

## Bio-Hydrogen Production from Food Waste through Anaerobic Fermentation (Pengeluaran Bio Hidrogen daripada Sisa Makanan melalui Fermentasi Anaerobik)

OSUAGWU CHIEMERIWO GODDAY\*, OSUAGWU UCHECHUKWU LEVI & AGAMUTHU PARIATAMBY

### ABSTRACT

*In order to protect our planet and ourselves from the adverse effects of excessive CO<sub>2</sub> emissions and to prevent an imminent non-renewable fossil fuel shortage and energy crisis, there is a need to transform our current 'fossil fuel dependent' energy systems to new, clean, renewable energy sources. The world has recognized hydrogen as an energy carrier that complies with all the environmental quality and energy security, demands. This research aimed at producing hydrogen through anaerobic fermentation, using food waste as the substrate. Four food waste substrates were used: Rice, fish, vegetable and their mixture. Bio-hydrogen production was performed in lab scale reactors, using 250 mL serum bottles. The food waste was first mixed with the anaerobic sewage sludge and incubated at 37°C for 31 days (acclimatization). The anaerobic sewage sludge was then heat treated at 80°C for 15 min. The experiment was conducted at an initial pH of 5.5 and temperatures of 27, 35 and 55°C. The maximum cumulative hydrogen produced by rice, fish, vegetable and mixed food waste substrates were highest at 37°C (Rice = 26.97±0.76 mL, fish = 89.70±1.25 mL, vegetable = 42.00±1.76 mL, mixed = 108.90±1.42 mL). A comparative study of acclimatized (the different food waste substrates were mixed with anaerobic sewage sludge and incubated at 37°C for 31 days) and non-acclimatized food waste substrate (food waste that was not incubated with anaerobic sewage sludge) showed that acclimatized food waste substrate enhanced bio-hydrogen production by 90 - 100%.*

*Keywords: Acclimatization; anaerobic sewage sludge; bio-hydrogen; food waste; initial pH*

### ABSTRAK

*Dalam usaha untuk melindungi planet dan diri kita daripada kesan pelepasan CO<sub>2</sub> yang berlebihan dan untuk mengelakkan krisis kekurangan bahan api fosil dan tenaga tidak boleh diperbaharui, terdapat keperluan untuk mengubah sistem semasa 'kebergantungan kepada tenaga bahan api fosil' kepada sumber tenaga baharu, bersih dan boleh diperbaharui. Dunia telah mengiktiraf hidrogen sebagai tenaga pembawa yang mematuhi permintaan terhadap kualiti alam sekitar dan keselamatan tenaga. Kajian ini bertujuan untuk menghasilkan hidrogen melalui fermentasi anaerobik dan menggunakan sisa makanan sebagai substrat. Empat substrat sisa makanan telah digunakan: Nasi, ikan, sayur-sayuran serta campuran. Pengeluaran bio hidrogen telah dijalankan pada skala reaktor makmal, menggunakan botol serum 250 mL. Pertama, sisa makanan tersebut dicampur dengan enap cemar kumbahan anaerobik dan dieram pada 37°C selama 31 hari (pengikliman). Enap cemar kumbahan anaerobik kemudiannya dirawat pada suhu 80°C selama 15 min. Kajian pemula telah dijalankan pada pH 5.5 dan suhu 27, 35 dan 55°C. Hidrogen terkumpul maksimum yang dihasilkan oleh beras, ikan, sayur-sayuran dan substrat sisa makanan campuran adalah tertinggi pada 37°C (beras = mL 26.97±0.76, ikan = mL 89.70±1.25, sayur-sayuran = mL 42.00±1.76 serta campuran = 108.90±1.42 mL). Suatu kajian perbandingan pengikliman (substrat sisa makanan berbeza telah dicampur dengan enap cemar kumbahan anaerobik dan dieram pada 37°C selama 31 hari) dan substrat sisa makanan tanpa pengikliman (sisa makanan yang tidak dieram dengan enap cemar kumbahan anaerobik) menunjukkan bahawa pengikliman substrat sisa makanan meningkatkan pengeluaran bio hidrogen sebanyak 90 - 100%.*

*Kata kunci: Bio hidrogen; enap cemar anaerobik kumbahan; pengikliman; pH awal; sisa makanan*

### INTRODUCTION

Fossil fuels used as energy sources are diminishing. Global warming is no longer a new issue to mankind. These have become some of the current problems of human. Soil, air and water pollution has been on the increase due to the continuous use of fossil fuels. This has driven humanity into looking for alternative sources of fuel that will not endanger the environment when used or combusted. A study by Mizuno et al. (2000) showed that hydrogen has

a high energy content (122 kJ/g), the combustion produces water which does not endanger the environment and it is environmentally friendly. Okamoto et al. (2000) reported that out of the various processes of producing hydrogen such as steam reforming, electrolysis, gasification and biological processes, the least expensive process is the biological process, which uses organic components of waste as resources.

Requirements such as abundance and availability are considered before choosing organic material as a potential substrate for sustainable bio-hydrogen production. Agricultural waste and food waste meet these requirements. A report (EU 2003) showed that about 0.7 billion tonnes of agricultural and forestry waste was generated in Western Europe between 1998 and 2001. In the year 2000, agricultural waste amounted to more than 175 million tonnes per year in Germany. A survey conducted in France from 1995 to 2006 showed the agricultural and forestry waste to be 374 tonnes, which is 43% of 849 million tonnes by 2006 (Mtui 2009). One of the components of this agricultural waste is the food waste which has high water and organic content amounting to 75-85% and 85-95%, respectively (Li et al. 2008).

In Malaysia, food waste is classified as a component of municipal solid waste. In 2008, Malaysia generated about 30000 tonnes per day of municipal solid waste of which 45% of the components was food waste (Fauziah & Agamuthu 2008). In 2009, Malaysia generated an average amount of about 0.5-0.8 kg/person/day and 1.7 kg/person/day in the rural and urban areas, respectively, of municipal solid waste (Manaf et al. 2009). Kuala Lumpur, the capital city of Malaysia generates about 1.2 kg/person/day (Iwan et al. 2012). Malaysians are good in eating out thus there are lots of restaurants in Malaysia and this accounts for the high food waste content of the municipal solid waste. Using food waste in bio-hydrogen generation, through a biological process called anaerobic fermentation, becomes very interesting because it will reduce the amount of waste that goes to the landfills, thus increasing the lifespan of the landfill. Furthermore, it will also be a cheap source of raw material used in production of hydrogen which is a way of waste reduction and reuse. In bio-hydrogen generation, many factors play vital roles such as pH, temperature, substrate concentration, pre-treatment and retention time (Fang et al. 2006; Wang & Wan 2009). Research has shown the importance of optimum pH values as a critical factor in bio-hydrogen production (Fang et al. 2006; Ramos et al. 2012).

In this study, the comparison of producing bio-hydrogen from acclimatized and non-acclimatized food waste substrates was investigated in lab scale batch reactors to determine which treatment is better. Additionally, studies were done on various temperatures to determine the optimum temperature for bio-hydrogen production.

#### MATERIALS AND METHODS

Anaerobic sewage sludge used as seed sludge in this study was obtained from the anaerobic digester of Pantai Dalam Sewage Treatment Plant, Kuala Lumpur, Malaysia. The sludge was then transported to the laboratory and sieved with a 1 mm sieve and stored in a refrigerator prior to use for experiments at 4°C. The sludge was pre-heated at 80°C for 15 min to inhibit the bioactivity of methane forming bacteria and other pathogenic microbes as well

as to promote the growth of hydrogen producing bacteria. A warring blender machine was used to grind the food waste in the laboratory after it was collected from cafes at the University of Malaya. It was then sealed in sterile plastic bags and stored in the freezer at -4°C. The food waste was defrozed before it was used for the experiment.

#### BATCH FERMENTATION

*Acclimatization* The food wastes were acclimatized with the anaerobic sewage sludge for 31 days at 37°C in an incubator. Thirty mL of acclimatized food waste were then inoculated into 250 mL serum bottle which was used as the fermenter. Eight grams of each food waste substrate was added to the reactor and 50 mL of anaerobic sewage sludge heated at 80°C for 15 min was added to the mixture. The initial pH was corrected to 5.5 using 1 N NaOH and H<sub>2</sub>SO<sub>4</sub>. To maintain an anaerobic condition, the headspace of the reactor was filled with pure Nitrogen gas. Mixing was done manually for 2 times a day. According to the design, 3 runs of the experiment were performed with 3 replicates.

*Non Acclimatization* The only mixture in this batch reactor was 50 mL anaerobic sludge heat treated at 80°C and 8 g of food waste substrates. The other conditions remained the same.

After the conditioning, the fermenters were placed in a water bath at 37±1°C till the end of the experiment. A transfusion needle at one end of the transfusion tube as shown in Figure 1 was connected to the fermenters and the open end of the transfusion tube was connected to a conical flask full of water. The conical flask was covered with a rubber cork and properly sealed with a sealing to avoid gas escape. Displaced water was collected in another conical flask and measured using a measuring cylinder. The amount of water displaced equals the amount of gas produced (Patil et al. 2011).

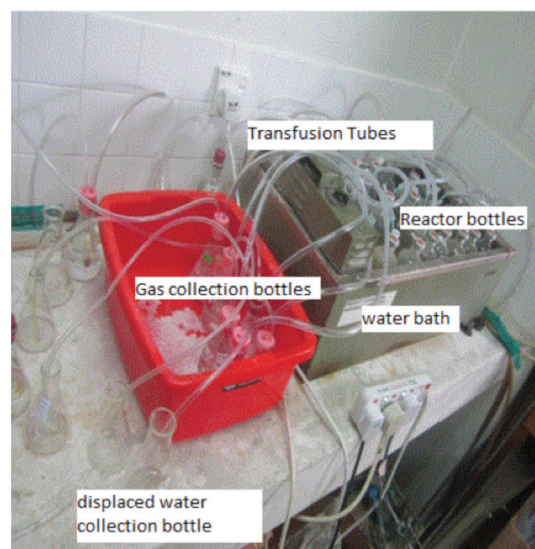


FIGURE 1. Biogas production by water displacement

## ANALYTICAL METHODS

The mass of the substrates were determined using a weighing balance. The volume of gas production in each bottle was measured and recorded through the water displacement method. One mL of the gas in the fermenters was injected into a gas chromatography (GC Shimadzu 8A) with thermal conductivity detector to analyze the biogas content. Helium gas was used as the carrier gas at a flow rate of 60 mL/min. The injector, detector and column were operated at 160, 130 and 130°C, respectively. A pure hydrogen gas was used as the calibration standard. The rate of hydrogen production was analyzed using the modified Gompertz equation (Zwietering et al. 1990).

$$H(t) = P \cdot \exp\left[-\exp\left\{\frac{R_m \cdot e(-t)}{p} + 1\right\}\right], \quad (1)$$

where  $H(t)$  is the cumulative hydrogen production (mL);  $P$  is the hydrogen production potential (mL);  $R_m$  is the maximum hydrogen production rate (mL/d);  $e = 2.71828$ ,  $\lambda$  is the lag phase (d) and  $t$  is the time (d).

## STATISTICAL ANALYSIS

Statistical analysis was performed using the Kuskal Wallis non test to compare the amount of cumulative biogas production produced by all 4 substrates between the acclimatized and non-acclimatized food waste substrates (rice versus rice, fish versus fish, mixed versus mixed and vegetable versus vegetable). The level of statistical significance was set at 5% post-hoc analysis and 95% confidence level.

## RESULTS AND DISCUSSION

## EFFECT OF TEMPERATURE

When the substrate was subjected to a temperature of 35°C, biogas production was recorded on day zero as opposed to H<sub>2</sub> gas which commenced on day one as opposed to H<sub>2</sub> gas which commenced on day one (Figure 2). There was a rapid increase in the production of biogas and H<sub>2</sub> reaching its peak on the 9<sup>th</sup> day (26.97±0.76 mL) before stabilizing from the 10<sup>th</sup> day onward. However, considering biogas and H<sub>2</sub> production at other temperatures, it was observed that at 27°C and 55°C, H<sub>2</sub> and biogas production commenced on the 4<sup>th</sup> day. It was accompanied by a slow increase in H<sub>2</sub> production observed for substrates at 27°C before reaching its peak on the 7<sup>th</sup> day with a H<sub>2</sub> yield of 7.76±1.25 mL. Furthermore, it was observed that the maximum H<sub>2</sub> production recorded at 55°C was 4.85±1.87 mL.

The higher hydrogen production at 35°C might be because it favors the proliferation of the H<sub>2</sub> producing bacteria (Wang & Wan 2009). This could also be because the temperature made sugar conversion easier for the hydrogenase which in turn increases H<sub>2</sub> production (Ma et al. 2008). Moreover it was statistically shown that the H<sub>2</sub> production from rice waste substrate was statistically

significant at 37°C ( $p < 0.001$ ) than at 27 and 55°C. The same was the case when statistical analysis was conducted for cumulative biogas production. Generally, the optimum temperature for bio-hydrogen production using rice waste was found to be 35°C in this study.

This agrees with previous report by Fang et al. (2006) which showed that the optimum temperature for H<sub>2</sub> production from rice waste was 35°C even though they recorded a higher maximum H<sub>2</sub> yield of 346 mL. This higher yield might be because the substrate used by Fang et al. (2006) was rice slurry which provides an enabling environment for the microbes. Nevertheless, our results disagree with that of Iyagba et al. (2009) and Lee et al. (2008) where the optimum temperature was 55°C which might be because the latter used rice husks and as such higher temperature was needed to get the nutrients out.

Hydrogen and biogas production commenced on the 6<sup>th</sup> day when the fish waste were subjected to a temperature of 27°C (Figure 3). A slight increase in H<sub>2</sub> production was observed from the 7<sup>th</sup> day before reaching its peak (7.56±1.18) on the 9<sup>th</sup> day after which it decreased to zero on the 10<sup>th</sup> day. At 35°C, biogas and H<sub>2</sub> production commenced on day one. A rapid increase was observed until it reached its peak on day 5, then a sudden fall in H<sub>2</sub> production was observed after the 5<sup>th</sup> day (89.70±1.25 mL). For 55°C, H<sub>2</sub> and biogas production commenced on the 1<sup>st</sup> day. We also observed maximum H<sub>2</sub> production of 63.74±2.23 which decreased gradually until no H<sub>2</sub> gas was produced. Furthermore, the amount of H<sub>2</sub> produced by Fish waste at 35°C was only statistically significant than the amount produced at 27°C ( $p < 0.05$ ). Likewise, the amount of biogas produced at 35°C was only statistically significant than the amount produced at 27°C ( $p < 0.001$ ).

Generally, the optimum temperature for H<sub>2</sub> production using fish waste substrate was 35°C. The low yield in fish waste could also be attributed to the amino acid which is the catalytic end product of protein; this also reduces the pH in the medium, thereby inhibiting H<sub>2</sub> producing bacteria.

This agrees with previous report by Zhu et al. (2011) where the optimum temperature for H<sub>2</sub> production using protein substrate was 35°C.

When vegetable waste substrate was subjected to 27 and 55°C, no gas production was recorded (Figure 4). Nevertheless, biogas and H<sub>2</sub> production commenced when the vegetable substrate was subjected to a temperature of 35°C. On day one, gas production was recorded. A rapid increase in H<sub>2</sub> production was observed from the second day until a maximum of 42.00±1.76 mL was reached on the 4<sup>th</sup> day. The subsequent days showed a gradual decrease in H<sub>2</sub> production until it stabilized from the sixth day onward. The amount of H<sub>2</sub> produced by vegetable waste substrate at 35°C was statistically significant than the amount produced at 27°C ( $p < 0.001$ ) and 55°C ( $p < 0.001$ ). This is mainly because no gas production was observed when vegetable substrate was used for bio-hydrogen production at 27 and 55°C.

According to Okamoto et al. (2000), H<sub>2</sub> producing bacteria are more active at mesophilic temperature; therefore 55°C might be too high and 27°C might favor

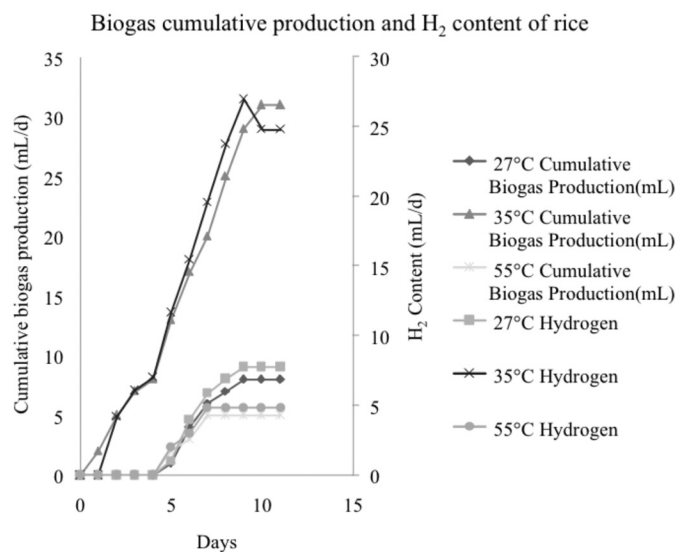


FIGURE 2. Effect of temperature on cumulative biogas and H<sub>2</sub> production of rice waste

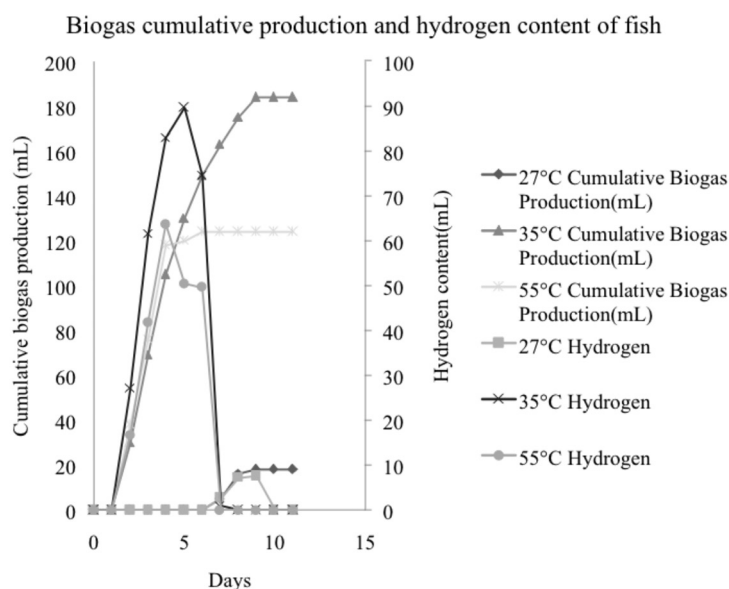


FIGURE 3. Effect of cumulative biogas production and H<sub>2</sub> content of fish waste

lactic acid formation and as such hydrogen producing bacteria are inhibited (Leon 2011). This was probably because the temperature favored H<sub>2</sub> producing bacteria.

This result disagrees with previous reports by Chu et al (2008) and Vijayaraghavan et al. (2007). They recorded gas production at 55°C. This was probably due to the type of vegetable waste substrate used or the longer acclimation period used in these studies. Nevertheless, this result agrees with a previous study by Okamoto et al. (2000) who reported 35°C as the optimum temperature for bio-H<sub>2</sub> production from vegetable waste substrate. As shown in Figure 5, the cumulative biogas and H<sub>2</sub> production of mixed food waste substrate at 27 and 35°C commenced on the 5<sup>th</sup> day. There was a slow but steady increase in the production of biogas and H<sub>2</sub> at 27°C until it reached its peak on the

8<sup>th</sup> day (25.22±0.76 mL). However, on the 9<sup>th</sup> day, no H<sub>2</sub> production was observed. Furthermore, a rapid increase in H<sub>2</sub> production was recorded at 35°C on the 5<sup>th</sup> day until a maximum cumulative H<sub>2</sub> production of 108.90±1.42 mL was recorded on the 7<sup>th</sup> day before dropping sharply to zero on the 8<sup>th</sup> day. We further recorded no biogas or H<sub>2</sub> production for mixed waste substrates at 55°C. Statistically, there was no significant difference in the amount of H<sub>2</sub> gas produced at the two different temperatures 27 and 35°C. Nevertheless, the amount of biogas produced by mixed food waste at 35°C was statistically significant than the amount produced at 27°C ( $p<0.05$ ) and 55°C ( $p<0.01$ ).

The five days lag period observed at 35°C could be because this waste has more than one component which has different reaction pathways thus affecting hydrogen

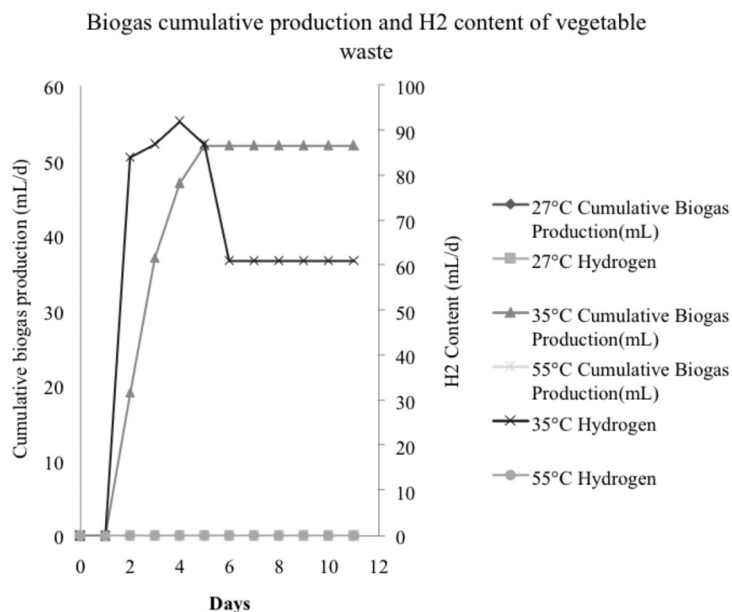


FIGURE 4. Effect of temperature on cumulative and H<sub>2</sub> production of vegetable waste

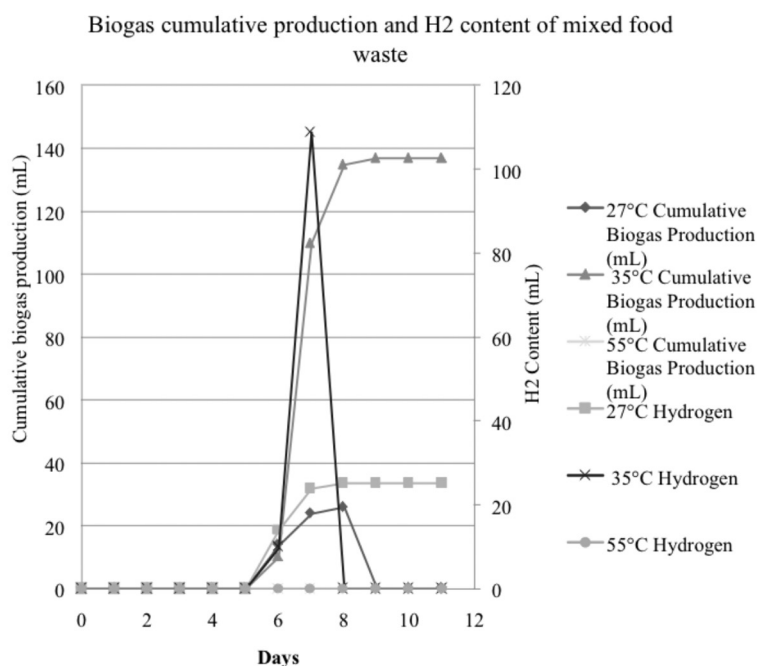


FIGURE 5. Effect of temperature on cumulative biogas production and H<sub>2</sub> content of mixed food waste

production. The higher cumulative biogas and H<sub>2</sub> yield recorded at 35°C could be because the H<sub>2</sub> producing bacteria were enhanced and the different components integrated effectively at this temperature but could not maximize production at 27°C (Singh et al. 2010).

This result agrees with the previous reports by Xiao et al. (2013) having optimum temperature of 37°C. It also agrees with that of Chen et al. (2006) having the maximum H<sub>2</sub> yield of 101 mL/d. This might be because sewage sludge from anaerobic digester was used in both studies (Chen et al. 2006). Similarly, a previous report by Pan et al. (2008)

recorded H<sub>2</sub> production at 50°C. This might be attributed to the temperature difference of 5°C which could be lethal to H<sub>2</sub> producing bacteria (Lin et al. 2008). Nevertheless, a report by Shimizu et al. (2008) agrees with this study, recording no gas production at 55°C.

#### EFFECT OF ACCLIMATIZATION

Statistical analysis between the H<sub>2</sub> produced by acclimatized and non-acclimatized food waste substrates showed a significance difference in the H<sub>2</sub> produced between

acclimatized vegetable waste and non-acclimatized vegetable waste ( $p < 0.0001$ ), not quite a significant difference ( $p = 0.05$ ) between that produced by comparing acclimatized rice waste and non-acclimatized rice waste, acclimatized mixed waste and non-acclimatized mixed waste. No significance difference was observed in comparing the  $H_2$  production of fish between the acclimatized and non-acclimatized food waste substrates. Statistical analysis performed using the Kuskal Wallis non test showed that there is a significant difference between the amounts of cumulative biogas production of acclimatized and non-acclimatized food waste substrates ( $p < 0.05$ ). However, further analysis in the Dunn's multiple comparisons test showed that the differences in cumulative biogas production was only statistically significant in the comparison between acclimatized rice waste and non-acclimatized rice waste ( $p = 0.0455$ ), fish and fish ( $p = 0.0006$ ), vegetable and vegetable ( $p = 0.0029$ ) while there was not quite a significance difference between that of mixed food waste substrate ( $p = 0.680$ ).

The significant differences might be due to the additional bacteria obtained from acclimatization, which enhanced the fermentation process in the acclimatized food waste substrates. Also, the bacteria in the system have adapted to the food waste substrate during acclimatization while the bacteria needs to adapt to the environmental conditions in the non-acclimatized food waste substrate and the  $H_2$  producing bacteria were not helped in anyway, therefore only the indigenous microbes performed the fermentation (Skonieczny & Yargeau 2009). Nevertheless, for rice that showed no significant difference in its  $H_2$  production, this might be attributable to its high carbohydrate component which is easily convertible to  $H_2$  with or without the additional microbes from acclimatization. Besides,

the rice used in this study is cooked rice which is easily decomposed by microbes. The significant difference in the biogas production was probably because acclimatization increased the amount of biogas and had little or no effect on the amount of  $H_2$  gas produced.

Figure 6 shows the effect of acclimatization on bio-hydrogen production from rice waste substrates. We observed that  $H_2$  production commenced on the second day for the acclimatized rice waste as opposed to the 4<sup>th</sup> day on which it commenced for the non-acclimatized rice waste. The maximum  $H_2$  production was recorded on the 10<sup>th</sup> day and on the 9<sup>th</sup> for the acclimatized ( $26.97 \pm 1.25$  mL) and non-acclimatized ( $13.6 \pm 2.64$  mL) rice waste, respectively.  $H_2$  production stabilized from the 11<sup>th</sup> day for the acclimatized and on the 9<sup>th</sup> day for the non-acclimatized rice waste. Nevertheless, we observed that for the acclimatized rice waste,  $H_2$  production decreased after the 10<sup>th</sup> day with increase in biogas production. This was not the case in the non-acclimatized,  $H_2$  and biogas production stabilized the same day.

Acclimatization introduces more bacteria into the system, thus speeding up the reaction. This could explain the shorter lag period observed in the acclimatized rice waste. The increasing biogas in the acclimatized rice waste could be as a result of the presence of methanogenic bacteria which were also enhanced by acclimatization even though they were affected by pre-heating (Ahn et al. 2005; Kim et al. 2006; Ueno et al. 2001).

As shown in Figure 7, cumulative biogas production and  $H_2$  production commenced on day 3 for acclimatized and non-acclimatized fish waste substrate. A rapid increase in biogas and  $H_2$  production was observed in the acclimatized fish waste as opposed to the slow increase observed in the non-acclimatized. The maximum  $H_2$

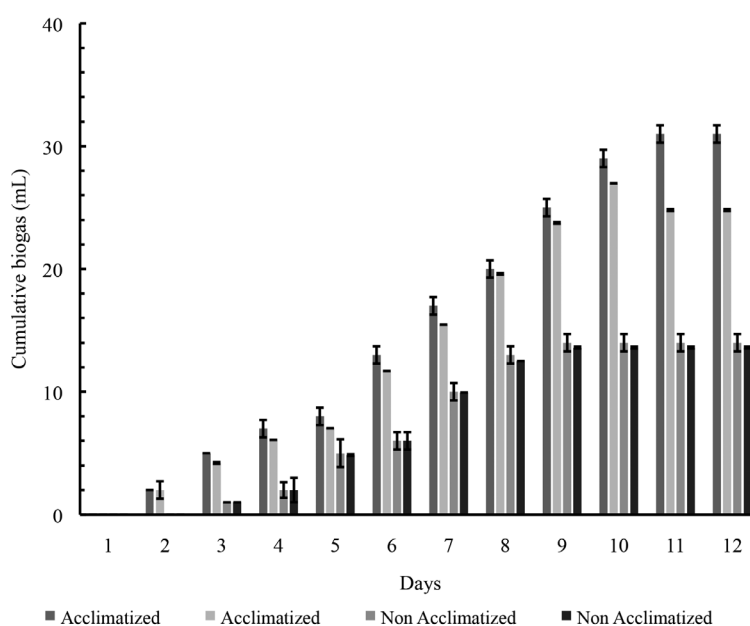


FIGURE 6. Effects of acclimatization on cumulative biogas and  $H_2$  production of rice waste

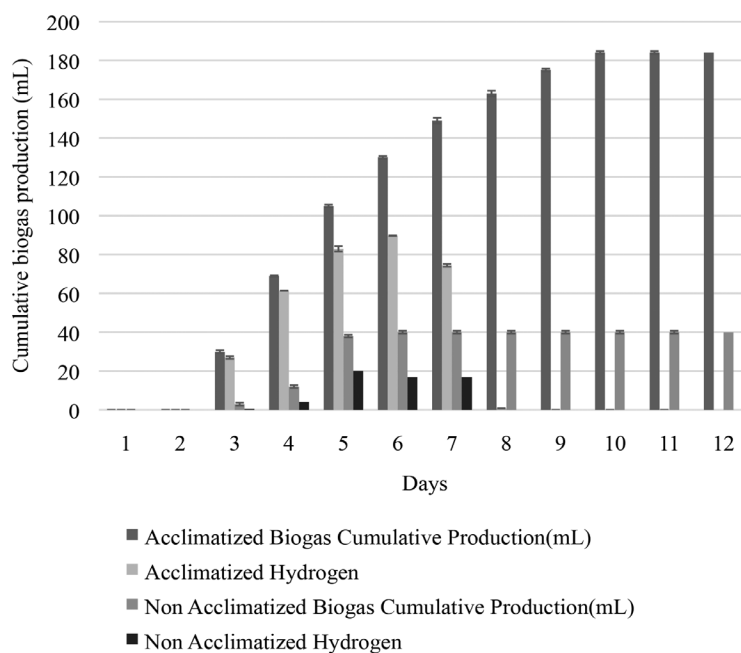


FIGURE 7. Effect of acclimatization on cumulative biogas and H<sub>2</sub> production of fish waste

production was  $89.7 \pm 2.34$  mL for acclimatized and  $20 \pm 1.75$  mL for the non-acclimatized fish waste substrate. We also observed that H<sub>2</sub> production stopped on the 8<sup>th</sup> day and on the 6<sup>th</sup> day for the acclimatized and the non-acclimatized fish waste, respectively. We equally observed that CO<sub>2</sub> production was increasing with a decrease in H<sub>2</sub> content of the biogas both in both experimental conditions.

The 3 day lag period in both conditions could be as a result of the acidic content of fish waste which acclimatized bacteria has to suppress for hydrogenase to be enhanced (Pan et al. 2008). This implies that in both conditions, H<sub>2</sub> production will commence on the first day but the difference is in the amount produced. It was also observed that the H<sub>2</sub> producing bacteria in the non-acclimatized were consumed earlier than that of the acclimatized. This is probably because it will take a longer time to consume more bacteria than fewer bacteria. Acclimatized fish waste has more bacteria sources than non-acclimatized.

As shown in Figure 8, it was observed that H<sub>2</sub> production commenced on the 3<sup>rd</sup> day in the acclimatized vegetable waste and on the 6<sup>th</sup> day in the non-acclimatized vegetable waste. It was observed that for the acclimatized vegetable waste, the H<sub>2</sub> content increased with increasing biogas production. It continued until a maximum of  $45.24 \pm 0.01$  mL in the 6<sup>th</sup> day as opposed to  $20.50 \pm 0.70$  mL in the non-acclimatized vegetable waste. Thus, the acclimatized vegetable waste produced twice as much hydrogen as that produced by the non-acclimatized vegetable waste. It might not be wrong to say that acclimatization reduces the formation of CO<sub>2</sub> when vegetable waste is used as substrate for bio-hydrogen production.

The reason for the lower yield in non-acclimatized vegetable waste might be because the H<sub>2</sub> producing bacteria were inhibited by the acidic nature of the vegetable

and no extra microbe was introduced to aid the process. Accumulated acidic medium will lower the pH of the reactor since the pH was not controlled. Thus, H<sub>2</sub> producing bacteria involved were unable to sustain its metabolic activity (Nazlina et al. 2009; Yap 2013).

Figure 9 shows the effect of acclimatization on cumulative biogas and H<sub>2</sub> production from mixed food waste substrate. We observed that biogas production commenced on the 5<sup>th</sup> day in the acclimatized as opposed to the non-acclimatized which commenced on the 6<sup>th</sup> day. A rapid increase in H<sub>2</sub> production was observed in the acclimatized and non-acclimatized experimental conditions from the 6<sup>th</sup> and 7<sup>th</sup> day, respectively. Furthermore, we observed that biogas and H<sub>2</sub> gas production seems to stabilize on the 9<sup>th</sup> day for acclimatized and on the 10<sup>th</sup> day for the non-acclimatized mixed food waste. The maximum H<sub>2</sub> production was  $130.95 \pm 0.007$  mL for acclimatized and  $33.3 \pm 0.14$  mL for non-acclimatized mixed food waste substrate.

This study agrees with previous studies (Fang et al. 2006; Massanet et al. 2008; Nazlina et al. 2011) where acclimatization was used to enhance bio-H<sub>2</sub>. Nevertheless, some studies also showed enhanced bio-H<sub>2</sub> production without acclimatization (Hao et al. 2010; Kim 2004; Pan et al. 2008; Wang 2010).

#### GOMPERTZ KINETIC MODEL

Gompertz kinetic model was also used to determine if acclimatized food waste has higher H<sub>2</sub> production potential than the non-acclimatized food waste. The maximum rate of hydrogen (R<sub>m</sub>) produced by acclimatized rice waste was almost twice that produced by non-acclimatized ( $19.715$  mL/d). Likewise, the cumulative hydrogen production

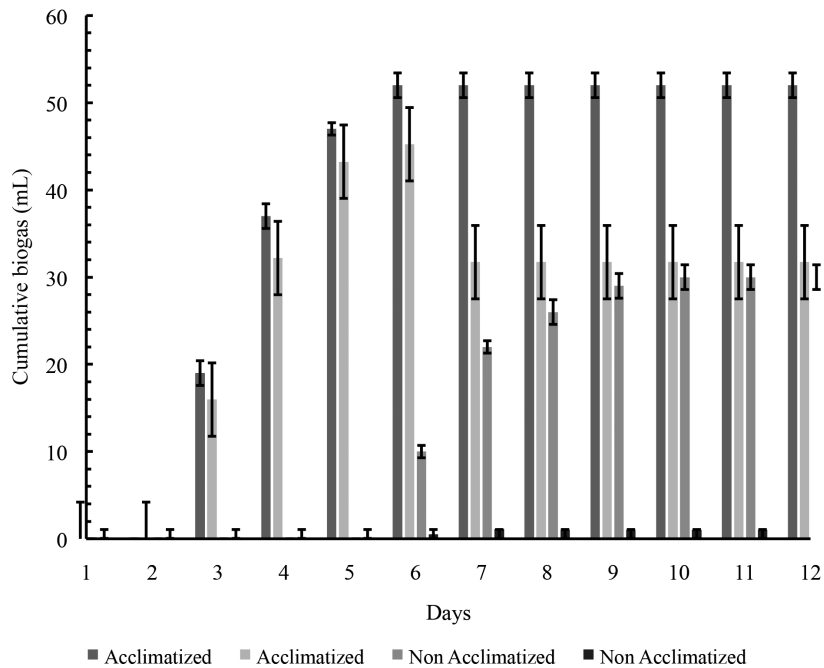


FIGURE 8. Effects of acclimatization on cumulative biogas and  $H_2$  production of vegetable waste

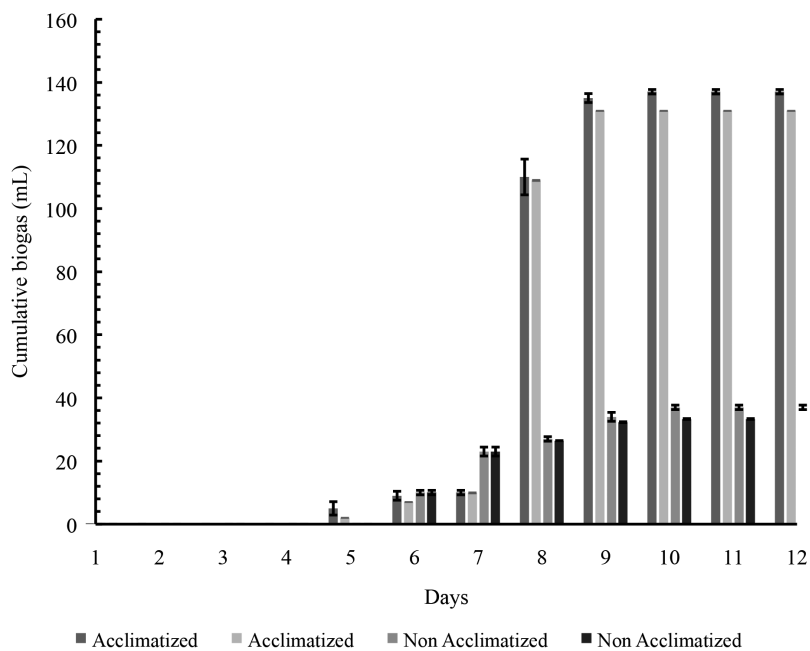


FIGURE 9. Effects of acclimatization on cumulative biogas and  $H_2$  production of mixed food waste

potential (P) of acclimatized rice waste was twice of the non-acclimatized (44 mL). For fish, the difference was 20.95 mL/d for Rm and 16.8 mL for P. That of vegetable was 15.55 mL/d for Rm and 31.9 mL for P. For mixed food waste was 49.5 mL/d for Rm and 57.9 mL for P. Generally, one could say that acclimatization enhanced hydrogen production by 90 - 100%.

This agrees with previous studies where anaerobic sewage sludge was used for acclimatization (Dong et al. 2009; Jayalakshmi et al. 2009; Karlsson et al. 2008).

## CONCLUSION

Bio-hydrogen production experiment was conducted in a laboratory scale using 250 mL batch reactor at three temperatures 27, 35 and 55°C at an initial pH of 5.5. Four different food waste substrates (rice, fish, vegetable and their mixture) were used. Across the various food waste substrates used, it was shown that the optimum temperature for bio-hydrogen production was 35°C. Furthermore, a study was done to determine the effect of acclimatization



on bio-hydrogen production. It was discovered that acclimatization with anaerobic sewage sludge enhanced hydrogen production by 90 - 100%. Using Gompertz kinetic model, it was shown that rice waste, fish waste, vegetable waste and mixed food waste substrates has the potential to produce hydrogen gas. Rice waste showed the highest rate of maximum hydrogen production while mixed waste showed the highest cumulative maximum hydrogen production. Therefore, in as much as rice waste produced hydrogen faster, mixed waste would be preferred for its cumulative production.

#### RECOMMENDATIONS

For further studies, we recommend that more studies can be done on determining how acclimatization periods can be reduced and at the same time improve bio-hydrogen yield. We also recommend that combination of aerobic sewage sludge and anaerobic sewage sludge be used for acclimatization to assess their combined effect on bio-hydrogen production.

#### ACKNOWLEDGEMENTS

The authors wish to acknowledge members and the management of the solid waste laboratory, Institute of post graduate studies, University of Malaya where this research was conducted and whose contributions and suggestions were very helpful in this research. We also want to express our sincere gratitude to University of Malaya for the research grant (Project No: PG025-2012B).

#### REFERENCES

- Ahn, Y., Park, E.J., Oh, Y.K., Park, S., Webster, G. & Weightman, A.J. 2005. Biofilm microbial community of a thermophilic trickling biofilter used for continuous biohydrogen production. *FEMS Microbiol. Lett.* 249(1): 31-38.
- Chen, W.H., Chen, S.Y., Khanal, S.K. & Sung, S. 2006. Kinetic study of biological hydrogen production by anaerobic fermentation. *International Journal of Hydrogen Energy* 31(15): 2170-2178.
- Dong, L., Zhenhong, Y., Yongming, S., Xiaoying, K. & Yu, Z. 2009. Hydrogen production characteristics of the organic fraction of municipal solid wastes by anaerobic mixed culture fermentation. *International Journal of Hydrogen Energy* 34(2): 812-820.
- Eu, E.C. 2003. *Waste Generated and Treated in Europe*. <http://europa.eu.int>.
- Fang, H.H.P., Li, C. & Zhang, T. 2006. Acidophilic biohydrogen production from rice slurry. *International Journal of Hydrogen Energy* 31(6): 683-692.
- Fauziah, S.H. & Agamuthu, P. 2008. Challenges and issues in moving towards sustainable landfilling in a transitory country - Malaysia. *Waste Manag. Res.* 29(1): 13-19.
- Hao, Q., Wang, C., Lu, D., Wang, Y., Li, D. & Lu, G. 2010. Production of hydrogen-rich gas from plant biomass by catalytic pyrolysis at low temperature. *International Journal of Hydrogen Energy* 35(17): 8884-8890.
- Iwan Budhiarta, Chamhuri Siwar & Hassan Basri 2012. Current status of municipal solid waste generation in Malaysia. *International Journal on Advanced Science Engineering Information Technology* 2(2): 16-21.
- Iyagba, E.T., Mangibo, I.A. & Mohammad, Y.S. 2009. The study of cow dung as co-substrate with rice husk in biogas production. *Scientific Research and Essay* 4(9): 861-866.
- Jayalakshmi, S., Joseph, K. & Sukumaran, V. 2009. Bio hydrogen generation from kitchen waste in an inclined plug flow reactor. *International Journal of Hydrogen Energy* 34(21): 8854-8858.
- Karlsson, A., Vallin, L. & Ejlertsson, J. 2008. Effects of temperature, hydraulic retention time and hydrogen extraction rate on hydrogen production from the fermentation of food industry residues and manure. *International Journal of Hydrogen Energy* 33(3): 953-962.
- Kim, S.H., Han, S.K. & Shin, H.S. 2006. Effect of substrate concentration on hydrogen production and 16S rDNA-based analysis of the microbial community in a continuous fermenter. *Process Biochemistry* 41(1): 199-207.
- Kim, S.H., Han, S.K. & Shin, H.S. 2004. Feasibility of biohydrogen production by anaerobic co-digestion of food waste and sewage sludge. *International Journal of Hydrogen Energy* 29(15): 1607-1616.
- Lee, K.S., Hsu, Y.F., Lo, Y.C., Lin, P.J., Lin, C.Y. & Chang, J.S. 2008. Exploring optimal environmental factors for fermentative hydrogen production from starch using mixed anaerobic microflora. *International Journal of Hydrogen Energy* 33(5): 1565-1572.
- Leon, A.T. 2011. *Health-promoting Properties of Fruit and Vegetables*. Cranfield University: CAB International.
- Li, M., Zhao, Y., Guo, Q., Qian, X. & Niu, D. 2008. Bio-hydrogen production from food waste and sewage sludge in the presence of aged refuse excavated from refuse landfill. *Renew Energy* 33(12): 2573-2579.
- Lin, C.Y., Wu, C.C. & Hung, C.H. 2008. Temperature effects on fermentative hydrogen production from xylose using mixed anaerobic cultures. *International Journal of Hydrogen Energy* 33(1): 43-50.
- Ma, J., Ke, S. & Chen, Y. 2008. Mesophilic biohydrogen production from food waste. *Bioinformatics and Biomedical Engineering Conference IEEE Xplore*. pp. 2841-2844.
- Manaf, L.A., Samah, M.A. & Zukki N.I. 2009. Municipal solid waste management in Malaysia: Practices and Challenges. *Waste Management* 29(11): 2902-2906.
- Massanet-Nicolau, J., Dinsdale, R. & Guwy, A. 2008. Hydrogen production from sewage sludge using mixed microflora inoculum: Effect of pH and enzymatic pretreatment. *Bioresource Technology* 99(14): 6325-6331.
- Mizuno, O., Dinsdale, R., Hawkes, F.R., Hawkes, D.L. & Noike, T. 2000. Enhancement of hydrogen production from glucose by nitrogen gas sparging. *Bioresource Technology* 73(1): 59-65.
- Mtui, G.Y.S. 2009. Recent advances in pretreatment of lignocellulosic wastes and production of value added products. *Afr. J. Biotechnol.* 8(8): 1398-1415.
- Nazlina, H.M.Y., Nor'Aini, A.R., Hasfalina, C.M., M.Z.M. Yusof. & Hassan, M.A. 2011. Microbial characterization of hydrogen-producing bacteria in fermented food waste at different pH values. *International Journal of Hydrogen Energy* 36(16): 9571- 9580.
- Nazlina, H.M.Y., Nor'Aini, A.R. Ismail, F., Yusof, M.Z.M. & Hassan, M.A. 2009. Effect of different temperature, initial pH and substrate composition on biohydrogen production from food waste in batch fermentation. *Asian Journal of Biotechnology* 1(2): 42-50.

- Okamoto, M., Miyahara, T., Mizuno, O. & Noike, T. 2000. Biological hydrogen potential of materials characteristic of the organic fraction of municipal solid wastes. *Water Sci. Technol.* 41(3): 25-32.
- Pan, J., Zhang, R., El-Mashad, H.M., Sun, H. & Ying, Y. 2008. Effect of food to microorganism ratio on biohydrogen production from food waste via anaerobic fermentation. *International Journal of Hydrogen Energy* 33: 6968-6975.
- Patil, J.H., MALourdu A.R. & Gavimath, C.C. 2011. Study on effect of pretreatment methods on biomethanation of water hyacinth. *International Journal of Advanced Biotechnology and Research* 2(1): 143-147.
- Ramos, C., Buitrón, G., Moreno-Andrade, I. & Chamy, R. 2012. Effect of the initial total solids concentration and initial pH on the bio-hydrogen production from cafeteria food waste. *International Journal of Hydrogen Energy* 37(18): 13288-13295.
- Shimizu, S., Fujisawa, A., Mizuno, O., Kameda, T. & Yoshioka, T. 2008. Fermentative hydrogen production from food waste without inocula. *5th International Workshop on Water Dynamics*, AIP Conf. Proc. 987: 171.174.
- Singh, S., Sudhakaran, A.K., Sarma, P.M., Subudhi, S., Mandal, A.K., Gandham, G. & Lal, B. 2010. Dark fermentative biohydrogen production by mesophilic bacterial consortia isolated from riverbed sediments. *International Journal of Hydrogen Energy* 35(19): 10645-10652.
- Skonieczny, M.T. & Yargeau, V. 2009. Biohydrogen production from wastewater by *Clostridium beijerinckii*: Effect of pH and substrate concentration. *Int. J. Hydrogen Energy* 34: 3288-3294.
- Ueno, Y., Haruta, S., Ishii, M. & Igarashi, Y. 2001. Changes in product formation and bacterial community by dilution rate on carbohydrate fermentation by methanogenic microflora in continuous flow stirred tank reactor. *Appl. Microbiol. Biotechnol.* 57(1-2): 65-73.
- Vijayaraghavan, K., Ahmad, D. & Soning, C. 2007. Bio-hydrogen generation from mixed fruit peel waste using anaerobic contact filter. *International Journal of Hydrogen Energy* 32(18): 4754-4760.
- Wang, J. & Wan, W. 2009. Factors influencing fermentative hydrogen production: A review. *International Journal of Hydrogen Energy* 34(2): 799-811.
- Wang, Y., Wang, H., Feng, X., Wang, X. & Huang, J. 2010. Biohydrogen production from cornstalk wastes by anaerobic fermentation with activated sludge. *International Journal of Hydrogen Energy* 35(7): 3092-3099.
- Xiao, L., Deng, Z., Fung, K.Y. & Ng, K.M. 2013. Biohydrogen generation from anaerobic digestion of food waste. *International Journal of Hydrogen Energy* 38(32): 13907-13913.
- Yap, S. 2013. Alkalinity For Better Health. *The Sun*. Malaysia: p. 21.
- Zhu, G.F., Li, J.Z. & Liu, C.X. 2011. Fermentative hydrogen production from soybean protein processing wastewater in an anaerobic baffled reactor (ABR) using anaerobic mixed consortia. *Applied Biochemistry and Biotechnology* 168(1): 91-105.
- Zwietering, M.H., Jongenburger, I., Rombouts, F.M. & van't Riet, K. 1990. Modeling of the bacterial growth curve. *Applied and Environmental Microbiology* 56(6): 1875-1881.

Osuagwu Chiemeiriwo Godday\* & Agamuthu Pariatamby  
Solid Waste Laboratory  
A307 Block A Level 3  
Institute of Post Graduate Studies  
University of Malaya, 50603 Kuala Lumpur  
Malaysia

Osuagwu Uchechukwu Levi  
Visual and Ophthalmic Optics Group  
(Chronic Disease & Ageing Domain),  
Institute of Health & Biomedical Innovation  
School of Optometry & Vision Sciences  
Queensland University of Technology Kelvin Grove,  
Brisbane 4059  
Australia

\*Corresponding author; email: chisvictory@yahoo.com

Received: 6 June 2013

Accepted: 16 April 2014