



Tropical Fruit Wastes as an Organic Nutrient Sources for the Cultivation of *Chlorella vulgaris* and *Haematococcus pluvialis*

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ABSTRACT

The possibility of replacing the inorganic medium with tropical organic fruit waste medium as a nutrient supplement was evaluated for the cultivation of *Chlorella vulgaris* and *Haematococcus pluvialis* in this study. Various concentrations of tropical fruit waste medium such as papaya, pineapple and mango were prepared to cultivate microalgae of *C. vulgaris* and *H. pluvialis*. The biomass concentration, productivity and specific growth rate were determined and compared with those grown in a fully inorganic medium. For *C. vulgaris*, the use of a 20% tropical fruit waste medium was found to yield higher biomass concentration (4.133-4.533 g/L) compared with cultivation in a fully inorganic medium (3.400 g/L). For *H. pluvialis*, the use of a 10% mango waste medium was found to yield a similar biomass concentration compared with cultivation in a fully inorganic medium (3.400 g/L). These results unveiled the potential of utilizing with tropical organic fruit waste medium as an effective strategy to reduce the cultivation cost of microalgae and treat the tropical fruit waste prior to discharge to the environment.

INTRODUCTION

The economic growth and population growth have rendered fast-growing global energy demand. Currently, energy generation through fossil fuels combustion is still incumbent in most developing countries (International Energy Agency 2019) albeit they have creaked about many environmental problems such as global warming stemming from greenhouse gases emission (BP 2019). Besides, overreliance on non-renewable fossil fuel accelerates resource depletion and increases economic burden due to the fluctuating price of fossil fuel. Given this scenario, alternative energy sources which are sustainable and greener have been proposed. Well-known biofuel such as biodiesel has been recognized as a potential energy source as compared to fossil fuels (Leong et al. 2018).

Biofuels can be derived from renewable sources including plant or microalgae (Raheem et al. 2018, Voloshin et al. 2016). The latter organisms have gain enormous attention recently due to their attractive advantages including high growth rate, high oil productivity, great photosynthetic efficiencies and less requirement of arable land (Bhalamurugan et al. 2018, Thawechai et al. 2016). Apart from the high oil productivity for biodiesel, its untapped potential to produce carbohydrate and protein can be explored for biogas (Ferreira et al. 2017, Tao et al. 2017) and agriculture application (Khatoon et al.

2010). Despite these advantages, the major challenges for scale-up biomass production are high capital and operational cost especially cultivation cost and high requirement for freshwater (Chia et al. 2018). Thus, more studies are needed to evaluate the strategy to remedy this bottleneck. Recently, wastewater (Acién Fernández et al. 2018, Ashokkumar et al. 2019, Wuang et al. 2016) and food waste (Chew et al. 2018, Lau et al. 2014, Zhang et al. 2018) have been formulated as cultivation medium since they are rich in nutrients which can promote microalgae growth while the microalgal growth can purify the waste before discharging into water bodies. This integration strategy renders a win-win situation by reducing environmental pollution and concurrently minimizing the cultivation cost of microalgae.

Tropical fruits are one of the important economic commodities in Malaysia. Despite consumed by Malaysian and exported to other countries, some tropical fruits are used for industrial processing for the production of fruit juice, flavouring and canned fruit. The worldwide popularity and demand for tropical fruits has shown an increasing trend from time to time (Rozhan 2017). Some tropical fruits such as pineapple and durian encompass more than 50% rind and seeds that are not consumable. These fruits wastes are rich in moisture and organic composition therefore the long-term disposal of these fruit wastes to the environment not only results in greenhouse gas emission but also environmental pollution

(Cheok et al. 2018). Hence, treatment of these fruit wastes before disposing of in the environment is necessary. In this context, the integration of tropical fruit waste medium for the cultivation of microalgae can be a potential strategy to solve the aforementioned problems.

The objective of this preliminary study was to investigate the potential of using tropical fruit wastes such as papaya, pineapple and mango as nutrient mediums for the cultivation of *C. vulgaris* and *H. pluvialis*. The optimum concentration for three fruit wastes was identified to evaluate the feasibility of replacing the inorganic medium with tropical fruit waste medium.

MATERIALS AND METHODS

Microalgae Strain Cultivation

Microalgae strain of *C. vulgaris* used in this study was derived from a local lake near Inti International University, while *H. pluvialis* used in this study was obtained from Algaetech Malaysia. Both microalgae were cultivated in a 250 mL conical flask containing 150 mL of Basal's basic medium (BB medium). Microalgae cultures were incubated at room temperature under illumination from cool-white fluorescent tubes for 16:8 hours of the light-dark cycle. During the cultivation, microalgae were manually shaken twice each day to prevent microalgal adherence and congregation.

Growth phases of the cells were investigated by determining cell density using a haemocytometer (Marienfeld-Superior, Neubauer) under a light microscope (Eclipse E-100 LED, Nikon).

Microalgae Cultivation with Fruit Waste Medium

Tropical organic fruits namely papaya, pineapple and mango were purchased from the local market. The fruits were washed and cut. The fruit waste including peel and core was cut, blended and filtered using a kitchen sieve. Then, the Duran bottles containing filtered fruit waste solution were heated in a microwave oven at 400 W for 10 minutes. The bottles were wrapped with aluminium foil and kept in the refrigerator at 4°C to avoid nutrient decomposition. To prepare 250 mL of different concentrations (5%, 10%, 20% and 25%) of papaya, pineapple and mango fruit waste mediums, each fruit waste was diluted with deionized water. Each diluted fruit waste medium was immediately cultivated with 4 mL of 2-days-old *C. vulgaris* and 4-days-old *H. pluvialis*. Microalgae cultivated in BB medium and deionized water was used as positive and negative control respectively. The microalgae were cultivated at the condition as previously described.

Determination of Microalgae Cell Growth

The dry cell weight (DCW) of the microalgae biomass was attained by vacuum filtering 5 mL aliquots of culture using pre-weighted mixed cellulose ester membrane filters with absorbent pads (0.45 µm pore size, 47 mm in diameter). Each loaded filter was dried at 80°C until the weight was constant. To obtain the dry cell weight of microalgae, the dry weight of the blank membrane filter was subtracted from that of the loaded membrane filter.

The DCW was used to calculate the microalgae growth. The biomass concentration, productivity and specific growth rate were calculated using the formulas below (Chew et al. 2018, He et al. 2018):

$$\text{Biomass concentration, } X = (\text{DCW}_t / \text{volume of aliquots}) - (\text{DCW}_0 / \text{volume of aliquots})$$

$$\text{Biomass productivity, } P_b = (X_f - X_0) / (t_f - t_0)$$

$$\text{Specific growth rate, } \mu = (\ln X_f - \ln X_0) / (t_f - t_0)$$

Where X_f and X_0 are the biomass concentration (g/L) on days t_f and t_0 (the end and beginning of the determined growth phase respectively).

Statistical Analysis

All experiments were conducted in triplicates and data were presented as means ± standard error of the mean.

RESULTS AND DISCUSSION

Prior to microalgae cultivation with tropical fruit waste mediums, the growth phase of two microalgae in BBM was determined based on the cell count. The results showed that the *C. vulgaris* entered the log phase during the 2nd day and then entered the stationary phase during the 4th day while *H. pluvialis* initiated log phase during 1st day and entered stationary phase during 8th day. The 2-day-old *C. vulgaris* and 4-day-old *H. pluvialis* had adequate cell density therefore they were inoculated into a 250 mL fruit waste medium. The inoculum of high cell density was imperative to ensure the survival of microalgae in the new environment (Gani et al. 2016).

Effect of Fruit Waste Medium Concentrations on Microalgae Growth

The growth of *C. vulgaris* and *H. pluvialis* in papaya, pineapple and mango fruit wastes at various dilutions and in BB medium are shown in Fig. 1 and Fig. 2. The results demonstrate that these two microalgae could grow in almost all concentrations (10, 15%, 20% and 25%) of papaya, pineapple and mango waste mediums compared to microalgae that grew in negative control which is

deionised water. Microalgae of *C. vulgaris* supplemented with 20% papaya, pineapple and mango yielded biomass concentration of 4.133 g/L, 4.533 g/L and 4.600 g/L respectively at the end of 7 days which is higher than the BB medium at 2.233 g/L (Table 1). *C. vulgaris* in 5% papaya, pineapple and mango waste did not grow well and the biomass concentration was very low, only at 0.667, 1.100 and 0.533 g/L respectively, while *C. vulgaris* in 25% papaya, pineapple and mango wastes yielded 1.500, 1.400 and 1.433 g/L respectively.

As shown in Fig. 2, *H. pluvialis* was able to grow in all concentrations of papaya, pineapple and mango fruit waste mediums. *H. pluvialis* in 10% mango waste yielded biomass concentration of 3.400 g/L at the end of 7 days which is comparable to positive control at 3.500 g/L. Although other concentrations of fruit waste medium yielded biomass concentration that lower than BB medium, a slow and increasing trend was observed.

Papaya, pineapple and mango peels have been discovered to contain organic carbon, protein, vitamins and trace metals (Abdul Aziz et al. 2012, Souza et al. 2016, Siti Roha et al. 2013, Suchiritha et al. 2017) which can be supplemented to support microalgae growth. However, the nutrient concentration must be monitored since concentrated nutrient would contrarily reduce microalgae growth. For all the concentration of fruit waste medium up to 25% including the positive control, the *C. vulgaris* grew rapidly during the

first 3 days and continued to attenuate slowly until the 7th day, indicating the fruit waste medium did not deflect the growth phase of *C. vulgaris*. The continuous decline or slow increment of biomass concentration after 3rd day probably stemming from the diminishing of nutrients and the cells instigated the stationary phase. In Table 1, the results showed that *C. vulgaris* in 20% fruit waste medium gave the highest biomass concentration and average biomass productivity when compared to BB medium.

For *H. pluvialis*, the cells grew slowly in BB medium and fruit waste mediums within 7th day. As shown in Table 2, *H. pluvialis* in 5% and 10% fruit waste mediums resulted in higher biomass concentration and productivity, indicating that lower fruit waste mediums are more suitable for *H. pluvialis* to grow. Compared to *H. pluvialis*, *C. vulgaris* showed higher biomass concentration and biomass productivity. It is probably because of their varied metabolisms mode. Besides, compared to *C. vulgaris*, *H. pluvialis* preferred growing in a low concentration of fruit waste medium. The high turbidity of the fruit waste medium may force microalgae to grow in heterotrophic or mixotrophic mode. Testaments from previous studies have revealed *C. vulgaris* was capable to grow with higher biomass concentration in various types of mixotrophic or heterotrophic medium (Gao et al. 2019, Lam et al. 2017, Li et al. 2019, Melo et al. 2018), whereas not many studies have unveiled capability of *H. pluvialis* in different types of mixotrophic medium (Sipaúba-Tavares et al. 2015). Further

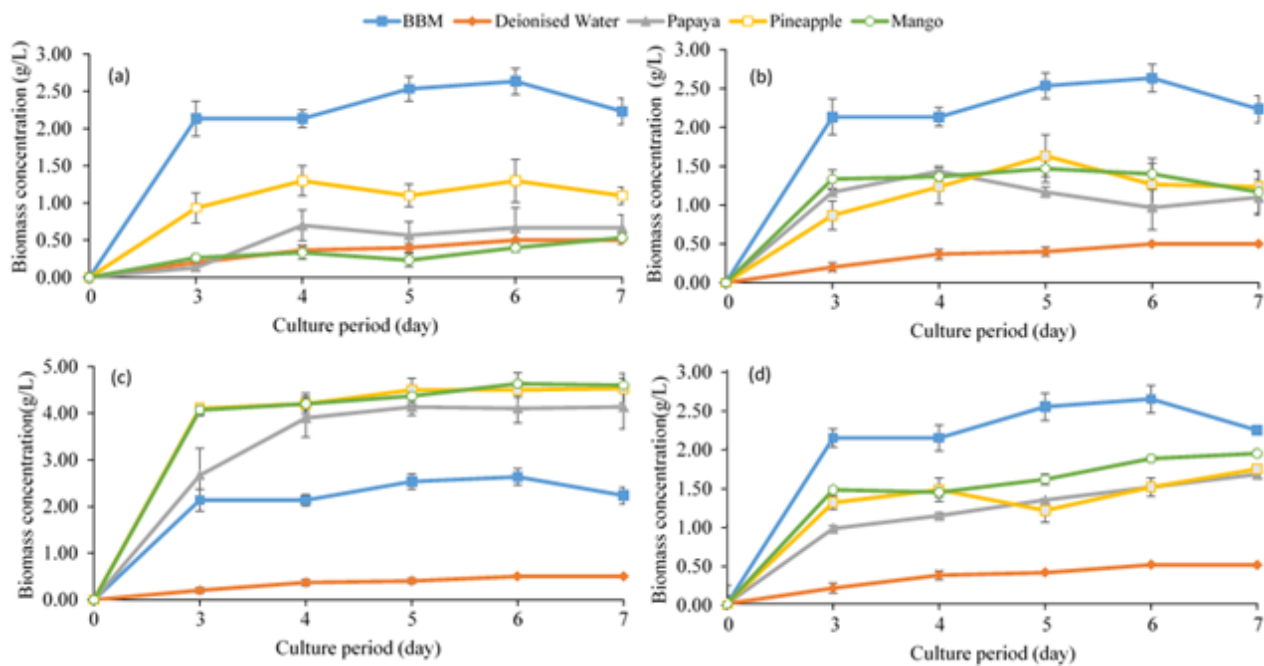


Fig. 1: Biomass concentration of *C. vulgaris* in (a) 5%, (b) 10%, (c) 20% and (d) 25% papaya, pineapple and mango wastes and in BB medium (n = 3).

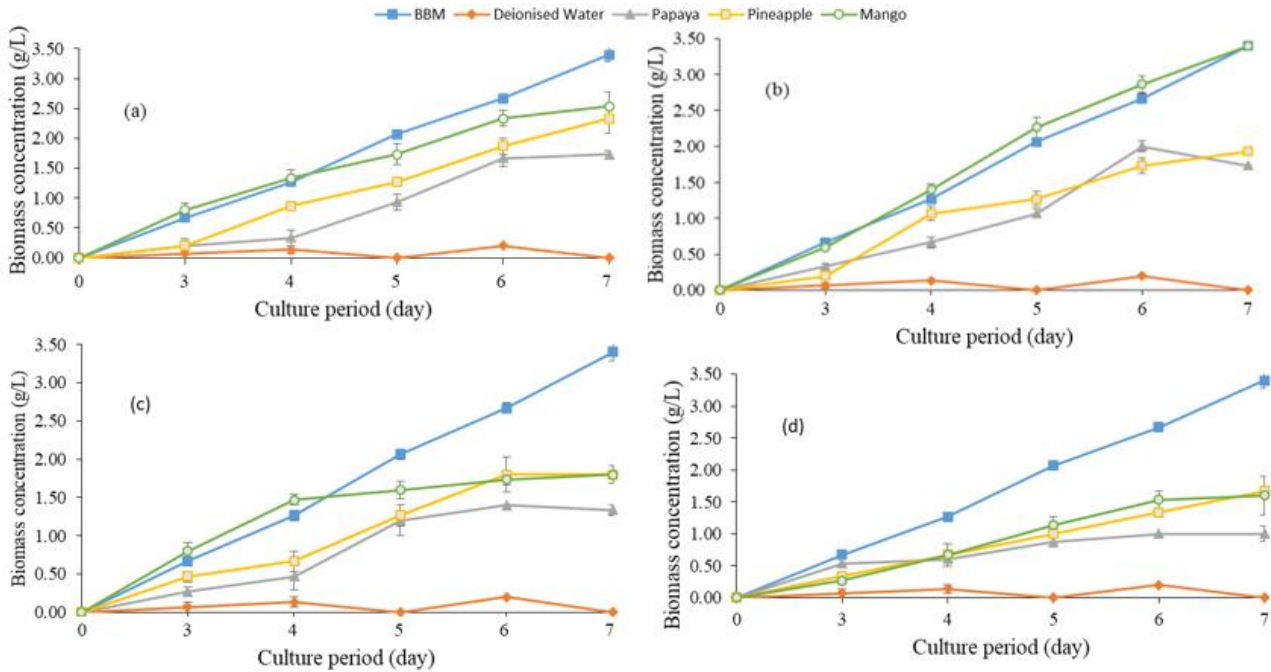


Fig. 2: Biomass concentration of *H. pluvialis* in (a) 5%, (b) 10%, (c) 20% and (d) 25% papaya, pineapple and mango wastes and in BB medium (n = 3).

Table 1: Final dry cell weight (DCW), average biomass productivity (P_b) and average specific growth rates (μ) of *C. vulgaris* in papaya, pineapple and mango waste medium at various concentrations and in BB medium.

Medium		DCW (g/L)	P_b	μ (d ⁻¹)
BBM		2.233 ± 0.176	0.172 ± 0.015	0.180 ± 0.022
DW		0.500 ± 0.000	0.022 ± 0.008	0.046 ± 0.003
Papaya	5%	0.667 ± 0.176	0.045 ± 0.013	0.040 ± 0.007
	10%	1.100 ± 0.231	0.110 ± 0.013	0.067 ± 0.012
	20%	4.133 ± 0.470	0.244 ± 0.047	0.208 ± 0.021
	25%	1.667 ± 0.333	0.076 ± 0.006	0.145 ± 0.016
Pineapple	5%	1.100 ± 0.115	0.084 ± 0.015	0.105 ± 0.013
	10%	1.233 ± 0.203	0.089 ± 0.009	0.069 ± 0.008
	20%	4.533 ± 0.318	0.319 ± 0.009	0.240 ± 0.034
	25%	1.733 ± 0.120	0.096 ± 0.005	0.164 ± 0.023
Mango	5%	0.533 ± 0.088	0.025 ± 0.004	0.046 ± 0.007
	10%	1.167 ± 0.273	0.113 ± 0.003	0.117 ± 0.004
	20%	4.600 ± 0.153	0.308 ± 0.005	0.365 ± 0.006
	25%	1.933 ± 0.030	0.103 ± 0.007	0.101 ± 0.012

research is required to discover the influence of metabolism mode on the growth of *H. pluvialis*. Moreover, *C. vulgaris* has a shorter log phase than *H. pluvialis*, indicating *C. vulgaris* has faster cell metabolism thereby their cell concentration grow quickly. Greater cultivation time is feasibly required for *H. pluvialis* to achieve higher biomass concentration and average specific growth rate.

The average specific growth rates (μ) during 3-7 days' culture are shown in Table 1. The increasing of papaya and mango waste medium concentration stimulated μ of *C. vulgaris* to increase until the concentration of 20%. μ was dropped in the concentration of 10% pineapple and then increased in the concentration of 20% followed by a decrease in the concentration of 25%. μ attained in papaya, pineapple

Table 2: Final dry cell weight (DCW), average biomass productivity (P_b) and average specific growth rates (μ) of *H. pluvialis* in papaya, pineapple and mango waste medium at various concentrations and in BB medium.

Medium		DCW (g/L)	P_b	μ (d ⁻¹)
BBM		3.400 ± 0.115	0.314 ± 0.013	0.239 ± 0.009
DW		0.000 ± 0.000	0.015 ± 0.006	0.022 ± 0.007
Papaya	5%	1.733 ± 0.067	0.144 ± 0.022	0.102 ± 0.018
	10%	1.733 ± 0.133	0.179 ± 0.017	0.133 ± 0.014
	20%	1.333 ± 0.115	0.145 ± 0.033	0.090 ± 0.001
	25%	1.000 ± 0.200	0.135 ± 0.023	0.046 ± 0.002
Pineapple	5%	2.333 ± 0.240	0.197 ± 0.021	0.125 ± 0.019
	10%	1.933 ± 0.176	0.192 ± 0.022	0.111 ± 0.017
	20%	1.800 ± 0.200	0.189 ± 0.040	0.077 ± 0.005
	25%	1.667 ± 0.115	0.156 ± 0.017	0.062 ± 0.006
Mango	5%	2.533 ± 0.240	0.283 ± 0.027	0.157 ± 0.004
	10%	3.400 ± 0.200	0.328 ± 0.022	0.161 ± 0.014
	20%	1.733 ± 0.115	0.250 ± 0.026	0.030 ± 0.001
	25%	1.600 ± 0.429	0.161 ± 0.045	0.033 ± 0.010

and mango waste mediums with the concentration of 20% were higher than in BB medium.

For *H. pluvialis*, μ was increased from the concentration of 5% to 10% and then decreased in the concentration of 20% and 25%. These results indicated that fruit waste with a concentration of 10% was ideal for *H. pluvialis* to grow compared with other concentrations while it was not optimised as in BB medium.

This research confirmed that some of the diluted papaya, pineapple and mango waste mediums were capable to grow these two microalgae. Similar results were reported by previous studies (Chew et al. 2018, Heller et al. 2015, Lau et al. 2014, Zhang et al. 2018), using food waste as a nutrient medium for *C. vulgaris*. A high concentration of food waste exhibited an inhibition effect on cell growth while a low concentration of food compost favoured the cell growth. In this study, only 20% of fruit waste medium with *C. vulgaris* have higher biomass concentration, productivity and specific growth rate than those in BB medium. Whereas only *H. pluvialis* in 10% mango waste medium has similar biomass concentration, productivity and specific growth rate compared to those in BB medium. Two reasons can elucidate this phenomenon. First, a high level of turbidity was observed in all fruit waste mediums, and the sediment may be a light barrier for microalgae cultivation. The high turbidity and sediment hinder the light to penetrate the fruit waste medium therefore microalgae were distracted from photosynthesis. This can be confirmed by comparing pigments colour of microalgae that grew in BB medium and fruit waste medium. The cell pigment in the fruit waste

medium was paler than those grown in the BB medium. Furthermore, the fruit product usually has a somewhat acidic pH which may not favour most microalgae growth (Difusa et al. 2015, Zhang et al. 2014).

Second, to reduce the cultivation cost, raw and unsterile fruit waste was used in the present study. As it was unsterile, unknown zooplanktons appeared. The presence of zooplanktons competed for nutrient sources thereby reduced the number and biomass concentration of algal cells. Besides, white fungi appeared in all concentration of papaya waste mediums during cultivation. Although combating white fungi was attempted to be removed using spatula, it regrew again and only white fungi in 20% papaya waste medium that growing *C. vulgaris* was successfully eliminated.

CONCLUSIONS

In this study, the recycling of nutrients from tropical fruit waste medium was investigated by the cultivation of *C. vulgaris* and *H. pluvialis* using papaya, pineapple and mango wastes with different concentrations. The highest biomass concentration for *C. vulgaris* and *H. pluvialis* was attained in 20% fruit waste mediums and 10% mango fruit waste respectively. This study demonstrated that papaya, pineapple and mango wastes have feasible to cultivate microalgae. The utilization of tropical fruit waste medium can be an effective strategy to minimize environmental pollution and the microalgae cultivation cost. Determination of nutrient removal rate and metabolites such as lipids, carbohydrates and proteins should be carried out in the future to investigate the economic value of this integrated strategy.

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