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# Protocol to compensate net evaporation and net precipitation in open-pond microalgal massive cultures and permit maximal steady-state productivities

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## ABSTRACT

Commercial cultivation of microalgae in open ponds can be strongly limited by evaporation. This is particularly the case in highly evaporative areas where production plants are likely to be located. We present a mathematical model to calculate native solute and exogenous nutrient concentrations as affected by net evaporation or net precipitation. The model takes into account the periodic compensatory addition of new feedstock water and/or removal of rain-originated excess water and the eventual recycling of the culture medium. We present a management protocol in which, for a wide variety of climates, it is possible to stabilise the native solute concentrations and minimise the exogenous nutrient washout. The protocol includes harvesting a minimum of 10% of the pond volume per day and replacing this volume and the evaporated water with new feedstock water. We test the approach against a 9-year daily weather data set for a locality with 1740 mm.y<sup>-1</sup> average net evaporation. We find that the native solute concentrations can be maintained between 1.2 and 1.5 times the feedstock water values, while the daily washout of exogenous nutrients is ≤3% and the pond volume is kept nearly constant. This protocol should prove particularly useful for the exploitation of marine microalgae growing in open ponds with brackish or seawater-based growth medium.

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## 1. Introduction

Solar radiation can be harvested as a sustainable source of energy by exploiting natural photosynthesis. Part of the visible light energy is stored in biomass, which can be used to

produce biofuels or a variety of industrial feedstocks. Diverse “energy crops” are available [1] but, noticeably, some microalgae have the highest productivities of biomass and oil [2]. Because of their high productivities, these organisms should be the best “energy crops” [3] and become the basis for a new “agriculture”. Furthermore, microalgae display a variety of

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advantages over classical crops, among others: (a) They do not need the supporting properties of a soil, and some can grow in sea or saline water, thus not competing for land or fresh water; (b) Their culture is hydraulic in nature, which allows the inoculation and harvest of an extended culture from a single pumping point and the continuous monitoring and provision of nutrients; (c) They can display year-round growth, whereas classical crops are productive only during a few months per year; (d) Microalgal growth can be fertilized with CO<sub>2</sub> derived from power plant flue gas or from the extraction of fossil oil and gas, or with CO<sub>2</sub> from soluble carbonates [3–5]. Other advantages of marine microalgae, when used in terrestrial industrial setups are: (a) Seawater has minimal sediment content in comparison with rivers. (b) Most non-marine algae and animals cannot tolerate seawater, which reduces contamination effects from the terrestrial surrounding environment. (c) Because most marine microalgae cannot tolerate a fresh water environment, any accidental spillage will have a lower environmental impact on neighbouring ecosystems.

A particular feature of microalgal industrial cultures is that all the nutrients needed for biomass generation are provided by the feedstock water and by the addition of fertilizers [6], here called “exogenous nutrients”.

Microalgal production facilities should be placed in areas of adequate insolation, moderate temperatures, and easy provision of water. But also, very importantly for the rational use of soil resources, they should be located in classically non-productive land, which typically involves areas of high evaporation and low rainfall. Moreover, the extended microalgal cultivation infrastructure has to be simple and cheap, which suggests the use of open ponds [7,8]. This however implies significant water loss through evaporation, in addition to the water removed for algal harvesting (which can be partly recycled). The addition of new feedstock water to compensate evaporation will result in increasing solute concentrations, which if not managed may ultimately force the replacement of the entire growth medium [9].

We present a mathematical model to predict the native solute and exogenous nutrient concentrations as affected by net evaporation or precipitation, initially utilising an idealised climate with constant daily conditions, manipulating the periodic input of feedstock water or removal of growth medium in combination with the harvesting regime. This model using constant daily weather is used to demonstrate the role of those variables, however it can also be directly applied to short-term periods of steady conditions. For a wide range of idealised climates from 4000 mm.y<sup>-1</sup> net precipitation to 4000 mm.y<sup>-1</sup> net evaporation, it is possible to stabilise the native solute concentrations even with the high level of water recycling which is required to minimise exogenous nutrient washout. The case of net precipitation is considered as, even in arid areas, such conditions will periodically occur and must be managed. The model assumes the harvest of a minimum of 10% of the pond starting volume per day, and the addition of new feedstock water to compensate both for harvesting and net evaporation. Growth curves of *Nannochloropsis salina* measured experimentally under laboratory conditions are used in the model. We then test the approach against a 9 year long daily weather data set for a locality with 1740 mm.y<sup>-1</sup> net

evaporation, which includes periods of both severe evaporation and precipitation. We find that by daily manipulation of the plant operating parameters, the native solute concentrations can be maintained between 1.2 and 1.5 times the starting values, while the daily washout of exogenous nutrients is ≤3% and the pond height increases to at most 20% higher than the starting value. The system, for the tested weather conditions, would ideally require the selection or genetic construction of a microalgal strain capable of proliferating well in a range of 1.0–1.5 relative feedstock solute concentrations.

## 2. Materials and methods

### 2.1. Growth of *N. salina* and outdoors growth simulation

The marine unicellular microalga *N. salina* [10], obtained as strain CS-190, CSIRO Collection of Living Microalgae, Hobart, Australia, was grown in *f*<sub>2</sub> medium, a half concentration of *f* medium [6], prepared with filtered natural seawater (Sydney, Australia). Cultures were performed in 2 L conical flasks containing 1.5 L of culture at 25 ± 2 °C, magnetically stirred, continuously illuminated with an average photon irradiance at the culture surface level of 150 μmol photons.m<sup>-2</sup>.s<sup>-1</sup>, and continuously bubbled with a 5% volume fraction of CO<sub>2</sub> in humidified air, at a flow rate of 0.7 L.h<sup>-1</sup>. Biomass concentration was evaluated turbidimetrically at 750 nm [11]. In the model, growth in outdoor conditions was simulated by interspersing 16 h of no growth (i.e. “night conditions”) per each 8 h of growth (i.e. “day conditions”) using the actual growth curve obtained under continuous light as described above. This simulation was subsequently modified to represent the pseudo steady-state growth in which a culture growing in the late linear phase is partially harvested each day by the withdrawal of 10% of the total volume. An 8 h period of linear growth (maximal rate) is followed by a harvest and addition of new feedstock water that brings the volume to the standard value (e.g. equivalent to 300 mm pond depth) and causes a 10% dilution. This dilution is followed by a period of no growth. The three phases, totalling 24 h, were repeated for many cycles representing the pseudo steady-state culture.

### 2.2. Model assumptions

#### 2.2.1. Pond type and mixing

We consider a standard pond depth of 300 mm. We impose the general requirement that the mixing mechanism for the open pond should be effective for assuring the homogenization through the pond of: a) changes in solute concentration due to evaporation or precipitation, b) addition of new feedstock water, and c) addition of recycled water. Raceway ponds with paddle wheel mixing are the most common commercial configuration [4], however our analysis is also applicable to other geometries and mixing devices. In applying this study to a real system, if mixing significantly increases evaporation above the local pan evaporation rate, the model is simply adjusted accordingly. As a variety of evaporation rates have been evaluated, including extreme ones, the general results of the model will remain applicable. Further refinement of the

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