



Potential of Fruit Peel Waste in Growing Cyanobacteria *Anabaena cylindrica*

Tan Yeong Hwang¹, Ho An Qi², Chai Mee Kin¹, Wong Ling Shing^{2*},
Sinouvassane Djearamane³, Chinnasamy Thirunavukkarasu⁴

¹College of Engineering, Universiti Tenaga Nasional, Jalan Ikram-Uniten, Kajang, Selangor 43000, Malaysia

²Faculty of Health and Life Science, INTI International University, Persiaran Perdana BBN, Putra Nilai, Nilai, Negeri Sembilan 71800, Malaysia

³Faculty of Science, Universiti Tunku Abdul Rahman, Kampar, Perak 31900, Malaysia

⁴Department of Biochemistry and Molecular Biology, Pondicherry University, Puducherry 605014, India

Abstract. Fruit peels are usually disposed of or treated as fertilizer. The peel, however, contains rich nutrients that can be used as a medium for growing microbes. Conventional culture medium is widely used for growing microbes, but the cost is very high and it is not suitable to produce cyanobacterium-based biofuel, biomass, and in other applications. Therefore, this study explores the potential for using fruit peels as a culture medium for *Anabaena cylindrica*. The fruit peels were dried, homogenized, and filtered to make different concentrations of fruit peel media (5%, 10% and 20%). For comparison, BG-11 medium acted as a positive control whereas deionized water serves as a negative control in this experiment. Growth of *A. cylindrica* in different types of media was analyzed after cell counting using a hemacytometer and biomass measuring. The cyanobacterial growth rate and biomass production were recorded in different types of fruit peel media with different concentrations. *A. cylindrica* have greater biomass yield when growing in 20% papaya peel medium and a higher growth rate when growing in 10% pineapple peel media compared to that growing in the BG-11 medium. In other words, the fruit peel media have more potential in growing cyanobacteria than conventional medium.

Keywords: Agriculture waste; Biomass production; Cell culture

1. Introduction

Anabaena cylindrica are cyanobacteria which belong to the *Anabaena* species and are found to appear singly or in a chain of cells. Their vegetative cells possess the capability to carry out carbon dioxide fixation and nitrogen fixation simultaneously. Besides vegetative cells, *A. cylindrica* can transform into two cell types that are heterocysts and akinetes (Hori et al., 2002). Nitrogen fixation will develop when nitrogen is limited (Heng et al., 2014). Heterocysts would suppress nearby cells from developing into another heterocyst, but adjacent vegetative cells will be procured to transform into akinetes, which have a thick cell wall (Qiu et al., 2018). Although both of these specialized cells cannot carry out photosynthesis, they can resist in the adverse environment and grow back into a vegetative cell in a favorable environment (Sukenik et al., 2019).

Many investigations have been done on cyanobacterium biomass, including the *Anabaena* species, to produce environmentally friendly biofuel products, such as bio-

*Corresponding author's email: lingshing.wong@newinti.edu.my, Tel.: +606-7182000 Fax.: +606-7997531
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hydrogen, bio-methane, and biodiesel (Patel et al., 2018; Vargas et al., 2018). Additionally, cyanobacteria have economic applications in the nutrition, cosmetic, and pharmaceutical industries due to their valuable co-products (Hamouda et al., 2017; Singh et al., 2017). However, there are challenges in growing cyanobacteria. One of them is the high cost of culture medium (deCastro et al., 2015). Large amount of media is needed in extensive practical studies for cultivation, streak plate or pour plate and other experiments but low cost media is less available. Therefore, there were studies finding alternatives to these expensive commercial medium (deCastro et al., 2015; Beyl et al., 2019; Jiang et al., 2019).

Due to extensive agricultural exercises nowadays, there have been many vegetables and fruit produced daily in Malaysia (Grünwald, 2021). Papaya, pineapple, mango, and banana are fruits that are most widely consumed by Malaysians or used for the food industry (Rozhan, 2017). About 40% of the fruit's total mass is made up from the inedible portion like peels, seeds, and pulps (Cheok et al., 2018). Some irresponsible people would dispose of these unwanted fruit parts improperly into the environment, leading to environment pollution (Gowe, 2015; Tonini et al., 2018). The dumping site that accumulates this fruit waste becomes the culture area of pathogenic bacteria, fungi, or yeast (Cheok et al., 2018), and produces leachate that pollutes ground water and affect aquatic life (Ali et al., 2014).

Fruit wastes are cheap organic ingredients because they are readily available as domestic waste. Moreover, they consist of high amounts of sugar that can be utilized by cell (Katiyar et al., 2019; Mohammed et al., 2020). Fruit also contains various kinds of minerals and other hydrocarbons in the form of carbohydrates, protein, and lipids (Septembre-Malaterre et al., 2016). Therefore, it would be better if there was potential for using fruit wastes to design a culture medium for cyanobacteria. It would eliminate the consequences associated with improperly disposed fruit residues.

To date, there are reports showing that fruit peels have been used for formulation in culture medium for microbial (Sarkar et al., 2019), fungal (Choi et al., 2015; Anbu et al., 2017), and yeast (Dhanasekaran et al., 2011) growth, but their capacity to cultivate cyanobacteria has not been confirmed yet. Hence, the research aims to find out the potential of growing cyanobacteria by biological waste from fruits such as mango, papaya, and pineapple. The growth of one commonly available cyanobacteria, *A. cylindrica*, on the medium produced from the fruit waste is determined as well.

2. Methods

2.1. Microalgae Strain Cultivation

An *A. cylindrica* culture (1403/2A) was obtained from CCAP, United Kingdom. The cells were cultured into two separate sterile Erlenmeyer flasks containing 200 mL of fresh BG-11 medium. The flasks were covered with gauze and a rubber band. The cultures were incubated in 22-24 °C under illumination from cool-white fluorescent tubes with 16:8 hours of light-dark cycle. As no air was pumping into the cell culture during the short period of the experiment, continuous shaking was not utilized (Hsia et al., 2015). Gentle shaking of liquid culture was done every day to avoid microalgae adherence and congregation. Growth phases of the cells were investigated by determining cell density (Equation 1) using a hemocytometer (Marienfeld-Superior, Neubauer) under a light microscope (Eclipse E-100 LED, Nikon):

$$\text{Cell density (cells/mL)} = \text{Average number of cells per field} \times 10^4 \times \text{dilution factor} \quad (1)$$

2.2. Fruit Peel Collection and Treatment

The pineapple, mango, and papaya fruits were purchased at a local supermarket. The fruit peels were removed and dried using a drying oven with temperatures between 40 and 50°C. The papaya peels were homogenized using a Faber blender. Then, the homogenized papaya peels were filtered by using filter paper to remove the large solid residues before being transferred into Duran glass bottles and labelled. They were heated in a microwave oven at 200 W for 5 minutes to reduce the potential microorganism activity. The bottles were wrapped with aluminum foil and kept in the refrigerator at 4°C to avoid nutrient decomposition. This procedure was repeated for pineapple and mango peels.

2.3. Microalgae Cultivation with Fruit Peel Medium

All of the fruit peel media were diluted into concentrations of 5%, 10%, and 20% with distilled water for a final volume of 200 mL with pH between 5.0–6.0. Each diluted fruit peel medium was cultivated with 3 mL or 4.7×10^7 cells/mL of 5-days-old *A. cylindrical*. Microalgae cultivation with BG-11 and deionized water was used as positive and negative controls, respectively. The microalgae were cultivated previously described.

2.4. Determination of Microalgae Cell Growth

The initial dry biomass of culture growing in every media was measured on their day zero of cultivation. Their dry biomass was also measured on every two days of cultivation. Ten milliliters of cell culture was taken out from each kind of medium and put on mixed cellulose ester membrane filters with absorbent pads (0.45 μm pore size, 47 mm in diameter). Then, they were vacuum-filtered by a Büchner funnel. Each loaded filter was dried in a drying oven at 70°C until the weight was constant. Then, the weight of the membrane filter with the cyanobacterial dry load was measured. Cyanobacterial dry biomass was obtained by calculating the difference of the weight between the pure dried membrane filter and the membrane filter with cyanobacterial dry load.

Dry biomass was used to calculate the microalgae growth by biomass concentration (Equation 1), productivity (Equation 2), and specific growth rate (Equation 3).

$$\text{Biomass concentration} = \frac{\text{Dry biomass}}{V_a} - \frac{\text{Dry biomass}}{V_a} \quad (2)$$

where V_a is the volume of aliquots.

$$\text{Biomass productivity, } P_b = \frac{(N_f - N_0)}{(t_f - t_0)} \quad (3)$$

where N_f and N_0 are the biomass concentration (g/L) on days t_f and t_0 (the end and beginning of the determined growth phase, respectively).

$$\text{Specific growth rate, } \mu = \frac{\ln(N_f - N_0)}{(t_f - t_0)} \quad (4)$$

2.5. Statistical Analysis

All experiments were conducted in triplicate and data were presented as means \pm standard error of the mean.

3. Results and Discussion

The biomass productivity of *A. cylindrical* grown in different concentrations of fruit peel media was illustrated in Figure 1. From Figure 1a, the biomass concentration in all 5% media is similar with BG-11 medium. Only the cells cultivated in 5% pineapple and mango

peel media showed slightly higher biomass concentrations. As illustrated in Figure 1b, the biomass concentration in 10% papaya peel media was observed much higher than those cells grew in the BG-11 medium whereas the biomass concentration in 10% mango and pineapple peel media was similar to BG-11 medium throughout the cultivation period. Although the greatest concentration of biomass was produced by cells cultivated in 10% pineapple peel media at day 1, the biomass concentration was dropped at day 3 and then slowly increased thereafter. This is probably due to the presence of contaminated microorganisms. These medium were not autoclaved to reduce the cultivation cost and therefore have the potential of contamination. Presence of contaminated microorganisms competed nutrient sources with *A. cylindrica* thereby reduced the biomass concentration. As shown in Figure 1c, the biomass concentration of cells cultivated in 20% papaya and pineapple peel media is higher than cells grown in BG-11, whereas the biomass concentration in 20% mango peel medium showed a similar trend compared to BG-11 medium.

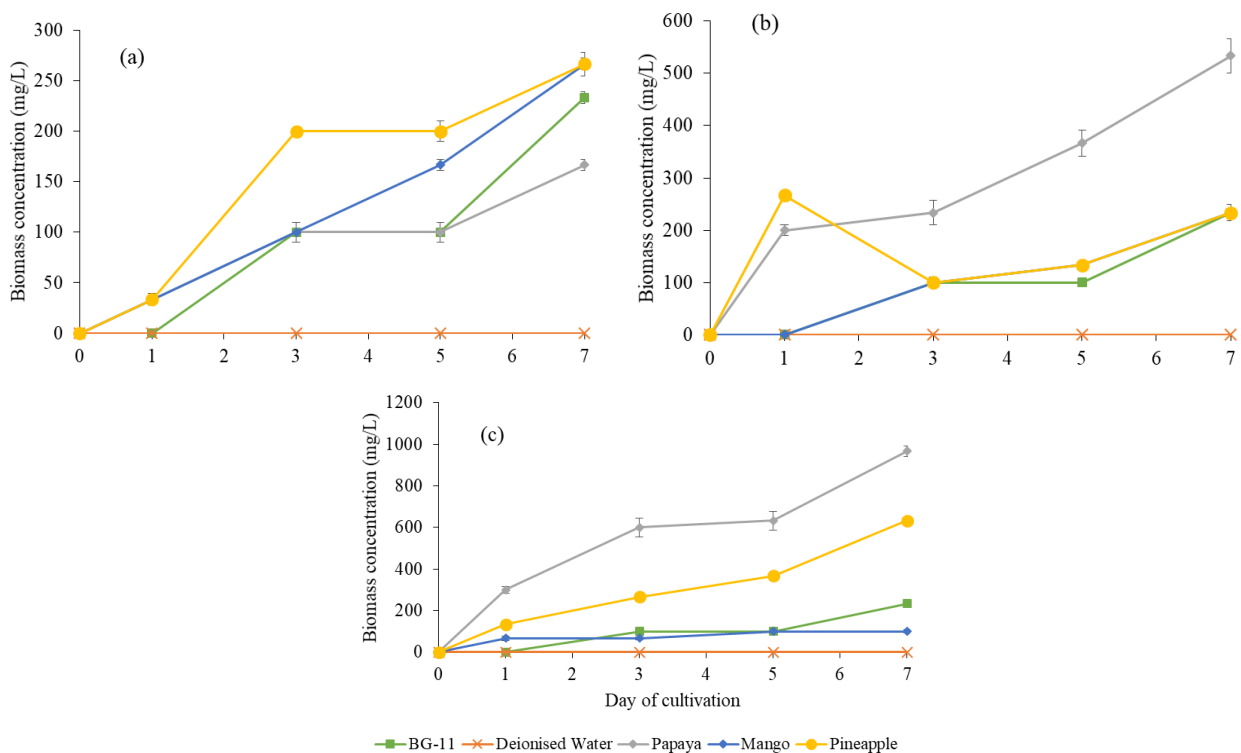


Figure 1 Biomass concentration of *A. cylindrica* in: (a) 5%; (b) 10%; and (c) 20% papaya, pineapple, and mango peel and BG-11 medium (n = 3)

Generally, papaya, pineapple, and mango peel are abundant with various nutrients, such as organic carbon, protein, vitamins, and trace metals (Abdul Aziz et al., 2012; Roha et al., 2013; Souza et al., 2016; Pavithra et al., 2017) which can be supplemented to support *A. cylindrica* growth. Previous studies revealed that high concentrated waste has negative impacts on some microalgal growth (Lau et al., 2014; Sloth et al., 2017). Concentrated waste medium is usually dark in color, which reduces the light penetration to it. As a result, the phototrophic or mixotrophic microalgae, which require light as part of their metabolism, may slow down their growth. Nevertheless, this study showed contrary results that high final biomass concentration with high specific growth rate and biomass productivity was observed in 10% and 20% pineapple and papaya peel media. On the other hand, the biomass productivity and specific growth rate in most concentrations of papaya and

pineapple peel media are better than the BG-11 medium (Table 1). This phenomena can be elucidated by two hypotheses. First, unlike the cells in the BG-11 medium which are green, the cells in high concentrated fruit peel medium showed low intensity of green and were almost colorless. This indicated that the chlorophyll content of the cell decreased and they probably did not perform photosynthesis. Hence, it can be deduced that *A. cylindrica* probably carried out heterotrophic growth. The literature has demonstrated that some of the cyanobacteria can become chemoorganoheterotrophic by using simple carbohydrates without a light supply (Stebegg et al., 2012). Although the mechanisms behind *Anabaena* assimilation of organic carbon and nitrogen have not been greatly determined, Herrero and Flores (2019) predict that, in a harsh environment, genes for heterocyst differentiation will be activated then followed by activation of genes involved in glycogen catabolism, glycolysis, and the pentose phosphate pathway. Wan et al. (2017) revealed that, during dark and heterotrophic conditions, an oxidative pentose phosphate pathway and oxidative phosphorylation were upregulated so that cyanobacteria was able to catabolize simple sugar in order to produce energy. In another study, Nieves-Mori3n and Flores (2018) found that the *Anabaena* species was able to grow in the presence of glucose, sucrose, and fructose by activating the ABC glucoside transporter and other ABC transporters which are responsible for sugar uptake. These studies suggest that cyanobacteria, including *Anabaena*, are capable of photoautotrophic, organoheterotrophic, and mixotrophic growth.

Second, the fruit peel media were not sterile, posing the possibility of contamination. Compared to the BG-11 medium, microorganisms are preferred to grow in fruit peel medium that is comprised of abundant organic nutrient. These microorganisms might have beneficial responses to *A. cylindrica* by exchange of nutrients, excreting growth-promoting compounds, vitamins, and protecting from competitors (Ma et al., 2014; Cho et al., 2015). Paddock et al. (2020) suggested that the presence of a complex microbial community in wastewater can enhance wastewater treatment and biomass production.

In summary, the cultivation of *A. cylindrica* with papaya, pineapple, and mango media showed promising results for cyanobacterial growth. Among all the experimental controls, the highest biomass concentration was performed by cells grown in 20% of papaya peel medium (933.33 mg/L) whereas the lowest biomass concentration was performed by cells grown in 20% of mango peel medium which was only 100.00 mg/L. On the other hand, the highest growth rate of cells was observed in 10% pineapple peel medium while the lowest growth rate of cells was observed in 20% mango peel medium. Moreover, the biomass concentration did not increase much in the mango peel medium even though the concentration of mango peel medium was increased from 5% to 20%. The biomass productivity and specific growth rate in mango peel medium was also lower than the same concentration of papaya and pineapple peel media (Table 1). This can be explained by a high level of polysaccharides, especially pectin or starch in mango peel (Iagher et al., 2002). The growth of microalgae was inhibited because the polysaccharides in the culture medium increased the concentration polysaccharide in the cell wall, thus disrupting the nutrient uptake or nutrient diffusion (Rotem et al., 1992). Inhibition of carbon uptake would deter the cells from performing photosynthesis.

Table 1 Final biomass concentration, biomass productivity, and specific growth rate of *A. cylindrica* in fruit peel and BG-11 medium

Medium		Final biomass concentration (mg/L)	P _b (mg/L/day)	μ (day ⁻¹)
BG-11		233.33 ± 5.77	17.33 ± 0.165	0.167 ± 0.032
DW		0.00 ± 0.000	0.00 ± 0.000	0.000 ± 0.000
Papaya	5%	166.67 ± 5.74	22.10 ± 1.72	0.122 ± 0.041
	10%	533.33 ± 32.15	85.46 ± 5.46	0.205 ± 0.077
	20%	933.33 ± 5.75	152.95 ± 9.04	0.344 ± 0.132
Pineapple	5%	266.67 ± 11.55	35.62 ± 1.88	0.191 ± 0.135
	10%	266.67 ± 15.28	72.00 ± 2.49	0.366 ± 0.039
	20%	633.37 ± 25.17	77.24 ± 2.84	0.116 ± 0.025
Mango	5%	266.67 ± 5.75	27.62 ± 2.22	0.135 ± 0.153
	10%	233.33 ± 57.74	18.67 ± 3.96	0.122 ± 0.024
	20%	100.00 ± 10.01	24.63 ± 2.98	0.084 ± 0.008

4. Conclusions

A. cylindrica grew successfully on the nutrient-rich fruit peel medium. The biomass concentration, productivity, and specific growth rate were enhanced when cultivating in fruit peel media. Fruit peel media have the potential to replace the expensive chemically-synthetic medium. From the experiment, *A. cylindrica* was able to produce its greatest biomass in a 20% papaya medium and possesses the highest growth rate in a 10% pineapple peel medium.

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