Evaporation reduction and salinity control in microalgae production ponds using chemical monolayers

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Abstract

Algae have great potential as a hyper-productive crop to produce food, fuels, and chemicals. However, freshwater availability limits their widespread application. Here we investigate whether chemical monolayers can reduce evaporation in microalgae cultures, and whether algal growth is affected. Thin-film monolayers were formed on the surface of freshwater (*Chlorella vulgaris*) and marine (*Nannochloropsis salina*) algae cultures using ethylene glycol monooctadecyl ether. Monolayers applied daily reduced evaporation in both cultures by 70% on the first day, and ~50% by day 3. The cause of the reduced performance was investigated but could not be directly attributed to any particular cellular activity or chemical change. *Nannochloropsis* was uninhibited by the monolayer, while the growth of *Chlorella* decreased by 38% over 3 days. There was no evidence that the monolayer reduced gas exchange (CO_2/O_2), but the reduced growth of *Chlorella* could have been caused by direct chemical inhibition by the monolayer or the slightly elevated temperature (1–2 ºC) resulting from the reduction in evaporative cooling. A techno-economic analysis indicated that water savings could make monolayers economically beneficial, especially in arid climates suited to algae production. In addition, monolayers enable control of salinity in marine production systems. Overall, the application of monolayers to reduce evaporation from outdoor algae cultures has great promise, with testing in outdoor trials an obvious next step.

Keywords: Microalgae production; monolayer film; evaporation reduction; *Chlorella vulgaris*; *Nannochloropsis*; biofuel.

1. INTRODUCTION

Microalgae are currently grown commercially at a limited scale to produce high-value antioxidant pigments and health food supplements. In the future, microalgae have great promise for mass production of bulk commodity products such as animal and aquaculture feed, biofuel, and chemical feedstocks owing to their ability to be cultivated in low-cost open ponds on non-arable land at much greater productivities than terrestrial plants. Cultivation of algae at such a large scale could provide major environmental benefits in terms of land and nutrient utilisation. However, economically viable production of low-value commodity products from microalgae is a major challenge that requires further research efforts to reduce the cost of cultivation and biomass processing **[1]**. One of the major factors currently limiting mass production of microalgae is their requirement for water **[2]**. As microalgae are cultivated as highly dilute suspensions (0.5 – 1 g/L), very large amounts of water are required relative to the amount of biomass that is produced. While the overall demand for water can be reduced considerably by recycling the growth media following harvest of the biomass, evaporation from open pond systems represents a major and unavoidable loss of water. For example, it has been estimated that approximately 530 L of water is lost by evaporation per litre of microalgal lipid produced in a large-scale open pond system producing 4×10^6 L/year of algal oil **[1]**. Although different species of microalgae can grow in fresh water, seawater or brackish water, the addition of fresh water is always required to compensate for the water lost from evaporation to maintain salinity levels for optimum algal growth. This requirement for fresh water to compensate for evaporation effectively nullifies one apparent advantage of cultivating marine microalgae. In addition to the cost of the fresh water itself, there is a cost associated with pumping this make-up water into algal ponds **[3]**, and therefore a need for mass cultivation sites to be situated near an abundant source of fresh water, even if a marine alga is grown near the coast using seawater. Unfortunately, many of the locations otherwise most suited for mass

cultivation of algae (i.e., regions with an abundance of cheap, flat land with high solar radiation per unit area), often have limited supplies of fresh water **[4]**. Owing to these challenges, it is recognised that water consumption is a major bottleneck to large-scale algae production **[2]**. While much research has been devoted to reducing the water requirements for algal cultivation by water recycling **[5, 6]** and high-density cultivation **[7]**, it does not appear that anyone has yet tried to mitigate evaporation, which is seen as an inevitable consequence of cultivation using open systems **[2]**.

The aim of this study was to investigate for the first time, the use of chemical monolayers to reduce evaporation rates in open microalgae production systems. Beyond microalgae cultivation, evaporation is a practical issue in the context of water storage in reservoirs and dams **[8]**. As such, a number of strategies have been developed to reduce evaporation from freshwater storage bodies, including mechanical methods such as floating spheres and suspended covers **[9]**. While these systems can be quite effective at reducing evaporation, the capital cost can be high, particularly when applied over a large surface area. Even more importantly in the context of algae cultivation, these devices block solar radiation into the water body, which would thereby reduce photosynthetic algal growth. Therefore, to be applied in algal cultivation, any water evaporation technology must not reduce the light available to the algae. Based on this requirement, it is proposed here that optically transparent chemical monolayer films could be a promising method to reduce water evaporation for large-scale algae cultivation (Fig. 1). In the context of evaporation reduction, a ‘monolayer’ is a closely-packed, single-molecule thin film of amphiphilic compounds, which spreads spontaneously to cover a water surface **[10]**. Such monolayers have been widely investigated for freshwater storage applications, including in numerous field trials that demonstrate the potential to reduce water evaporation by as much as 40% **[8]**. In addition to their effectiveness and light transparency, advantageous features of monolayers for algae applications are the low capital costs and their

ability to be applied only when required, specifically in times of high evaporation. Various amphiphilic chemicals have been used in monolayer applications, including hexadecanol, octadecanol and ethylene glycol monooctadecyl ether (referred to as C18E1). As C18E1 has been shown to be particularly effective for evaporation reduction **[11]**, it was chosen for this study.

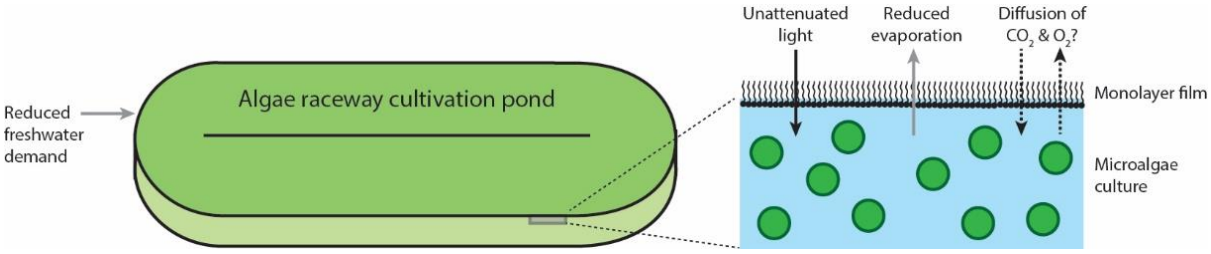


Fig. 1 Illustration of the proposed approach of applying a monolayer film to reduce evaporation from large-scale outdoor algae cultivation ponds.

The novel application of chemical monolayers to algae cultures presents new research questions that have not been addressed in previous studies for pure water applications such as reservoir preservation **[12]**. In particular, the aim of the current study was to investigate whether monolayers could successfully reduce evaporation in the presence of concentrated algae cultures, and conversely, whether the presence of a monolayer adversely affects the growth of algae. It is possible that the algae and associated bacteria could degrade the monolayer chemicals or interfere with the intermolecular packing to reduce performance. On the other hand, the monolayer could directly or indirectly inhibit algal growth. For instance, as photosynthetic organisms, microalgae assimilate atmospheric CO_2 and release oxygen during photosynthesis. The exchange of gaseous CO_2 and oxygen between the atmosphere and culture is important, in particular to facilitate photosynthesis and to avoid the build-up of dissolved oxygen which can be toxic to algae **[13, 14]**. In this respect, one important physical effect of monolayers is an increase in the boundary layer thickness at the air/water interface, which could decrease the rate of gas diffusion **[8]**.

The present study investigates the evaporation reduction performance and potential impacts of C18E1 monolayers during the growth of both a marine and freshwater algal species (*Nannochloropsis salina* and *Chlorella vulgaris*, respectively) of industrial relevance. The economic feasibility of using monolayers to control evaporation in algal ponds is also investigated and a sensitivity analysis is applied to explore the impact of variations in water price and climate on the cost savings. The results from the present study provide insights into a novel application of monolayer films as an innovative means for reducing water requirements for commercial algal cultivation.

2. MATERIALS AND METHODS

2.1 Preparation of algae seed cultures and chemical monolayer

A seed culture of *Nannochloropsis salina* (CCMP 1776, obtained from the University of Melbourne Culture Collection) was grown in an aerated 2 L Schott bottle containing 1500 mL of f/2 medium **[15]** at 21 ± 2 °C under a light:dark cycle of 14:10 h with a light intensity of 60 – 70 µmol photons m^{-2} s^{-1}. Similarly, a seed culture of a freshwater species, *Chlorella vulgaris* (obtained from CSIRO, Australia), was grown in 1500 mL of MLA medium[16]. The nitrogen source was sodium nitrate for both f/2 and MLA media, provided at a concentration of 0.1 g/L or 0.17 g/L, respectively. The molar concentrations of the individual nutrient stock solutions of f/2 and MLA media are provided in Supplementary File S.1-a.

Monolayer solutions were prepared by dissolving ethylene glycol monooctadecyl ether (C18E1) in chloroform (RCI Labscan Ltd) at a final concentration of 10 mg/mL, which was applied to the surface daily unless specified to form a film.

2.2 Experimental set-up

Evaporation experiments (with or without monolayer) were conducted in duplicate algae cultures grown in open round glass dishes (diameter = 11.5 cm, height = 6.5 cm). For this, the

algae seed cultures (Section 2.1) of *N. salina* and *C. vulgaris* were centrifuged at 2000 g for 5 min and the resulting pellets were resuspended into 100 mL of fresh f/2 or MLA media, respectively to obtain an initial biomass concentration of 0.16 g/L or 0.13 g/L, respectively. These 100 mL cultures were then added to the open round glass dishes, which were placed on individual digital balances (Mettler-Toledo Limited). These algae cultures were then grown for 3 days under constant light (24:0 h) with an intensity of approximately 70 μmol photons m^{-2} s^{-1}. At the start of each experiment, 36.4 μL of the monolayer solution was added to the test cultures, and this addition was repeated daily over the course of the experiment. Parallel duplicate cultures without monolayer films were included as controls. The experimental design is depicted in Supplementary File S.1-b.

2.3 Evaporation analysis

Evaporation rates were measured gravimetrically by recording the mass loss from the cultures over time (the cultures remained in place on individual digital balances during the 3-day cultivation period). In the cultures with monolayer film, the mass was recorded automatically every minute using a weight reading program (BalanceLink®). Due to a limited availability of automatic recording, the mass loss from cultures without monolayer (i.e., the control cultures) was manually recorded intermittently during the cultivation period. To reduce the cumulative concentrating effect of evaporation, deionised water was added daily to replenish the water lost due to evaporation. Immediately following the daily water replenishment, 36.4 μL of the monolayer solution was reapplied. Subsequently, the average daily evaporation rates in the control and monolayer cultures were calculated.

A separate evaporation analysis (with or without monolayer) was conducted using algal culture supernatants. Briefly, 400 mL of *N. salina* (in f/2 media) and *C. vulgaris* (in MLA media) cultures were grown for 3 days in 225 cm^2 Corning flasks having 0.2 μm vented caps, under

the same growth conditions as those described above and shaken at 100 rpm using an orbital shaker. On day 3, *N. salina* and *C. vulgaris* cultures were centrifuged at 2000 g for 5 min and each of the obtained supernatants (i.e. used f/2 or MLA media) was divided into four and placed in open round glass dishes (duplicate controls and monolayer dishes), which were placed on individual digital balances for determination of the evaporation rate. As with the algae culture evaporation experiments, monolayer was reapplied daily over the 3 day experiment.

2.4 Measurement of algal growth and culture pH, temperature, and dissolved oxygen

To monitor algal growth, culture samples (3 mL) were collected daily and the absorbance at 750 nm measured using a Cary 3E UV-Vis spectrophotometer (Agilent Technologies, Australia). The absorbance was converted into dry weight biomass concentrations (g/L) using a standard curve prepared separately for each species. As the absorbance was measured prior to replenishing the evaporated water, the biomass concentrations were adjusted based on the amounts of water evaporated in the monolayer and control dishes. Also prior to replacement of evaporated water, the culture pH (Metler-Toledo InLab® Science), dissolved oxygen concentration (LDO^{TM} probe, HQ40d, Hach) and temperature (infrared thermometer) were measured periodically. Results are presented as the average of biological duplicate culture dishes.

2.5 Techno-economic analysis

2.5.1 Net cost savings calculations and assumptions

Calculations of cost savings resulting from the use of monolayer films were based on the cost of purchasing replacement water and associated pumping costs. The cost associated with pumping the replacement water was based on the economic evaluation by Rogers et al. **[3]** ($2.07 million/year, for 5 mm evaporation/day over an area of 4875 ha, which works out to be $0.23/(year.ha.mm)). Water demand was based on the amount of evaporation (mm/year) with

and without the film, as determined experimentally in this study. Estimates of the evaporation rate were obtained for different climate regions from Guieysse et al. **[17]**. The different regions used in this study were 'Tropical', 'Sub-tropical', 'Temperate', 'Mediterranean', and 'Arid' climates. The allocated rate of process water disposal (3230 mm/year) and the leak loss (860 mm/year) were also obtained from Guieysse et al. **[17]**. For the cost of the monolayer, a price of $10 per kg was used in the calculations at the same loading rate as the lab trials (388 g/(ha.day)) based on the total surface area of the water. Variation in water depth will not impact the amount required, as the monolayer forms on the water surface. Chloroform was used in these small-scale experiments to improve the spreading of monolayer film and to facilitate accurate application of small amounts. Chloroform would not be used in field trials or large-scale applications, with the monolayer added directly to the surface in these cases. Therefore, chloroform is not considered in the techno-economic analysis. For economic calculations, an inflation rate of 1.5% was assumed.

2.5.2 Salt concentration analysis and assumptions

An analysis of the effect of monolayer evaporation reduction on the salinity of marine algae growth ponds was performed using *Nannochloropsis* as a reference marine alga, where 35 g/L is the optimum salinity for growth and 45 g/L was used as the upper range for growth **[18, 19]**. The algae media solution was assumed to be withdrawn at a rate of 10% of the pond volume per day to balance new algae growth with algae removed to maintain the concentration of algae at 0.5 g/L **[3]**. The algae stream was then concentrated via a two-stage process going through a clarifier where it was concentrated to 30 g/L **[3, 18]**. This stream was then concentrated to 200 g/L via a centrifuge. The water removed was then collected and sent back to the pond with a fraction being purged at varying rates. Salt water was used as the inlet water supply to make up for water lost to evaporation and removed with the algae. This was set at 35 g/L in the simulation unless otherwise stated.

2.5.3 Impact of evaporation suppression on pond temperature

Calculations of the increase in pond temperature due to the reduced evaporation by the monolayer were performed on the basis of a pond with a depth of 30 cm. Energy lost from the system through evaporation was calculated based on the latent heat capacity of water at 25 °C (2442 kJ/kg) and the difference in evaporation through use of the monolayer. The corresponding temperature change was calculated based on the equivalent energy embedded in water at 25 °C with a specific heat capacity of 4.19 kJ/(kg.K).

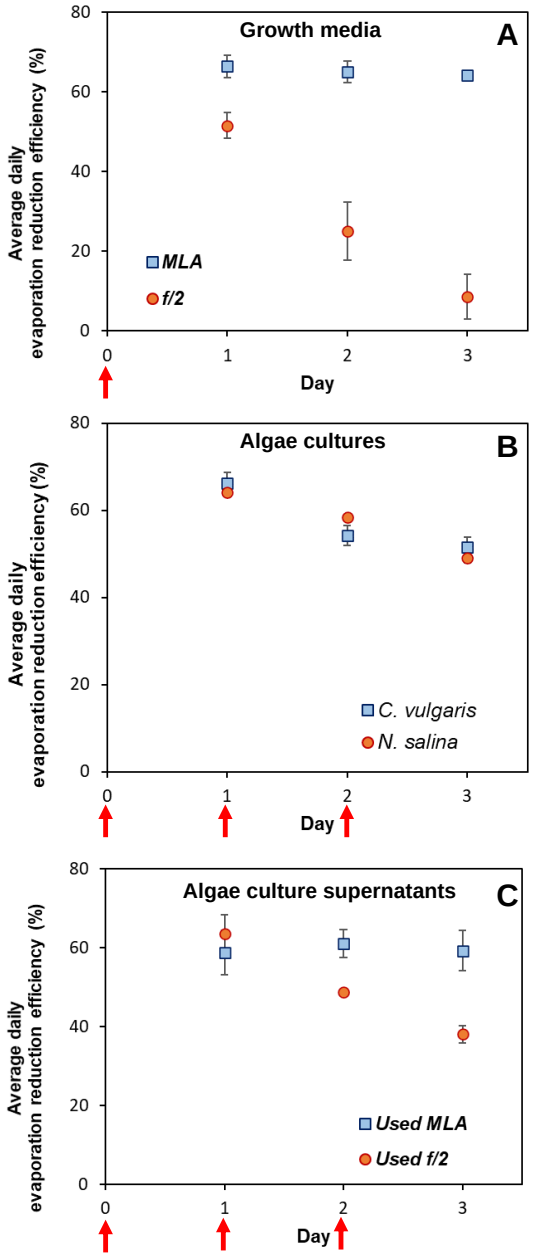
3. RESULTS AND DISCUSSION

3.1 Evaporation reduction in fresh water and marine algae cultures

While chemical monolayers are usually applied to fresh water, monolayer dissociation can be affected by environmental conditions such as pH, temperature, salt type and concentration **[20]**. As such, the evaporation reduction performance of a single dose of C18E1 monolayer was first tested on freshwater and marine algae growth media in the absence of algae cells to determine the influence of salt and media components (Fig. 2A). In the freshwater medium (MLA), evaporation was reduced by approximately 66% without decline over the three days of testing (Fig. 2A), representing similar performance to that on deionised water (approximately 64%, data not shown). However, in the marine growth medium (f/2), evaporation performance progressively declined from >50% over the first day to <10% during the third day. The reduction in evaporation performance was not found to occur in the presence of either the sea salt (30 g/L) alone or with the other major medium components included individually (Supplementary File S.2), suggesting some complex interactions with the monolayer occurred.

To address this issue in algae cultures, the monolayer was replenished each day, consistent with standard practice in large-scale water reservoir applications **[21]**. As discussed later, daily

228 reapplication of a monolayer is economically feasible. The reduction in water evaporation in
229 freshwater *C. vulgaris* and marine *N. salina* cultures was tested, with the average daily
230 evaporation rate measured over three days (Fig. 2B). The impact of the monolayer on algal
231 growth was investigated in parallel (Fig. 3). Overall, the monolayer film was found to
232 significantly reduce the evaporative water loss from both freshwater and marine algae culture
233 (by 50 – 70%) (Fig. 2B), consistent with previously published results for pure water systems
234 **[22].** There was no apparent difference in evaporation performance between the two cultures,
235 despite the different algae and the different longer-term performance of the freshwater and
236 marine media (Fig. 2A).



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238 **Fig. 2** Average daily evaporation reduction efficiency (percentage of evaporation prevented by
239 the presence of the monolayer relative to controls) in (A) freshwater (MLA) and marine (f/2)
240 growth media (blue square and orange circles, respectively), (B) *Nannochloropsis salina*
241 (orange circles) and freshwater *Chlorella vulgaris* (blue squares) cultures, and (C) cell-free,
242 used media (supernatants) from *Nannochloropsis salina* (orange circles) and freshwater
243 *Chlorella vulgaris* (blue squares) cultures. As indicated by the red arrows, monolayer solution
244 (36.4 µL; 10 mg/mL) was added at day 0, and for (B) and (C) reapplied on days 1 and 2. Results
245 are presented as the average and standard error of biological duplicate cultures.

While evaporation reduction remained high over three days for both cultures, there was an observable decrease in the reduction efficiency from 70% to 50% during this period. The decline in performance (Fig. 2B) despite daily replenishment of the monolayer on the surface of algal cultures could be due to various reasons. An increase in the concentration of algal cells during batch cultivation would result in more frequent collisions of the cells with the water surface, which could temporarily create localised interruptions in the film. However, a separate experiment using *N. salina* cultures at a range of initial biomass concentrations (0.12 – 0.57 g/L) showed a negligible effect of cell concentration on monolayer performance (data not shown). During batch cultivation, extracellular products released by algal cells could also accumulate, increasing the surface pressure **[23]** and reducing the packing density and performance of the monolayer film **[22]**. To investigate this, the performance of monolayer was tested in cell-free f/2 and MLA media (i.e., supernatants taken from *N. salina* and *C. vulgaris* cultures, respectively; without monolayer) (Fig. 2C). Interestingly, the *N. salina* supernatant resulted in a progressive decline in the evaporation reduction efficiency of the monolayer film, despite daily replenishment, while the impact of the *C. vulgaris* supernatant was negligible. These trends (Fig. 2C) are consistent with the differential impact of the fresh and marine growth media (Fig. 2A) rather than the cultures (Fig. 2B), suggesting that extracellular algae products cannot explain the diminishing monolayer performance in both cultures.

The results from the experiments using culture supernatants would also have been influenced by other culture-induced changes to the growth medium such as pH shifts or nutrient uptake. As such, these factors do not appear sufficient to explain the differential decline in monolayer performance in *N. salina* and *C. vulgaris* supernatants. However, interestingly monolayer performance in the first day was worse in the marine growth media (Fig. 2A) than in the *N. salina* supernatant (Fig. 2C), suggesting that some growth media components impair

monolayer packing and that these media components are reduced by the algal growth. The better performance of monolayer film with *N. salina* cultures (Fig. 2B) than in the corresponding supernatant (Fig. 2C) could also indicate a potential protective behaviour of algal cells in shielding the monolayer from these interfering components.

In the current study, the pH increased from approximately 8.5 to 10 and 10.5 in *N. salina* and *C. vulgaris* cultures, respectively (Fig. 3). While previously published literature has shown that the structure, phase behaviour, and function of monolayers with acidic head groups can be negatively impacted by high pH conditions such as pH 10 **[24-26]**, the monolayer used here (C18E1) does not contain an acidic head group and should not therefore be severely affected by pH. This was verified in a separate experiment in which a monolayer on f/2 media (free of algal cells) was adjusted to pH 8.5 and 10.5, with no apparent difference in the evaporation rates (data not shown). As discussed below, the presence of the monolayer slightly increased the temperature of the cultures (1–2 ºC) due to suppression of evaporative cooling. However, this does not appear to be a factor in the gradual decline in performance, as the elevation in temperature remained quite constant throughout the experiments.

It has previously been suggested that some chemical monolayer compounds such as hexadecanol and octadecanol could be degraded by bacteria **[10, 12]**, the rate of which could increase over time. However, microscopic analysis revealed no obvious increase in the abundance of bacterial cells in the cultures with monolayer film present (Supplementary File S.3), consistent with previously published field trials using C18E1 monolayer film **[23]**. Monooctadecyl ether-based monolayers have been previously shown to be at risk of photodegradation **[23]**, although only a very low light intensity (compared to sunlight) of 70 μmol photons m^{-2} s^{-1} was applied. In addition, the monolayer was replenished each day, meaning that the impact of bacterial or light degradation of monolayer films is not likely to

account for the decline in performance observed here. However, in environments with higher light intensities, the rate of photodegradation of the monolayer will likely be higher. Pittaway et al., demonstrated that photodegradation was a function of cumulative solar radiation and the concentration of components in the media (e.g. iron and aromatic compounds) **[12]**. The rate of addition of monolayer may need to be adjusted for these environments.

In summary, the reason for the decline in monolayer performance in both cultures over 3 days cannot be attributed to any obvious singular cause. Although the performance declined, the evaporation reduction was still around 50% after 3 days of cultivation, which is consistent with outdoor trials on pure water and highly significant from a practical standpoint as discussed below. The rate of decline is sufficiently slow in relation to periodic harvesting of the cells, which would remove interfering cell materials and thus restore monolayer performance. Nonetheless, detailed research into the direct interactions between algal cell materials and monolayers is recommended to improve stability and performance. In addition, given the decline observed over 3 days, it would be worthwhile investigating monolayer performance over prolonged periods in future studies, which are of relevance to extended batch cultivations and implementation of nutrient deprivation strategies for lipid accumulation.

3.2 Algae growth in the presence of monolayer

Similar experiments were used to assess the potential impact of the monolayer on algal growth (Fig. 3). For *N. salina* cultures grown in marine f/2 media, the biomass productivities in both the monolayer and control cultures were not significantly different over the 3-d growth period (75 ± 7 and 78.5 ± 0.3 mg/L/d, respectively). The pH increased from 8.5 to 9.8 in both the monolayer and control cultures (Fig. 3), due to the consumption of nitrate and CO_2 in the media. Monolayers have previously been reported to reduce oxygen diffusion (by 10 - 15%) **[27]**. Here, the dissolved oxygen concentrations were similar in the control and monolayer cultures,

319 at around 7.5 - 8 mg/L, indicating that the monolayer did not prevent oxygen egress from these
320 cultures. The lack of any appreciable effect of the monolayer on the pH and dissolved oxygen
321 concentration are consistent with the unimpaired productivity in these cultures.

322 The reduction in evaporation by the monolayers also reduces evaporative cooling, which can
323 increase the temperature of the water body **[28]**. The temperature in the monolayer cultures
324 (21.2– 24.2 °C) of *N. salina* was on average 1.1 ± 0.5 °C higher than the control cultures (21.2
325 – 23 °C), the impact of which on algal growth is likely negligible (Fig. 3), consistent with the
326 constant growth rate of the *Nannochloropsis* cultures at elevated temperatures **[29, 30]**.

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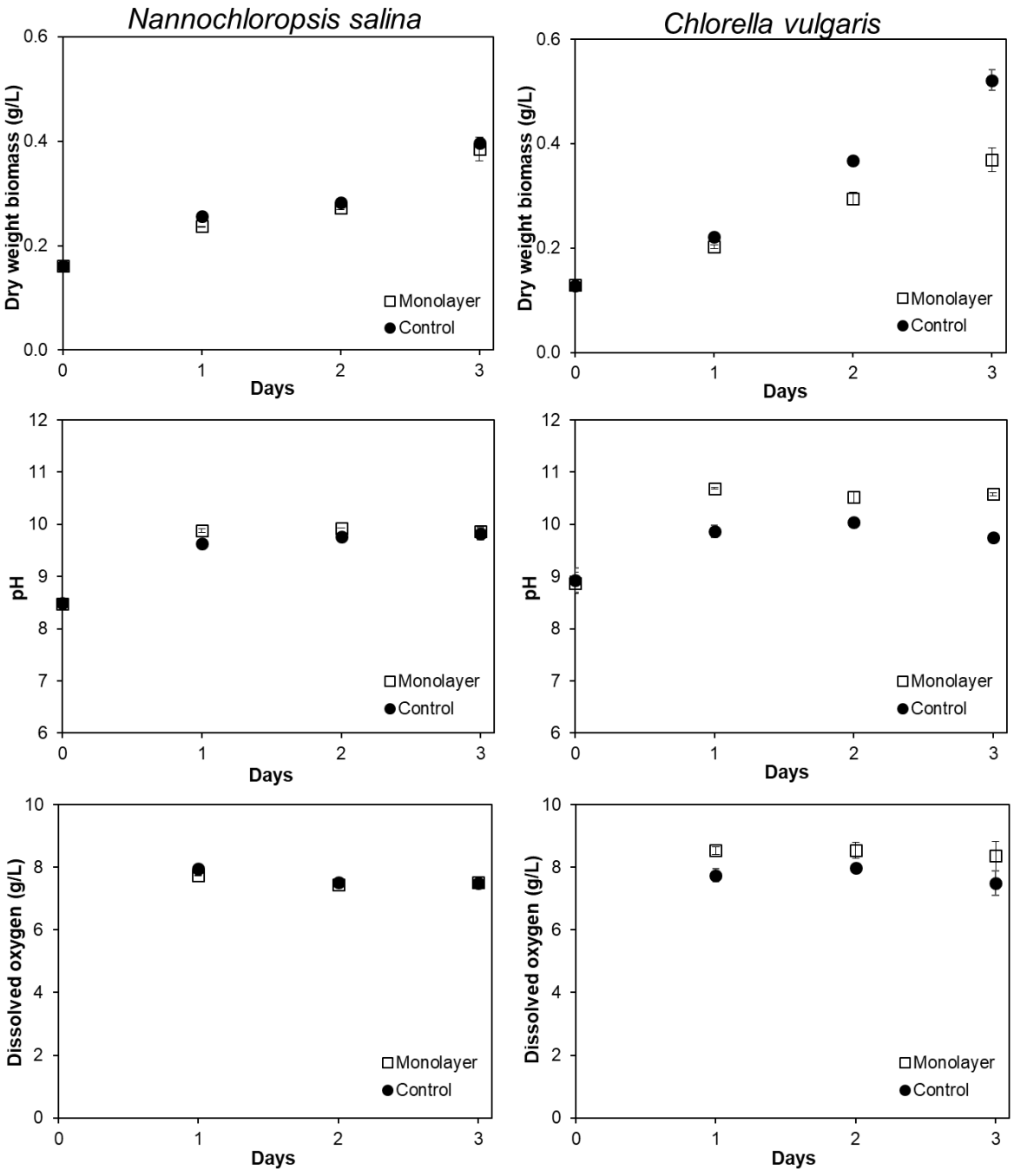


Fig. 3 Dry weight biomass, pH, and dissolved oxygen concentration in *Nannochloropsis salina* (left hand panels) and *Chlorella vulgaris* (right hand panels) cultures grown in open cultures for 3 days in the presence (monolayer; empty square) or absence (control; filled circle) of a monolayer film. All the parameters were measured before compensating for evaporated water. Biomass is normalised to the proportional water evaporation in the monolayer and control

cultures. Results are presented as the average and standard error of biological duplicate cultures.

Continual growth was observed in both the monolayer and control cultures of freshwater *C. vulgaris*; however, the biomass productivity in the control cultures was higher than that of the cultures containing a monolayer (131 ± 7 and 80 ± 8 mg/L/d, respectively). There are a number of possible reasons why *Chlorella* growth was negatively impacted by the presence of the monolayer while *Nannochloropsis* was not. Firstly, it is conceivable that *Chlorella* was directly inhibited by either the chloroform or the monolayer chemical, while *Nannochloropsis* was not. However, chloroform was only applied to the surface of the cultures in very small amounts (~36.4 μL on 100 mL cultures). As a water-immiscible and volatile solvent it would not have mixed into the cultures but rather would have been rapidly evaporated from the surface, preventing any possible inhibition of the algae. On the other hand, the monolayer was present for the duration of the experiments, and although predominantly on the surface, a proportion of the molecules could partition into the culture. The differential effect on the two algae indicates that if chemical inhibition was a factor, it is species specific. Direct chemical inhibition of algae will also be dependent on the chemical monolayer used, meaning this is a complex and important topic that warrants future investigation.

Considering other factors, the temperature of *C. vulgaris* monolayer cultures (22.6 – 24 ºC) was on average 1.4 ± 0.5 ºC higher than that of the control cultures (20 – 23 ºC), similar to the *N. salina* cultures. Although the growth of *Nannochloropsis* was evidently not affected by the small increase in temperature, some algal species like *C. vulgaris* (Fig. 2) have a strict temperature range, outside of which growth efficiency can drop significantly **[31]**. It is therefore possible that the increase in temperature of just a few degrees Celsius could have negatively impacted the growth rate of *Chlorella*. The ability to start and stop the application

of the monolayer provides a means of controlling evaporative cooling, and thereby influencing the culture temperature. For example, in cooler periods the monolayer could increase the temperature to enhance growth, whereas it may be preferable to not apply monolayer on extremely hot days to avoid reducing evaporative cooling and to keep the temperature within the preferred limits of the desired algae strain.

In the current experiments, the cultures were not aerated and relied solely on CO_2 uptake from the atmosphere. The pH increased from 8.9 to 9.8 - 10.2 in the *Chlorella* culture without monolayer and increased to >10.5 in the first 24 hours of cultivation with the monolayer. Meanwhile, the dissolved oxygen was approximately 8.5 mg/L and 8 mg/L in the monolayer and control cultures, respectively (Fig. 3). The slightly higher pH and decreased biomass productivity in the freshwater monolayer cultures could reflect a reduction in CO_2 mass transfer into the media caused by the monolayer, while the increase in dissolved oxygen could similarly result from a reduction in outgoing O_2 mass transfer. Decreased gas diffusion resulting from the presence of a monolayer has previously been proposed in freshwater systems **[8]**. To investigate whether the monolayer film reduced the rate of CO_2 mass transfer from the atmosphere, separate experiments were performed using a novel method based on the Wilbur Anderson assay (Supplementary File S.4). The results showed no reduction in the rate of CO_2 mass transfer due to the presence of monolayer film for either freshwater or marine growth media. The reduced growth rate of *C. vulgaris* (Fig. 2) in the presence of a monolayer cannot therefore be explained by a reduction in gas exchange. The most likely remaining factors appear to be direct chemical inhibition by the monolayer or a reduced growth rate due to the slightly elevated temperature resulting from reduced evaporation. Future research to understand the potential direct (e.g. chemical) and indirect (e.g. thermal) effects of this and other monolayer chemicals on *Chlorella* and other algae species is warranted. In particular, the current study showed that the ethylene glycol monooctadecyl ether monolayer may have

384 negatively impacted *Chlorella* but not *Nannochloropsis*, suggesting the value of further
385 research involving different combinations of monolayer chemicals and algal species.

386 **3.3 Techno-economic analysis of monolayer films for algae cultivation**

387 ***3.3.1 Impact of monolayers on water demand for large-scale algae cultivation***

388 The experimental results from this study show that a monolayer can reduce water evaporation
389 from algae ponds by 50 - 70%. This information was used to investigate the effect of this
390 reduction on the overall water demand in large-scale algae cultivation (including contributions
391 from evaporation, leak losses, and process water disposal **[17]**) in relation to climate. Assuming
392 a 60% reduction in evaporation it was found that monolayer films can reduce the overall water
393 demand of freshwater algae cultivation by 6 - 21% depending on the climate (Table 1), with
394 arid regions seeing larger benefits from using the monolayer. This is due to evaporation losses
395 making up a smaller percentage of the water demand (10.4%) in regions with low evaporation
396 rates and high rainfall, such as tropical climates, compared to arid regions (35.7%). The impact
397 in arid regions is likely to be further enhanced given higher water prices associated with lower
398 water availability. This is particularly relevant to algae cultivation, as warm, sunny, arid regions
399 are typically most suited for large-scale algae cultivation.

400 **Table 1** Comparison of the water demand of standard algae production pond operation (WD
401 base) with that of using a monolayer film (WD film)*.

Climate	Evaporation (mm/year)		WD base (mm/year)	WD film (mm/year)	Reduction in WD (%)	Rainfall (mm/year)
	Base	*With film*				
Tropical	476	190	4,566	4,280	6.3	4,250
Temperate	740	296	4,830	4386	9.2	1,150

Sub-tropical	1,150	460	5,240	4550	13.2	1,010
Mediterranean	1,320	528	5,410	4618	14.6	1,280
Arid	2,275	910	6,365	5000	21.4	54.9

*WD base and WD film both include process water disposal (3,230 mm/year) and leak losses (860 mm/year). Data for base evaporation rates, rainfall, and other water losses were obtained from [17].

3.3.2 Impact of monolayer on pond temperature

The application of a monolayer was seen to increase the temperature of the experimental algae cultures, which can be attributed to the reduction in thermal energy lost through evaporation. To investigate the temperature impact of reduced evaporation in different climates, calculations were performed based on the change in energy lost via evaporation and the corresponding temperature change assuming this energy was present as the specific heat of water in the pond, again assuming a 60% reduction in evaporation. The results are summarised in **Table 2**. For tropical and temperate climates, the calculated increase in pond temperature is between 1.5 to 2.4 °C, which is similar to the temperature increase seen in the experimental results in this study. For regions with higher evaporation rates, the temperature increases. For example, in arid areas the temperature increase could be as high as 7.3 °C. Further trials would need to be conducted in these areas to demonstrate the impact of evaporation suppression on pond temperatures and algal growth rates in higher evaporation climates. As mentioned above, periodic application of the monolayer may be needed in these situations to minimise temperature stress.

Table 2 Comparison of the impact of evaporation suppression on the rate of evaporative energy loss and pond temperature in different climates for a pond with a depth of 30 cm.

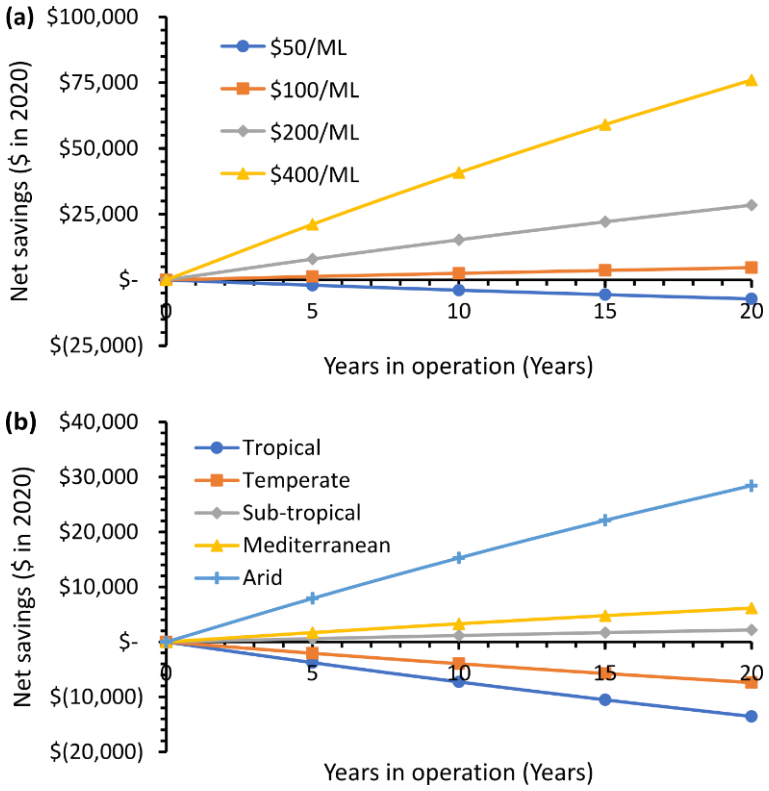
Climate	*Water evaporation (m^3/day)*		*Change in conditions*	
	No monolayer	*Monolayer*	*Rate of energy lost (W/m^2)*	*Pond temperature (°C)*
Tropical	13.0	5.2	-22.0	1.5
Temperate	20.3	8.1	-34.3	2.4
Sub-tropical	31.5	12.6	-53.3	3.7
Mediterranean	36.2	14.5	-61.1	4.2
Arid	62.3	24.9	-105.4	7.3

3.3.2 Economic evaluation

The application of monolayers to reduce evaporation in algae production ponds could have both economic and operational benefits. Firstly, the economic benefit was assessed for the reduced amount of water required to replace water lost and a reduction in associated pumping costs. A monolayer price of $10/kg was assumed, with the same loading as the small-scale laboratory trials applied daily. The cost of applying the monolayer was not included, based on the assumption that the ponds will be actively managed, and monolayer application could be simply integrated into daily operations. Based on the performance of the monolayer in the algae trials, an evaporation reduction of 60% was assumed.

The first major factor that was considered was the price of water, which can range from $50/ML up to $400/ML in arid regions (Fig. 4a**)**. The analysis shows that even without considering any operational benefits, monolayer application could be profitable for water prices >$80/ML in arid regions. At a water price of $200/ML, net savings were estimated at $15,280 per hectare after 10 years. For a hypothetical commercial algae cultivation facility of 800 ha this would result in $12.2 million in net savings over the life of a project **[1]**.

The economic viability of the monolayer film was also considered for a range of different climates to show the impact of different evaporation and rainfall rates (Fig. 4b). Assuming a water price of $200/ML, the economic benefit of monolayer films depends on the climate and is highest in regions where annual rainfall is below annual evaporation losses. Net cost savings are predicted for regions with medium to high rates of evaporation, such as sub-tropical and arid regions, which importantly are typically the regions most suited to algae production. It is also possible that monolayer addition could be avoided during times of the year when evaporation losses are below the economically viable rates, further improving the savings.



443

444 **Fig. 4** Economic analysis of using monolayer films on a one hectare basis over a 20 year period:
445 a) in arid regions with water prices between $50/ML and $400/ML, b) in regions with different
446 climates at a water price of $200/ML.

447 ***3.3.3 Operational benefits of applying monolayers for marine algae cultivation***

448 Beyond direct water savings, the ability to reduce evaporation using monolayers has the
449 potential to positively impact the conditions and operation of the algae ponds. In particular,
450 evaporation during the cultivation of marine microalgae either results in increases in salinity
451 or the requirement for a freshwater source to maintain salinity. In this analysis, we investigate
452 the impact of monolayer application on the salt concentration in batch and continuous
453 operations.

454 First, the increase in salt concentration caused by evaporation during batch algae cultivation
455 was calculated with and without a monolayer in arid and tropical regions (Fig. 5). In the arid

climate base case, the salt concentration reached 45 g/L (a potentially inhibitory level of salts for some species) within 11 days, while the addition of monolayer increased this time to 27 days. This indicates that the addition of monolayer would help to keep the ponds closer to optimal salt concentrations when there is limited or intermittent fresh water available.

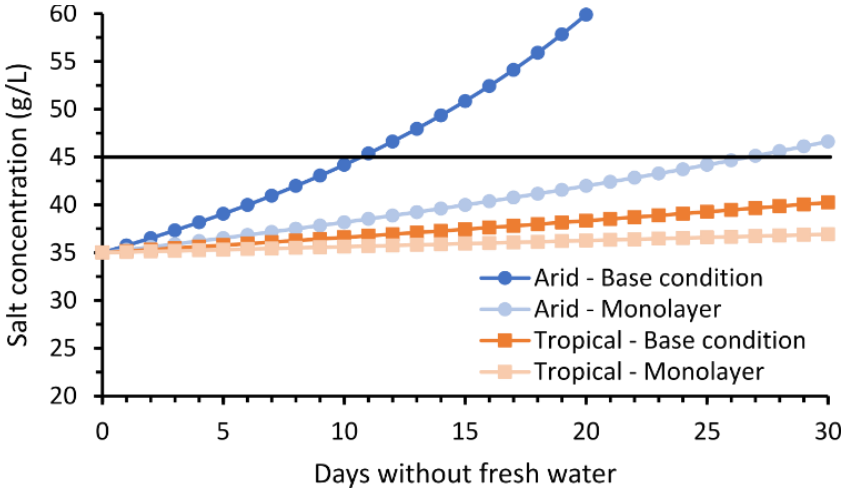


Fig. 5 Concentration of salt in a marine algae pond in arid and tropical climates with and without monolayer. The initial salt concentration is 35 g/L (seawater), and operation is conducted without any outflow or water top-up. An indicative upper threshold for algae growth (45 g/L) is shown for reference.

More commonly, algae will be cultivated in a continuous or semi-continuous manner (Fig. 6), which includes streams of make-up water/growth medium (a), evaporated water (b), recycled growth medium (c), a high-salt purge (d), and harvested and thickened algae (e). Without a purge stream the salt level will increase as with batch operation, with the monolayer reducing the rate of increase. Incorporating a purge stream into the system is more practical for continuous algae growth as it allows the salt concentration to be maintained at a desired level **[32]**.

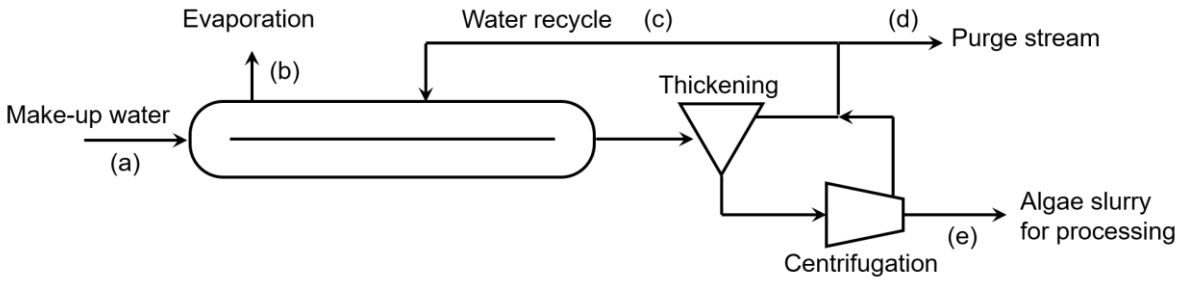


Fig. 6 Process flow diagram of a raceway pond with concentration steps and a water recycle stream.

The impact of evaporation and water recycle on the maximum salt concentration in the make-up water (stream (a) in Fig. 6) was investigated to determine the effect of the monolayer on pond operation. The results (calculated based on an upper limit salt concentration in the pond of 45 g/L) showed that at higher recycled stream percentages the upper limit salt concentration in the make-up water stream must be reduced (Fig. 7). Higher evaporation rates (i.e., in arid regions or without the monolayer) reduce the concentration of salt that can be included in the make-up, reflective of the need to replace evaporated water with fresh water. For example, at 90% recycle rates the base case for an arid region required a make-up stream concentration less than 12 g/L (about 1/3 the concentration of seawater), whereas in a tropical region up to 25 g/L can be tolerated. Adding the monolayer to the surface significantly increased the maximum make-up salt concentration. For example, in the arid region, this salt concentration increased from 12 to 19 g/L. For regions where the make-up water is from salty or brackish water sources, lower levels of purification will be required before use, potentially reducing processing costs or even enabling algae cultivation in otherwise non-viable locations.

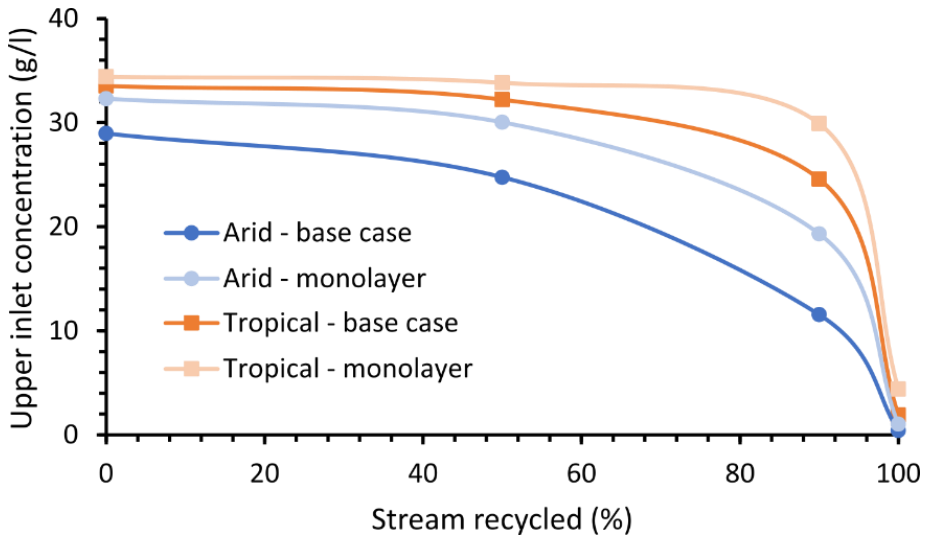


Fig. 7 Upper inlet stream salt concentration (g/L) as a function of the percentage stream recycled comparing the use of monolayer and no monolayer in arid and tropical climates. The maximum salt concentration in the pond was set at 45 g/L.

As a result of adding a monolayer the amount of water recycled for a given inlet salt concentration can be increased. In Fig. 8, this impact is illustrated for all climate regions. The impact of the monolayer is significant in arid regions with more evaporation, again otherwise suited for algae production. With an inlet salt concentration of 35 g/L, the addition of a monolayer enables 71% of the recovered water to be recycled compared with 28% in the base case.

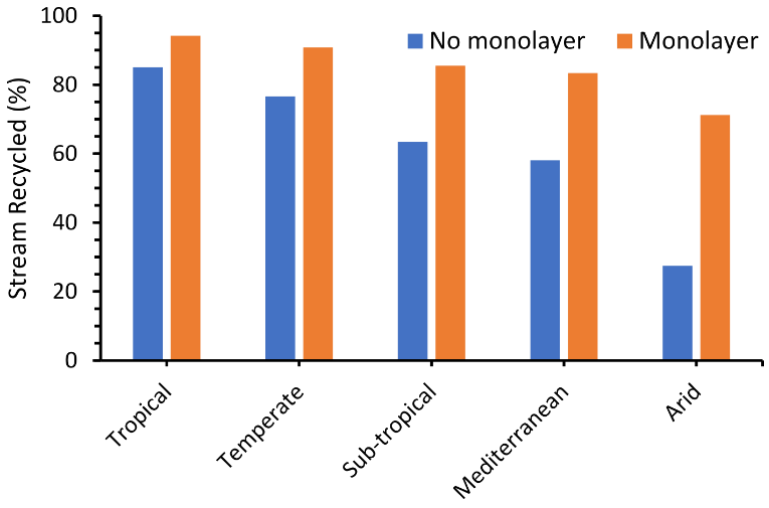


Fig. 8 Percent of recycle stream to maintain an equilibrium operation with a pond concentration at 45 g/L and an inlet saltwater stream of 35 g/L comparing operation with and without a monolayer.

3.3.4 Additional considerations for large-scale implementation

The implementation of monolayer technology in large-scale, outdoor algae production facilities, will present a range of practical challenges that are beyond the scope of this study and will require future outdoor testing. For instance, the effects of turbulent mixing and CO_2 sparging on monolayer performance can be investigated in pilot raceways. In addition, the potential impact of the monolayer material on the need for post-treatment prior to water discharge, recycling or biomass utilisation should be considered. Studies on the use of monolayers for freshwater storage dams **[10][11][21, 23]** have concluded that the chemicals had no potential toxicity on the biodiversity of phytoplankton, and chemical monolayers such as cetyl alcohol and stearyl alcohol were found to be naturally biodegraded by bacteria **[10]**. Nonetheless, depending on the choice of monolayer and the system used, there will need to be studies on the potential degradation products from the monolayer materials. The effect of the presence of monolayer compounds in the resulting algal products may also require additional consideration for certain applications such as animal and aquaculture feed.

4. CONCLUSIONS

This study has shown that an ethylene glycol monooctadecyl ether monolayer could reduce evaporation by 50-70% in both freshwater and marine algae cultures. Although performance declined, this evaporation reduction efficiency remained above 50% for 3 days. Despite extensive investigations, the reduction in performance could not be attributed to any singular cause. Freshwater *Chlorella* and marine *Nannochloropsis* algae were able to grow effectively

in the presence of the monolayer. The growth rate of *Chlorella* was somewhat lower in the presence of a monolayer, the reasons for which remain unconfirmed but could be due to chemical inhibition by the monolayer or a slight increase in culture temperature (1–2 ºC). Importantly, CO_2 mass transfer from the atmosphere was shown to not be reduced by the monolayer. A techno-economic analysis indicated that the application of the monolayer could be economically beneficial, solely on the basis of water savings, particularly in arid climates in which algae are most suited for cultivation. In addition, reduction of evaporation using monolayers offers a means of controlling salinity in marine algae production systems. It is suggested that future investigations are conducted to understand the performance of different combinations of algae and monolayer chemicals, and that performance is tested in outdoor raceway ponds trials considering other operating factors such as turbulent mixing and excess CO_2 supply.

ASSOCIATED CONTENT

Supporting Information

Composition of f/2 and MLA media, performance of monolayer on sea salt and major components of f/2 medium, microscopic images of *N. salina* and *C. vulgaris* cultures with or without monolayer film, and measurement of surface CO_2 mass transfer.

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AUTHOR STATEMENT

The authors declare no commercial or proprietary interest in any product or concept discussed in this article.

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ABBREVIATIONS USED

CO_2, carbon dioxide; O_2, oxygen; C18E1, ethylene glycol monooctadecyl ether; f/2, marine growth media; MLA, freshwater growth media.

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