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Review article

## Mini-review: A priori considerations for bacteria–algal interactions in algal biofuel systems receiving municipal wastewaters

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## ABSTRACT

Bacteria may be critical for the functionality, associated productivity, and risk profile of wastewater based algal systems. Both wastewaters and algal stocks act as sources of bacteria effectively creating a tripartite system where it is reasonable to expect interactions between algae, bacteria from wastewater, and algae associated bacteria. The functionality associated with this complex system will be critically derived from the synergistic activities of its biological members, which in turn will be affected by the induced trophic status of the very same system. It is critical that, as algal biofuel research expands into large-scale cultivation trials and production, these interactions and associated functions are assessed, monitored, and considered when management options are being developed.

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### 1. Introduction

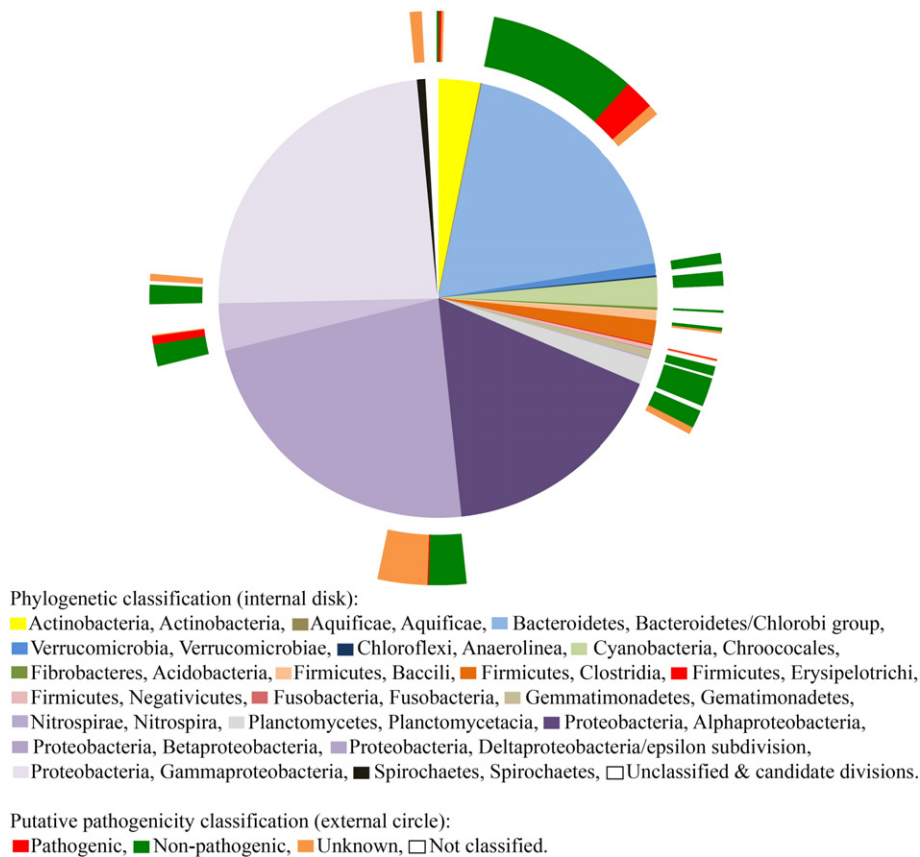
Microalgae have been long considered as a wastewater treatment (WWT) option principally due to their perceived capacity to deplete available nitrogen (N) and phosphorus (P) before discharge to the environment. As the quest for renewable energy sources draws more attention to the potential of autotrophic organisms to produce biofuels, WWT facilities are touted once more as plausible economical sources of nutrients for biofuel algae.

However beside nutrients and easily available carbon compounds, wastewater, even treated wastewater, contains a large diversity of microbial organisms (e.g. Fig. 1 and [1]). Members of Proteobacteria,

Bacteroidetes and Actinobacteria phyla generally dominate wastewaters. This is a reflection of the combined environmental and fecal sources that make-up such wastewaters. Treatment options are expected to affect diversity and richness in the final effluent. While additional use of algae as a treatment option is also expected to impact final bacterial populations available surveys [1] offer an image rather similar to the one presented in our Fig. 1. Partly this is a function of the relatively low resolution of the current metagenomic protocols, partly an indication of the likelihood that at least some of these bacteria might persist in the presence of algae, and partly, as discussed further below, likely a representation of algal associated bacterial populations. Some of these may be human, animal or even plant pathogens. Post treatment re-growth of many of these organisms has been shown to readily occur [2]. Wastewater associated microbes may interact directly or indirectly with the biofuel algae strains through a range of mechanisms ranging from commensalism to mutualism, to parasitism or to antagonism. These interactions may enhance or inhibit persistence of certain pathogens.

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**Fig. 1.** Example of an order level summary of bacterial diversity in effluent derived from an advanced secondary wastewater treatment plant that includes two aerobic treatment stages followed by chlorination/dechlorination. A total sample pyrosequencing dataset for 16 rDNA fragments that include the V1 through V3 hypervariable regions was used. The wastewater was sampled in February 2010 in southern New Mexico (32°19'11"N 106°45'55"W). Methodology and sampling details are as described by Sigala and Unc, 2013 [3]. Taxonomic and putative microbial properties assignments (i.e. pathogenicity) were carried out in MEGAN 4 [4].

Currently we know little about the significance and activity potential of the likely complex tripartite interactions between algae, their associated microbial consortia [5], and wastewater associated microbes for the growth and utilization stages of the biofuel algae. This limits our capability to develop coherent hypotheses to be tested in such systems. Physical and chemical characteristics of the biofuel algae growth systems are expected to govern any such interaction. Algae-associated bacterial populations may play critical roles in algal adaptation and survival [5]. DeLuca and McCracken [6] have shown *Oscillatoria* spp. associated bacteria (*Pseudomonas*, *Flavobacterium* and *Xanthomonas*) to either inhibit or stimulate the growth of other algal species. Moreover, there is a paucity of information on the role of the interactions between algae and bacteria on the persistence of contaminant microorganisms including the ones originating in wastewaters. Here we review environmental research that may shed light on the potential impact of using treated municipal wastewaters on the transfer and persistence of wastewater bacteria in biofuel algal systems. A similar logical treatment might be applicable to animal farm wastewaters.

To cogently evaluate relevant information on potential risk we first have to define the ecological status of algal growth systems to identify similarities with environmental phenomena. Growing biofuel algae in enclosed systems is akin to farming where the goal is to enhance algal biomass and the associated array and concentration of lipids. Such systems are to be designed to maximize the growth of algae to the detriment of other organisms. This is achieved through enhanced nutrient availability, and thus by promoting conditions analogous to naturally encountered eutrophic conditions (see Table 1 [7–22]). It is by now commonly accepted for aquatic environments that phytoplankton production to eutrophic levels correlates directly to nutrient loading and that N, P or even Silicon (Si), can be the key rate limiting nutrients

[23–25]. Hence nutrient limitation became the keystone concept at the base of eutrophication research which implies that (a) one key nutrient is a primary limiting factor for algal growth, (b) growth of algae in a given ecosystem is proportional to the rate of supply of this nutrient and thus (c) eutrophication can be controlled by restricting the loading of this key nutrient to the ecosystem [2,5]. Nevertheless autotrophic index (AI) calculations (AI and ash-free dry biomass vs. chlorophyll *a* ratio) suggest that nutrient enrichment not only stimulates the growth of algae but also the growth of fungi and bacteria [10].

Correlations between inorganic nutrients, of both natural and anthropogenic origin [26], and periphyton chlorophyll *a* levels suggest that, for benthic algae, P acts more often as the limiting factor than N (nitrate or ammonium) [9]. While P is generally considered as the primary limiting nutrient in fresh waters, N may often be the primary limiting nutrient in marine ecosystems [27,28]. Changes in N availability under constant P content of freshwater systems might not necessarily affect the total chlorophyll production but may effect a shift in the dominant autotrophic species [23] with N fixing, possibly toxic, cyanobacteria dominating N depleted waters [24]. Similarly Si availability governs diatom growth in nutrient rich systems with species that have lower Si requirements being selectively enriched as available Si declines [25]. Thus when P is not limiting variations in the availability of Si or N, can induce shifts in the diversity of species in algal blooms while not necessarily affecting total productivity of the ecosystem [23,28]. Nutrient load driven eutrophication also induces significant changes in other physical, chemical and biological parameters of aquatic environments. Hypoxia, a symptom of acute eutrophication [30], affects both nutrient availability patterns and microbial diversity [31].

For deep waters, diminished light penetration with depth may be controlling chlorophyll *a* even when nutrient concentrations are not

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