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Application of an Acclimated Microbial Consortium as Biocatalyst for Rapid Determination of Biochemical Oxygen Demand

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ABSTRACT

Biochemical Oxygen Demand (BOD) is an important parameter for water pollution monitoring. Since the conventional BOD assay by American Public Health Association (APHA) takes 5 days to complete (termed BOD_5), development of an alternative method that can rapidly measure the amount of biodegradable organics in wastewater is highly desirable. In the present study, single and mixed microbial cultures isolated from different environments in Malaysia were tested as potential biocatalyst in an assay called Ferricyanide Mediated-Biochemical Oxygen Demand (FM-BOD) used for rapid BOD detection. Unlike the BOD_5 assay, the FM-BOD is based on biochemical degradation of organic compounds via reduction of ferricyanide as an alternative terminal electron acceptor to dissolved oxygen. The increase in concentration of microbially produced ferrocyanide during incubation of the microorganisms in the presence of ferricyanide and organic substrates was measured voltammetrically using a 10 μm platinum working electrode. Measurements were conducted using linear sweep voltammetry at potential 0-600 mV versus Ag/AgCl reference electrode. Limiting currents were determined by voltammetry at E_{app} = +450 mV (vs Ag/AgCl). The amount of ferrocyanide produced was found to be proportional to the amount of biodegradable organics. From the study, it is shown that mixed microbial consortium acted as a better biocatalyst for rapid BOD detection compared to both single cultures and a commercial consortium. The microbial consortium consists of four species of microorganisms. FM-BOD assays were conducted in optimized conditions with microbial consortium at final absorbance of OD_{600} 2.5 and ferricyanide at concentration 60 mM. Results showed that the FM-BOD assay was able to detect biodegradable organics in 1 h compared to the current BOD_5 assay of 5 days. This FM-BOD assay with optimized conditions is applicable to food wastewater consisting of easily degradable carbon source.

Key words: Biochemical oxygen demand (BOD), ferricyanide, ferrocyanide, FM-BOD assay, microbial consortium

INTRODUCTION

Water quality is assessed in terms of the amount of organic compounds present in it. Biochemical Oxygen Demand (BOD) is an important and widely used parameter for water bodies pollution monitoring (Akpoveta *et al*., 2011; Alquwaizany *et al*., 2011; Aluyi *et al*., 2006;

Ayeni *et al.*, 2011; Khan *et al.*, 2003; Mahre *et al.*, 2007; Norizan *et al.*, 2011; Ogunfowokan *et al.*, 2005; Zainudin *et al.*, 2010). The BOD load of wastewater needs to be determined to ensure it is within the parameter limits of effluents that can be discharged into inland waters under the Sewage and Industrial Effluents Regulations 1979 (How, 2003). According to the American Public Health Association (APHA, 1992), the standard method for BOD_5 assay requires 5 days of incubation time at ±20°C in the dark. This conventional method of BOD measurement is time consuming, irreproducible and labour intensive with questionable accuracy. Measurement depends on temperature, oxygen concentration, presence of toxins, as well as the type, quantity and quality of seeding microorganism (Pasco *et al.*, 2000, 2004). The long duration of BOD_5 assay is not suitable for on-line monitoring especially where rapid feedback is essential for environmental monitoring and/or process control (Morris, 2005).

To overcome the shortcomings in the traditional BOD_5, various rapid microbial biosensors have been developed. Microbial BOD biosensor was first discovered by Karube *et al.* (1977), where the sensor consists of a combination of an oxygen electrode as a transducer and a bio-film (Nakamura *et al.*, 2008). Microorganisms immobilized in the bio-film were placed in close, intimate contact with an amperometric oxygen electrode. When organic compounds present in the samples are degraded, dissolved oxygen is consumed. Dissolved oxygen consumed is proportional to the amount of biodegradable organics and designated as BOD_5 value.

To date, most BOD biosensors still focus on measuring oxygen uptake by immobilized cells attached to an oxygen electrode (Riedel *et al.*, 1990; Tan and Wu, 1999; Lehmann *et al.*, 1999; Liu *et al.*, 2000). The history of BOD sensor developments has been well summarized in two literatures (Nakamura *et al.*, 2007, 2008). But these BOD sensors are limited either by the availability of oxygen, or by pure microbial cultures with a narrow substrate range, or they require calibration to a BOD_5 standard solution.

The conventional 5-day BOD assay can achieve an average of 60.5% of organic compounds degradation while using Glucose-Glutamic Acid (GGA) as the standard check solution (Pasco *et al.*, 2000; Morris *et al.*, 2001). For the classic microbial BOD sensor, only less than 1% of the organic compounds are degraded (Morris *et al.*, 2001). To increase the percentage degradation of organic compounds for rapid BOD biosensor, a novel technique employing mediator (redox dye) such as ferricyanide ion and an amperometric transducer was introduced by Pasco *et al.* (2000). This technique replaces oxygen as final electron acceptor for the biochemical reaction in the detection process. More recently, this kind of mediated microbial sensors have received much attention for rapid BOD measurement (Yoshida *et al.*, 2000, 2001; Catterall *et al.*, 2001, 2003; Trosok *et al.*, 2001; Morris *et al.*, 2001; Pasco *et al.*, 2004; Morris, 2005).

Ferricyanide is an efficient redox mediator to shuttle electrons from the redox center of reduced microbial enzymes to the electrode in the presence of excess Glucose/Glutamic Acid (GGA). Thus, ferricyanide can be used instead of oxygen as an electron acceptor during microbial catabolism of organic compounds. The microbially reduced ferricyanide is then quantified electrochemically upon re-oxidation at the electrode surface. The amount of ferricyanide reduced is directly proportional to the amount of organic material present in a sample. The mechanism of this process is represented in the following equation:

$$\text{Substrate(reduced)} + [Fe(CN_6)]^{3-} \xrightarrow{\text{Microorganism}} \text{Substrate(oxidised)} + [Fe(CN)_6]^{4-}$$

Ferricyanide is about 10,000 times more soluble than oxygen in water. Moreover, higher population of microbial cells can be used without the depletion of the electron acceptor. Hence, large microbial consumption of organic matter can be achieved in much shorter time (Pasco *et al.*, 2000; Morris *et al.*, 2001).

FM-BOD (Ferricyanide-mediated determination of BOD) approaches that have been reported can be divided into two general types. The first type is the bioreactor assay that includes a separate incubation and detection step (Pasco *et al.*, 2000). The second type is ferricyanide-mediated amperometric BOD biosensors in which the microbial was immobilized directly on the tip of the working electrode (Yoshida *et al.*, 2000, 2001; Trosok *et al.*, 2001).

The first approach was employed by Pasco *et al.* (2000), where it is named as MICREDOX®. In this study, the first approach was employed too and it is named as FM-BOD assay. FM-BOD assay comprises of two steps that occur independently. Firstly, FM-BOD assay involves incubation of the sample solution which consists of microorganisms, ferricyanide and the sample organic substrate. In the second step, detection of accumulation of the reduced form of mediator (ferrocyanide) or the decrease in the oxidized form of mediator (ferricyanide) is involved. Pasco *et al.* (2000) quantified the microbially reduced mediator by measuring the charge required for its re-oxidation with a coulometric transducer. Coulometric detection was found to be labour-intensive and time consuming. Thus, several improvements of BOD detection have been reported afterwards Morris *et al.* (2001), Catterall *et al.* (2001, 2003), Pasco *et al.* (2004) and Morris (2005).

The second method is the ferricyanide-mediated amperometric BOD biosensors described by Yoshida *et al.* (2000, 2001) and Trosok *et al.* (2001). This FM-BOD biosensor method is similar with traditional BOD biosensors in which the biocatalyst is immobilized on the tip of the working electrode to eliminate the entry of biocatalyst into the sample solution. The difference is that the detection principle involves microbially produced ferrocyanide being re-oxidized back to ferricyanide at the electrode surface. The FM-BOD biosensor needs to be calibrated to a standard organic solution before analyzing.

Though offering simple and rapid monitoring of BOD, FM-BOD biosensor has its limitations. Firstly, sensor fouling may occur due to constant contact of samples with the sensor resulting in constant sensor recalibration. The total BOD value may also be overestimated since only the most easily biodegradable portion of a sample will be degraded in the short reaction time. Furthermore, due to the variations in biodegradable organics content between samples frequent recalibration of the BOD biosensor is needed.

To demonstrate the feasibility of the FM-BOD approach, this study sought to employ a locally developed microbial consortium as biocatalyst to measure the BOD load of synthetically prepared organic solutions and real industrial waste water. The use of acclimated microorganisms is expected to extend the application of the FM-BOD assay to a broad range of wastewater.

MATERIALS AND METHODS

Reagents: BOD standard solution, Glucose Glutamic Acid (GGA) (150 mg L^{-1} glucose, 150 mg L^{-1} glutamic acid) was prepared according to APHA procedures (APHA, 1992). This solution was assigned a BOD value of 198±30 mg BOD_5^{-1}. Potassium ferricyanide solutions (analytical reagent grade) were prepared in Phosphate Buffer Saline (PBS), pH 7. The Organization for Economic Cooperation and Development (OECD) synthetic wastewater solution comprised of 16 g L^{-1} peptone, 11 g L^{-1} meat extract and 3 g L^{-1} urea (diluted to100-fold) (Morris, 2005). All solutions were prepared in deionized water.

Microbial consortium preparation: The microbial consortium used in this study comprised of microorganisms isolated from various environmental sources in Malaysia. Microorganisms were grown in Tryptic Soy Broth (TSB) at 37°C in a shaking incubator for 16-18 h. Cells were then harvested by centrifugation at 4000 rpm for 15 min at room temperature, washed twice with phosphate buffer (pH 7) and then resuspended in phosphate buffer (pH 7). Cells were adjusted to a final absorbance of 5 at 600 nm using a 100 VIS Spectrophotometer (BUCK Scientific).

Sample preparation: Three sample solutions: blank, endogenous control and real sample were prepared in a final volume f 10 mL in serum bottles. Real sample contained 5 mL of appropriate bacterial suspension (OD_{600} 5), 1.5 mL of 400 mM potassium ferricyanide and 3.5 mL of standard GGA solution/OECD solution (or real wastewater). Blanks were prepared by replacing ferricyanide with Phosphate Buffer Saline (PBS). Endogenous control solutions were prepared by replacing GGA standard solution with sterile deionized water. All solutions were spurged with oxygen free nitrogen for 15 min. For real samples, two further controls were employed: (1) Sample in the absence of ferricyanide and microorganisms to determine if there were any species present that undergo electrochemical oxidation and (2) Sample in the absence of microorganisms to determine if there were any species present capable of reducing ferricyanide to ferrocyanide.

Incubation: The samples prepared were incubated at 37°C in a shaking water bath. Sample (1 mL) was removed at hourly intervals for 2 h and the microbial reaction was terminated by centrifugation at 14,000 rpm for 15 min. The supernatant solution was then added into Phosphate Buffered Saline (PBS) to a total volume of 30 mL and bubbled with nitrogen gas for 30 sec before being analysed for microbially produced ferrocyanide using voltammetry.

Voltammetric detection: Measurements were conducted using a linear sweep voltammetry at potential 0-600 mV versus Ag/AgCl reference electrode. A 10 μm platinum microelectrode was used as the working electrode and platinum gauze auxiliary electrode was employed to complete the three electrode electrochemical cell. The limiting current value obtained for a given sample at a given incubation time was taken to determine the ferrocyanide concentration and thus the amount of organics degraded.

Calculation of FM-BOD_5 equivalent values: As in BOD_5 assay, GGA solution was used as a standard check solution for the FM-BOD assay. In all cases, the limiting current values of the endogenous control solution were subtracted from the sample and GGA limiting current values prior to calculation. Limiting current (i_{lim}) values obtained throughout the FM-BOD incubation were divided by values obtained from GGA standard solution. This value is termed the normalized limiting current and is a dimensionless parameter. This parameter can be converted to a FM-BOD_5 equivalent value by multiplication of 198 mg L^{-1} (average accepted BOD_5 value for the GGA solution) (Catterall *et al*., 2003; Morris, 2005).

When the OECD synthetic wastewater standard was used as calibration standard, limiting current (i_{lim}) values obtained throughout the FM-BOD incubation were divided by values obtained from OECD standard solution. In all cases, the limiting current values of the endogenous control solution were subtracted from the sample and OECD limiting current values prior to calculation. This normalized limiting current was converted to a FM-BOD_5 equivalent value by multiplication of 170 mg L^{-1} (the average accepted BOD_5 value for the OECD solution following 100-fold dilution) (Morris, 2005).

Statistical analysis: Experiments were typically done in triplicates and the standard deviation of the mean values were calculated accordingly. The analysis of data were conducted at 5% level of confidence ($p<0.05$).

RESULTS AND DISCUSSION

Use of Ferricyanide by microbial consortium and commercial consortia for GGA degradation: Locally developed microbial consortium and commercial consortia were tested for their ability to use ferricyanide for degradation of GGA. Apart from its use in standard BOD_5 assay, GGA is also the most commonly employed calibration standard for BOD biosensors. The locally developed microbial consortium mainly consists of four bacteria species from various environmental sources in Malaysia. The advantage of the microbial consortium is that it has bacteria from various sources, thus could degrade greater type of organic compounds in the real wastewater samples (Liu *et al*., 2000; Liu and Mattiasson, 2002).

A commercial consortium was assessed to compare the response of our locally developed microbial consortium in the FM-BOD assay. The limiting current values obtained from repeated application of commercial consortium in FM-BOD assay were very low and not reproducible compared to our locally developed microbial consortium. This is because commercial consortium is already premixed and it is grown in TSB and adjusted to OD_{600} 5, the exact concentration of each bacterium is unknown. On the contrary, our locally developed microbial consortium was adjusted to OD_{600} 5 prior to mixing. Each bacterium has the same concentration and reproducibility of results was proven in repeated test. The results indicate that commercial consortium was not able to utilize ferricyanide as the final electron acceptor in the biochemical reaction for organic biodegradation.

From Fig. 1, a commercial consortium was replicated in this study to compare with locally developed microbial consortium. It can be seen that the limiting current response obtained for the microbial consortium is higher compared to both commercial consortiums at the first and second hour incubation. This indicates that the microbial consortium has rapidly degraded GGA in the first and second hour. It is also shown that significant degradation of GGA can already be observed in the first hour, showing that a rapid BOD detection within 1 h is possible.

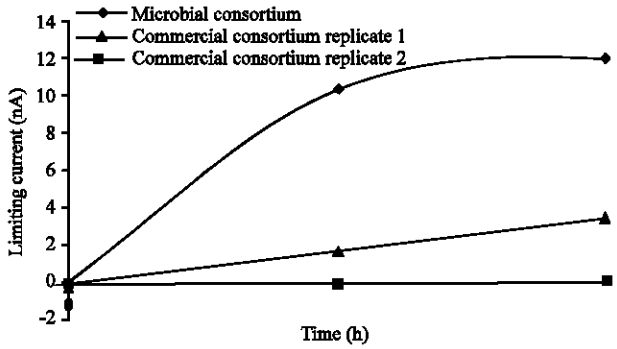

Fig. 1: Limiting current values obtained at various incubation times for locally developed microbial consortium and commercial consortium in GGA standard solution. Each point represents the average of response from three replicate measurements. Endogenous control values have been subtracted. Microbial consortium final absorbance = 2.5. Ferricyanide final concentration = 60 mM. Limiting currents determined by voltammetry at E_{app} = +450 mV (vs Ag/AgCl)

From the limiting current values, the extent of GGA degradation can be determined. The extent of GGA degradation, expressed as percent conversion, was calculated as previously described by Morris (2005). The data of microbial consortium in Fig. 1 indicates that more than 45% of the GGA solution had been degraded in the first hour of incubation. This compares favourably with both the BOD_5 assay (~60% GGA degraded in 5 days) and that previously reported by Morris *et al.* (2005) (~40% GGA degraded in 1 h). Hence, in the FM-BOD method, the BOD of a sample can be determined in 1 h compared to 5 days with the standard BOD_5 assay.

Linear dynamic range for standard calibration solutions: GGA standard solution and OECD synthetic solution were tested for their ability as a calibration solution for the FM-BOD assay in 1 h incubation. GGA is a standard solution for the conventional BOD_5 assay and also used extensively for BOD biosensors. However, OECD synthetic solution is the preferred calibration solution for BOD biosensors due to more complex and low degradable organic compounds (Liu *et al.*, 2000). OECD synthetic solution is made synthetically to mimic the real sample.

Figure 2 shows that the limiting current values are directly proportional to the substrate concentration at approximately 400 mg L^{-1} for GGA solution and to approximately 250 mg L^{-1} for the OECD solution. Comparing both lines, OECD shows a lower limiting current value which is indicative of slower biodegradation rate. This explains that although OECD synthetic wastewater is often the preferred calibration solution, most commonly employed calibration solution for BOD biosensors is GGA (Morris, 2005). In addition, in rapid BOD biosensor, only certain amount of assimilable organic compounds in real samples can be degraded in such a short period. GGA with only two simple components meets this requirement. Consequently, GGA with better performance was chosen as the calibration solution for the experiments that follow. Real wastewater samples were diluted to approximately 200 mg L^{-1} prior to starting FM-BOD assay.

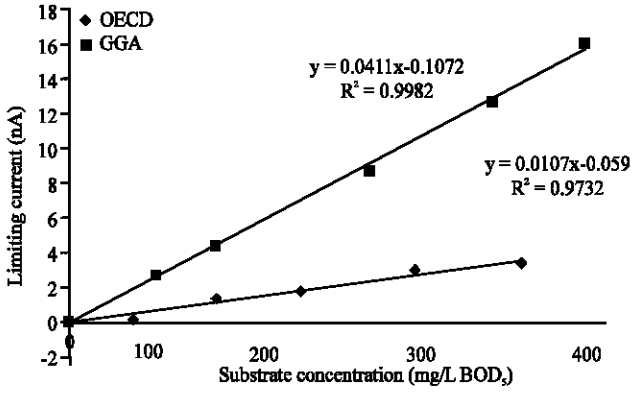

Fig. 2: Limiting current values obtained at various substrate concentrations for mixed microbial consortium in GGA standard solution and OECD synthetic wastewater. Each point represents the average of response from three replicate measurements. Endogenous control values have been subtracted. Microbial consortium final absorbance = 2.5. Ferricyanide final concentration = 60 mM. Limiting currents determined by voltammetry at E_{app} = +450 mV (vs Ag/AgCl)

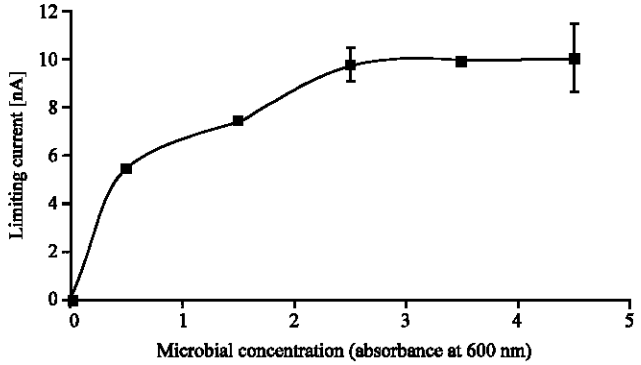

Fig. 3: Limiting current values obtained at various microbial concentrations for mixed microbial consortium in GGA standard solution. Each point represents the average response of three replicate measurements. Endogenous control values have been subtracted. Microbial consortium final absorbance = 0.5, 1.5, 2.5, 3.5, 4.5. Ferricyanide final concentration = 60 mM. Limiting currents determined by voltammetry at Eapp = +450 mV (vs Ag/AgCl)

Effect of concentration of the mixed microbial consortium: The influence of microbial concentration towards the BOD measurements is important as it could affect the biochemical reaction. For instance, when microbial population is too low, only a small amount of organic substrate in the samples could be oxidized, thus making the biocatalyst a limiting factor. On the contrary, when the microbial population is too high, electron acceptor (oxygen in BOD_5, ferricyanide for FM-BOD) may become the rate limiting reactant in order to fully degrade the organic substrate. Therefore, microbial with appropriate concentration is vital for the BOD measurement in this study.

Figure 3 shows the limiting current values obtained at various concentration of mixed microbial consortium after incubation of one hour with the standard GGA solution in the presence of ferricyanide. From the plot, it is observed that the limiting current values increased gradually at concentrations lower than OD_{600} 2.5, before reaching a stable limiting current of approximately 10 nA at concentration above 2.5. The optimum concentration with a final absorbance of OD_{600} 2.5 was then selected for the remaining study and this finding is in agreement with previous study by Morris (2005).

Effect of mediator concentration: As previously described, solubility of oxygen in water is low and is a limiting reactant for BOD_5. Oxygen which acts as final electron acceptor is rapidly reaction rate, ferricyanide which is approximately 10 000 times more soluble than oxygen was employed in FM-BOD assay. This approach is used to shorten the reaction time for the purpose of rapid BOD detection. The effect of varying the ferricyanide concentration was investigated after incubation of one hour in GGA standard solution.

As shown in Fig. 4, limiting current values were dependent on ferricyanide concentration below 40 mM and independent above that. The reaction of ferricyanide concentration below 40 mM was limited by the availability of electron acceptor. Above 40 mM, the reaction was limited by substrate concentration which higher concentration of substrate is needed to reduce ferricyanide microbiall (Morris *et al*., 2001). Ferricyanide concentration with 60 mM was employed in all subsequent experiments to ensure that ferricyanide does not limit the microbial process.

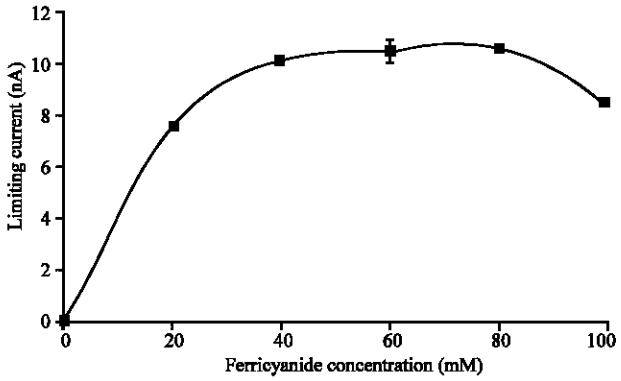

Fig. 4: Limiting current values obtained at various ferricyanide concentrations for mixed microbial consortium in GGA standard solution. Each point represents the average of response from three replicate measurements. Endogenous control values have been subtracted. Microbial consortium final absorbance = 2.5. Ferricyanide final concentration = (20, 40, 60, 80, 10) mM. Limiting currents determined by voltammetry at E_{app} = +450 mV (vs Ag/AgCl)

Table 1: Ferricyanide-mediated BOD5 equivalent values for organic standard solutions

		FM-BOD_5 equivalent values (mg BOD_5 L^{-1})		
Solution	BOD_5 value (mg BOD_5 L^{-1})	1 h	2 h	3 h
GGA	198±30	198±10	198±8	198±1
Glucose (350 mg L^{-1})	200±24*	243±10	228±8	280±5
Sucrose (320 mg L^{-1})	200±43*	263±17	231±12	260±9
Glutamic Acid (344 mg L^{-1})	200±24*	59±1	79±4	121±1
Glycine (370 mg L^{-1})	200±6	12±2	10±3	26±2

Each point represents the average of response from three replicate measurements. Endogenous control values have been subtracted and the BOD5 equivalent values have been calculated by comparison with the GGA standard. Microbial consortium final absorbance = 2.5. Ferricyanide final concentration = 60 mM. Limiting currents determined by voltammetry at E_{app} = +450 mV (vs Ag/AgCl). *Catterall *et al*. (2001) and Morris *et al*. (2001)

Application of FM-BOD assay to organic standard solution: The purpose of this study is to determine the ability of the microbial consortium together with FM-BOD assay in predicting BOD_5 values of several organic solutions (glucose, sucrose, glutamic acid and glycine). All organic standard solutions were prepared at concentrations equivalent to BOD_5 values of 200mg BOD_5^{-1}. Limiting current values were obtained voltametrically and converted to FM-BOD_5 equivalent values by assigning general standard solution BOD^{198} GGA as calibration standard. Table 1 shows FM-BOD_5 equivalent values obtained from microbial consortium employed in various organic solution after 1, 2 and 3 hour incubation. The FM-BOD_5 equivalent values of microbial consortium for the simple sugars glucose and sucrose were within the ±15% of the standard 5-day method. However the amino acids i.e., glutamic acid and glycine were significantly underestimated as the FM-BOD_5 equivalent values were significantly outside the acceptable range. The effect of incubation time was also investigated in this test. For the simple sugars (glucose and sucrose), FM-BOD_5 equivalent values slightly increased within the allowable range over 3 h incubation time except for glucose at 3 h incubation where it gives overestimated value. For glutamic acid, a

Table 2: BOD_5, FM-BOD_5 equivalent values and percentage degradation of real samples

Real samples	BOD_5 (mg L^{-1}) (diluted value)	FM-BOD_5 equivalent (mg L^{-1})	Percentage degradation (Morris, 2005)
Pineapple	195±2.09	251±19	93
Cafeteria 1	195±4.20	133±7	49
Cafeteria 2	253±1.20	260±16	71

significant increase in limiting current over 3 h incubation time was observed but it is still underestimated compared to the actual BOD_5 value. Glycine has shown only a slight increase and underestimated.

Evidently, microbial consortium used in this study does not have the capacity to degrade a broad range of substrates. It is more suitable for simple sugars i.e., glucose and sucrose as substrate. Amino acids i.e., glutamic acid and glycine are found not suitable.

Application of FM-BOD assay to industrial wastewater: The FM-BOD assay employing the microbial consortium was applied to real industrial wastewater and river water samples (Table 2) with BOD values of 1-120000 mg BOD_5/L. It has been reported that the linearity of the FM-BOD method is approximately 200 mg L^{-1} for the standard GGA of solution (Morris *et al.*, 2001). Hence, the industrial wastewater sample used in this study was diluted accordingly prior to FM-BOD measurement.

Table 2 presents the conventional BOD_5 values, FM-BOD_5 equivalent values and percentage degradation for the microbial consortium in the diluted waste waters. FM-BOD_5 equivalent values were obtained after 1 h of incubation. The percentage degradation of food wastewater samples such as pineapple and cafeteria 1 and 2 in 1 h incubation FM-BOD assay were 93, 49 and 71%, respectively which compared favorably with the GGA standard solution BOD_5 assay (~60% GGA degraded in 5 days). On some occasions, food waste such as coffee has shown significant underestimated BOD_5 equivalent value in 1 h incubation FM-BOD assay. Other industrial wastewaters such as palm oil mill effluent, POME (raw and treated), textile and petrochemical were also applied in the FM-BOD assay. The percentage degradation were all underestimated which are only 5-10% (data not shown). These types of wastewater were hardly assimilable and the microbial consortium used was not able to degrade the compounds in such a short time. Similar to previous discussion microbial consortium is more suitable for degrading food wastewater samples with simple compounds such as glucose and sucrose. Therefore, this FM-BOD assay with the microbial consortium is found to be more suitable for wastewaters that are related to food with easily degradable carbon source.

Some river waters from different places were taken and applied on the FM-BOD assay. BOD_5 values of river waters are usually <10 mg L^{-1} and contain less biodegradable organic compounds such as humic acid, lignin, tannic acid, gum arabic and surfactants (Chee *et al.*, 1999). River water with its BOD_5 values <10 mg L^{-1} is thus not suitable as it is limited by the sensitivity of this FM-BOD assay.

CONCLUSION

The present study has shown that a locally developed microbial consortium can be used as biocatalyst in the FM-BOD assay for rapid BOD determination. A commercial consortium was unable to utilize ferricyanide as the final electron acceptor compared with locally developed microbial consortium in FM-BOD assay. All experiments in this study were conducted by using microbial consortium with each bacterium mixed at the same optical density (OD_{600}) of 5 and incubated with 60 mM of ferricyanide for 1 h with GGA as the standard solution. FM-BOD assay

was applied on synthetic organic solutions as well as real wastewater samples. In conclusion, BOD of food samples with easily degradable carbon source can be determined within 1 h using FM-BOD assay compared to 5 days standard BOD_5 assay.

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