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Title:

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

Date:

2022-07

Citation:

Poddar, N., Scofield, J., Shi, S., Prime, E. L., Kentish, S. E., Qiao, G. G. & Martin, G. J. O. (2022). Evaporation reduction and salinity control in microalgae production ponds using chemical monolayers. *Algal Research*, 66, <https://doi.org/10.1016/j.algal.2022.102783>.

Persistent Link:

<https://hdl.handle.net/11343/332377>

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

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14 **Abstract**

15 Algae have great potential as a hyper-productive crop to produce food, fuels, and chemicals.
16 However, freshwater availability limits their widespread application. Here we investigate
17 whether chemical monolayers can reduce evaporation in microalgae cultures, and whether algal
18 growth is affected. Thin-film monolayers were formed on the surface of freshwater (*Chlorella*
19 *vulgaris*) and marine (*Nannochloropsis salina*) algae cultures using ethylene glycol
20 monooctadecyl ether. Monolayers applied daily reduced evaporation in both cultures by 70%
21 on the first day, and ~50% by day 3. The cause of the reduced performance was investigated
22 but could not be directly attributed to any particular cellular activity or chemical change.
23 *Nannochloropsis* was uninhibited by the monolayer, while the growth of *Chlorella* decreased
24 by 38% over 3 days. There was no evidence that the monolayer reduced gas exchange
25 (CO₂/O₂), but the reduced growth of *Chlorella* could have been caused by direct chemical
26 inhibition by the monolayer or the slightly elevated temperature (1–2 °C) resulting from the
27 reduction in evaporative cooling. A techno-economic analysis indicated that water savings
28 could make monolayers economically beneficial, especially in arid climates suited to algae
29 production. In addition, monolayers enable control of salinity in marine production systems.
30 Overall, the application of monolayers to reduce evaporation from outdoor algae cultures has
31 great promise, with testing in outdoor trials an obvious next step.

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

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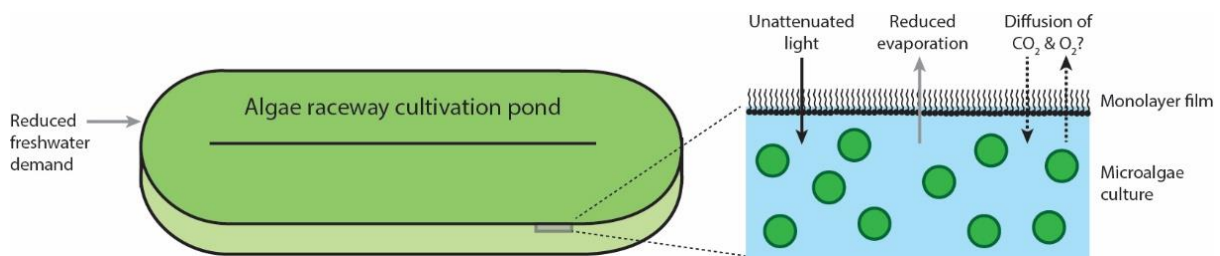
35 1. INTRODUCTION

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

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

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

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



90
91 **Fig. 1** Illustration of the proposed approach of applying a monolayer film to reduce evaporation
92 from large-scale outdoor algae cultivation ponds.

93 The novel application of chemical monolayers to algae cultures presents new research
94 questions that have not been addressed in previous studies for pure water applications such as
95 reservoir preservation [12]. In particular, the aim of the current study was to investigate
96 whether monolayers could successfully reduce evaporation in the presence of concentrated
97 algae cultures, and conversely, whether the presence of a monolayer adversely affects the
98 growth of algae. It is possible that the algae and associated bacteria could degrade the
99 monolayer chemicals or interfere with the intermolecular packing to reduce performance. On
100 the other hand, the monolayer could directly or indirectly inhibit algal growth. For instance, as
101 photosynthetic organisms, microalgae assimilate atmospheric CO₂ and release oxygen during
102 photosynthesis. The exchange of gaseous CO₂ and oxygen between the atmosphere and culture
103 is important, in particular to facilitate photosynthesis and to avoid the build-up of dissolved
104 oxygen which can be toxic to algae [13, 14]. In this respect, one important physical effect of
105 monolayers is an increase in the boundary layer thickness at the air/water interface, which could
106 decrease the rate of gas diffusion [8].

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

115 **2. MATERIALS AND METHODS**

116 **2.1 Preparation of algae seed cultures and chemical monolayer**

117 A seed culture of *Nannochloropsis salina* (CCMP 1776, obtained from the University of
118 Melbourne Culture Collection) was grown in an aerated 2 L Schott bottle containing 1500 mL
119 of f/2 medium [15] at 21 ± 2 °C under a light:dark cycle of 14:10 h with a light intensity of 60
120 – 70 $\mu\text{mol photons m}^{-2} \text{s}^{-1}$. Similarly, a seed culture of a freshwater species, *Chlorella vulgaris*
121 (obtained from CSIRO, Australia), was grown in 1500 mL of MLA medium[16]. The nitrogen
122 source was sodium nitrate for both f/2 and MLA media, provided at a concentration of 0.1 g/L
123 or 0.17 g/L, respectively. The molar concentrations of the individual nutrient stock solutions
124 of f/2 and MLA media are provided in Supplementary File S.1-a.

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

128 **2.2 Experimental set-up**

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

131 algae seed cultures (Section 2.1) of *N. salina* and *C. vulgaris* were centrifuged at 2000 g for 5
132 min and the resulting pellets were resuspended into 100 mL of fresh f/2 or MLA media,
133 respectively to obtain an initial biomass concentration of 0.16 g/L or 0.13 g/L, respectively.
134 These 100 mL cultures were then added to the open round glass dishes, which were placed on
135 individual digital balances (Mettler-Toledo Limited). These algae cultures were then grown for
136 3 days under constant light (24:0 h) with an intensity of approximately 70 $\mu\text{mol photons m}^{-2}$
137 s^{-1} . At the start of each experiment, 36.4 μL of the monolayer solution was added to the test
138 cultures, and this addition was repeated daily over the course of the experiment. Parallel
139 duplicate cultures without monolayer films were included as controls. The experimental design
140 is depicted in Supplementary File S.1-b.

141 **2.3 Evaporation analysis**

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

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

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

161 **2.4 Measurement of algal growth and culture pH, temperature, and dissolved oxygen**

162 To monitor algal growth, culture samples (3 mL) were collected daily and the absorbance at
163 750 nm measured using a Cary 3E UV-Vis spectrophotometer (Agilent Technologies,
164 Australia). The absorbance was converted into dry weight biomass concentrations (g/L) using
165 a standard curve prepared separately for each species. As the absorbance was measured prior
166 to replenishing the evaporated water, the biomass concentrations were adjusted based on the
167 amounts of water evaporated in the monolayer and control dishes. Also prior to replacement of
168 evaporated water, the culture pH (Metler-Toledo InLab® Science), dissolved oxygen
169 concentration (LDO™ probe, HQ40d, Hach) and temperature (infrared thermometer) were
170 measured periodically. Results are presented as the average of biological duplicate culture
171 dishes.

172 **2.5 Techno-economic analysis**

173 ***2.5.1 Net cost savings calculations and assumptions***

174 Calculations of cost savings resulting from the use of monolayer films were based on the cost
175 of purchasing replacement water and associated pumping costs. The cost associated with
176 pumping the replacement water was based on the economic evaluation by Rogers et al. [3]
177 (\$2.07 million/year, for 5 mm evaporation/day over an area of 4875 ha, which works out to be
178 \$0.23/(year.ha.mm)). Water demand was based on the amount of evaporation (mm/year) with

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

192 **2.5.2 Salt concentration analysis and assumptions**

193 An analysis of the effect of monolayer evaporation reduction on the salinity of marine algae
194 growth ponds was performed using *Nannochloropsis* as a reference marine alga, where 35 g/L
195 is the optimum salinity for growth and 45 g/L was used as the upper range for growth [18,
196 19]. The algae media solution was assumed to be withdrawn at a rate of 10% of the pond
197 volume per day to balance new algae growth with algae removed to maintain the concentration
198 of algae at 0.5 g/L [3]. The algae stream was then concentrated via a two-stage process going
199 through a clarifier where it was concentrated to 30 g/L [3, 18]. This stream was then
200 concentrated to 200 g/L via a centrifuge. The water removed was then collected and sent back
201 to the pond with a fraction being purged at varying rates. Salt water was used as the inlet water
202 supply to make up for water lost to evaporation and removed with the algae. This was set at 35
203 g/L in the simulation unless otherwise stated.

204 **2.5.3 Impact of evaporation suppression on pond temperature**

205 Calculations of the increase in pond temperature due to the reduced evaporation by the
206 monolayer were performed on the basis of a pond with a depth of 30 cm. Energy lost from the
207 system through evaporation was calculated based on the latent heat capacity of water at 25 °C
208 (2442 kJ/kg) and the difference in evaporation through use of the monolayer. The
209 corresponding temperature change was calculated based on the equivalent energy embedded in
210 water at 25 °C with a specific heat capacity of 4.19 kJ/(kg.K).

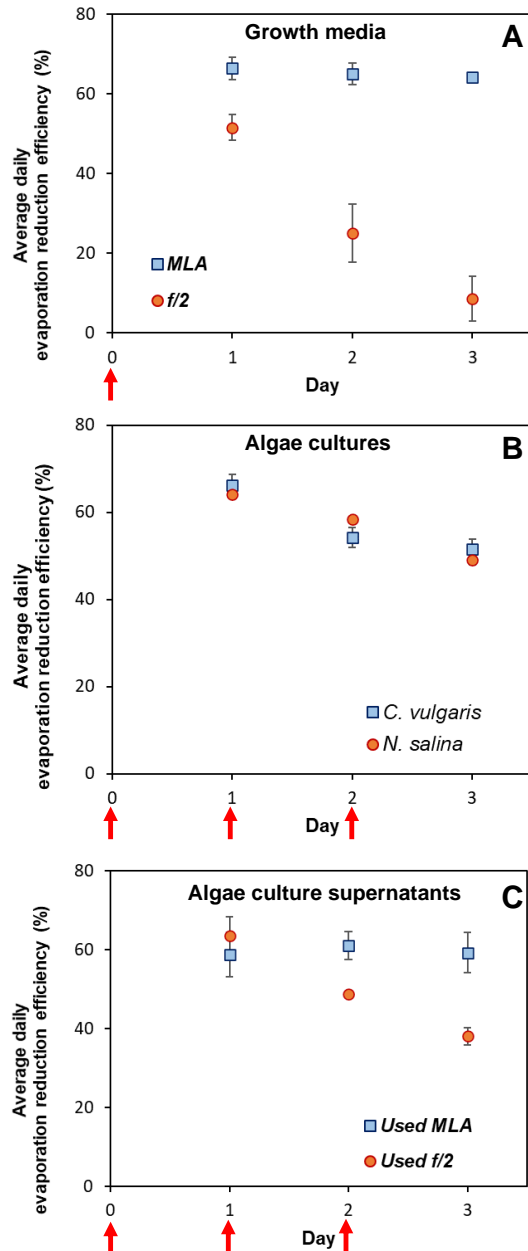
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212 **3. RESULTS AND DISCUSSION**

213 **3.1 Evaporation reduction in fresh water and marine algae cultures**

214 While chemical monolayers are usually applied to fresh water, monolayer dissociation can be
215 affected by environmental conditions such as pH, temperature, salt type and concentration [20].
216 As such, the evaporation reduction performance of a single dose of C18E1 monolayer was first
217 tested on freshwater and marine algae growth media in the absence of algae cells to determine
218 the influence of salt and media components (Fig. 2A). In the freshwater medium (MLA),
219 evaporation was reduced by approximately 66% without decline over the three days of testing
220 (Fig. 2A), representing similar performance to that on deionised water (approximately 64%,
221 data not shown). However, in the marine growth medium (f/2), evaporation performance
222 progressively declined from >50% over the first day to <10% during the third day. The
223 reduction in evaporation performance was not found to occur in the presence of either the sea
224 salt (30 g/L) alone or with the other major medium components included individually
225 (Supplementary File S.2), suggesting some complex interactions with the monolayer occurred.
226 To address this issue in algae cultures, the monolayer was replenished each day, consistent
227 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).



237

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 μ L; 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.

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

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

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

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

286 It has previously been suggested that some chemical monolayer compounds such as
287 hexadecanol and octadecanol could be degraded by bacteria [10, 12], the rate of which could
288 increase over time. However, microscopic analysis revealed no obvious increase in the
289 abundance of bacterial cells in the cultures with monolayer film present (Supplementary File
290 S.3), consistent with previously published field trials using C18E1 monolayer film [23].
291 Monoctadecyl ether-based monolayers have been previously shown to be at risk of
292 photodegradation [23], although only a very low light intensity (compared to sunlight) of 70
293 $\mu\text{mol photons m}^{-2} \text{ s}^{-1}$ was applied. In addition, the monolayer was replenished each day,
294 meaning that the impact of bacterial or light degradation of monolayer films is not likely to

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

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

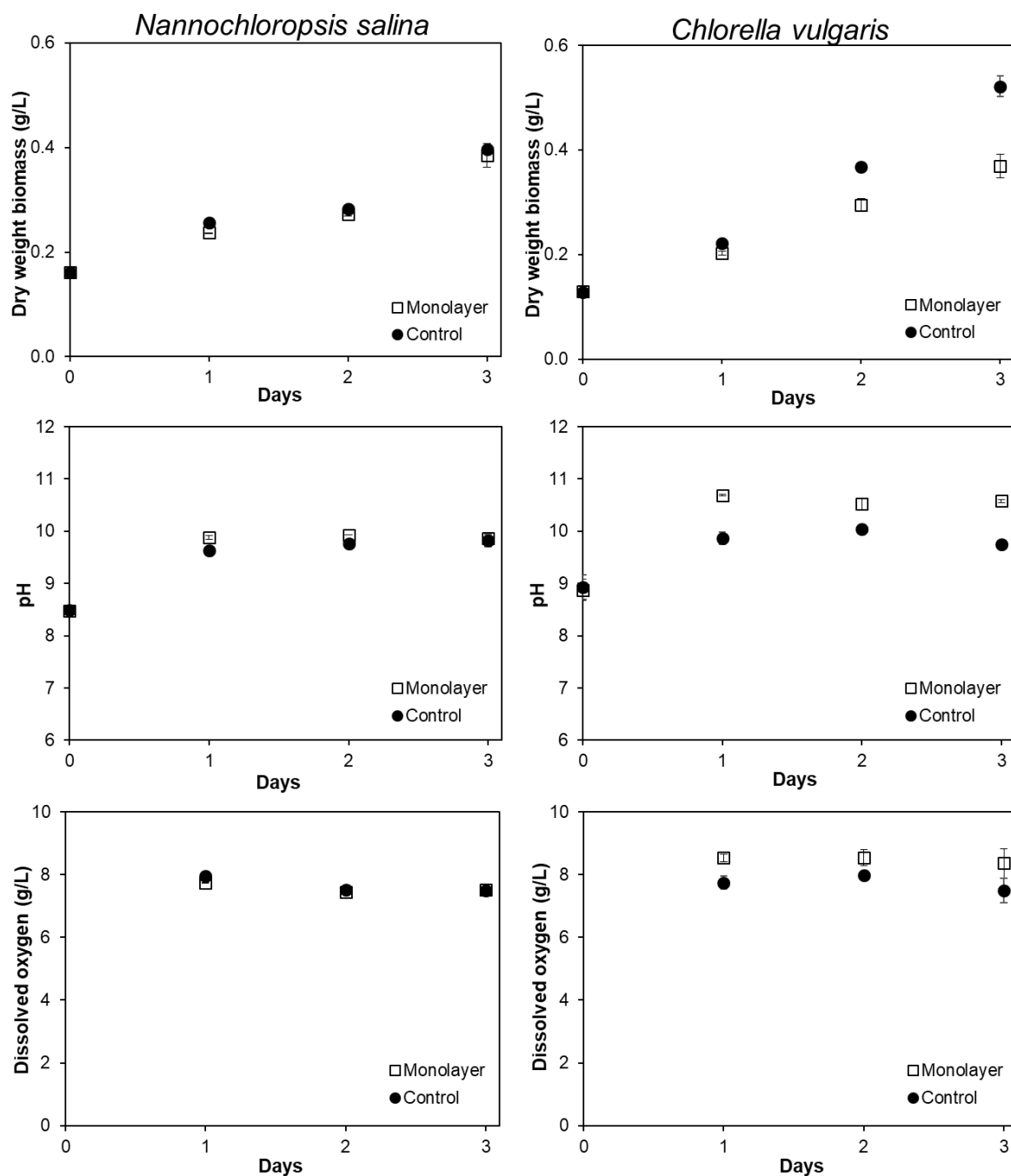
311 **3.2 Algae growth in the presence of monolayer**

312 Similar experiments were used to assess the potential impact of the monolayer on algal growth
313 (Fig. 3). For *N. salina* cultures grown in marine f/2 media, the biomass productivities in both
314 the monolayer and control cultures were not significantly different over the 3-d growth period
315 (75 ± 7 and 78.5 ± 0.3 mg/L/d, respectively). The pH increased from 8.5 to 9.8 in both the
316 monolayer and control cultures (Fig. 3), due to the consumption of nitrate and CO₂ in the media.
317 Monolayers have previously been reported to reduce oxygen diffusion (by 10 - 15%) [27].
318 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].

327



328
 329
 330 **Fig. 3** Dry weight biomass, pH, and dissolved oxygen concentration in *Nannochloropsis salina*
 331 (left hand panels) and *Chlorella vulgaris* (right hand panels) cultures grown in open cultures
 332 for 3 days in the presence (monolayer; empty square) or absence (control; filled circle) of a
 333 monolayer film. All the parameters were measured before compensating for evaporated water.
 334 Biomass is normalised to the proportional water evaporation in the monolayer and control

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

337 Continual growth was observed in both the monolayer and control cultures of freshwater *C.*
338 *vulgaris*; however, the biomass productivity in the control cultures was higher than that of the
339 cultures containing a monolayer (131 ± 7 and 80 ± 8 mg/L/d, respectively). There are a number
340 of possible reasons why *Chlorella* growth was negatively impacted by the presence of the
341 monolayer while *Nannochloropsis* was not. Firstly, it is conceivable that *Chlorella* was directly
342 inhibited by either the chloroform or the monolayer chemical, while *Nannochloropsis* was not.
343 However, chloroform was only applied to the surface of the cultures in very small amounts
344 (~ 36.4 μ L on 100 mL cultures). As a water-immiscible and volatile solvent it would not have
345 mixed into the cultures but rather would have been rapidly evaporated from the surface,
346 preventing any possible inhibition of the algae. On the other hand, the monolayer was present
347 for the duration of the experiments, and although predominantly on the surface, a proportion
348 of the molecules could partition into the culture. The differential effect on the two algae
349 indicates that if chemical inhibition was a factor, it is species specific. Direct chemical
350 inhibition of algae will also be dependent on the chemical monolayer used, meaning this is a
351 complex and important topic that warrants future investigation.

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

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

364 In the current experiments, the cultures were not aerated and relied solely on CO₂ uptake from
365 the atmosphere. The pH increased from 8.9 to 9.8 - 10.2 in the *Chlorella* culture without
366 monolayer and increased to >10.5 in the first 24 hours of cultivation with the monolayer.
367 Meanwhile, the dissolved oxygen was approximately 8.5 mg/L and 8 mg/L in the monolayer
368 and control cultures, respectively (Fig. 3). The slightly higher pH and decreased biomass
369 productivity in the freshwater monolayer cultures could reflect a reduction in CO₂ mass transfer
370 into the media caused by the monolayer, while the increase in dissolved oxygen could similarly
371 result from a reduction in outgoing O₂ mass transfer. Decreased gas diffusion resulting from
372 the presence of a monolayer has previously been proposed in freshwater systems [8]. To
373 investigate whether the monolayer film reduced the rate of CO₂ mass transfer from the
374 atmosphere, separate experiments were performed using a novel method based on the Wilbur
375 Anderson assay (Supplementary File S.4). The results showed no reduction in the rate of CO₂
376 mass transfer due to the presence of monolayer film for either freshwater or marine growth
377 media. The reduced growth rate of *C. vulgaris* (Fig. 2) in the presence of a monolayer cannot
378 therefore be explained by a reduction in gas exchange. The most likely remaining factors
379 appear to be direct chemical inhibition by the monolayer or a reduced growth rate due to the
380 slightly elevated temperature resulting from reduced evaporation. Future research to
381 understand the potential direct (e.g. chemical) and indirect (e.g. thermal) effects of this and
382 other monolayer chemicals on *Chlorella* and other algae species is warranted. In particular, the
383 current study showed that the ethylene glycol mono-octadecyl 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] .						

402 *3.3.2 Impact of monolayer on pond temperature*

403 The application of a monolayer was seen to increase the temperature of the experimental algae
404 cultures, which can be attributed to the reduction in thermal energy lost through evaporation.
405 To investigate the temperature impact of reduced evaporation in different climates, calculations
406 were performed based on the change in energy lost via evaporation and the corresponding
407 temperature change assuming this energy was present as the specific heat of water in the pond,
408 again assuming a 60% reduction in evaporation. The results are summarised in **Table 2**. For
409 tropical and temperate climates, the calculated increase in pond temperature is between 1.5 to
410 2.4 °C, which is similar to the temperature increase seen in the experimental results in this
411 study. For regions with higher evaporation rates, the temperature increases. For example, in
412 arid areas the temperature increase could be as high as 7.3 °C. Further trials would need to be
413 conducted in these areas to demonstrate the impact of evaporation suppression on pond
414 temperatures and algal growth rates in higher evaporation climates. As mentioned above,
415 periodic application of the monolayer may be needed in these situations to minimise
416 temperature stress.

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

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

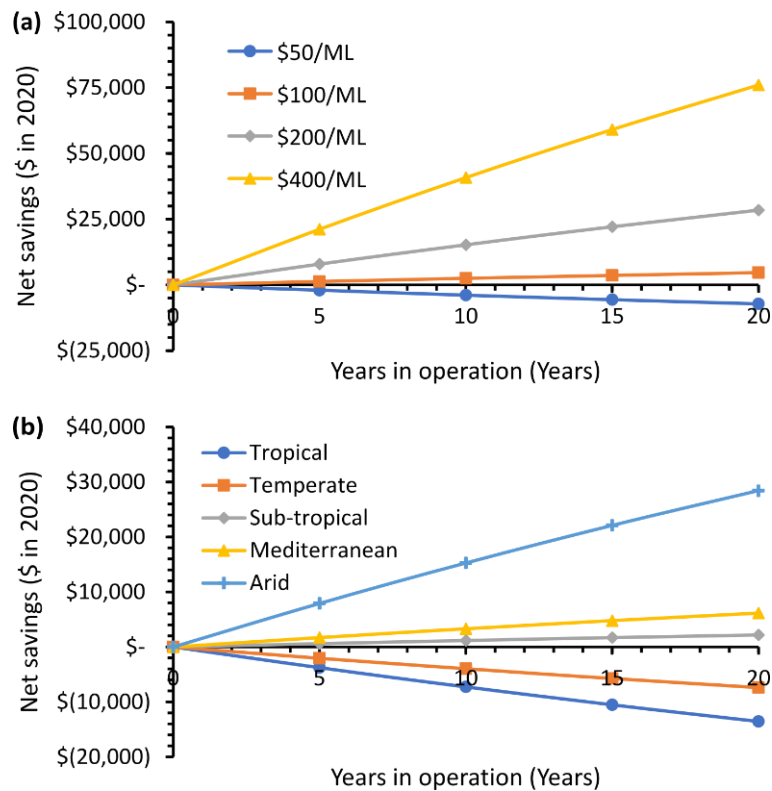
419

420 **3.3.2 Economic evaluation**

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

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

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



443

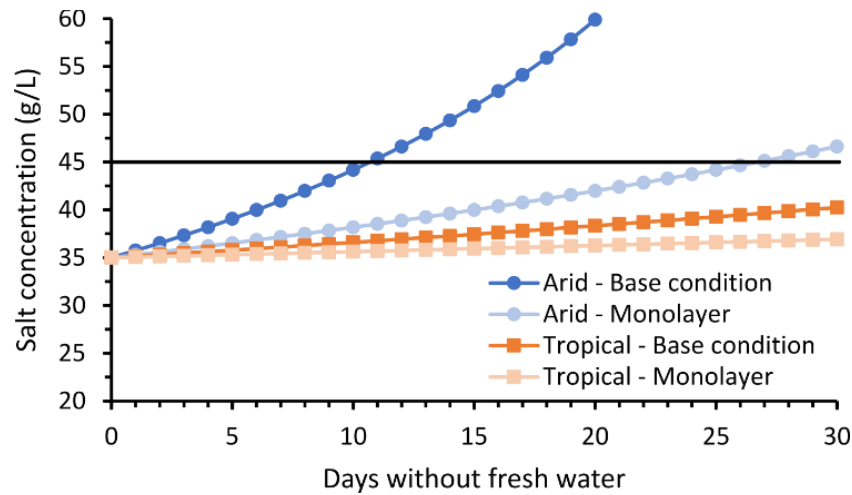
444 **Fig. 4** Economic analysis of using monolayer films on a one hectare basis over a 20 year period:
 445 a) in arid regions with water prices between \$50/ML and \$400/ML, b) in regions with different
 446 climates at a water price of \$200/ML.

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

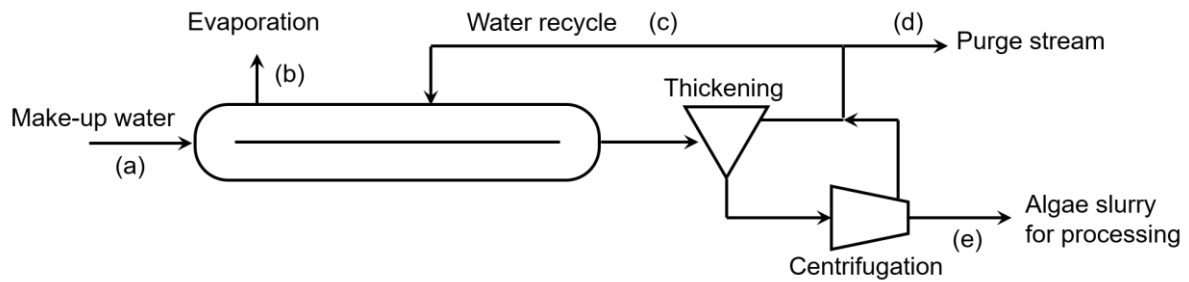
456 climate base case, the salt concentration reached 45 g/L (a potentially inhibitory level of salts
457 for some species) within 11 days, while the addition of monolayer increased this time to 27
458 days. This indicates that the addition of monolayer would help to keep the ponds closer to
459 optimal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



460

461 **Fig. 5** Concentration of salt in a marine algae pond in arid and tropical climates with and
462 without monolayer. The initial salt concentration is 35 g/L (seawater), and operation is
463 conducted without any outflow or water top-up. An indicative upper threshold for algae growth
464 (45 g/L) is shown for reference.

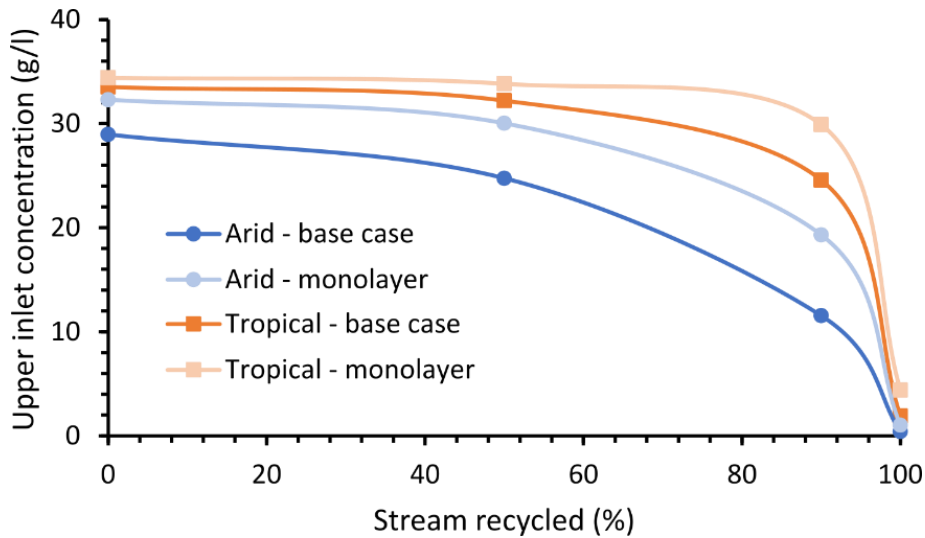
465 More commonly, algae will be cultivated in a continuous or semi-continuous manner (Fig. 6),
466 which includes streams of make-up water/growth medium (a), evaporated water (b), recycled
467 growth medium (c), a high-salt purge (d), and harvested and thickened algae (e). Without a
468 purge stream the salt level will increase as with batch operation, with the monolayer reducing
469 the rate of increase. Incorporating a purge stream into the system is more practical for
470 continuous algae growth as it allows the salt concentration to be maintained at a desired level
471 [32].



472

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

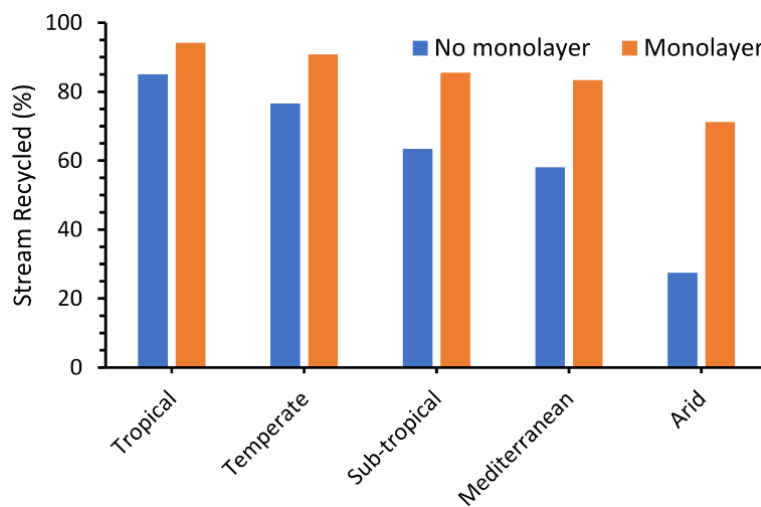
475 The impact of evaporation and water recycle on the maximum salt concentration in the make-
 476 up water (stream (a) in Fig. 6) was investigated to determine the effect of the monolayer on
 477 pond operation. The results (calculated based on an upper limit salt concentration in the pond
 478 of 45 g/L) showed that at higher recycled stream percentages the upper limit salt concentration
 479 in the make-up water stream must be reduced (Fig. 7). Higher evaporation rates (i.e., in arid
 480 regions or without the monolayer) reduce the concentration of salt that can be included in the
 481 make-up, reflective of the need to replace evaporated water with fresh water. For example, at
 482 90% recycle rates the base case for an arid region required a make-up stream concentration less
 483 than 12 g/L (about 1/3 the concentration of seawater), whereas in a tropical region up to 25 g/L
 484 can be tolerated. Adding the monolayer to the surface significantly increased the maximum
 485 make-up salt concentration. For example, in the arid region, this salt concentration increased
 486 from 12 to 19 g/L. For regions where the make-up water is from salty or brackish water sources,
 487 lower levels of purification will be required before use, potentially reducing processing costs
 488 or even enabling algae cultivation in otherwise non-viable locations.



489

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

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



499

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

503 ***3.3.4 Additional considerations for large-scale implementation***

504 The implementation of monolayer technology in large-scale, outdoor algae production
505 facilities, will present a range of practical challenges that are beyond the scope of this study
506 and will require future outdoor testing. For instance, the effects of turbulent mixing and CO₂
507 sparging on monolayer performance can be investigated in pilot raceways. In addition, the
508 potential impact of the monolayer material on the need for post-treatment prior to water
509 discharge, recycling or biomass utilisation should be considered. Studies on the use of
510 monolayers for freshwater storage dams [10][11][21, 23] have concluded that the chemicals
511 had no potential toxicity on the biodiversity of phytoplankton, and chemical monolayers such
512 as cetyl alcohol and stearyl alcohol were found to be naturally biodegraded by bacteria [10].
513 Nonetheless, depending on the choice of monolayer and the system used, there will need to be
514 studies on the potential degradation products from the monolayer materials. The effect of the
515 presence of monolayer compounds in the resulting algal products may also require additional
516 consideration for certain applications such as animal and aquaculture feed.

517

518 **4. CONCLUSIONS**

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

524 in the presence of the monolayer. The growth rate of *Chlorella* was somewhat lower in the
525 presence of a monolayer, the reasons for which remain unconfirmed but could be due to
526 chemical inhibition by the monolayer or a slight increase in culture temperature (1–2 °C).
527 Importantly, CO₂ mass transfer from the atmosphere was shown to not be reduced by the
528 monolayer. A techno-economic analysis indicated that the application of the monolayer could
529 be economically beneficial, solely on the basis of water savings, particularly in arid climates
530 in which algae are most suited for cultivation. In addition, reduction of evaporation using
531 monolayers offers a means of controlling salinity in marine algae production systems. It is
532 suggested that future investigations are conducted to understand the performance of different
533 combinations of algae and monolayer chemicals, and that performance is tested in outdoor
534 raceway ponds trials considering other operating factors such as turbulent mixing and excess
535 CO₂ supply.

536 **ASSOCIATED CONTENT**

537 **Supporting Information**

538 Composition of f/2 and MLA media, performance of monolayer on sea salt and major
539 components of f/2 medium, microscopic images of *N. salina* and *C. vulgaris* cultures with or
540 without monolayer film, and measurement of surface CO₂ mass transfer.

541 **ACKNOWLEDGEMENTS**

542 This work was supported by the Australian Research Council (ARC) through the Discovery
543 Projects Grant Scheme (DP200101230).

544 **AUTHOR STATEMENT**

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

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550 **ABBREVIATIONS USED**

551 CO₂, carbon dioxide; O₂, oxygen; C18E1, ethylene glycol mono-octadecyl ether; f/2, marine
552 growth media; MLA, freshwater growth media.

553

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