concentration throughout the study while there was no significant difference in the concentration of *Chlorella* sp. (Figure 10). The establishment of microalgae in the macro–micro cultures despite prior harvest through centrifugation and filtration could be due to the presence of autosporing produce by both *Chlorella* and *Scenedesmus* sp. which are much smaller than parent cells (Hoek et al., 1995; Sharma, 1986; Yamamoto et al., 2004).

![Figure 10](image.png)

**Figure 10**: Cellular concentration of the microalgae consortium co-cultivated with *Cladophora* sp.

Overall, the co-cultivation of the macroalgae, *Cladophora* sp. and the microalgae consortium together is a single growth vessel under controlled conditions was found not viable as it resulted in the death of the macroalgae over time. Moreover, similar outcomes were also observed for *Cladophora* grown in post-harvest ADPE that were initially without any microalgae. Overtime, microalgae grew and overtook the cultures resulting in the decline of the macroalgae.
3.2 Outdoor co-cultivation of microalgae and macroalgae using an inclined reactor system.

The previous indoor study clearly showed that the co-cultivation of macroalgal and microalgae to be ineffective and unsustainable due to the dominancy of microalgae which resulted in the decline of the macroalgae cultures. Based on this outcome, an outdoor inclined reactor was customized to evaluate the potential use of attached macroalgal culture as a way of scrubbing the nutrients from ADPE post microalgal treatment. This inclined reactor was designed and operated as a flow-through system whereby a continuous and constant flow of ADPE (with and without microalgae) was channeled through cultures of macroalgae that were attached onto filter mats and positioned from the start to the end of the reactor (Figure 11). In this flow-through system, ADPE (with or without microalgae) at desired concentrations were positioned in storage tank at the start of the inclined reactor and was allowed to flow (Figure 11-D) through encapsulated chambers of the reactor containing filter mats that hosted the macroalgae consortium (Figure 11-E). The aim of this continuous flow-through system was to improve the interaction of both these organisms (e.g. light availability), reduce contact period between both groups of algae and to maximize the nutrient removal efficiency of ADPE. Flow-through set up such as this allow for the characterization of effluent entering and exiting the system at a certain flowrate that can be used to maximize nutrient removal rates on a given area as well as the chronological sequence of events.
Figure 11: The customized inclined reactor setup for the co-cultivation of macro- and micro-algae: A) compartments to house the macroalgae filter mats and culture, B) baffles separating each macroalgae compartment and holes in the middle to allow the flow of ADPE, C) shade cloth used to cover compartments, D) storage bucket and tap to store the ADPE, E) macroalgae consortium attached to the filter mats and F) fully functional inclined reactor system.
3.2.1. Environmental conditions

As this study was conducted under natural climatic conditions, the influence of prevailing and fluctuating environmental parameters have to be taken into account. Daily solar radiation during daylight hours of the cultivation period ranged between 2 and 1178.4 W m$^{-2}$ while the daily ambient air temperature was between 5.6 and 33.2 °C (Figure 12). Decrease in solar irradiance correlated with a decrease in ambient temperature, most likely due to cloudy weather and rain (Figure 12). The maximum rainfall recorded during the study was 2.75mm (Figure 12).

High solar irradiance (up to 1178.4 W m$^{-2}$) and temperature (up to 33.2 °C) was expected and unavoidable as this study was carried out during the Austral season of summer. Thus, to reduce the occurrence of photoinhibition, shade cloths reducing approximately 50% of incoming solar irradiation was used to cover the macroalgae cultivation compartments (Figure 11-C) (Häder et al., 1998).

In order to evaluate the growth viability of the isolated macroalgae consortium (Spirogyra sp. and Rhizoclonium sp.) on ADPE and the nutrient removal efficiency of the inclined system, the macroalgae consortium was initially grown on different concentrations of ADPE ranging from 10 – 55 mg L$^{-1}$ of NH$_4^+$. It is important to note that during the first week of the experiment, a significant decline in the biomass of the macroalgae consortium was observed. This decline in biomass is believed to be due to the death of a significant portion of Spirogyra sp. which has been previously shown to be intolerant to even low concentrations of ADPE. Nevertheless, biomass yield of the macroalgae improved during subsequent weeks of cultivation under the increasing concentration of ADPE. Due to the initial decline in biomass, the progress of the macroalgal cultures in ADPE was only evaluated through photo-physiology and nutrient removal measurements.
Figure 12: The average daily rainfall, solar irradiance and ambient air temperature during the cultivation period
Table 4: Nutrient removal rates of the macroalgae consortium when cultivated in different concentrations of ADPE. The same letter above value indicates no significant differences (one-way repeated measures ANOVA P > 0.05)

<table>
<thead>
<tr>
<th>Ammonium Concentration (mg NH₄⁺ L⁻¹)</th>
<th>Ammonium Removal Rates (mg NH₄⁺ L⁻¹ m⁻²)</th>
<th>Total Phosphate Removal Rates (mg PO₄³⁻ L⁻¹ m⁻²)</th>
<th>Nitrate Removal Rates (mg NO₃⁻ L⁻¹ m⁻²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADPE 10</td>
<td>6.5 ± 0.8ᵃ</td>
<td>2.0 ± 0.3ᵃ</td>
<td>18.2 ± 6.3ᵃ</td>
</tr>
<tr>
<td>ADPE 15</td>
<td>7.5 ± 0.3ᵃ</td>
<td>1.6 ± 0.5ᵃ</td>
<td>29.8 ± 5.1ᵃ</td>
</tr>
<tr>
<td>ADPE 25</td>
<td>11.5 ± 0.9ᵇ</td>
<td>2.1 ± 0.4ᵃ</td>
<td>67.0 ± 7.2ᵇ</td>
</tr>
<tr>
<td>ADPE 40</td>
<td>15.5 ± 1.2ᶜ</td>
<td>1.6 ± 0.3ᵃ</td>
<td>193.1 ± 11.8ᶜ</td>
</tr>
<tr>
<td>ADPE 55</td>
<td>22.6 ± 1.1ᵈ</td>
<td>2.9 ± 0.4ᵇ</td>
<td>218.5 ± 17.0ᶜ</td>
</tr>
<tr>
<td>ADPE 55 with microalgae consortium</td>
<td>11.2 ± 2.4ᵇ</td>
<td>3.6 ± 0.4ᵇ</td>
<td>118.2 ± 22.4ᵈ</td>
</tr>
</tbody>
</table>

Table 4 highlights the nutrient removal efficiency of the macroalgal consortium per amount of cultivated area of the inclined reactor under the different ADPE concentrations. The ammonium removal rates varied from 6.5 ± 0.8 to 22.6 ± 1.1 mg NH₄⁺ L⁻¹ m⁻² and was found to be significantly higher at ADPE concentration of 55 mg L⁻¹ of NH₄⁺ (Table 4). Therefore, ADPE with 55 mg L⁻¹ of NH₄⁺ was selected for the experiment for scrubbing nutrients from the microalgae consortium. However, the ammonium removal rates of ADPE 55 with microalgal consortium (11.2 ± 2.4 mg L⁻¹m⁻²) was significantly lower than those recorded for ADPE 40 and ADPE 55 without microalgae (Table 4).

In terms of phosphate removal rates, no significant differences were observed between values recorded for ADPE 55 (2.9 ± 0.4 mg PO₄³⁻ L⁻¹ m⁻²) and the ADPE 55 with microalgae (3.6 ± 0.4 mg PO₄³⁻ L⁻¹ m⁻²). Nitrate removal rates trended higher with an increase in ADPE concentration where no significant differences were observed when ADPE 40 (193.1 ± 11.8) and ADPE 55 (218.5 ± 17.0) were used (Table 4). However, values were significantly lower when ADPE 55 with microalgae was used (Table 4). Above results indicates a decline in
physiological status and nutrient removal efficiency of both the algal groups when integrated together.

The distribution and photo-physiology of the microalgae consortium at different lengths (compartment) of the inclined reactor was evaluated during the co-cultivation study to identify the interaction between the microalgae and macroalgae cultures. As illustrated in Figure 13, there was a significant decrease in the $F_{q'}/F_{m'}$ values of the microalgae cultures as it travelled from the storage container (initial) through four inclined reactor compartments. Initial $F_{q'}/F_{m'}$ values of cultures in the storage bucket was $0.51 \pm 0.04$ while values of cultures exiting the 4th compartment of the reactor decreased to $0.14 \pm 0.04$ (Figure 13).

The decline in photo-physiology of the microalgae consortium was in line with the loss of biomass as cultures moved through the inclined reactor. There was approximately 73.5% reduction in the organic biomass of cultures from the storage bucket ($0.67 \pm 0.03 \text{ g L}^{-1}$) to that recorded at the end of the 1st compartment ($0.18 \pm 0.01$) (Figure 13). This loss in biomass was observed to be primarily caused by the attachment of microalgae cells to macroalgae filaments and also onto the sponge filter itself (Figure 8). We also observed microalgal attachment to macroalgae in our indoor study (see section 3.1.2). There was approximately $90.4 \pm 3.8 \%$ and $93.1 \pm 2.1 \%$ reduction in the microalgal cell density of *Chlorella* and *Scenedesmus* sp. respectively between the storage container and the 4th compartment of the reactor (Figure 13).
Figure 13: The cellular composition, biomass yield and photo-physiology of the microalgae consortium at the different sampling compartment of the inclined algae reactor. The same letter above each column indicates no significant differences (one-way repeated measures ANOVA $P > 0.05$)
Figure 14: Photo-physiology of the macroalgae consortium during the different treatments. The same letter above each column indicates no significant differences (one-way repeated measures ANOVA $P > 0.05$)

Figure 14 summarizes the $F'_q/F'_m$ values of the macroalgae consortium during growth in the different concentrations of ADPE and also during the co-cultivation with microalgae. Despite the continuous increase in ammonium concentration from 10 to 55 mg L$^{-1}$, no significant difference in $F'_q/F'_m$ value were recorded for the macroalgae consortium when grown by itself (Figure 14). However, values were found to be significantly lower during the co-cultivation study (macro + micro), indicating an immediate decline in the physiological status of the macroalgae. The decline in macroalgae photo-physiology is believed to a direct result of shading brought forward by the formation of microalgae layers that covered the surface of the
cultivation area (Figure 15). The presence of microalgae layers above the macroalgae cultures also explains the loss of microalgae biomass as it travelled through the inclined reactor due to the attachment and entrapment of cells in these biological layers (Figure 13).

Figure 15: Shading of macroalgae caused by free floating microalgae on the surface of the co-cultivation compartments of the inclined reactor.

The inverse co-relationship and inability of micro- and macro-algae to grow together is most certainly a result of competition for space, nutrients and availability of light between both algal groups (Armitage et al., 2005; Huisman et al., 1999; Smith & Horne, 1988). This was evident in this study as not only the photo-physiological of both the micro- and macro-algae were
negatively affected during co-cultivation but also ammonium removal rates were found to be significantly lower than that of macroalgae cultures grown by itself in ADPE (Table 4). Despite morphological variation, different algae groups still essentially require the same set of nutrients, which they obtain from the surrounding environment (Smith & Kalff, 1982). Faster growing microalgae are well documented for their ability and efficiency in utilizing available nutrient and light for unlimited growth. In general, microalgae have been shown to be more efficient in acquiring nutrients such as phosphate and also nitrogen (at elevated concentrations) from the external environment when compared to macroalgae (Nan & Dong, 2004; Smith & Kalff, 1982). Contrasting outcomes regarding the competitive dominance of different algal groups have been reported at different nutrient availabilities. For example, Pedersen and Borum (1996) reported the superiority of microalgae over macroalgae at high nutrient availability and the dominancy of macroalgae over microalgae at lower nutrient concentration whereas the contrary have been reported by Fong et al. (1993a) and Fong et al. (1993b).

3.3 General Discussion

Classical ecology theories on competition predicts that, under idealized conditions, the one species capable to best acquire and use available limiting resources can significantly determine the distribution of natural populations by displacing all other competing species (Roughgarden, 1983; Titman, 1976). Similarly, under our controlled laboratory conditions, such competitive displacement was observed in the co-cultivated algal cultures and is believed to be a result of inter- actions between both groups of algae. The strong inverse competition for nutrients and available resources significantly favoured the smaller unicellular microalgae cells with improved surface area to volume ratios over the larger macroalgae in all our experimental conditions (Grover, 1989; Stolte & Riegman, 1995). Studies looking into the natural
distribution of macroalgae and smaller phytoplankton have also reported negative correlation between the abundance of both groups that varied spatially and seasonally (Fong et al., 1986, Rudnicki et al., 1986). Moreover, correlative evidence on competition between different algal groups in shallow semi-enclosed systems have been established for groups that remain in proximity for an extended period of time. The outcome observed in this study during the co-cultivation of micro- and macro-algae is on par to that of pelagic and benthic algae inhabiting shallow water bodies and competing for available limiting resources (Flöder et al., 2006; Pasternak et al., 2009). Pelagic algae are generally dissolved (floating) in water columns while benthic algae are typically distributed on the bottom/bed of the waterbody. In general, pelagic algae are more efficient in utilizing dissolved nutrients in the water column for propagation of biomass which in turn can negatively affect the distribution and availability of light for benthic algae at the bottom (Flöder et al., 2006; Pasternak et al., 2009). Prolonged dominance of pelagic algae in eutrophic rich systems over benthic algae has been shown to result in the loss of benthic communities even in shallow lakes (Flöder et al., 2006; Vadeboncoeur et al., 2003).

Especially when competing for light, these local interactions of both these organism are observed to be inversely correlated as the organisms closest to the light source are seen to be more profitable dominant. This was evident in our studies as the smaller microalgae cells suspended in the ADPE and positioned closer to the light source was able to harvest and utilize incident light more successfully than the ‘heavier’ macroalgae cultures which sank to the bottom of the Erlenmeyer flasks or were attached to the filter mats of the inclined reactor. Furthermore, shading brought forward by the increase in microalgae biomass and also the prevailing turbidity of the ADPE was seen to further significantly limit the availability of light for the macroalgae cultures (Figure 15).

Moreover, the epiphytic behaviour of the microalgae adhering closely on the macroalgae consortium is also believed to have been major factor toward the decline of the macroalgae
culture as similar outcomes on microalgal epiphytism have been observed to potentially kill crustose forms (Steneck, 1982).

**4.0 Conclusion**

Through this study and previous Pork CRC funded projects, we have successfully isolated and demonstrated the ability of local species of microalgae and macroalgae that were capable of growth and efficient nutrient removal in various concentrations of ADPE when cultured separately. In order to further improve the efficiency and economics of ADPE phytoremediation, we evaluated the viability of co-cultivating both micro- and macro-algae together in ADPE in this work. Nevertheless, despite multiple different approaches and cultivation systems, both algal groups were unable to co-exist for efficient growth in ADPE due to direct completion for available resources and the negative interaction of both algal groups. From the work carried out here, it is evident that a strategy can be adopted to first grow microalgal consortium on untreated ADPE using open ponds. Microalgae could be used to reduce the ADPE concentration down to a range suitable for macroalgae (50 to 150 mg NH$_3$ L$^{-1}$). At this concentration, microalgae grown in ADPE could be potentially scrubbed and harvested using attached or floating macroalgae for the collection of biomass.

There is surprising very little information and experimental evidence available in the literature highlighting the interaction and competition between both these groups of algae. Through this work, we have also briefly highlighted the detrimental changes brought upon to the integrated algal groups due to resource competition and their respective interactions in ADPE. Nevertheless, we believe the outcome of competition identified in this study may change according to available resources and algal species.
5.0 Recommendations

Based on the outcome of this study, our main recommendations to industry are as follow:

1) This study highlights the ineffectiveness of co-cultivating microalgae and macroalgae together in ADPE.

2) It is more efficient and viable to either cultivate microalgae by itself in undiluted or macroalgae in diluted ADPE for the bioremediation of ADPE and for biomass production.

3) Macroalgae biomass can be used as a cost effective harvesting way for harvesting microalgae grown in ADPE.

4) There may be alternative methods to for co-cultivation systems to integrate the cultivation of microalgae and macroalgae in ADPE for efficient bioremediation and biomass production. These needs to be tested.

5) Evaluation of more efficient methods of removing/harvesting microalgae from ADPE for subsequent use of effluent for the cultivation of macroalgae.


