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Small doses, big troubles: Modeling growth dynamics of organisms affecting microalgal production cultures in closed photobioreactors

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HIGHLIGHTS

- Model realistically simulates growth of contaminants in a production photobioreactor (PBR).
- "Sudden" onset of contamination can be attributed to exponential growth.
- PBR management protocols can reduce the risks of serious contamination.
- Small numbers of sufficiently fast-growing contaminants can lead to loss of algal cultures in days.
- A simple and cheap protocol for short-term prediction of severe contaminants in PBRs is presented.

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ABSTRACT

The destruction of mass cultures of microalgae by biological contamination of culture medium is a pervasive and expensive problem, in industry and research. A mathematical model has been formulated that attempts to explain contaminant growth dynamics in closed photobioreactors (PBRs). The model simulates an initial growth phase without PBR dilution, followed by a production phase in which culture is intermittently removed. Contaminants can be introduced at any of these stages. The model shows how exponential growth from low initial inocula can lead to "explosive" growth in the population of contaminants, appearing days to weeks after inoculation. Principal influences are contaminant growth rate, PBR dilution rate, and the size of initial contaminant inoculum. Predictions corresponded closely with observed behavior of two contaminants, *Uronema* sp. and *Neoparamoeba* sp., found in operating PBRs. A simple, cheap and effective protocol was developed for short-term prediction of contamination in PBRs, using microscopy and archived samples.

1. Introduction

Just like terrestrial plant crops, microalgae in large-scale biomass cultures can be beset by pests and weeds. Grazing organisms, including protozoa and microinvertebrate animals, can be devastating pests. Complete destruction of microalgal crops has been recorded in as little as 48 h from first detection of an aggressive grazer (Moreno-Garrido and Cañavate, 2001). This phenomenon is consistent with what has been observed for microalgae in nature (Sherr and Sherr, 2002; Narwani and Mazumder, 2010). Fast-growing non-target algal weeds may degrade the quality of the product and can even displace the target strain entirely, as has been observed in natural populations (Sieracki et al., 1993).

Despite the obvious risks, little has been published on this aspect of microalgal cultivation, and the research and development needs are considerable (Day et al., 2012a). Most grazers have been identified to the genus level at best, with many known to no greater accuracy than (for example) 'amoeba', 'ciliate', or 'rotifer' (Post et al., 1983; Moreno-Garrido and Cañavate, 2001). Without an accurate identification, information on the distribution in nature, life history, growth rate, and prey choice of the contaminating organism is not accessible – assuming that such information even exists – leaving a production team to guess whether a particular contaminant poses a risk to cultivation, and if it does, how grave is the situation. Chemical control of an established, aggressive contaminant is problematic (Moreno-Garrido and Cañavate, 2001), and other means of control have hardly been investigated (Day et al., 2012a).

Given the large number of contaminant species that can infest algae production cultures (Post et al., 1983), and the lack of information on the biology and control of practically all of these

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contaminants, a production unit currently is unlikely to be able to "cure" a mass algal culture of an established contaminant. At present, mitigation strategies attempt to prevent contaminant entry, and manage cultures to minimize growth of contaminants present relative to target algae. To accomplish this mitigation for any given production system requires the following: information on the means by which a contaminant enters the production system (vector), the amount (rate) of contaminant entry via the relevant vector(s) (inocula), the time (latency) it takes for a contaminant, from a given inoculum (or inoculation rate) to reach a set threshold – typically, a contaminant detection limit or a product quality control metric.

Latency, is likely a function of the specific growth rate of the contaminant in the production system. Therefore, given an inoculum and a specific growth rate, it should be possible to produce a simple mathematical model that will predict the behavior of a contaminant in culture and thereby provide a tool for culture management where contamination is present, without the need for an accurate taxonomic identification of the contaminant(s). The objective of this research was to develop such a model.

2. Methods

2.1. Context and assumptions

As the starting condition for the model, a closed cultivation system or photobioreactor (PBR) of the type commonly used in microalgal production systems to maintain cultures at large volumes for extended periods of time was postulated. The target alga is kept under nutrient-replete conditions, and therefore is in exponential-phase growth throughout. The PBR is operated in semi-continuous culture mode, with a portion of the culture volume being removed and replaced with fresh medium as needed to keep the target alga in exponential-phase growth. A key characteristic of all PBR designs is the isolation of the target culture from contaminants, but we postulate that the air and water supplies for our PBR are slightly leaky, and this leakiness provides the vector for contaminant entry. The lower bound for the initial inoculum via these vectors (combined) was set at 1 cell per 1000 L of culture medium – an empirically derived figure. A contaminant-free starter culture of the target alga used to initiate the PBR at time $t = 0$ was assumed. The threshold value for contaminant cell numbers (for the purposes of determining latency) was set at 2×104 cells L^{-1} – also an empirically-derived figure representing the lowest contaminant cell number that could consistently be detected in a microscopy-based screening system.

Microscopy of PBR cultures was performed daily by trained and experienced personnel, using a Zeiss AxioObserver A1 inverted microscope at a magnification of up to 400×. Freshly collected samples of culture were viewed both live and after fixing with Lugol's Iodine.

Simple exponential cell growth of the contaminant was assumed with no resource limitation for most of the culture's life. Growth of the contaminant was assumed to not be significantly influenced by growth of the target alga, except as noted for Eq. (2) below.

Amoebae (*Uronema* sp.) were obtained from KPF operations (Cellana LLC, Kona Pilot Facility, Kailua-Kona, Hawaii, USA), and transported to Friday Harbor Laboratories (Friday Harbor, Washington, USA), where they were isolated into uniprotist cultures by single-cell picking. The cultures were maintained in sterile seawater medium solidified with 1.5% agar with mixed bacteria. The bacteria were also obtained from KPF operations and grown on medium 2216 (Difco) solidified with 1.5% agar. Cell counts were achieved by placing single amoebal cells in the wells of 24-well spot plates and incubating them with sterilized seawater to which bacteria from the agar cultures were added. Ciliate cell growth was monitored daily with an inverted microscope. Samples were taken from individual wells daily over a 5-day period, fixed with Lugol's iodine, and ciliates were counted with a hemocytometer.

2.2. Terms of the model

The concentration of the contaminants after the time interval $\Delta t = t_1 - t_0$ is given by:

$$n_1 = n_0 e^{\mu(t_1 - t_0)} \tag{1}$$

where μ is the specific growth rate of the contaminant in units of d^{-1}, n_0 and n_1 denote the concentration (in cells L^{-1}) of the contaminant at times, t_0 and t_1.

Contaminants were assigned a reduced rate of growth during the initial grow-up period following inoculation of the PBR Eq. (2). For heterotrophic contaminants (grazers), this equation represented resource-limited growth due to the small rate of encounters with algal prey in a sparse culture. For autotrophic contaminants (algae), the reduced growth rate represented the lag period before exponential growth. The growth rate increased each day during the grow-up period:

$$\mu_{su}(t) = \mu \frac{t}{t_{su}} \tag{2}$$

where $\mu_{su}(t)$ is the specific growth rate of the contaminant on day t; μ is the (resource sufficient) specific growth rate of the contaminant; and t_{su} is the number of days with no dilution for grow-up. Calculation of contaminant population density was performed for each hour. During grow-up:

$$cc_{(h)} = e^{\frac{\mu_{su}(t)}{24}} c_{(h-1)} \tag{3}$$

where $c_{(h)}$ is the cell density at the end of the current hour; and $c_{(h\text{-}1)}$ the cell density at the end of the previous hour.

In normal production mode, the growth rate of the contaminant was assumed to be maximal and growth was continuous over 24 h. Food resources for the contaminant in the dense culture of microalgae was assumed to be limitless. Each production day was divided into an initial 12-h period without a change in volume, where:

$$c_{(h)} = e^{\frac{\mu}{24}} c_{(h-1)} \tag{4}$$

followed by 6 h where a number of cells were removed during the removal of some fraction of the medium containing the culture:

$$c_{(h)} = e^{\frac{\mu}{24}} (c_{(h-1)} - r c_{(h-1)}) \tag{5}$$

where r is the hourly rate of drain/refill. The actual volume of culture medium was not used in these calculations. $c_{(h)}$ is the concentration of the population of cells in the total volume of the PBR.

For a further 6 h, the lost volume was replaced with fresh (cell free) medium and the population was again calculated by using equation (Eq. (4)). Once the refill was complete, the volume of the culture was left unchanged until the next drain/refill cycle.To simulate an ineffective water filtration system, a term for the addition of contaminants at a particular concentration (a) with the refill was added:

$$c_{(h)} = e^{\frac{\mu}{24}} (c_{(h-1)} + a) \tag{6}$$

The model was coded and graphs generated in the R environment (R Development Core Team, 2011).