Preliminary assessment of Malaysian micro-algae strains for the production of bio jet fuel

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Abstract. Malaysia is the main hub in South-East Asia and has one of the highest air traffic movements in the region. Being rich in biodiversity, Malaysia has long been touted as country rich in biodiversity and therefore, attracts great interests as a place to setup bio-refineries and produce bio-fuels such as biodiesel, bio-petrol, green diesel, and bio-jet fuel Kerosene Jet A-1. Micro-algae is poised to alleviate certain disadvantages seen in first generation and second generation feedstock. In this study, the objective is to seek out potential micro-algae species in Malaysia to determine which are suitable to be used as the feedstock to enable bio-jet fuel production in Malaysia. From 79 samples collected over 30 sites throughout Malaysia, six species were isolated and compared for their biomass productivity and lipid content. Their lipid contents were then used to derived the require amount of micro-algae biomass to yield 1 kg of certifiable jet fuel via the HEFA process, and to meet a scenario where Malaysia implements a 2% alternative (bio-) jet fuel requirement.

1. Introduction
Malaysia has long been touted as country rich in biodiversity and therefore, attracts great interests as a place to setup bio-refineries and produce bio-fuels such as biodiesel, bio-petrol, green diesel, and bio-jet fuel Kerosene Jet A-1. Numerous studies have been performed in Malaysia to determine suitable waste biomass, and among them, suitable sustainable biomass waste, as a feedstock to convert to other valued products such as bio-fuel [1]. However, the consensus of these studies seems to be in relative competition with each other for the waste biomass, especially since the industrial players' involvement to utilize a “waste to wealth” business model is only just maturing. In essence, the waste biomass in Malaysia are gaining traction as a means to create or enhance current business models. As fuel is relatively cheap and requires large amounts of volume, segregating biomass for the purpose of fuel production will not yield a high return of investment in comparison to other utilization of bio-based products [2, 3]. Therefore, a new sustainable biomass source needs to be realised to enable energy security that is not in competition with food and other bio-based economy. Micro-algae and macro-algae, as third generation feedstock, could be an alternative that provides the energy security, non-food competing and creates its own value chain rather than competing with other economic drivers [4].
The aviation industry is unique in its initiatives to be more sustainable, which was committed under the Aviation CO₂ Roadmap during the Aviation and Environment Summit organised by ATAG (Air Transport Action Group) in 2008 [5]. Major industrial aviation players have pledged to reduce their collective emissions that contributes to roughly 2% of global man-made CO₂ [6]. These pledges are: to increase fuel efficiency by 1.5 % per year (average) between 2009 and 2020, to achieve carbon neutral growth from 2020 onwards and to reduce net emissions by 50 % by 2050 (from 2005 levels) [5]. In order to achieve such ambitious targets, the aviation industry has catalysed developments across the world to search for the best sustainable biomass feedstock, its processing and conversion technology, and to support testing and certification of these bio-derived jet fuels. At present, the most widely used aviation jet fuel is Kerosene Jet A-1, which is governed by international standards such as ASTM D 1655 “Standard Specification for Aviation Turbine Fuels”. In anticipation of the synthesized fuel to be widely used, ASTM D 7566 was released in 2009 that enables blending of synthetic fuel (bio-derived fuel) and conventional commercial jet fuel. ASTM D 7566 is known as “Specification for Aviation Turbine Fuel Containing Synthesized Hydrocarbon” [7, 21].

A research and development collaboration between University of Malaya, Aerospace Malaysia Innovation Centre (AMIC) and Airbus Group is aimed at searching and selecting suitable micro-algae strains in Malaysia for the production of bio-jet fuel. Micro-algae strains were screened primarily for their lipid content to determine on the viability of having mass scale micro-algae cultivation for the purpose for bio-jet fuel production. This paper assesses the selected micro-algae strains using Hydro-processed Esters and Fatty Acid (HEFA) conversion process to produce synthetic jet fuel in order to determine if this combination is able to fit Malaysia’s jet fuel consumption needs.

2. Materials and methods
The Malaysian micro-algae strains were sampled from freshwater, marine, and polluted (palm oil mill factory) sources. The samples were isolated to yield the dominant strain and the isolated strains were characterized for their lipid content. Table 1 tabulates the micro-algae considered in this study.

| No | Type of algae/ habitats     | Enrichment media used                      |
|----|----------------------------|-------------------------------------------|
| 1  | Freshwater algae           | Bold’s Basal Medium (BBM) [8]             |
| 2  | Marine algae               | Provasoli 50 Medium (Prov50) [9]          |
| 3  | Blue-green algae           | Kosaric Medium (modified after [10])      |
| 4  | Diatoms                    | Diatom Medium [11]                        |

2.1. Algae isolation
A number of isolation techniques can be employed to isolate dominant species from a cocktail of strains to obtain unialgal cultures. In order to have clonal cultures, the culture were propagated from a single cell of a few cells. Samples collected from the variety of environments (freshwater, marine, and polluted) were subjected to manipulations before isolation was carried out. The following techniques of algae isolation was used in this study: selective enrichment media technique and techniques for isolation of algae. The use of selective enrichment media was chosen to promote the growth of the particular dominant species in order to grow sufficient cells density for isolation. For isolation of algae, a number of varied techniques were used, including centrifuge-washing, streak-plating and taxis techniques [12]. Isolates were purified using antibiotic Kanamycin and Penicillin G treatment as mentioned in [12].

2.2. Culture maintenance
Purified micro-algae cultures were maintained in sterile Bold’s Basal Medium (BBM) for freshwater algae and Provasoli 50 (Prov50) Medium for marine algae. The cultures were placed on illuminated shelves, incubated at 25±1 °C under 12:12 hour light-dark cycle with an irradiance of 40 – 60 µmol photon/s/m².
2.3. Determination of microalgae dry weight

A known sample volume containing the algae was filtered through a pre-weighed dried 0.45 μm glass fiber cellulose (GF/C-47mm) filter paper. The pre-weighed filters were heated prior to the filtration in order to remove any organic contaminants. The used filters containing algae were dried at 100±1°C in an oven (ULM-600 Memmert Oven, Schwabach, W. Germany) for 24 hours, cooled in a dessicator filled with silica gel and weighed. The algae dry weight is determined through Equation 1.

\[
\text{DW (mg/L)} = \frac{\text{weight of filters with dried algae biomass, (mg)}}{\text{Volume of algae culture (L)}} - \frac{\text{weight of blank filters, (mg)}}{\text{Volume of algae culture (L)}}
\]  

(1)

2.4. Determination of total lipid content in micro-algae

Total lipid content of the microalgae was determined using gravimetric measurements as outlined by [13]. A known volume of filtered algae (filtered on 0.45 μm GF/C-47mm filter paper) was pounded with 5 ml Methanol-Chloroform (2:1 v/v) solution using glass hand-homoginiser and transferred into a screw-cap centrifuge plastic tube. The sample was then centrifuged (using Kubota 2100 centrifuge, Kubota Corporation, Japan) at 3000 rpm for 10 minutes. The residue was re-extracted again in 5ml Methanol-Chloroform (2:1 v/v) solution as followed in above procedures. The supernatant obtained was pooled in a new centrifuge tube and 2 mL of chloroform was added followed by 2 mL of distilled water. The tube was thoroughly shaken using a vortex mixer (S0200-230-UK Vortex mixer, Labnet International Inc, USA) until the mixture turned into a milky green colour. The sample was then centrifuged at 3000 rpm for 10 minutes (using Kubota 2100 centrifuge, Kubota Corporation, Japan). The lower layer was then removed using a Pasteur pipette and transferred into a new screw-cap glass tube. The sample was then blow-dried with stream of nitrogen gas. After drying, the extracted sample was dissolved again in 1 mL of chloroform and transferred into a 3.5 mL borosilicate glass vial (pre-weight). The extract was then blow-dried and the dry extract was placed in a dessicator containing the silica gel for 24 hours. After that, it was weighed and retained for fatty acid transesterification. The lipid content in percentage of lipid per dry weight unit was calculated using Equation 2.

\[
\text{Lipid content} = \frac{[\text{Weight of lipid + vial (final weight)}] - [\text{Weight of empty vial}]}{\text{X (g) biomass (DW microalgae)}} \times 100\%
\]  

(2)

3. Results and assessment

A total of 79 samples were collected across 30 collection sites in Malaysia. They were identified and isolated, and then investigated for its lipid productivity to meet the needs of jet fuel conversion [14]. The strains of interests were narrowed down to the ones listed in Table 2.

Table 2. Micro-algae strain of interest for high lipid productivity for the purpose of bio-jet fuel conversion [14]

| Location         | Species       | Reference  |
|------------------|---------------|------------|
| Hotspring        | *Chlorella* sp.| UMACC 326  |
| POME             | *Chlorella* sp.| UMACC344   |
| POME             | *Chlamydomonas* sp.| UMACC 352 |
| Freshwater (lake)| *Chlorella* sp.| UMACC 348  |
| Marine           | *Chlorella* sp.| UMACC 345  |
| Marine           | *Chlorococcum* sp.| UMACC 346 |

A total of five species were noted to be of interest, with the majority of the species isolated is the *Chlorella* sp from various sources [14]. These five species were compared in terms of their biomass productivity potential and lipid content. This is shown in Table 3.
Table 3. Comparison micro-algae biomass productivity, lipid content and its productivity, and dry weight after 12 days of incubation [14]

| Type   | Biomass Productivity (mg/L/Day) | Lipid content (%wt) | Lipid Productivity (mg/L/Day) | Dry weight (mg/L) |
|--------|---------------------------------|---------------------|--------------------------------|-------------------|
| UMACC 326 | 34.43 ± 8.29.                  | 40.08 ± 2.74.       | 13.78 ± 4.33.                  | 190.00 ± 8.00.    |
| UMACC 344 | 76.27 ± 11.08.                 | 40.38 ± 7.89.       | 30.57 ± 6.63.                  | 114.00 ± 14.00.   |
| UMACC 352 | 32.95 ± 7.23.                  | 43.34 ± 2.44.       | 14.03 ± 3.08.                  | 222.67 ± 12.06.   |
| UMACC 348 | 49.43 ± 2.07.                  | 26.72 ± 6.20.       | 12.71 ± 3.14.                  | 506.00 ± 133.55.  |
| UMACC 345 | 44.90 ± 6.44.                  | 30.48 ± 5.85.       | 13.48 ± 2.80.                  | 328.67 ± 22.03.   |
| UMACC 346 | 57.93 ± 14.00.                 | 39.28 ± 1.96.       | 22.55 ± 5.70.                  | 294.00 ± 24.25.   |

These species were grown in standard medium, autotrophic (12 hours light, 12 hours dark), in 2.5 L flasks for 12 days in the incubators, as mentioned in section 2.2 [14]. The results from Table 3 were obtained after 12 days of incubation and they can be ranked as follows:

- **Biomass Productivity**: UMAC344 > UMACC 346 > UMACC 348 > UMACC 345 > UMACC 326 > UMACC 352
- **Lipid content**: UMACC 325 > UMACC 344 > UMACC 326 > UMACC 346 > UMACC 345 > UMACC 348
- **Lipid Productivity**: UMACC 344 > UMACC 346 > UMACC 352 > UMACC 326 > UMACC 345 > UMACC 348
- **Dry Weight**: UMACC 348 > UMACC 345 > UMACC 346 > UMACC 352 > UMACC 326 > UMACC 344

As observed in the above ranking breakdown of the four criteria, the correlation between biomass productivity, lipid content and its productivity and dry weight is not apparent. Lipid content is the amount of lipid that is contained within the micro-algae cell after 12 days of incubation while lipid productivity calculates the change of lipid content within the cell across the 12 days of incubation. High lipid productivity does not mean that it also contains high lipid content. This is similar to the biomass productivity and the ensuing dry weight. Ideally, there should be high biomass productivity, lipid productivity and lipid content, which corresponds to fast growing algae that accumulates high amounts of lipid yielding plenty of biomass (dry weight) that contains large amounts of lipid. Further study can be carried out to determine the best conditions for each of these micro-algae species and they are later compared once again, including the economics.

4. Production calculation

Malaysia’s consumption of aviation jet, Kerosene Jet A-1, is numbered around 3 billion litres per year (2010) and in comparison to global consumption, this is roughly 0.01% [15]. Malaysia’s neighbour, Indonesia, has already introduced a biofuel mandate to introduce alternative jet fuel of 2% by 2018, increasing to 5% by 2025 [16]. IATA (International Air Transport Association) is keeping a keen eye on Indonesia and its developments, noting that other member states around the world will soon start to reflect on this move as well. In lieu of this, it can be calculated that for Malaysia to introduce a 2% alternative jet fuel, 60 million litres of sustainable bio-jet fuel has to be produced. For 5%, this number increases to 150 million litres.

HEFA has been certified in 2011 as an approved pathway to produce alternative jet fuel, which can be “dropped in” and blend up to 50% with the conventional jet fuel [17]. The HEFA process is most suitably used to process oil based feeds, such as vegetable oil, animal fat and recycled oils. In this scenario, HEFA as a process can be used as a means to convert micro-algae into jet fuel. From the literatures, it can be gathered that a maximum conversion efficiency from micro-algae oil to jet fuel is 60% (by weight) [18, 19, 20]. The process can be simply summed up in Figure 1.
**Figure 1.** Simplified box-process of algae biomass to jet fuel via the HEFA process [20]

The HEFA process starts off with a hydrogenation process, after the micro-algae’s lipid content has been extracted. Hydrotreatment involves oxygen removal, which achieves a paraffinic middle distillate (linear $nC_{16}$, $nC_{17}$, and $nC_{18}$, and hydroisomerization) that transforms a near linear-paraffinic diesel like product into iso-paraffins. Iso-paraffins are important for low temperature performance seen in aviation fuel. After hydroisomerization, a distillation step is required to remove the gasoline range hydrocarbons to maximize the aviation jet range product. To convert micro-algae oil to jet fuel, 1.67 kg of micro-algae oil is required for process that yields 1 kg of jet fuel (based from 60% conversion). The calculated values in Table 4 are obtained by micro-algae species based on lipid (oil) percentage found per kg of micro-algae biomass.

| Type         | Lipid content (%wt) | Per 1 kg of micro-algae biomass | For 1 kg of micro-algae oil |
|--------------|---------------------|---------------------------------|-----------------------------|
| UMACC 326    | 40.08 ± 2.74        | 40.08 g ± 2.74                  | 2.50 kg ± 0.23              |
| UMACC 344    | 40.38 ± 7.89        | 40.38 g ± 7.89                  | 2.48 kg ± 0.41              |
| UMACC 352    | 43.34 ± 2.44        | 43.34 g ± 2.44                  | 2.31 kg ± 0.13              |
| UMACC 348    | 26.72 ± 6.20        | 26.72 g ± 6.20                  | 3.74 kg ± 0.70              |
| UMACC 345    | 30.48 ± 5.85        | 30.48 g ± 5.85                  | 3.28 kg ± 0.53              |
| UMACC 346    | 39.28 ± 1.96        | 39.28 g ± 1.96                  | 2.55 kg ± 0.13              |

From Table 4, based on the lipid content, it was calculated that UMACC 326 requires 2.50 kg ± 0.23 of biomass to yield 1 kg of micro-algae oil; UMACC 344 requires 2.48 kg ± 0.41 of biomass to yield 1 kg of micro-algae oil; UMACC 352 requires 2.31 kg of biomass ± 0.13 to yield 1 kg of micro-algae oil; UMACC 348 requires 3.74 kg ± 0.70 of biomass to yield 1 kg of micro-algae oil; UMACC 345 requires 3.28 kg ± 0.53 of biomass to yield 1 kg of micro-algae oil; and lastly, UMACC 346 requires 2.55 kg ± 0.13 of biomass to yield 1 kg of micro-algae oil. From this tabulation, Table 5 can be calculated by determining the amount of micro-algae biomass required to have 1.67 kg of micro-algae oil to yield 1 kg of jet fuel.

| Type         | For 1 kg of micro-algae oil | For 1 kg of jet fuel (via HEFA) |
|--------------|----------------------------|---------------------------------|
| UMACC 326    | 2.50 kg ± 0.23             | 4.18 kg ± 0.38                  |
| UMACC 344    | 2.48 kg ± 0.41             | 4.14 kg ± 0.68                  |
| UMACC 352    | 2.31 kg ± 0.13             | 3.86 kg ± 0.22                  |
| UMACC 348    | 3.74 kg ± 0.70             | 6.25 kg ± 1.17                  |
| UMACC 345    | 3.28 kg ± 0.53             | 5.48 kg ± 0.89                  |
| UMACC 346    | 2.55 kg ± 0.13             | 4.26 kg ± 0.22                  |
For the highest lipid containing micro-algae species, UMACC 352, it requires 3.86 kg ± 0.22 to yield 1 kg of jet fuel via the HEFA process. This means that to yield 1 kg of jet fuel, it would take approximately 17,300 L of micro-algae culture. It was mentioned that to meet the 2% alternative jet fuel, Malaysia would require 60 million litres of alternative jet fuel. Kerosene Jet A-1 having a density between 775 – 840 g/L [7], this would mean between 46.5 to 50.4 million kgs or 46,500 – 50,400 tons of alternative fuel. Ultimately this would equate to Table 6.

Table 6. Calculation of micro-algae biomass required per year to meet the expected demand of 2% alternative (bio-) jet fuel

| Type      | For 1 kg of jet fuel (via HEFA) | 2% Bio-jet Fuel for Malaysia |
|-----------|---------------------------------|-----------------------------|
| UMACC 326 | 4.18 kg ± 0.38                  | ca. 194 – 212,000 tons     |
| UMACC 344 | 4.14 kg ± 0.68                  | ca. 192 – 225,000 tons     |
| UMACC 352 | 3.86 kg ± 0.22                  | ca. 179 – 189,500 tons     |
| UMACC 348 | 6.25 kg ± 1.17                  | ca. 290 – 345,000 tons     |
| UMACC 345 | 5.48 kg ± 0.89                  | ca. 254 – 296,000 tons     |
| UMACC 346 | 4.26 kg ± 0.22                  | ca. 198 – 208,000 tons     |

For the highest lipid containing micro-algae species, UMACC 352, it requires 179,000 to 189,500 tons of biomass per year to meet the expected 2% alternative fuel (60 million litres) for Malaysia’s consumption. For the lowest lipid containing micro algae species, UMACC 348, it requires 290,000 to 345,000 tons of micro-algae biomass per year. Typically, open pond systems are the easiest and low cost way of mass production of micro-algae. Conventionally, open ponds have a productivity of 30 – 50 ton/ha/year [22], using the data obtained from the selected micro-algae strain the following can be calculated. From Table 7, it is evident that the land size required is comparatively large. Nonetheless, compared to the entirety of Malaysia, this accounts to roughly 0.1 – 0.2 % of its land mass.

Table 7. Calculation of micro-algae biomass required to yield 1 kg of jet fuel via the HEFA process

| Type      | 2% Bio-jet Fuel for Malaysia | Approximate Land Size Required (ha) |
|-----------|------------------------------|-------------------------------------|
| UMACC 326 | ca. 194 – 212,000 tons       | 4,300 – 4,720                       |
| UMACC 344 | ca. 192 – 225,000 tons       | 4,280 – 4,990                       |
| UMACC 352 | ca. 179 – 189,500 tons       | 3,990 – 4,210                       |
| UMACC 348 | ca. 290 – 345,000 tons       | 6,450 – 7,660                       |
| UMACC 345 | ca. 254 – 296,000 tons       | 5,660 – 6,570                       |
| UMACC 346 | ca. 198 – 208,000 tons       | 4,400 – 4,620                       |

5. Conclusions
Malaysia has one of the highest air traffic movements in the South-East Asian region, and the need to have a blend of bio-jet fuel with conventional Kerosene Jet A-1 is just a matter of time. Having a rich biodiversity is a key enabler for Malaysia to have biomass waste to wealth economies. However, the conversion of biomass waste to bio-fuel has still yet to gain much traction in Malaysia due to many competing factors. Micro-algae is poised to alleviate certain disadvantages seen in first generation and second generation feedstock. Potential of Malaysia’s micro-algae has been determined as a possible feedstock to enable bio-jet fuel production. UMACC 326, UMACC 344, UMACC 352, UMACC 348, UMACC 345, and UMACC 346 were selected from 79 samples collected over 30 sites throughout Malaysia. These species were selected based upon their high biomass productivity and lipid content. Their lipid contents were then used to derived the require amount of micro-algae biomass to yield 1 kg of certifiable jet fuel via the HEFA process and subsequently the amount of micro-algae biomass
required to meet a scenario where Malaysia implements a 2% alternative (bio-) jet fuel requirement. Finally, the total amount of land size required to sustain a micro-algae cultivation plant that can cater to Malaysia's jet fuel consumption for a 2% alternative (bio-) jet fuel blend is also estimated.

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