JOURNAL OF PURE AND APPLIED MICROBIOLOGY, Nov 2015. Vol. **9**(Spl. Edn. 2), p. 533-537

Growth Performance, Waste Reduction and Efficiency of Conversion of Digested Food Waste by *Hermetia illucens* Larvae Via Bioconversion

Shamsul Rahman Mohamad Kutty[1]*, Leong Siew Yoong[1,2] and Tan Chew Khun[2]

[1]Department of Civil and Environmental Engineering, Universiti Teknologi PETRONAS, 32610 Bandar Seri Iskandar, Perak, Malaysia.
[2]Department of Petrochemical Engineering,Universiti Tunku Abdul Rahman, Jalan Universiti, Bandar Barat, 31900 Kampar, Perak, Malaysia.

(Received: 02 September 2015; accepted: 10 November 2015)

In this study, *Hermetia illucens* (Diptera: Startiomyidae) a decomposer by nature was used as an agent for converting food waste and this process is known as bioconversion. Bioconversion is a transformation process of waste into valuable product using microorganism or higher biota organisms. In this study, waste reduction index (WRI), efficiency of conversion of digested food (ECD), growth rate (GR, g. d^{-1}) and survival of the *H. illucens* larvae were determined. The experiment was designed with three different feed loading of 1, 5 and 25 g and. Food wastes as the feed were supplied to the larvae daily. The highest waste reduction index (WRI) value was 4.35 ± 0.17 fed with 1 g of feed, followed by 3.91 ± 0.15 with 5 g of feed and the lowest WRI value was 1.98 ± 0.28 fed with 25 g of feed. Growth rate of the larvae was determined with the changes in the biomass per day. The highest larvae biomass gained was achieved at 78.3 wt% feeding with 25 g of food waste. The one-way ANOVA showed the significant difference between the mean larvae biomass with different loading ($P < 0.05$; $F = 15.82$; d.f = 2, 48). We conclude that, food waste fed is a suitable feed for the larvae proliferation and at the same time successfully reduces the waste.

Key words: Bioconversion, Food waste, Hermetia illucens larvae, waste reduction.

Waste constituents are mainly generated from manufacturing processes, industries, municipal solid wastes (MSW) and agro-based waste. Regardless of its energy content, most of these wastes are discarded on the landfill or by the roadside, which leads to environmental pollution and affecting public hygiene and health. Hence, it would be ideal if these wastes could be transformed into valuable product to solve this problem. A solution would be embedding insect's larvae via bioconversion of organic waste.

Bioconversion process is a sustainable approach for organic waste management using insects' larvae such as *H. illucens* that serve as an individual "waste factories" to managing waste. Moreover, bioconversion process not only paves its way for sustainable treatment process option but the amount of lipid harness from the larvae biomass has the potential use for biodiesel production. Majority of the *H. illucens* larvae biomass are well known to contain at least 30 % of lipid in dry weight basis. Therefore, the lipid from the larvae could be one of the feedstock for biodiesel.

Other related bioconversion study using lipid accumulation microorganism or oleaginous microorganism such as yeast and fungi grown on various sources of wastewater had been studied because of their ability to accumulate humongous quantity of cellular lipids[1-6]. Therefore, this paper

* To whom all correspondence should be addressed.
Tel.: +6 017 572 4633;
E-mail: shamsulrahman@petronas.com.my

is aimed to evaluate the performances of *H. illucens* larvae through transformation of food waste in terms of waste reduction, efficiency of converting digested food (ECD), growth rate (GR, g. d^{-1}) and survival.

MATERIALS AND METHODS

Feed Preparation

Food waste (FW) was collected from the cafeteria located in university campus. The organic waste samples were prepared in a stock and kept in refrigerator at "18°C.

Farming of *H. illucens* larvae

Adult *H. illucens* used in this study was acquired from wild population. The set up was located near to the forest edge. The adult fly was lured into a cage (H x W x L (cm): 120 x 40 x 40) using fruit waste collected from cafeteria fruit stall. Thereafter, the *H. illucens* populations were reared in an enclosure for at least 3 generations for consistency of the population wellbeing prior experiments. The eggs oviposit by the adult flies were left to hatch until larvae emerged 4 to 6 days later. Finally, the larvae were allowed to feed on the fruit waste for 7 days prior being used in the experiments.

Experiment designs

In this study, each treatment was done in triplicates containing 200 individuals' larvae per container. Food wastes were used as the feed for the larvae. Feed was supplied to the larvae in three different amounts: 1, 5 and 25 g. The dimension of the container was (15 x 10 x 7) cm with perforated lid. Feed was fed daily. The individuals were sampled daily to evaluate growth in weight, for this purpose the captured larvae is cleaned and blotted dry prior weight measurement. Pre-pupae were isolated from their container, weighed and placed in another dry container to allow them to complete their life-cycle as pupa and adult. The rate of waste consumption by the larvae was evaluated in terms of waste reduction index (WRI). The efficiency of the larvae to consume and metabolize the waste was evaluated in terms of efficiency of conversion of digested food (ECD). The growth rate (GR, g. d^{-1}) was determined too using the method described by Stefan et al., (2009) [7]. Eqn. (1) - (3) were used for determination of WRI, ECD and growth rate.

$$\mathrm{WRI} = \frac{D}{t} \times 100 \qquad \text{...(1)}$$

$$\text{where } D = \frac{(W - R)}{W}$$

W = Total amount of feed applied, g

R = Residual of feed, g

D = Overall degradation

t = Duration, days

$$\mathrm{ECD} = \frac{B}{(I - F)} \qquad \text{...(2)}$$

B = prepupae biomass, g

I = total feed offer, g

F = the residual feed leftover, g

$$\mathrm{GR} = \frac{\text{(Final body weight - Initial body weight)}}{\text{Rearing durations in days}} \qquad \text{...(3)}$$

Statistical analysis

The data were analyzed using one way ANOVA from Microsoft Excel with 95 % confidence level to establish whether a statistical significant difference occurred between feed loading of 1, 5 and 25 g.

RESULTS AND DISCUSSION

Hermetia illucens larvae development

Food waste (FW) loading of 1, 5 and 25 g were supplied daily to the *H. illucens*. The biomasses gained by the *H. illucens* were measured throughout 16 days. Figure 1 shows the biomass gained by *H. illucens* against feed loading.

Larvae biomass when fed with 1 g of food waste daily showed a slow weight increment as compared to 5 and 25 g of food waste. Final weight increment by *H. illucens* when fed with 1 g of feed was achieved at 35.7 wt%. Larvae fed with 5 g of food waste showed an increasing trend of biomass until day 14 which achieved at 65.52 wt%. Subsequently, 6.75 wt% of larvae weight reduction was perceived from day 14 onwards. Growth of the larvae is rapid when feeding with 25 g of feed as compared to 1 and 5 g feed loading. Their biomasses were achieved at 78.3 wt% and thereafter the weight was reduced by 9.95 wt% from day 14 onwards.

From the observation in Figure 1, the biomass reduction begun on the 15th day for 5 and 25 g of feed loading. Cessation of feeding, wandering and isolation of *H. illucens* larvae from their feed were observed during this period. This indicates the sign of pupation and is part of metamorphosis process to become adult flies. The most obvious indication is evidently perceived in the larvae appearance from beige into dark brown colour.

It was perceived that, at certain period of days, there were minor weight increased by 0.03 g (0.57 wt%) and 0.02 g (1.5 wt%) which were observed between the 5 to 6th day and 10 to 11th day, respectively for 5 g of feed. Similarly, this trend was also observed in 25 g of feed where weight increased by 0.02 g (0.4 wt%) and 0.1 g (1.1 wt%) which were observed between 5 to 6th day and 9 to 10th day, respectively (Figure 1). The minor weight gained is due to ecdysis, a metamorphic process of the larvae or pre-pupae. Callier and Nijhout (2011)[8] suggests that, ecdysone event is triggered in every instar, that a larval-larval or the pupal are preparing for ecdysis. This is a common mechanism for the invertebrates to shed their exoskeleton including their mouthparts once a particular size is reached. The larvae at this moment was seen inactive and cease feeding and that led to minimal weight increment.

Conversely, *H. illucens* fed with 1 g of feed, the development into pre-pupae were prolonged but no mortality was observed during the entire 16 days of feeding period. Increasing of feed loading from1, 5 and 25 g, showed an increased in growth rate of 0.10 ± 0.01, 0.25 ± 0.04 and 0.38 ± 0.01 $g.d^{-1}$, respectively.

Moreover, one-way ANOVA was conducted to evaluate the significance of larvae biomass gained between three different loadings: 1, 5 and 25 g. The ANOVA results obtained from the study is shown in Table 1.

Table 1 shows the analyses of variance (ANOVA) on the differences of mean larval biomasses among the various feed loading were highly significant ($p < 0.05$). Mean weight of the larvae with feed loading of 1, 5 and 25 g were 3.84,

Table 1. Summary of one-way ANOVA

Loading	Count	Sum	Average	Variance		
1g	17	65.30	3.84	0.23		
5g	17	91.87	5.40	2.04		
25g	17	121.10	7.12	6.39		
ANOVA						
Source of Variation	*SS	*df	*MS	F	P-value	Fcrit
Between loading	91.40	2	45.69	15.82	5.26×10^{-6}	3.2
Within loading	138.60	48	2.88			
Total	230	50				

*Note: SS is Sum of Square df is degree of freedom MS is Mean Square

Table 2. Summary of the *H. illucens* performances in growth, waste reduction and conversion efficiency

Food waste loading	*Growth rate, $g.d^{-1}$	*WRI	*ECD,%
1g	0.10 ± 0.01	4.35 ± 0.17	0.02 ± 0.01
5g	0.25 ± 0.04	3.91 ± 0.15	0.04 ± 0.01
25g	0.38 ± 0.01	1.98 ± 0.28	0.05 ± 0.01

*Note: mean ± S.E.

5.4 and 7.12 g, respectively. The significance of the feed loading on biomasses gained was supported by the p-value and F_{value} obtained in this analysis. From the analysis, p-value of 5.26×10^{-6} is lesser than 0.05 ($p < 0.05$) and the F_{value} of 15.82 is greater than 3.2 obtained from $F_{critical}$. In this case, based on p-value of 5.26×10^{-6}, it can be concluded that the effect of feed loading on weight of the larval are significant due to á = 0.05 is lesser than 5.26×10^{-6} and $F_{value} > F_{critical}$. The study inferred that the biomasses gained by the larvae fed with food waste was significant ($p < 0.05$; $F = 15.82$; d.f = 2, 48).

Larval growth, waste reduction and efficiency of digested waste by *H. illucens* larvae

The results obtained from Table 2, summarizes the larvae performances in growth, waste reduction and conversion efficiency of digested waste.

Food waste of 1, 5 and 25 g were supplied daily to the larvae. This study demonstrate that the growth of the larvae were influence by the feed loading. Larvae supplied with minimal feed of 1 g grew the slowest (0.10 ± 0.01 g.d^{-1}) than those fed with 5 and 25 g of feed where their growth rate achieved at 0.25 ± 0.04 and 0.38 ± 0.01 g.d^{-1}, respectively. The larval growth rates are corresponds with the biomasses gained. It was observed that, growth rate at slower pace depicts lower biomass gain by the larvae. For instance, comparing the larvae biomasses achieved at its maximum day 14 and between the feed loadings of 1 and 5 g, the biomasses increased were 66.83 and 36.97 % between loadings of 5 and 25 g. Therefore, feed loading is one of the factors influencing the growth and biomasses gained of the larvae.

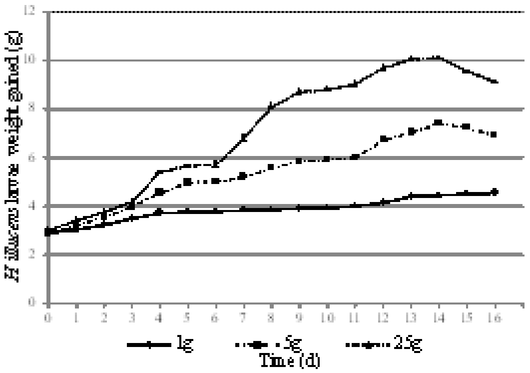

Fig. 1. *H. illucens* larvae weight gained fed with food waste.

The amount of waste reduced and the efficiency to digest the waste by the larvae were indicated as waste reduction index (WRI) and efficiency of conversion of digested waste (ECD). As shown in Table 2, the highest waste reduction index (WRI) value was 4.35 ± 0.17 when fed with 1 g of feed, followed by 3.91 ± 0.15 with 5 g of feed and the lowest WRI value was 1.98 ± 0.28 when fed with 25 g of feed. Higher WRI indicates that the larvae are effective at reducing with lower amount of feed[9]. The results showed that, when the larvae were fed with lower loading, it indicates that the larvae were more efficient in converting the waste as compared to higher loading. However, the highest WRI value indicated in 1 g of feed did not commensurate with the ECD value obtained at 0.02 ± 0.0 %. The ECD value indicates how efficient of the larvae converts the feed supplied into their own biomasses. As observed, the lowest ECD value (0.02 ± 0.01 %) when fed with 1 g of feed showed the lowest larvae biomass with average biomass of 3.84 g (Table 1). Therefore, lower feed loading did not contribute to larvae biomass gain. Suggesting that, lower ECD value could be due to the insufficient of feed supplied which can be related with the growth rates of larvae. Comparatively, higher feed loading relates to higher ECD value which interrelates with higher growth rates as indicated in 5 g of feed where the ECD value obtained was 0.04 ± 0.01 % with growth rate of 0.25 ± 0.04 g.d^{-1} and ECD value of 0.05 ± 0.01 % was obtained for 25 g feed loading with growth rate of 0.38 ± 0.01 g.d^{-1}. Hence, the growth rates were dependent on the efficiency of the larvae ingesting the feed and converts into their body mass (Table 2).

In summary, the waste transformation by the larvae showed similarity with the results obtained from Trudgill et al. (2005)[10] where the duration for the larvae to complete life cycle can differ depending on the types of waste. This revealed that, the period for insect's development and proliferation is very much depending on the quality of waste supplied. A shorter time of development is required when larvae fed with better quality waste than those on lower quality. The results obtained were congruent with the finding from Almaraj et al. (2005)[11] and Hass et al. (2006)[12].

CONCLUSION

It was conclude that, the study showed the potential of the larvae to serve as an ecological agent in managing organic waste. The feed loading showed a significant difference in mean of the larvae biomass. The results indicated that, higher feed loading contribute to higher weight gained and is clearly indicated from the ECD determination in Table 2. Therefore, food waste provides a suitable feed source for pupae production, thus allowing the proliferation of *H. illucens* population.

REFERENCES

1. Mondala, A., Liang, K., Toghaini, H., Hernandez, R. and French, T, "Biodiesel Production by In Situ Transesterification of Municipal Primary and Secondary Sludges", *Biosource Technology,* 2009; **100**: 1203-1210.
2. Angerbauer, C., Siebenhober, M., Mittelbach, M. and Guebit, G. M, "Conversion of sludge into lipids by *Lipomyces starkeyi* for Biodiesel Production", *Bioresource Technology*, 2008; **99**: 3051-3056.
3. Chen, X. F., Huang, C., Chen, X. D., Chen, Y. and Ma, L. L, "Oil Production on Wastewater after Butanol Fermentation by Oleagineous Yeast *Trichosporon coremiiforme*". *Bioresource Technology*, 2012; **118**: 594 -597.
4. Cheirsilp, B., Suwannarat, W. and Niyomdecha, R, "Mixed Cultured of Oleagineous Yeast *Rhodotorula glutinis* and Microalga *Chlorella vulgaris* for Lipid Production from Industrial Waste and its use as Biodiesel Feedstock", *New Biotecnology,* 2011; **28**: 362-368.
5. Seo, Y. H., Lee, I. G. and Han, J. I, "Cultivation and Lipid Production of Yeast *Cryptococus curvatus* using Pretreated Waste Active Sludge Supernatant". *Bioresource Technology*, 2013; **135**: 304-308.
6. Liu, Z., Ruan, Z., Xiao, Y., Yi, Y., Tang, Y., Liao, W. and Liu, Y, "Intergration of Sewage Sludge Digestion with Advance Biofuel Synthesis". *Bioresource Technology*, 2013; **132**: 166-170.
7. Stefan. D., Christian, Z. and Klement, T, "Conversion of Organic Material by Black Soldier Fly Larvae: Establishing Optimal Feeding Rates", *Waste Management and Research*, 2009; **27**: 603-610.
8. Callier, V. and Nijhout, H. F, "Control of body size by oxygen supply reveals size-dependent and size-independent mechanisms of molting and metamorphosis", PNAS, 2011; **108**: 14664-14669.
9. Leong, S.Y., Kutty, S. R. M., Amirhossein, M. and Tan, C. K, "Feasibility study of biodiesel production using lipids of *Hermetia illucens* larvae fed with organic waste", Waste Management, Article In Press (2015), doi:10.1016/j.wasman.2015.03.030
10. Trudgill, D. L., Honek, A., Li, D. and Van Straalen, N. M, "Thermal Time-concepts and Utillity", *Annals of Applied Biology*, 2005; 1-14.
11. Amalraj, D.D., Sivagnaname, N. and Das, P. K, "Effect of Food on Immature Development, Consumption Rate, and Relative Growth Rate of *Toxorhynchites splendens* (Diptera: Culicidae), a Predator of Container Breeding Mosquitoes", *Mem. Inst. Oswaldo Cruz* 2005; **100**, 893-902
12. Hass, E. M. D., Wagner, C., Koelmans, A. A., Karaak, M. H. S. and Admiraal, W, "Habitat Selection by Chironomid Larvae: Fast Growth Requires Fast Food", *Journal of Animal Ecology* 2006; **75**: 148-155.