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Cross-study analysis of factors affecting algae cultivation in recycled medium for biofuel production

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ABSTRACT

Current high costs of commercial-scale algal biofuel production prevent the widespread use of this renewable fuel source. One cost-saving approach is the reuse of algae cultivation water after biomass harvesting, which reduces water pumping and treatment costs. However, dissolved compounds, cell debris, and microorganisms remaining in the water could affect subsequent algae generations. Previous studies demonstrate a variety of effects of recycled medium on algae growth, yet their results have not been collectively analyzed. Here we integrate data across 86 studies to determine the relative importance of different factors influencing algae growth in recycled medium. We found that algae taxa can have the greatest influence, while the harvesting method is less influential on growth outcomes. This meta-analysis identifies favorable taxa and thus provides a tool for algae cultivation decision-making when medium reuse is an important driver. Results can also aid in estimating relative algae yield and growth rates for technoeconomic assessments that incorporate water recycling.

1. Introduction

While benefits of using microalgae as a feedstock for biofuels and co-products are numerous (e.g., carbon capture, ability to use non-arable land and non-freshwater resources, higher areal productivity than other oil-based crops [1]), algal biofuels are currently not cost-competitive with conventional fuels [2] and have an impractical energy return on investment [3]. Increasing the profitability of algal fuels requires improvements to algae cultivation, among other production processes.

Using wastewater or recycled growth medium for algae cultivation is necessary for financially and environmentally sustainable algae production, especially for low-value products such as biofuels [4,5]. Using a variety of wastewater sources for cultivation has been extensively researched to reduce freshwater and nutrient demand [6]. Some studies demonstrate energetic feasibility of such systems [7,8], although obstacles remain such as biological contaminants in wastewater [9] and co-location of cultivation facilities with wastewater sources [1]. An alternative approach is to reuse cultivation water to reduce costs and energy associated with pumping input water, adding nutrients, and treating discharged cultivation water [4].

After harvesting, however, remaining water can contain dissolved compounds, cell debris, and microorganisms that may affect algae growth. The composition and concentration of extracellular organic matter in recycled medium will depend on the previously grown algae strain, its growth phase when harvested, the harvesting process, microbial co-habitants, and growth conditions [4,10–13]. Algal organic matter is released by excretion and cell lysis and is composed of all major macromolecules, but is mostly polysaccharides by weight [14]. These compounds can be photosynthetic waste products, signaling molecules, allelochemicals, colony formation and motility aids, or metal chelators, among others [15–18]. Released organic compounds could be directly inhibitory, such as fatty acids [19,20] released from lysed cells, or may alter physical and chemical aspects of the water (e.g., viscosity [21]) that could influence nutrient availability and uptake, motility, gas exchange, cell aggregation, and shading [22,23].

Algae growth experiments in spent, or conditioned, medium date back to the 1940s [24], while similar experiments for biotechnological applications have been reported mostly within the last five years. Collectively these studies provide a wide range of conclusions about the effects of recycled medium and extracellular compounds on algae growth, likely because of the vast array of algal exudates and their relative concentrations across studies. Recently, Farooq et al. [4] reviewed the topic of water use in algae cultivation, and described about a dozen studies testing algae growth in recycled medium. The review identified harvesting methods and organic matter as influential factors affecting growth in recycled water, but a comprehensive, quantitative analysis of many more studies and factors is warranted.

Here, we present a meta-analysis to evaluate how recycled growth medium affects algae growth and what factors affect suitability of the recycled medium. The objectives of this cross-study analysis are to 1) determine which cultivation and harvesting factors explain variation

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in algae growth response in recycled medium, and 2) provide a recommendation of cultivation and harvesting strategies for successful reuse of cultivation water.

We hypothesize that factors strongly influencing dissolved organic matter concentration and composition explain variation in algae growth success in recycled medium. These factors include a) harvesting method, which affects the removal of organic matter [25,26] and potential contaminants [27] from the water, as well as the degree of algae cell disruption during harvest; b) taxa of microalgae previously grown in the medium (hereafter termed "source algae"); and c) growth stage when harvested. We predict that relative algae growth success is negatively correlated with the number of medium reuses, because dissolved waste products or toxins may accumulate and affect growth.

Commodity industry needs recommendations for cultivation strategies that make long-term, low-cost reuse of growth medium a reality. Although a demonstration facility has successfully performed long-term water recycling [27], understanding how different factors influence algae growth in recycled medium can provide recommendations to make medium recycling more widespread. This analysis can also provide relative algae growth data for life cycle and technoeconomic assessments so that they reflect any changes in algae yield caused by recycled medium.

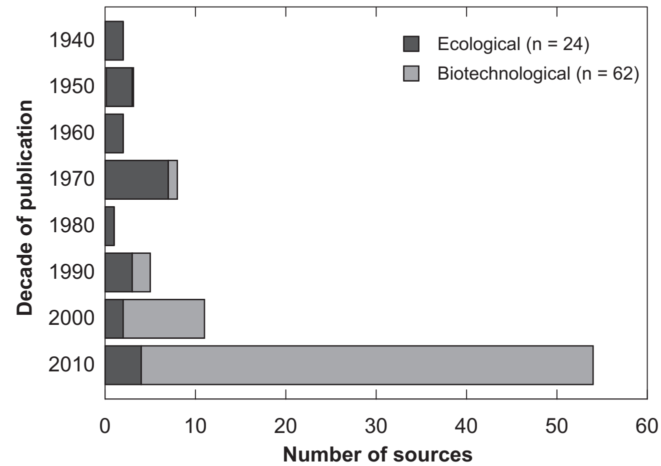

Fig. 1. Sources used for meta-analysis grouped by publication decade and research purpose (ecological or biotechnological).

2. Materials and methods

2.1. Resource search and inclusion criteria

Relevant data sources are defined as those that: a) include an experimental treatment in which microalgae are grown in water that was previously used to grow microalgae, b) provide quantitative growth results (e.g., biomass concentration, specific growth rate, biomass productivity) from both the experimental treatment and a control in fresh medium, and c) use microalgae taxa that are not associated with harmful algae blooms and are not dinoflagellates or *Prochlorococcus* (which are unlikely to be used for biofuel production). Sources that sterilized the recycled medium (by autoclaving, ozonation, or filtering with ≤0.2 μm pore size) were common (39% of total experiments analyzed) and were included, though experiments that used outdated methods such as boiling were excluded. Sources that used activated carbon filtration to treat the recycled medium were included as a comparison to other harvesting methods.

Comprehensive searches were done using Google Scholar and Web of Science databases with the terms: [conditioned OR spent OR recycled OR reuse] AND medium AND [algae OR microalgae OR phytoplankton]. Database searches accounted for 51 relevant sources used in the meta-analysis. Manual forward and backward citation searches were conducted on all relevant sources, which revealed 31 additional relevant sources. Three sources not found through these methods were either recommended by colleagues (e.g., student theses) or acquired at conferences, and an additional source was found as a citation in Wu, et al. [13]. Searches were performed up until November 12, 2016. A total of 86 sources were included in the meta-analysis from both ecological and biotechnological sources (Table S1 and Fig. 1) [15,17,22–26,28–106]. An additional 67 sources deemed potentially relevant were excluded because they did not meet the inclusion criteria or the full text in English was unavailable (Table S2).

2.2. Response variables and data extraction

Three response variables were chosen to represent algae growth: 1) biomass concentration (cells/mL, g/L dry weight, or optical density), 2) specific growth rate (day^{-1}), and 3) biomass productivity (g/L/day). Biomass measurements for all variables were based on cell counts (via microscopy or Coulter counters), dry weight, or optical density. Packed cell volume and long-term carbon uptake were also accepted as biomass measurements, used in three data sources. A Data Extraction Protocol was developed to extract data and information objectively and consistently from each source, and is available in the Supplementary Information (SI). Response variable data were recorded directly from text or tables when available, but were mostly available in figures only. The web-based application Webplot Digitizer [107] was used to extract data from figures, including standard deviations when error bars were present and distinguishable.

The first response variable, 'biomass concentration,' is defined here based on operation mode of the culture. For batch cultures (and semicontinuous cultures if a full growth curve is available), 'biomass concentration' is defined as the maximum biomass concentration reached in the growth curve, or the final biomass concentration if maximum is unavailable. Because some studies likely stopped experiments before algae reached their maximum biomass, the difference between biomass concentration in recycled and fresh medium could be influenced by experiment duration. Relatively short experiments are still included and may even be more realistic, however, since industrial-scale cultures are typically harvested on relatively short time scales to prevent biological contamination and maximize harvest yield [108]. For continuous cultures, 'biomass concentration' is the available biomass concentration data averaged over the experimental period. For semicontinuous cultures harvested on short time-scales (e.g., once a day), 'biomass concentration' is the peak biomass concentrations averaged over the experimental period.

The 'specific growth rate' response variable applies to batch cultures, as well as semicontinuous cultures with a full growth curve available. When figures of growth curves were available, digitized data were used to calculate the specific growth rate. Complete details on growth rate calculation are available in the Data Extraction Protocol in the SI.

'Biomass productivity' applies to continuous cultures and semicontinuous cultures harvested on short time-scales. When biomass productivity was obtained from 'biomass concentration versus time' figures, it was calculated from digitized biomass concentration data and averaged across the experimental period.

Many study descriptors were recorded where available, including growth conditions such as initial nitrogen (N) and phosphate (P) concentrations, initial pH, initial biomass concentration, light level, hours of light per day, temperature, culture volume, aeration, number of reuses of the medium, and percent dilution of the recycled medium with fresh medium or water. Categorical variables were also recorded such as the growing algae strain, the source algae strain, growth stage at harvest time, harvesting method, growth stage of the inoculum, sterilization method of recycled medium if applicable, whether the culture was axenic or not, and the growth measurement method.