Impacts of fungal disease on algal biofuel systems: Using life-cycle assessment to compare control strategies.

by

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Abstract

While the global community is looking for more sustainable sources of energy to combat climate change, algae has positioned itself as a both promising and problematic biofuel feedstock. On one hand, algae have higher photosynthetic efficiencies and higher lipid proportions than terrestrial crops and don’t compete for arable land. On the other hand, a major challenge to algae becoming an economically viable option is its vulnerability to diseases that lead to highly variable production output. Fungal pathogens are one of the most common forms of disease that cause algal feedstocks to crash. In turn, crashes of feedstocks cost significant losses of time and resources. Despite disease being a major bottleneck to commercial production, the life cycle assessments (LCA) used to analyze the viability of algal feedstocks for biofuel have yet to consider the impact of pathogens on life-cycle metrics. Here, we incorporate a disease model into a well-documented LCA for algal biorefineries to compare two sustainability metrics, energy return on investment (EROI) and global warming potential (GWP). We begin by showing the obvious – that failure to consider disease leads to overly optimistic LCA metric outputs. We then go on to compare two different control strategies of disease – chemical and biological. Our analyses show that biological engineering of a multi-species consortium of algae has a greater positive impact on LCA metrics than chemical control of the fungal pathogen using a fungicide. In fact, the addition of a second algal species to a disease susceptible monoculture, when able to engage in interspecies competition, can improve EROI by 68% compared to the 41% improvement of simply applying the most effective fungicide over a 1-year time frame. Biologically engineered algal bi-cultures are advantageous because of what is called the ‘dilution effect’ whereby species that are differentially susceptible to disease exhibit compensatory dynamics that stabilize feedstock production. Collectively, our results emphasize the importance of considering the impacts of disease on algal feedstocks, and suggest that mixed species algal feedstocks can be biologically engineered to reduce greenhouse gas emissions and improve economic viability of biofuel.
Introduction

Countries and companies alike are expanding interest in biofuels to conserve energy and reduce greenhouse gas emissions (GHGs) in an attempt to curb climate change (Jeswani et al., 2020; World Energy Outlook 2020, 2020). The algal biofuel industry's response to increasing biofuel demand has shown itself to be both promising and problematic over the last few decades. While algae are a viable candidate to improve biofuel production efficiency due to their higher photosynthetic efficiencies and higher lipid proportions than terrestrial crops, one outstanding challenge to making algal-based biofuel economically viable is optimizing the nexus between biological productivity, resilience to pathogens, and cost of infrastructure and upkeep (Borowitzka & Moheimani, 2013; Georgianna & Mayfield, 2012; Raheem et al., 2018).

The more cost-efficient open pond systems that are used to grow algae are constantly challenged with the introduction of diseases, such as fungal pathogens, which are readily introduced into ponds by wind, rain, animals, or other vectors. Once contaminated, fungal infections proliferate rapidly and can cause algal feedstocks to turn brown and crash within a matter of days (Carney & Lane, 2014). Pond crashes cost significant time and money to empty, clean and restart the cultivation pond and contribute to the difficulty in making algal biofuel an economical biofuel resource (Harmon et al., 2021; Richardson et al., 2014).

The large-scale cost of diseases on algal biofuel production has incentivized the investigation of two primary management strategies (Hannon et al., 2010; Nagi et al., 2021). Strategies for improving the resilience of the algal cultures are a priority for practitioners in order to maintain higher productivity over longer spans of time (Brentner et al., 2011; Harmon et al., 2021; Narala et al., 2016). One strategy is chemical control, which involves using pesticides and fungicides to help maintain cultures. The risks of chemical control are that it can lead to increased resistance in the pathogens, contribute to pollution, inadvertently harm other species, and pose health risks to humans (McMahon et al., 2012). Another strategy is biological control, which involves using multi species consortia, or polycultures, to stabilize crop yields by reducing the frequency of unwanted crashes caused by diseases (Hietala et al., 2017; Narwani et al., 2016). A potential mechanism for this stabilization is called the dilution effect, which occurs when diverse communities of hosts reduce the opportunity for effective transmission of a disease.
due to variance in host competency decreasing the overall density of viable hosts (Johnson & Thieltges, 2010; Newby et al., 2016).

Life-cycle assessment (LCA) is the tool most commonly called on to quantify and compare the impact of biofuel production decisions on pollution and productivity, but diseases have yet to be incorporated into an algal biofuel LCA. LCAs are useful because they mathematically model complex production systems and identify high impact design choices, often with the goal of informing policies and improving the sustainability of industry decisions (Curran et al., 2016). To compare algal management practices and determine if a dilution effect improves resilience without sacrificing productivity or cost, the total life cycle of outdoor algal culture ponds must be evaluated with respect to their cost, energy return, and greenhouse gas emissions. However, most LCAs are limited in their biological realism, and factors that greatly impact biological growth, such as disease, have been overlooked and omitted.

In this study, we utilize and improve upon an existing life cycle assessment by incorporating a disease model to demonstrate how the impact of disease on algal productivity translates to two sustainability metrics, energy return on investment (EROI) and greenhouse gas emissions (GHGs), and how chemical and biological control strategies might improve these metrics. The crashes that result from disease have a significant impact on the time and energy needed to grow a feedstock and must be recognized in order to make educated management decisions and potentially identify which improvements warrant the most efficient algal feedstock production methods. By incorporating a disease model, we not only go further than any other LCA to represent the stochasticity and growth dynamics of an algal community, but we are able to compare the effectiveness of the management strategies in order to communicate their impact on the ecological system and on the production of biofuel (Carruthers et al., 2019; Curran et al., 2016; Frank, Han, et al., 2011).
Materials and Methods

For purposes of this study, we started with the Greenhouse gases, Energy Use, and Emissions in Transportation (GREET) model, an existing LCA database that is widely used for analyses of algal biofuel systems and then modified it to include a common ecological model of disease as a driver of algal feedstock production. We then compared the outputs of systems with vs. without a disease to determine how fungal pathogens of algae influence life cycle metrics, and how those metrics might improve with different forms of chemical or biological control of the pathogen. The following sections describe how the disease model was integrated into an LCA and what methods were used to simulate controlled life cycle comparisons.

Base model for life cycle assessment

This study builds on a well-documented life cycle database called GREET, which was first created by the Argonne National Laboratory to analyze energy and emissions of transportation systems. The GREET model takes inputs that describe various vehicle technologies, fuels, products, and energy systems and calculates the renewable and non-renewable energy demand, greenhouses gas and air pollution emissions, and water consumption of an energy or vehicle system through a series of interconnected conversion equations.

The Argonne National Laboratory’s GREET model has been used as a template to produce several subsequent modifications (Carruthers et al., 2019; Frank, Han, et al., 2011; M. Wang et al., 2005). One of these modifications was made by Carruthers et al. (2019), who incorporated a logistic growth model and used Monte Carlo simulations across a standard distribution of growth rates to better represent stochasticity that occurs when growing algae. Though Carruthers's work began to better represent variation in an otherwise static LCA model, it omitted one important consideration to the accuracy of the LCA outputs, disease.

Incorporation of a disease model into AHM-GREET

Using the AHM-GREET model published by Carruthers et al. (2019), we increased this LCA’s biological realism to include the influence of disease on algal growth dynamics. The disease model is sufficiently general to represent any infectious agent that challenges the growth
and survivorship of algae. However, the formulation used in this paper is intended to describe infection of algae by pathogenic fungi like those of the families Chytridae or Asselidae, which are common infectious agents that attack and destroy algal feedstocks in outdoor cultivation ponds (Rasconi et al., 2012; Zhu et al., 2020).

To incorporate disease into the AHM-GREET model, the existing stochastic algal growth equation (a simple logistic growth equation) of Carruthers et al. (2019) was replaced with a coupled equation that describes growth and rate of spread of an infectious agent of algae (e.g., a fungal pathogen). We chose to use a density dependent model of disease transmission (i.e. transmission that is proportional to the density of the infected host) because fungal pathogen transmission in aqueous environments is most often assumed to be density-dependent (Dettner et al., 1997). Changes in biomass of a focal algal species $1$, $N_1$ (infected and susceptible), through time are given by:

$$\frac{dN_1}{dt} = N_1 r_1 \left( 1 - \frac{N_1 + c_2 N_2}{K_1} \right) - a_1 I_1$$

All rates are in units of day$^{-1}$ and are governing rates for changes in biomass, which are in units of ash-free dry weight (AFDW) of algae per liter to correspond to units of feedstock biomass used in AHM-GREET. In equation 1, $r$ is the intrinsic rate of biomass growth (the per capita birth minus death rate), $c$ is the competition effect of species 2 on species 1 (a unitless term equal to 0 for an algal monoculture, and $> 0$ for a mixed species culture of algae used to explore biological control options), $K$ is the biomass carrying capacity, $a$ is the pathogen induced mortality rate and $I$ is the infected biomass (Rudolf & Antonovics, 2005).

A growth rate $r$ of 0.5 day$^{-1}$ and carrying capacity $K$ of 1 g AFDW / L with standard deviations of 0.05 and 0.1 respectively were constants throughout our analysis, based on the productivity of *Selenastrum capricornutum* (X) and *Chlorella sorokiniana* (Y) grown in outdoor ponds (Godwin et al., 2018; Widin et al., 2022). Like Carruthers’s model, Monte Carlo simulations were used to embody variation over time based off a standard distribution for the $r$ and $K$ values. While the parameters $r$ and $K$ in equation 1 are based off previous experiments to calculate realistic LCA metrics, the parameters $a$ and $c$ were treated as variables in our analyses to explore a range of natural variability.

Eq. 1, which gives the dynamics of biomass for a focal host species of algae, was complemented with eq. 2, a density dependent disease transmission equation:
\[
\frac{di_1}{dt} = S_1B_{11}(I_1) + S_1B_{12}(I_2) + d_1I_1 \quad (2)
\]

where \( S \) is the number of susceptible individuals equal to \( N-I \) and \( B \) is the intra or inter-specific transmission rate (Rudolf & Antonovics, 2005). The term \( d \) is equal to \((a + v)\) with \( v \) representing the recovery rate of infected biomass. In this study, we assume that all cell death occurs from infection. This is a reasonable assumption because algal feedstocks are harvested at sufficient intervals that natural mortality is unlikely to contribute to population dynamics. Eq. 2 assumes that disease spread is proportional to the density of susceptible individuals, rather than to the number of infected individuals that contribute to the total population. Though these equations are simplistic in some regards (e.g., they do not incorporate resource consumption or multiple pathogens), they were chosen to achieve a balance between incorporating the basic dynamics of disease interactions into predictions of the AHM-GREET model without adding so much complexity that the model becomes intractable or uninterpretable. We feel this balance is appropriate for an initial study of disease like ours.

For each day without disease in Eq. 1 & 2, we assumed the growth ponds were subject to some risk of initial introduction of a pathogen (infection risk) to the system (eq. 3):

\[ P(I_1 + .001) | (I_1 = 0) = \text{risk of initial infection} \quad (3) \]

The infection risk represents the chance that 0.001 g AFDW / L of an infectious agent could enter the system per day via dispersal from wind, rain, insects, birds, or similar pathway of introduction.

Beyond the insertion of the disease model (eq 1-3), the AHM-GREET model remained structurally unchanged. We relied on the same parameter values of equation 1 that were used by Carruthers et al. (2019) for algae because (a) those parameters were derived from an extensive set of experiments, and (b) our goal in this paper is to examine how consideration of disease changes conclusions of Carruthers et al. (2019) from their analyses of the AHM-GREET model. Our revision of the model relied on the downstream data for the best performing bi-culture grown in variable temperature environments in outdoor ponds from Carruthers’s analysis as the
foundation of our LCA analysis. The species *Selenastrum capricornutum* (X) and *Chlorella sorokiniana* (Y) grown together resulted in a higher EROI than the best performing monoculture, *Selenastrum capricornutum*, due to the bi-culture’s ability to optimize trade-offs among multiple aspects of feedstock production, stability, and fuel quality (Carruthers et al., 2019). We used the characteristics of the XY bi-culture to parameterize the remaining AHM-GREET portion of the model for all analyses reported in this paper and focused on testing the impacts of the consideration of disease.

The disease model (eq. 1-3) informs the AHM-GREET model through three parameters: average biomass produced (g AFDW / L), average productivity or rate that biomass is produced (g AFDW / m² / day), and number of feedstock crashes per year. These outputs of the disease model served as inputs to the AHM-GREET model, replacing the same three parameters where Carruthers et al.’s simple logistic growth equation had connected to AHM-GREET and communicated the quantity of algal feedstock that was able to go toward making fuel. The oil yield per biomass was governed by the XY species combination and used as a constant throughout our analysis and any other chemical and mechanical inputs unmentioned specifically in this paper were all unchanged to be consistent with Carruthers et al.’s AHM-GREET model. We report on two outputs from the AHM-GREET model: Energy Return on Investment (EROI) and Global Warming Potential (GWP) because they represent an economic and environmental unit of comparison. GWP is equivalent to the greenhouse gas emissions (GHGs) output from GREET, which represents the impacts from CO₂, N₂O and CH₄ since the GREET model outputs the sum of these GHGs in kg CO₂ equivalents. To get to the outputs of EROI and GWP, any parameters in AHM-GREET that have not been mentioned can be assumed were constant default values.

Crashes

The primary effect of fungal disease is to induce a ‘crash’ in an algal feedstock pond. The term crash is used to reference a “natural introduction of predator or pest to [algal culture] typically following high algal biomass accumulation resulting in significant to total algal biomass losses” (Hamilton, 2014). The term ‘crash’ is subjective in the sense that growers often have a qualitative sense of the point at which a fungus has induced sufficient mortality to algae
that a pond must be dismantled and reset (e.g., when a green pond turns brown). Carruthers et al. (2019) took a more objective approach in their formulation of the AHM-GREET model, defining a crash as any point in time where the culture feedstock fell below a threshold of 50 mg biomass/L. For purposes of our study, we prefer to define a crash not in terms of standing biomass, since biomass at any single point in time can be influenced by things other than disease (e.g., stochastic variation in growth rates or carrying capacity). Instead, we define a crash based on the proportion of algal biomass that is infected by fungi. We use a threshold of 20% infection, meaning if there was greater than 20% of the biomass of algae infected one day after harvest, the culture was deemed to have “crashed” and then the pond would go through the process of being drained, cleaned, and inoculated again over 7 days before reinoculation. The 20% crash threshold was chosen based on a study of algal bi-cultures grown in outdoor ponds by Godwin et. al. (2018). That study showed that the bi-cultures' 25th and 75th percentiles of the maximum biomass crash magnitude (% reduction in 7 days) ranged from 20 – 75%. Additionally, each of the bi-cultures experienced crashes of at least 20% of their biomass (Godwin et al., 2018). By using 20% as our crash threshold, we consider the loss of 1/5 of the feedstock’s biomass as detrimental enough to warrant the process of draining, cleaning, and re-inoculation. As we will show later using sensitivity analyses, the specific threshold of infection does not impact the qualitative conclusions of our analysis, as the general trends are qualitatively robust.

Simulations

We conducted three simulations of the AHM-GREET model with disease (eq. 1-3) to examine i) how a fungal pathogen impacts EROI and GWP; ii) how chemical control of the pathogen using a fungicide impacts these LCA metrics, and iii) how biological engineering of a multi-species consortium of algae impacts the fungal pathogen and, in turn, LCA metrics. The purpose of these simulations was to identify the extent to which disease alters conclusions about feedstock viabilities, and then to compare two disease management strategies to see which one has the most potential to improve the life cycle metrics affected by disease.

The first simulation tested the impact of incorporating disease into the AHM-GREET model and how the parameters in the governing equations affected EROI and GWP. Ten replicate simulations were run for each variable combination to generate variance of the outputs.
and depict the range of potential outcomes. The list of tests and their corresponding parameters are listed in Table 1. Sensitivity analyses were conducted for the following parameters: transmission rate (B), recovery rate (v), pathogen induced mortality rate (a) and the crash threshold since these values are not yet available from empirical studies.

Based on the first simulations, a disease baseline condition of a highly susceptible disease and a control-AHM was established as the relative scenarios to which the two treatments, chemical and biological, would be compared. A disease with a transmission rate (B) of 3 day^{-1}, pathogen induced mortality rate of 0.756 day^{-1}, and initial infection risk of 0.04 was determined to represent a disease baseline in which a single species system of algae produces the least desirable LCA metrics due to disease. The control-AHM was the output of the AHM-GREET model that does not incorporate any disease. By running 200 simulations of the control-AHM and disease baseline, benchmark values of 0.519 EROI, 152.4 GWP for the control-AHM and 0.310 EROI, 259.6 GWP for the disease baseline were established (Table 2). In the following simulations, the % change in EROI or GWP was calculated using the difference between a treatment scenario and the disease baseline scenario. By reporting % change relative to the disease baseline, we were better able to communicate the relative improvement each treatment has as well as efficiently compare the effectiveness of the treatments.

Our second simulation created a scenario where a chemical fungicide was applied to the feedstock ponds to try and prevent a feedstock crash. One day after harvest, if the proportion of algal biomass infected by the fungus exceeded the 20% crash threshold, the infection was reduced by adding a fungicide. The percent decrease in infected algal biomass, which was called the fungicide effectiveness, was varied in the simulations. In addition, we tested the impact of variable dose limits, which is the number of consecutive weeks the fungicide was able to be added to the feedstocks, ranging from a single dose of 1 to a limit of 52 (1 per week for a year). The effect of the fungicide effectiveness and dose limits are presented in % change from the disease baseline. Both the first and second simulations of the disease model LCA were run with a monoculture represented in eq (1-3).

The third simulation tested the impact of a biological treatment on fungi in which a biculture of algae was grown, and therefore interspecies transmission and competition with a second algal species was included in the disease model LCA. This simulation allowed us to determine if the interaction with a second species might dilute the disease’s ability to spread and
improve LCA metrics. To compare chemical and biological treatment’s impact, we reduced the variables describing “susceptibility” down to transmission rate, holding \( v = 0 \) and \( a = .756 \) constant. The susceptibility of species 2 to infection was varied by changing interspecies transmission rate and intraspecies transmission rate simultaneously (\( B21 = B22 \)) while species 1’s parameters were held constant (\( B12 = B11 = 3 \)) to be consistent with the disease baseline. Competition among algal species was tested by holding the species 1 competition coefficient constant at \( c_{21} = 1 \) and varying the species 2 competition coefficient (\( 0 > c_{12} \geq 1 \)). By maintaining parameter values for species 1 consistent with the disease baseline, we were able to facilitate comparison between the chemical and biological community treatments.
Results and Discussion

Effect of fungal disease on LCA metrics

Our analyses show that failure to consider the impacts of disease in an algal biofuel life-cycle assessment leads to overly optimistic estimates about the Energy Return on Investment (EROI) and Global Warming Potential (GWP) of algal biofuel systems. This conclusion is evident from Figure 1a, which shows how EROI (green dots) and GWP (orange dots) change on the y-axis as a function of increasing probabilities of initial fungal infection on the x-axis. For comparison, we show outputs of the original AHM-GREET model by Carruthers et al. (2019), which we termed the control-AHM since that model did not include fungal disease (symbolized with *’s in the figure). Our disease baseline model from equations 1 through 3 is symbolized with +’s in the figure. Note that at zero infection risk on the x-axis, AHM-GREET outputs are identical between the control-AHM and disease baseline models, confirming that our new disease model produces identical results as the original published by Carruthers et al. (2019). However, as the probability of infection increases along the x-axis, EROI decreases to an asymptotic mean of 0.31 as infection risk passes 0.04. At the same time, GWP increases to an asymptotic mean of 260 as infection risk passes 0.04. These results show that inclusion of a fungal pathogen in the AHM-GREET model can reduce EROI by as much as 40% and increase GWP by 70% relative to the control-AHM where no disease is considered.

The decrease in EROI and increase in GWP shown in Figure 1a is driven by a decrease in algal feedstock biomass and productivity over the yearlong growing period when disease is incorporated. Figure 1b shows the biomass of algae over 200 days of the year with repeated harvest periods (evident by the drops in cell biomass) when there is no disease in the system. To contrast, Figure 1c shows the same metrics but under the scenario where even a small fungal infection risk of 0.04 allows for a high transmission of the disease to dampen growth and cause feedstock crashes (evident in the cell biomass falling to 0 after a rise in infected biomass proportion). These crashes worsen the LCA metrics because it takes time and energy to empty, clean, and restart the feedstock ponds which in turn reduces the overall productivity of the feedstock and worsens EROI and GWP (Carruthers et al., 2019).
The magnitude of impact of fungal disease on EROI and GWP is controlled by biological ‘thresholds’ that determine whether the host – pathogen system experiences a feedstock crash. As transmission rates (B) of the pathogen increase there is a threshold rate of 1.72 at which LCA metrics are negatively impacted (Figure 2a). At this transmission rate, EROI suddenly drops from 0.52 to 0.31 (-40%) and GWP increases from 152 to 260 (+70%) due to the disease’s rate of spread exceeding the rate at which the healthy population of algae can reproduce. Figure 2b shows that as the recovery rate (v) increases along the x-axis, the LCA metrics improve (EROI increases and GWP decreases) until a threshold of 0.23 where the recovery rate is sufficiently large to prevent the spread of disease. One counter-intuitive result of these analyses is shown in Figure 2c where an increase in the pathogen-induced mortality rate is associated with an improvement in EROI (increase) and GWP (decrease), which stabilize at a pathogen-induced mortality rate of 0.96. The value of 0.96 represents the point at which the spread of the fungal disease cannot keep up with the rate of algal mortality and, as a result, the fungus goes extinct from the culture system. This dynamic is illustrated further in Figures 2d and e, which show the biomass of algal feedstock and proportion of algal biomass infected for a low mortality rate of 0.40 (Figure 2d) and a high mortality rate of 1.16 (Figure 2e). At the low mortality rate, the fungus persists in the algal population, whereas at a high mortality rate, infected algae die faster than they can transmit the fungal disease ultimately driving infection toward 0. The threshold effects displayed in Figure 2 are key to understanding the relationship between algal characteristics and their resilience toward a pathogen that prevents feedstock crashes.

The threshold trends shown in Figure 2 are qualitatively robust, as supported by a sensitivity analysis (see SI). The values for pathogen induced mortality, transmission, recovery rate and crash threshold were varied three times and then modeled to see if our conclusions would change when using different parameter values. Even when varying the parameter values used to generate Figure 2a-c, we found not only that a threshold model consistently fit the outcomes better than any alternate regression model, but that the overall pattern of the outputs remained consistent even as the exact threshold values shifted left or right along the x-axis. The location of the threshold changes as the parameters change. So, the relationship between the parameters, especially between transmission and pathogen induced mortality, that drive crashes needs to be explored further especially in the context that the EROI and GWP change significantly before and after the threshold.
Our comparison of the disease baseline to the control-AHM model quantifies the intuitive expectation that disease significantly worsens the life cycle metrics of an algal feedstock system. Thus, predictions from published LCA’s like that by Carruthers (2019), which improved the biological realism of the AHM-GREET model for algae but did not consider the chronic problems of disease, are likely to provide overly optimistic estimates of life-cycle metrics for algal biofuel production. There are, however, at least two limitations of our disease model that need to be considered. First, while we used a common density dependent disease model that successfully incorporates the consideration of disease, there are other models such as the frequency dependent transmission model that might better describe some empirical systems (Rudolf & Antonovics, 2005). Refinement of the modes of disease transmission could modify our conclusions and should be explored in the future. Second, while this study improves the use of empirically supported parameterization of an algal LCA, there is a limitation associated with parameter values chosen and a continued need for empirical studies to support the parameters that go into the model. Understanding the magnitude of disease impact, the disease parameter interactions and patterns, and the limitations of our model are imperative to make more educated management decisions because the crashes that result from disease have a significant impact on the time and energy needed to grow a feedstock. Given the impacts of disease on the feedstock system, we must figure out how to control the challenge of pathogens. We explore this in the next two sections.

Chemical treatment of disease

To reduce the risk of feedstock crashes caused by fungal disease, there are two primary options for management: chemical treatment and biological control. In this section we explore the effectiveness of chemical treatment by simulating the application of fungicide to algal growth ponds. The fungicide reduces the biomass infected by the pathogen by a percentage, deemed the fungicide effectiveness, when the culture would have otherwise crashed. When considering the impact of the fungicide, we must separate how effective the fungicide is at reducing the pathogen for any single dose and how many times we can dose the system. The number of sequential doses could be limited by externalities such as ecosystem and human health impacts. Therefore, we look at both the fungicide effectiveness and the dose limit and compare how these variables
affect the EROI and GWP relative to the disease baseline, (i.e., the high transmission disease system established in Figure 1). By doing so, we see how much EROI and GWP can be recovered by applying fungicide to the system.

Our analyses show that chemical treatment of algal feedstock with fungicide can recover some of the lost EROI caused by a fungal pathogen, but even the most effective chemical treatment does not recover the system fully relative to the control-AHM scenario with no disease. In Figure 3a-b, we show the % change in EROI and GWP from the disease baseline (y-axis) as a function of the fungicide’s effectiveness in reducing the pathogen (x-axis) for multiple dose limits (the number of consecutive weeks the feedstock is dosed with fungicide). When there is no limit on the number of consecutive weeks the feedstock system can be dosed with fungicide (i.e. 52 doses, 1 per week for a year) and the % reduction of infected cells (the effectiveness) by the fungicide is 99%, EROI increases by 39.4% relative to the disease baseline. If, however, the fungicide dose limit is one, the recovery of EROI is drastically reduced to a maximum of 10.5% relative to the disease baseline. This result holds true even with a high fungicide effectiveness of 99% reduction in infected cells. The GWP is reduced by a maximum of 29.2% when the dose limit is 52 but only by a maximum of 10.1% when the dose limit is 1 (absolute values for EROI and GWP can be found in the SI). These improvements do not compare to the 67.6% increase in EROI and 41.3% decrease in GWP needed to match the control-AHM scenario of LCA model outputs without any disease.

The primary reason why chemical treatment does not fully recover the LCA metrics is that fungicide acts as a temporary measure to prevent feedstock crashes, but does not address the core driver of feedstock crashes, which is the biological susceptibility of the algae to disease. For example, a system with a dose limit of 1 will prevent 1 crash, but if the disease’s spread exceeds the healthy algal population’s reproduction rate, then the feedstock will crash in the following weeks with no further control from fungicide. This results in minimal improvement to the LCA metrics. Additionally, a system with a high dose limit of 52 and fungicide effectiveness of 99% respectively might not experience a feedstock crash anytime during a year of growth. But if the disease model parameters still allow for the spread of disease, there remains substantial biomass lost due to pathogen-induced mortality. This, in turn, reduces the EROI and increases GWP compared to a system without disease.
While our analyses suggest that chemical treatment has only modest impact on recovery of LCA metrics, there are several limitations of how we consider fungicide treatment in our model that are worth noting. First, our analyses of fungicide application do not account for externalities of higher fungicide dose limits such as high handling risks, persistence time of chemicals in the environment, or cleanup and disposal costs. Prior studies suggest the commonly used fungicide Fluazinam® is an effective deterrent of fungal infection at an average dosing frequency of once every 1.5 weeks and a concentration of 1 ppm over three months (Davis & Laurens, 2020). However, Fluazinam® is a known carcinogen considered “very toxic to aquatic life with long-lasting effect” according to the Global Harmonized System of chemical classifications. If not metabolized, the chemical compound has a hydrolysis half-life of 42 days at pH 7” (Komyoji et al., 1995; Li et al., 2020; “PubChem Compound Summary for CID 91731, Fluazinam,” 2022; Witono et al., 2019). Chemical fungicides, like Fluazinam®, might be able to improve life-cycle metrics in the short term, but the externalities such as the cost to human and ecosystem health are not represented in LCA’s like the GREET model (X. H. Wang et al., 2018). Thus, our results for chemical control may be overly optimistic. Additionally, our analyses do not consider any form of evolution in the host-pathogen system. Fungi can adapt to chemical control and resistance can develop over time, especially in high-density growth situations (Ghaderiardakani et al., 2020; Jensen, 2016; Niquil et al., 2011; Vanden Bossche et al., 1998). Changes in the pathogenic risks can lead to a coevolutionary arms race that poses challenges for management, and such scenarios are not represented in our LCA model (Langerhans, 2008; McGowen, 2021; Wilson et al., 2002).

**Biological control of disease**

Biological control is sometimes posed as an alternative to chemical control of fungal pathogens. In this section we explore the impact of biological control of disease by simulating disease transmission of fungi in a system composed of two algal species grown together as a bi-culture. In order to understand the impact of adding a second species to the algal feedstock we hold species 1’s characteristics consistent with the disease baseline characteristics. In turn, by varying only the parameters for the 2nd algal species we see how altering competition among algae, and how changing pathogen transmission rates independently impact the potential for
biological control of fungi to improve the EROI and GWP life cycle metrics relative to the disease baseline (the biomass comparisons of the two treatments vs the control and disease baseline can be found in the SI).

Our analyses suggest that biological engineering of the algal feedstock system has potential to recover EROI and GHG’s well above what can be achieved by a chemical treatment alone. However, effective biological control requires a specific combination of algal species traits to achieve good results. Figure 4a-b shows the % change in EROI and GWP from the disease baseline (y-axis) as a function of the species 2 disease transmission rates (x-axis) with various competition coefficients. When species 2 has transmission rates (B22, B21) of 0 (it is not susceptible to fungal pathogen) and a competition coefficient of 1 (competitively identical to the first species, resulting in strong competition), biological control of the fungal disease is nearly able to recover all the EROI that would otherwise be lost to disease. It is in this case we see the maximum improvement of LCA metrics where EROI increases by 68.1% and GWP decreases by 41.4% relative to the disease baseline. The high levels of recovery occur because, given equal competitive ability ($c_{12} = c_{21} = 1$), as species 1 succumbs to disease species 2 is able to grow in place of any biomass lost by species 1 lost to disease. This phenomenon is referred to as ‘compensatory dynamics’ in ecology (Cardinale et al., 2012).

The ability for a feedstock to exhibit compensatory dynamics depends on the strength of competition among the two species, as competition determines the magnitude of growth by an inferior species after being released from competition. Figure 4c illustrates compensatory dynamics with an example of algal biomass (left y-axis) and proportion of biomass infected (right y-axis) over a period of 200 days (x-axis). When species 2 is slightly less competitive than species 1 ($c_{12} = .85$) but not susceptible to disease ($B_{22} = B_{21} = 0$), we see greater compensatory dynamics (shown in Figure 4d) where disease grows in the system along with the more competitive species 1 until the disease begins to inhibit species 1. Death of algal species 1 releases species 2 from competition, giving species 2 the opportunity to increase in biomass and dominate the feedstock. The growth of species 2 then dilutes the spread of fungal disease since species 2 is less susceptible. As the fungal disease is diluted and decreases in prevalence in the system, species 1 recovers and again begins to exhibit its competitive advantage over species 2. This starts the cycle over again.
In contrast to the scenario with compensatory dynamics, Figure 4e shows what happens if species 2 is far less competitive than species 1 ($c_{12} = .25$). Even if species 2 is not susceptible to the fungal disease, in this scenario it is both unable to maintain enough biomass when the proportion of infected biomass is low to slow the spread of disease, and it is unable to grow fast enough once disease reduces the biomass of species 1 to prevent crashes.

Results shown in Figure 4 illustrate a phenomenon known as the ‘dilution effect’ in disease ecology (Schmidt & Ostfeld, 2001). The dilution effect is a biological mechanism where multi-species communities of hosts inhibit the spread of a disease as competition among hosts decreases the overall density of viable (competent) hosts, in turn, impeding transmission of the pathogen (Civitello et al., 2015). The dilution effect is particularly visible in the compensatory dynamics shown in Figure 4d where Species 2 is less competitive, but also not susceptible to the pathogen, and thus increases in biomass when the disease is challenging Species 1. This eventually decreases the density of Species 1 until the spread of disease is staved off. Because of compensation in biomass, the dilution effect has potential to improve feedstock stability and productivity by reducing the frequency of crashes (Newby et al., 2016). Fewer crashes equate to better stability and productivity of the feedstock system, which in turn serve to improve EROI and GWP because more feedstock is converted to fuel and less energy is used to empty, clean, and restart the feedstock ponds.

Our simulations serve to identify several limitations of the dilution effect in an algal biofuel system. The addition of a second algal species does not mean that the community will always dilute the prevalence of a disease. Instead, the dilution effect only operates if the two algae have large differences in susceptibility to the disease (one high, one low) and when competition among the species is sufficiently high to produce strong compensatory dynamics. Experiments that have searched for a dilution effect in the past may have failed to find evidence of this phenomenon because two biological characteristics were not met. For example, Widin et al. (2022) studied a three-species algal biofuel feedstock and saw one competitively dominant and non-susceptible species drive the other two less competitive and less susceptible species out of the system when a fungal disease was introduced (Widin et al., 2022). The presence of a single species that was both highly competitive and less susceptible to disease is different than what is required for a dilution effect to operate. Our study shows that one susceptible and competitively dominant species must be combined with a non-susceptible but closely
competitive species for a dilution effect to work. If species 2 is not susceptible to the disease \((B_{22} = B_{21} = 0)\), the positive impact on the LCA metrics increases as \(c_{12}\) gets closer to 1.

Our disease model analyses provide an introduction to the importance of disease considerations in algal feedstock growth and management decision implications, but the complexity of the process extends beyond what is captured in these analyses alone. Our model does not take into account differing oil contents of algal species, which could impact the EROI and GWP of the biological treatment scenario with two competing species. Future models will need to incorporate various oil contents to answer the question— if the oil content per biomass of bi-cultures is lower than monocultures, does the benefit of increase resilience to disease make up for this loss? Even with understanding the role of competition and the examples of the dilution effect in other settings, there also remains the challenge of ecologically engineering the algal system to incorporate species with the optimal characteristics (Civitello et al., 2015; He et al., 2019; Khalil et al., 2016; Smith et al., 2015). The results of our study could help direct ecological engineers to design algal systems with advantageous species combinations, but preliminary tests must be conducted to learn more about the susceptibility and interspecies competition of algal species to figure out which species might form the optimal design.
Acknowledgments

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Funding Sources
U.S. Department of Energy’s Co-Optima Program
### Tables

#### Table 1. List of parameters used for the simulations depicted in Figures 1 and 2.

<table>
<thead>
<tr>
<th>Figure</th>
<th>Pathogen Induced Mortality</th>
<th>Transmission Rate</th>
<th>Recovery Rate</th>
<th>Infection risk</th>
</tr>
</thead>
<tbody>
<tr>
<td>1a</td>
<td>.756</td>
<td>3</td>
<td>0</td>
<td>variable</td>
</tr>
<tr>
<td>1b</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>0</td>
</tr>
<tr>
<td>1c</td>
<td>.756</td>
<td>3</td>
<td>0</td>
<td>.04</td>
</tr>
<tr>
<td>2a</td>
<td>.756</td>
<td>variable</td>
<td>0</td>
<td>.04</td>
</tr>
<tr>
<td>2b</td>
<td>.756</td>
<td>2.225</td>
<td>variable</td>
<td>.04</td>
</tr>
<tr>
<td>2c</td>
<td>variable</td>
<td>2.225</td>
<td>0</td>
<td>.04</td>
</tr>
<tr>
<td>2d</td>
<td>.4</td>
<td>2.225</td>
<td>0</td>
<td>.04</td>
</tr>
<tr>
<td>2e</td>
<td>1.2</td>
<td>2.225</td>
<td>0</td>
<td>.04</td>
</tr>
</tbody>
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#### Table 2. Summarized LCA outputs for the control AHM and disease baseline

<table>
<thead>
<tr>
<th></th>
<th>EROI</th>
<th>St. Deviation</th>
<th>St. Error</th>
<th>GWP</th>
<th>St. Deviation</th>
<th>St. Error</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control-AHM</td>
<td>.5188</td>
<td>.01041</td>
<td>.0007363</td>
<td>152.36</td>
<td>3.117</td>
<td>2.204</td>
</tr>
<tr>
<td>High Transmission Disease Baseline</td>
<td>3096</td>
<td>.02881</td>
<td>.001441</td>
<td>259.55</td>
<td>23.649</td>
<td>1.1825</td>
</tr>
</tbody>
</table>
Figure Legends

Figure 1. a) comparison of the EROI and GWP outputs from the original AHM-GREET model outputs and a disease model with a single species that is highly susceptible to the disease. b) excerpt from the growth model of the AHM-GREET model used as the control system in this analysis. c) excerpt from the disease model showing the impact of disease increasing the infected biomass proportion and leading to the crashes that worsen EROI and GWP.

Figure 2. a) variable transmission rate effect b) variable recovery rate effect c) variable pathogen induced mortality rate effect d) excerpt from the disease model showing the increase in infected biomass proportion due to a low pathogen induced mortality rate e) excerpt from the disease model showing the non-prevalence of infected biomass proportion due to a high pathogen induced mortality rate.

Figure 3. a) change in EROI from the baseline for various fungicide dose limits as a function of fungicide effectiveness. b) change in GWP from the baseline for various fungicide dose limits as a function of fungicide effectiveness.

Figure 4. a) change in EROI from the baseline for various species 2 competitive abilities as a function of species 2’s susceptibility, represented by transmission rate. b) change in GWP from the baseline for various species 2 competitive abilities as a function of species 2’s susceptibility. c-e) excerpts from the disease model showing the dynamics of infected biomass proportion and the two algae species biomass with species 2 being completely non-susceptible (transmission rates = 0) and having various competitive abilities.
Figures

Figure 1.
Figure 2.

Figure 3.
Figure 4.
Supplemental Information (SI)

Fitting the threshold model

In order to test figure out which regression model fit the data shown in Figure 2, a linear, exponential, logarithmic and threshold model were fit to the data in R and then the Akaike Information Criterion (AIC) was used to indicate which had the best fit (Portet, 2020). The R package chngpt was used to model the threshold regressions (Fong et al., 2017). For each variable (B11, A1, V1), a threshold model provided the best fit (Table S1).

Sensitivity to each parameter

Sensitivity analyses were conducted for the following parameters: transmission rate (B), recovery rate (v), pathogen-induced mortality rate (a), and the crash threshold because these values have yet to be supported through empirical studies. To test the impact of our assumed variables, we simulated changes in each variable to determine how the location of thresholds in the data changed. Pathogen-induced mortality rate (a1) and transmission rate (B11) were both modeled with a 10% increase and 10% decrease from their assumed values reported in the main results (.756 and 2.225 respectively). Recovery rate (V1) and crash threshold were both modeled with at least two other increments intended to cover a range of plausible values.

We found that in every situation, threshold models fit the data better than an alternate regression model and the overall form of the data remained constant. The output series show a shift in the location of the threshold on the x-axis (Figures S1 – S4) and the precise value of the location as a result of the chngpt package is reported in Tables S2 – S5. Both the EROI and the GWP analysis graphs are included for each figure and show that the threshold trends remain consistent for both those LCA outputs.

Cumulative Harvest Biomass of each primary scenario
The respective EROI and GWP outputs from each scenario are driven by the disease impacts on overall algal biomass outputs as well as the number of times the culture crashes. To illustrate the difference in biomass output for the control AHM, disease baseline, chemical control and biological control scenarios, we plot the cumulative harvested biomass (y-axis) over a one-year period (x-axis) in Figure S5. For a 400 L pond, the cumulative biomass of the control AHM and the biological treatment scenario where the second species competition coefficient is .85 and the transmission rates are 0 (unsusceptible) are about the same at roughly 16 kg of AFDW in one year. The disease baseline scenario returned about 12 kg and the best chemical treatment scenario with a dose limit of 52 and effectiveness of 99% returned about 13.5 kg AFDW in one year. These accumulated biomass amounts are then factored into the amount of biofuel able to be made through the process of hydrothermal liquefaction (HTL). While Figure S5 shows the impact of crashes on biomass, it is omitting the impact of the crashes on the overall process directly. The similarity in the control AHM and the biological treatment's biomass accumulation supports the ability for biological treatment to recover most or all of the EROI and GWP lost to disease by producing a similar amount of biomass over a year.
### SI Tables

**Table S1. AIC values of each regression model**

<table>
<thead>
<tr>
<th>AIC values</th>
<th>Linear</th>
<th>exponential</th>
<th>log</th>
<th>Threshold</th>
</tr>
</thead>
<tbody>
<tr>
<td>B11</td>
<td>-2118.8</td>
<td>-1194.7</td>
<td>-1959.7</td>
<td>-2538.6</td>
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<tr>
<td>v1</td>
<td>-1491.8</td>
<td>-972.5</td>
<td>n/a</td>
<td>-1531.5</td>
</tr>
<tr>
<td>a1</td>
<td>-1319.3</td>
<td>-578.8</td>
<td>-1320.0</td>
<td>-1816.8</td>
</tr>
</tbody>
</table>

**Table S2. Threshold location value (B11) on the x-axis for variable pathogen-induced mortality rate, a1**

<table>
<thead>
<tr>
<th>a1</th>
<th>Threshold (B11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>.680 (-10%)</td>
<td>1.52</td>
</tr>
<tr>
<td>.756</td>
<td>1.72</td>
</tr>
<tr>
<td>.832 (+10%)</td>
<td>1.88</td>
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</table>

**Table S3. Threshold location value (a1) on the x-axis for variable transmission rate, B11**

<table>
<thead>
<tr>
<th>B11</th>
<th>Threshold (a1)</th>
</tr>
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<tbody>
<tr>
<td>2.003 (-10%)</td>
<td>.880</td>
</tr>
<tr>
<td>2.225</td>
<td>.960</td>
</tr>
<tr>
<td>2.447 (+10%)</td>
<td>1.04</td>
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</table>

**Table S4. Threshold location value (B11) on the x-axis for variable recovery rate, V1**

<table>
<thead>
<tr>
<th>V1</th>
<th>Threshold (B11)</th>
</tr>
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<tbody>
<tr>
<td>0</td>
<td>1.72</td>
</tr>
<tr>
<td>.1</td>
<td>1.96</td>
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<tr>
<td>.2</td>
<td>2.20</td>
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**Table S5. Threshold location value (B22,B21)) on the x-axis for variable Crash Threshold while C1 = C2 = 1 and B = 3**

<table>
<thead>
<tr>
<th>Crash Threshold</th>
<th>Threshold (B22,B21)</th>
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</thead>
<tbody>
<tr>
<td>.1</td>
<td>0.920</td>
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Table S6. Excerpt of absolute values of EROI and GWP (kg CO2 eq / mmBTU) with the standard error and the % change of EROI and GWP for chemical treatment scenario

<table>
<thead>
<tr>
<th>99% effectiveness</th>
<th>EROI</th>
<th>St. Error</th>
<th>GWP</th>
<th>St. Error</th>
<th>% change EROI</th>
<th>% change GWP</th>
</tr>
</thead>
<tbody>
<tr>
<td>F limit 1</td>
<td>.342</td>
<td>.00655</td>
<td>233</td>
<td>4.30</td>
<td>10.5</td>
<td>-10.1</td>
</tr>
<tr>
<td>F limit 30</td>
<td>.436</td>
<td>.00183</td>
<td>182</td>
<td>.770</td>
<td>40.9</td>
<td>-29.9</td>
</tr>
<tr>
<td>F limit 52</td>
<td>.431</td>
<td>.00194</td>
<td>184</td>
<td>.828</td>
<td>39.4</td>
<td>-29.2</td>
</tr>
<tr>
<td>Control</td>
<td>.316</td>
<td>.00996</td>
<td>254</td>
<td>8.20</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>80% effectiveness</th>
<th>EROI</th>
<th>St. Error</th>
<th>GWP</th>
<th>St. Error</th>
<th>% change EROI</th>
<th>% change GWP</th>
</tr>
</thead>
<tbody>
<tr>
<td>F limit 1</td>
<td>.321</td>
<td>.00520</td>
<td>249</td>
<td>3.97</td>
<td>3.64</td>
<td>-4.12</td>
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<tr>
<td>F limit 30</td>
<td>.383</td>
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<td>.904</td>
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<td>-20.1</td>
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<tr>
<td>F limit 52</td>
<td>.411</td>
<td>.00534</td>
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<td>32.6</td>
<td>-25.4</td>
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<tr>
<td>Control</td>
<td>.309</td>
<td>.00704</td>
<td>259</td>
<td>5.68</td>
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</tr>
</tbody>
</table>

Table S7. Excerpt of % change of EROI and GWP for biological treatment scenario with each competition coefficient of species 2.

<table>
<thead>
<tr>
<th>C12 = 1</th>
<th>EROI % change max</th>
<th>GWP % change max</th>
<th>EROI % range</th>
<th>GWP % range</th>
</tr>
</thead>
<tbody>
<tr>
<td>68.1</td>
<td>-41.4</td>
<td>65.5</td>
<td>-37.5</td>
<td></td>
</tr>
<tr>
<td>C12 = .85</td>
<td>58.8</td>
<td>-37.7</td>
<td>54.4</td>
<td>-33.7</td>
</tr>
<tr>
<td>C12 = .70</td>
<td>44.2</td>
<td>-26.0</td>
<td>38.5</td>
<td>-31.4</td>
</tr>
<tr>
<td>C12 = .25</td>
<td>33.4</td>
<td>-25.6</td>
<td>18.6</td>
<td>-13.1</td>
</tr>
</tbody>
</table>
SI Figure Legends

Figure S1. The EROI and GWP changing along a variable transmission rate (x-axis) and simulated holding three iterations of pathogen-induced mortality constant. The vertical lines represent the threshold of each simulation group.

Figure S2. The EROI and GWP changing along a variable pathogen induced mortality rate (x-axis) and simulated holding three iterations of transmission rate constant. The vertical lines represent the threshold of each simulation group.

Figure S3. The EROI and GWP changing along a variable transmission rate (x-axis) and simulated holding three iterations of recovery rate constant. The vertical lines represent the threshold of each simulation group.

Figure S4. The EROI and GWP changing along a variable transmission rate (x-axis) and simulated holding three iterations of crash threshold constant. The vertical lines represent the threshold of each simulation group.

Figure S5. Summary of the cumulative harvest biomass for a 400 L algal pond over 1 year under the following scenarios: the control AHM, the disease baseline, chemical treatment with dose limit of 52 and effectiveness of 99%, and biological control with a second species competition coefficient of .85 and transmission rates of 0.
Figure S1.

Figure S2.
Figure S3.

Figure S4.
Figure S5.
References


