standard deviation. To minimize the discrepancy, the environmental conditions should be monitored to shift biochemical reaction toward mineralization reactions, rather than biomass production. Increasing temperature to 28.5°C improved average ODB to 81% with 10% standard deviation. Optimal conditions of the four factors together for ODB test were achieved at 400 mg L^{-1} concentration, 37°C temperature, 7.5 pH, and an initial seed of 30% of total sample volume. These conditions resulted in an average ODB of 82% with 1% standard deviation.

Keywords: Biodegradability; Biochemical oxygen demand; Environmental conditions; Taguchi method; Principle component analysis

1. Introduction

Biodegradation of organic matter is a natural process in which micro-organisms utilize organic matter to get the needed carbon and energy for growth and reproduction [1–3]. Biodegradation, which includes an aerobic (presence of oxygen) and an anaerobic (absence of oxygen) processes, is determined by the type of the organisms and the environmental conditions [4]. The importance of the biodegradation process stems from its ability to protect the environment, soil and water, from organic matter contamination. However, human disposal of municipal and industrial organic wastes can exceed the environmental capacity for degradation, which induces the need for interference to limit and deal with this pollution.

The human interference attempts to mimic the natural biodegradation process by using this very concept to develop measurement techniques and treatment technologies to determine and remove, respectively, organic pollutants. On the one hand, the

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most widespread and applied measurement technique is the biochemical oxygen demand (BOD_5) test, which is used to measure organic pollution [5–10]. On the other hand, one of the most applied treatment technologies is the activated sludge process which is used to treat organic-polluted water [2–4]. The core of both the BOD_5 test and the activated sludge process is the aerobic biological process. The performance of this process depends on the type of organic substrate [11,12], type of micro-organisms [6], availability of oxygen, biodegradation period [12], and environmental conditions. Furthermore, the latter is characterized by the presence of toxic compounds and nutrients, the concentrations of the initial substrate and micro-organism, the temperature, and the pH [4].

The BOD_5 test determines the biodegradability of the organic matter. It does so by measuring the biochemical oxygen, demanded by the micro-organism to degrade the organic matter [2,3,8]. The BOD_5 test procedure has been standardized [7] to the following conditions:

- A five-day incubation period [12,13],
- Incubating temperature of 20˚C, and pH of 6.5–7.5 [1] which could reach up to 8.5 [4],
- Adding macro and micronutrients, pretreatment of the sample for toxic compound, acclimatization of a mixture of seeds (micro-organisms) to degrade the organic matter, and supply of enough oxygen through dilution and mixing. Dilution and mixing mitigate toxicity [2–4,7].

It is important to notice here that, [7] emphasizes that the initial seed should be supplied in enough quantity in order not to limit the biodegradation process. Nevertheless, the standardized procedure has not overcome the main test limitations, which are the long duration of the test and the discrepancy in the results [4,6,14]. The latter limitation has serious implication on the accreditation of the test results. To shorten the incubation times, a respirometric method [9,10,15] and a biological sensor [14,16] were developed, but the stability of both performances is still uncertain. To mitigate the discrepancy in the results, [7] recommends that each laboratory has to establish its own correction by conducting the BOD_5 test several times and then applying statistical analysis to account for this problem. Nevertheless, [7] considers up to 30% discrepancy in the biodegradability results as acceptable. Therefore, this correction procedure suggested by [7] still has not totally eliminated the discrepancy and besides the fact that it is time- and effort-consuming.

The oxygen demand-based biodegradability (ODB) test measures biodegradability based on the amount of oxygen demanded for the biochemical reactions. Therefore, in order to understand the discrepancy in ODB results, it is important to know the causes of discrepancy in the amount of oxygen demanded in this process. The ODB discrepancy could be attributed to the variation in the ratio of the amount of CO_2 to the biomass that is produced during the test period. More specifically, the amount of oxygen that is demanded to biologically convert a certain amount of organic matter to CO_2 is different from that demanded to convert the same amount of the organic matter to biomass. Thus, the total amount of demanded oxygen depends on the ratio of CO_2 to the biomass. However, the BOD_5 test "standardized conditions" has neglected the above cause of discrepancy along with the interactions between the recommended standardized conditions (pH 6.5–8.5, initial substrate, initial seed, and temperature), which could be another source of variation in the BOD_5 and so in ODB test results. A third source of discrepancy that is also overlooked by the recommended standardized conditions has to do with to the used temperature. According to the current BOD_5 test conditions, the recommended temperature should be 20˚C, while the optimal temperature range of the mesophilic micro-organisms is 25–40˚C [3]. This raised the possibility that increasing the temperature from 20˚C to the optimal range could reduce the discrepancy in the ODB results.

Therefore, this paper is targeting the ODB test, i.e. biodegradability that is determined based on the amount of oxygen demanded in the biochemical reactions, and thus it aims at minimizing the discrepancy in DOB results by: (1) checking the effect of applying the standardized ranges of the different factors, pH, initial substrate concentration, initial seed volume, and temperature on the ODB results; (2) identifying the effect of increasing temperature to the mesophilic range; and finally (3) optimizing the ODB test conditions.

2. Methodology

2.1. Taguchi design of experiment and optimization

In order to achieve the first and the fourth objectives of the paper, initially 81 experiments were needed to be carried out. This number results from using three levels of each of the four factors (Table 1), and then taking all possible combinations of the these four factors. However, as conducting such number of experiments is time-, money- and effort-consuming, Taguchi method [17] was applied to reduce the number of carried out experiments to a manageable

Table 1
Summary of the four factors and the studied interactions

Controllable factors	Level 1	Level 2	Level 3	Justification
(A) Initial substrate concentration (mg L^{-1})	400	600	800	Oxygen availability
(B) Initial micro-organism "Seed" (% of total volume)	10	20	30	Past experience (further justification)
(C) Temperature (°C)	20	28.5	37	Optimal mesophilic range (25–40°C)
(D) Initial pH	6.5	7.5	8.5	Optimal aerobic biodegradation range

number, which was 27 experiments. Taguchi method [17] utilizes orthogonal arrays (OAs) to reduce the number of experiments under permissive reliability. The signal-to-noise (S/N) ratio is then used to measure quality. Although Taguchi method is widely applied to improve a product/process performance, it is only efficient for optimizing a single quality response. As the fourth objective of this paper is to optimize the ODB test conditions, there is a need to deal with a multiple response case. To deal with multiple responses for the optimization in the Taguchi method, several approaches have been developed [18–20]. Among these approaches is the principal component analysis (PCA) introduced by Pearson and Hotelling [21]. PCA is a multivariate statistical technique for forming new variables or so called principal components, which are linear combinations of the original variables, such that they are uncorrelated with each other. Generally, in a robust design, if the objective is to reduce the number of responses in the data set to a few principal components that are linear combinations of the original ones, then it is imperative that the number of principal components be less than the number of responses. The PAC analysis applied the following [18].

2.2. The CO_2 to biomass ratio

The biodegradability process has two main critical outputs (responses; ODB and Biomass) which result from the conversion of the organic matter. The ODB actually represents the *biochemical* oxygen demanded to mineralize the organic matter and to produce biomass (Eqs. (1) and (2)) [3]. To achieve objective two of this paper, a new term is used that is real biodegradability (RB), which represents the *chemical* oxygen demanded to mineralize the organic matter Eq. (3), and desynthesize the biomass (Eq. (3)). Thus the basis of the calculation is that all organic matter is mineralized, which means that the carbon to oxygen ratio is one to one and can be calculated by Eq. (4). Hence this new term (RB) should eliminate the effect of the CO_2 to biomass ratio on the basis of the biodegradability results.

Mineralization:

$$\mathrm{COHNS} + \mathrm{O_2} + \mathrm{Bacteria} \rightarrow \mathrm{CO_2} + \mathrm{H_2O} + \mathrm{NH_3} + \mathrm{Other\ end\ products} + \mathrm{Energy} \quad (1)$$

Synthesis:

$$\mathrm{COHNS} + \mathrm{O_2} + \mathrm{bacteria} \rightarrow \mathrm{Energy} + \mathrm{C_5H_7NO_2\ (biomass)} \quad (2)$$

Endogenous respiration:

$$\mathrm{C_5H_7NO_2\ (biomass)} + 5\mathrm{O_2} \rightarrow 5\mathrm{CO_2} + \mathrm{NH_3} + 2\mathrm{H_2O} \quad (3)$$

The organic matter used in this paper is glucose which is a completely dissolvable and biodegradable. Therefore, for each of the experimental conditions (see Section 2.3 for the details), the target was to find the percentage of glucose that was converted into RB, ODB, and biomass. To determine the fraction of organic matter that was converted to RB, ODB, and biomass, Eqs. (4)–(6) were used. The three terms were calculated as percentages of the initial dissolved glucose concentrations [$COD_{t=0}$].

Real biodegradability calculation: RB represents the chemical oxygen demanded to oxidize all converted organic matter to CO_2 including the fraction that is initially converted into biomass. The basis of the calculations is that all the converted organic matter, including the synthesized biomass, is mineralized according to Eqs. (1) and (3) [3]; considering that the oxygen utilized in Eq. (3) is five times the oxygen utilized in Eq. (2) [3], then RB as percentage can be calculated by Eq. (4).

$$\text{Real biodegradability (RB)}\% = 100\% \times \left[\frac{(\mathrm{COD}_{t=0} - \mathrm{COD}_{t=5})_{\mathrm{dissolved}}}{(\mathrm{COD}_{t=0})_{\mathrm{dissolved}}}\right] \quad (4)$$

Outputs (Responses) calculations: The biomass and the ODB percentages were calculated using Eqs. (5) and (6), respectively.

$$\text{Biomass } (\%) = \frac{100\% \times [(\text{COD}_{\text{total}} - \text{COD}_{\text{dissolved}})_{t=5} - (\text{COD}_{\text{total}} - \text{COD}_{\text{dissolved}})_{t=0}]}{(\text{COD}_{\text{dissolved}})_{t=0}} \quad (5)$$

It is important to notice here, that the ODB was calculated in this paper by Eq. (6), rather than measured as done conventionally.

$$\text{Oxygen demand-based biodegridability (ODS) } (\%) = 100\% \left[\frac{5 \times (\text{COD}_{t=0} - \text{COD}_{t=5d})_{\text{total}}}{(5 \times \text{COD}_{\text{dissolved}})_{t=0}} + \frac{((\text{COD}_{\text{total}} - \text{COD}_{\text{dissolved}})_{t=5} - (\text{COD}_{\text{total}} - \text{COD}_{\text{dissolved}})_{t=0})}{(5 \times \text{COD}_{\text{dissolved}})_{t=0}} \right] \quad (6)$$

where t = time at zero and after five days.

2.3. Lab experiments

The four three-level factors were investigated according to Taguchi method by OA L_{27} (3^{13}), which resulted in 27 experiments (Table 2). Each experiment was carried out twice, so 54 experiments were operated between February and May 2010. The experiments were conducted in the labs of the Water, Energy and Environment Center at the University of Jordan.

2.3.1. Solutions preparations

The inputs of the lab experiments were:

Glucose stock solution: Glucose of analytical grade was dried at 105°C and then used to prepare a 4,000 mg L^{-1} stock solution. The concentration of the stock solution was checked by the COD test. Macronutrients and micronutrients were prepared according to [7].

Seed source: The seed was obtained from the plug flow activated sludge (AS) process in Abu-Nusier

Table 2
The preparation of the ODB-bottles solutions

Number of experiments per run based on Taguchi method	Substrate concentration (mg L^{-1})	Seed (% of total volume)	Glucose volume (ml)	Distilled water volume (ml)	Seed volume (ml)
1,2,3	400	10	50	400	50
4,5,6	400	20	50	350	100
7,8,9	400	30	50	300	150
10,11,12	600	10	75	375	50
13,14,15	600	20	75	325	100
16,17,18	600	30	75	275	150
19,20,21	800	10	100	350	50
22,23,24	800	20	100	300	100
25,26,27	800	30	100	250	150

wastewater treatment plant (WWTP). A 1.5–2.0 liter of the AS effluent was transported to the lab, filtered through 8 μm Whatman grade, 40 filter papers. The filtrate was the seed of the experiments in this research. The concentration of seed solution was measured by the COD test and to check the consistency of the seed solution through the whole test period, the total counts of *Escherichia coli* (EC) and total coliform (TC) were determined using the 9223 B enzyme substrate test method [7].

Tested solution: There were 27 solutions prepared as specified in Table 2; each solution was prepared in 500 ml volumetric flasks. The pH value of each solution was measured (pH 330i/SET WTW) and then adjusted to the required value by 1 N of NaOH or 1 NH_2SO_4. After that, each of the 27 solutions had 1 ml of micro and 1 ml of macronutrients added. For each of the test solutions, two controllers were prepared in the same manner, except that: (1) distilled water replaced glucose in the seed-controller solution; and (2) distilled water replaced seed in the blank-controller solution.

2.3.2. Experiment setup

Each of the 54 experiments (27×2) consisted of four 550 ml dark bottles. The biodegradability was tested in a glucose solution, where the volume of the test solution was chosen to assure the experiment was not oxygen limited, with 97 ml of the sample poured in 550 ml bottle as specified in the instruction manual of [22]. Two bottles of the four were filled with the test solution and the other two were filled as following: one with the seed-controller solution and the other with the blank-controller solution. In each bottle, a rod magnetic stirrer and a rubber sleeve were inserted. In the sleeves, two tablets of sodium hydroxide were added to adsorb CO_2 and the bottles were sealed tightly with parafilm and continuously stirred in a controlled temperature incubator for five days. At the end of the incubation period, the solutions in the bottles were measured for pH and total and dissolved COD. The samples were analyzed immediately and those that were not analyzed immediately were kept in a refrigerator at 4 °C, to be analyzed within 24 h.

The initial and final organic matter concentrations were measured by the chemical oxygen demand (COD) test following [7]. The COD concentrations were measured for total and dissolved COD, with the latter, filtrated through 0.45 μm-membrane filter paper [23].

2.3.3. Experiment validation

The procedure that was carried out in Section 2.3.2, was applied to test: (1) the ODB of glucose in the presence of 44.5 mg/l of phenol; and (2) the ODB of domestic wastewater that was obtained from Abu Nusier treatment plant, using the test conditions optimized in this study. Each sample was tested in triplicate.

3. Results and discussion

3.1. The ODB results vs. the standard conditions

The results of applying standard conditions are variations in the ODB results, (Table 3) as measured by the standard deviation of 21%, which was less than the accepted standard deviation of 30% in BOD_5 test [7]. Furthermore, the results showed a glucose average biodegradability, in percentages, calculated by RB and ODB of 63 and 46% with standard deviations of 26 and 21%, respectively. Therefore, the RB gave a better estimation of the average biodegradability than the ODB. This result was expected as the RB was introduced in this paper to overcome the ODB problem by measuring oxygen consumption as a chemical oxygen demand, rather than a biochemical oxygen demand; this means a 1:1 ratio of carbon to oxygen (Eqs. (1) and (3)), instead of 1:5 (Eqs. (1) and (2)). Hence the RB is not affected by the type of biochemical reaction.

More light needed is to be shed on the RB compared with ODB. Technically, the ODB is measured by monitoring the depletion in oxygen consumption that is determined by titration or by the respirometric method [9,10]. However, the RB was measured, in this paper, by monitoring the depletion of oxygen by measuring the total and dissolved COD. Therefore, the RB is applicable when the content of the test sample does not include particulate matters, which limits the applicability of the RB test. In light of this, in order to improve the estimation of the ODB test results to reflect the actual results, the standard experiment conditions should be optimized to encourage mineralization reaction (Eq. (1)) rather than biomass production (Eq. (2)). Theoretically, the optimal conditions are defined as the test conditions that comprise between minimizing the discrepancy in ODB values, while maximizing the ODB values to be closer to the RB values. Practically, that means the optimal test conditions are the conditions that support the mineralization reaction over the biomass production reaction and thus, lead to a minimum discrepancy in the results.

It is important to notice here that the variation in the results of the ODB test has nothing to do with the initial seed and time duration, since the mineralized amount of the organic matter was not limited by the seed or time. For example, adding the same amount of seed (30%) to two different initial substrate

Table 3
Taguchi and PCA

Experiment runs	Factors				ODB		Biomass		RB		SNR		Normalization of SNR		PC1
	Substrate	Seed %	Temp	pH	Run 1 (%)	Run 2 (%)	Run 1 (%)	Run 2 (%)	Run 1 (%)	Run 2 (%)	ODB	Biomass	ODB	Biomass	ODB + Biomass
1	400	10	20	6.5	44	45	19	22	60	63	−6.98	13.66	0.62	0.40	0.72
2	400	10	28.5	7.5	80	83	7	10	86	91	−1.77	21.41	0.95	0.88	1.29
3	400	10	37	8.5	35	38	24	29	55	61	−8.77	11.48	0.51	0.26	0.54
4	400	20	20	7.5	62	60	44	43	97	95	−4.30	7.20	0.79	0.00	0.56
5	400	20	28.5	8.5	85	83	10	10	92	91	−1.55	20.28	0.97	0.81	1.26
6	400	20	37	6.5	88	89	11	9	97	96	−1.06	19.80	1.00	0.78	1.26
7	400	30	20	8.5	42	45	11	15	51	56	−7.26	17.69	0.60	0.65	0.88
8	400	30	28.5	6.5	85	89	15	13	96	99	−1.26	17.31	0.99	0.62	1.14
9	400	30	37	7.5	83	81	7	6	89	85	−1.76	23.41	0.96	1.00	1.38
10	600	10	20	7.5	27	29	16	11	40	38	−11.07	17.33	0.36	0.62	0.69
11	600	10	28.5	8.5	53	53	18	16	67	66	−5.46	15.47	0.72	0.51	0.87
12	600	10	37	6.5	67	64	17	15	80	85	−3.68	16.00	0.83	0.54	0.97
13	600	20	20	8.5	64	70	22	23	82	88	−3.50	12.95	0.84	0.35	0.85
14	600	20	28.5	6.5	86	86	10	9	93	94	−1.28	20.39	0.99	0.81	1.27
15	600	20	37	7.5	58	62	19	22	74	80	−4.42	13.62	0.78	0.40	0.83
16	600	30	20	6.5	82	84	12	12	92	94	−1.62	18.43	0.96	0.69	1.17
17	600	30	28.5	7.5	83	83	10	8	91	89	−1.62	21.06	0.96	0.85	1.29
18	600	30	37	8.5	73	72	14	18	84	87	−2.79	15.94	0.89	0.54	1.01
19	800	10	20	8.5	28	23	14	12	40	32	−12.06	17.68	0.29	0.65	0.66
20	800	10	28.5	6.5	84	84	11	10	93	92	−1.53	19.43	0.97	0.75	1.22
21	800	10	37	7.5	85	88	13	11	96	97	−1.23	18.32	0.99	0.69	1.18
22	800	20	20	6.5	16	14	8	10	23	21	−16.63	20.98	0.00	0.85	0.60
23	800	20	28.5	7.5	86	86	11	12	95	96	−1.30	18.82	0.98	0.72	1.20
24	800	20	37	8.5	89	86	11	12	98	96	−1.16	18.64	0.99	0.71	1.20
25	800	30	20	7.5	48	50	37	40	77	82	−6.27	8.28	0.67	0.07	0.52
26	800	30	28.5	8.5	84	79	12	16	94	92	−1.76	17.09	0.95	0.61	1.11
27	800	30	37	6.5	61	58	12	13	71	68	−4.52	18.16	0.78	0.68	1.03

concentrations, 400 and 600 mg L^{-1}, resulted in mineralizing 160 and 477 mg L^{-1} of the substrate, respectively. Thus, the pH, temperature, and the ratio of substrate to seed were the factors that contributed to the discrepancy in the test results.

3.2. Temperature effect

Focusing on temperature effect alone while operating within the standard conditions for the other three factors, it was found that increasing the standard temperature from 20 to 28.5˚C improved the ODB results by increasing the estimation (Table 3) and reducing the variations. The average ODB result was 46% and its standard deviation was 21% at 20˚C compared with an average of 81% and a standard deviation of 10% at 28.5˚C. In addition, the average RB result was 63% and its standard deviation was 25% at 20˚C compared with an average of 90% and a standard deviation of 9% at 28.5˚C. Increasing the temperature from 20 to 28.5˚C to be within the optimal range (25–40˚C) of the mesophilic temperature, on the one hand, increased the average mineralization from 42 to 78%, which improved the estimated values of ODB and RB. On the other hand, it reduced the biomass production from 21 to 12% and hence, improved the estimation of the ODB and RB and reduced their variations and narrowed the gap between the in the ODB and RB results.

Based on this, increasing the temperature from 20 to 28.5˚C mitigated the impact of the environmental factors on the ODB results. Here also, it should be noticed that the ODB was not limited by the availability of seed, as based on the calculation in Table 3 and 30% seed biodegraded 704 out of 800 mg L^{-1} of glucose.

The question that came next: what was the effect of changing the temperature within the mesophelic range on the ODB test results? To answer this question, the experiments were conducted at 37˚C. The results showed that at this temperature the average ODB was 71% with a standard deviation of 16%, the average mineralization was 68% with a standard deviation of 17%, the average RB was 83% with a standard deviation of 13% and finally the average biomass conversion was 15% with a standard deviation of 6%.

The comparison of the average ODB at 20, 28.5, and 37˚C showed that better estimation and less variation were achieved at 28.5˚C followed by 37˚C and the worst results were achieved at 20˚C. Increasing the temperature from 20 to 28.5˚C and from 20 to 37˚C increased the ODB and RB and decreased the biomass production (Table 3), except for the optimal case that was discussed later in Section 3.4. A comparison of the results at 28.5 with those at 37˚C, showed that the biological conversion was shifted further toward biomass production at 37˚C, which deteriorated ODB results at that temperature. The interpretation of this is that increasing the temperature speeds up the mineralization rate, the biomass production rate, and the die-off rate. Therefore, it seems that the ratio of mineralization to biomass production rates (not measured in this study) is higher at 28.5˚C, rather than at 37˚C.

3.3. Taguchi method and principle component analysis

In the search for the optimal experiment conditions of all four factors together, Taguchi method was applied to convert each response to its signal to noise ratio (S/N). In the context of the current paper, the ODB S/N was the larger, the better and the biomass S/N ratio was the smaller, the better. As the two S/N ratios may tend to move together in some phases as shown in Fig. 1(a), it would be interesting to examine if it would be possible to reduce the two variables into one factor using the principle component analysis (PCA).

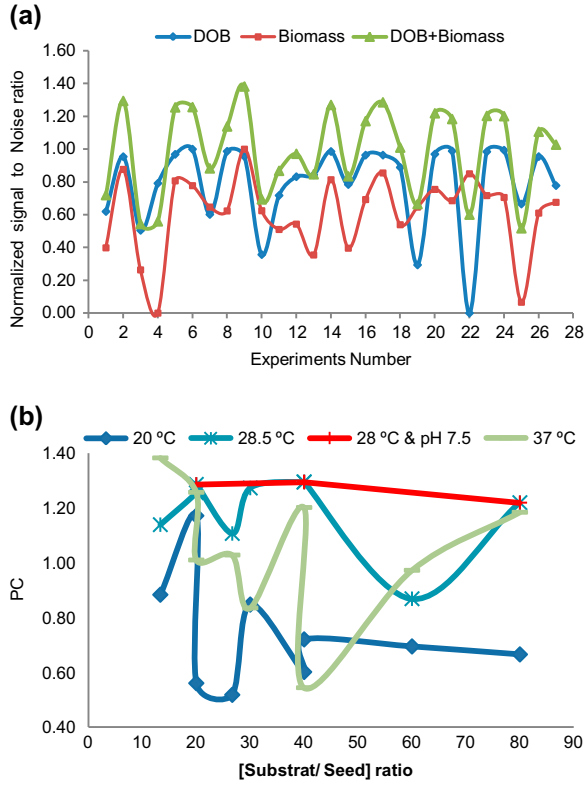

Fig. 1. (a) Plot of Normalized S/N ratio vs. experiment number for ODB, biomass, and reduced factor, (b) Plot of PC vs. ratio of substrate to seed.

Table 4
ODB test conditions and results for glucose, glucose with phenol and domestic wastewater

Parameters	Initial concentrations (mg/L)	Initial seed (%)	pH	Time (day)	DOB at 37°C	DOB at 20°C
Domestic wastewaters	1,250	30	7.5	5	63 ± 3	55 ± 3
Diluted domestic wastewaters	430	30	7.5	5	73 ± 4	62 ± 5
Glucose and phenol	400	30	7.5	5	54 ± 2	49 ± 2
Glucose	400	30	7.5	5	88 ± 2	81 ± 5

Applying the PCA on the ODB and biomass S/N ratios resulted in the following two eigenvalues 1.205 and 0.7951 and the associated eigenvectors [0.707; 0.707] and [0.707; −0.707], respectively. As one of the two eignevalues is more than one, the two responses are reduced to one factor (PC1) as shown in Table 3 and depicted in Fig. 1(a), referred to as ODB + biomass. The two S/N ratio curves intersected at many points. Interestingly, the highest point of intersection of the two ratios coincided with the maximum value of the reduced factor (ODB + biomass) PC. The maximum value of the PC1 was the maximum value of the combination of the two responses: the ODB the larger, the better and the biomass which was the smaller, the better could be considered as the optimal conditions. This maximum PC1 value, i.e. the optimal conditions for the ODB test, achieved under the following conditions were: concentration of 400 mg L^{-1}, temperature of 37˚C, pH of 7.5, and initial seed of 30% of the total sample volume. These conditions resulted in an average ODB test results of 82% with a standard deviation of 1% and an average biomass production of 7% with a standard deviation 1. The values were in the range of applying the ODB test at 28.5˚C at the standard conditions of the other three factors i.e. 81 ± 10% for ODB and 11 ± 3% for biomass production.

A further robustness check of the optimal conditions arrived at above, is provided by Fig. 1(b). The biodegradability is a function of the substrate to seed ratio as was established in the biological treatment literature by the ratio of food to micro-organisms "F/M" [3]. The curve that represents the relationship between the ratio of F/M and PC1, as shown in Fig. 1 (b), was almost horizontal at 28.5˚C and 7.5 initial pH, and it shows that the PC1 values are high at these conditions. In other words, regardless of the F/M ratio values, almost the same high value of PC1 can be achieved at 28.5˚C and 7.5 initial pH. These conditions compared with the optimal conditions determined above have a better estimation of RB and ODB, but with worse discrepancy in the results i.e. average RB of 91% with a standard deviation of 4% and an average ODB of 84% and with a standard deviation of 2% compared with the 87, 1% and 82, 1%, respectively, at the optimal conditions. Therefore, even though a better estimation was achieved at 28.5˚C and 7.5 initial pH, there was more discrepancy in the results which confirms the appropriateness of the optimal conditions that were arrived at above.

3.4. Test-validation

Table 4 shows the ODB test results of the glucose, in the presence of phenol, and the ODB test results of domestic wastewater, under the conditions optimized in this study; namely 400 mg/l, 37˚C and 30% seed, and pH 7.5. Diluting domestic wastewater from 1,250 to 430 mg/l, enhances the biodegradability at 20˚C, from 55 up to 66%, and at 37˚C, from 63% up to 37%. Although the presence of phenol as a toxic compound inhibited the biodegradability of glucose, the optimized conditions identified by this study, enhanced the biodegradability from 49 up to 54%. Thus the environmental conditions optimized in this study, enhanced the results of biodegradability (ODB) of the glucose in the presence of phenol as a toxic compound and the ODB of the raw domestic wastewater.

4. Conclusion

Under standard environmental conditions, average ODB was 46% with a 21% standard deviation and average biomass production was 21% with 12% standard deviation. Further tests showed that changing or increasing the temperature of the standard environmental conditions to 28.5˚C improved average ODB to 81% with a standard deviation of 10%. Furthermore, it was found that optimal test conditions of the four factors together for ODB test were achieved at 400 L^{-1} concentration, 37˚C temperature, 7.5 pH, and an initial seed of 30% of total sample volume. These conditions resulted in an average ODB of 82% with 1% standard deviation.

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