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Defining wastewaters used for cultivation of algae

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ABSTRACT

Employing algae to remove wastewater nutrients, as a treatment option, and employing wastewaters for algal cultivation for the production of biomass and bio-products are a growing field of research. Nevertheless, wastewaters are of a wide range of sources and consequently have a wide range of properties. Synthetic wastewaters of various nutrient profiles are often employed, likely as a means to normalize experimental results. Considering that the capability of an algal species to utilize nutrients in a certain chemical form and at certain concentrations and ratios may vary, an adequate understanding of both wastewater parameters and algal requirements, both kinetic properties, would benefit algal cultivation. A review of the available literature shows that reporting of experimental results does not follow a standardized protocol and thus much of the information available in the peer-reviewed literature cannot be always easily explained, compared and replicated. Moreover, while the often employed motivation for such research is the integration of wastewater treatment and production of algal biomass, the results commonly support the utility of algae for removal of wastewater nutrients paradigm and less so the conjoint paradigm of wastewaters as a reliable source of nutrients for algal production. This, arguably, leads to inconsistencies in reporting of experimental results which limits a concerted approach and thus a more rapid advancement of the technology. It would be recommendable that reporting of experimental conditions include a clear characterization of nitrogen and phosphorus concentrations and ideally also offer an account of the speciation of these nutrients in wastewater substrates.

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1. Introduction

Wastewaters are the by-product of a wide range of domestic, industrial, commercial or agricultural activities and consequently are of highly variable chemical and biological properties. The content of nitrogen and phosphorus in wastewaters is most concerning from an environmental point of view and extensive research has been directed towards their removal from wastewater [1]. One option is recovery of nutrients by algae or microalgae with the added benefit of producing bio-products and biofuels [2–5]. Consequently, a significant body of scientific literature is dedicated to the capacity of algae to remove nitrogen or phosphorus from wastewaters or to the capacity of wastewaters to sustain algal growth [6]. A query in the SCOPUS database for ["wastewater treatment" AND "algae" AND "biofuels" OR "fuels"] reveals a rapid increase in publications from 5 in 2007 to 87 in 2015, while the

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["wastewater" AND "algae"] query shows an increase from 51 in 2000 to 379 in 2015 (Fig. 1). A number of peer-reviewed articles describe algal research in artificial wastewaters (e.g. [7–10].), not necessarily always specifying the characteristics of the wastewater or the similarity of the said artificial wastewater to actual wastewaters. The reader is too often left to assume as to what wastewater type is the artificial version alleged to replicate.

Removal of nitrogen is described as the balance between the before and after cultivation of either total nitrogen, or the available forms of ammonia or nitrate. Removal of phosphorus is commonly described as the before and after cultivation balance of the total phosphorus. Changes in concentrations in the supernatant are commonly described in terms of absolute mass decline or in terms or proportional mass removal. We decided to not cite any one peer reviewed article, in support of the statements in the previous two sentences given the very large "wastewater and algae" body of literature [6] and to avoid any perception of undue selectivity.

Given that assimilation of nitrogen and phosphorus is coupled, the N:P ratio of wastewaters is obviously an important parameter to consider. It might be argued that for adequate nutrient removal the N:P ratio in wastewater ought to match the optimal algal species-specific ratio (Fig. 2). The rate of generation of biomass is maximized at optimal N:P ratio [11] but the specific range of concentrations for the unique optimal ratio are not well defined. Published research results might seem to offer divergent information, likely a feature of the inherent variability in the experimental conditions including variability in algal species and strains. As the N:P ratio diverge from the optimal value, algae might accumulate nutrient without biomass production. Biomass productivity might be static at luxury consumption [12]; Wu et al. [13] have shown that while *Scenedesmus* sp. consumed more phosphorus under nutrient replete condition this did not translate into more biomass. A batch study growing *Chlorella kessleri* on artificial wastewater has shown similar cell concentrations independent of the initial nitrate concentrations in the substrate [14]. Nevertheless, in general, augmentation of nutrient quantities is expected to increase biomass productivity, as seen for algae grown long term in continuous culture systems [15]. To further contextualize such nutrient removal-accumulation experimental results it is worth noting that the capacity to store nutrients vary among species and are dependent of environmental conditions [16]. Therefore, for a sound interpretation of results of investigations into biomass productivity and nutrient removal or availability, the distinction between the rate of assimilation into cell constituents, uptake from the substrate, and total accumulation in the algal cell of nutrients in organic and inorganic forms should be considered.

Such inconsistencies complicate directly comparison of results across experiments carried out in wastewaters of variable nutrient ratios, nutrient concentrations and especially nutrient availability profiles. Synthetic wastewaters are employed as a means to normalize experimental conditions and to simplify nutrient mass balance evaluations. The parameters of these synthetic wastewaters ought to reflect the nutrient availability in a reference wastewater type. Nevertheless, even a casual review of the make-up of synthetic wastewaters can point to inconsistent elements. Firstly, synthetic wastewaters lack an active wastewater microbial population [17]. Secondly, real wastewaters have complex organic matter chemistries that vary widely with source types and extent of treatment [18–20], rather challenging to replicate synthetically.

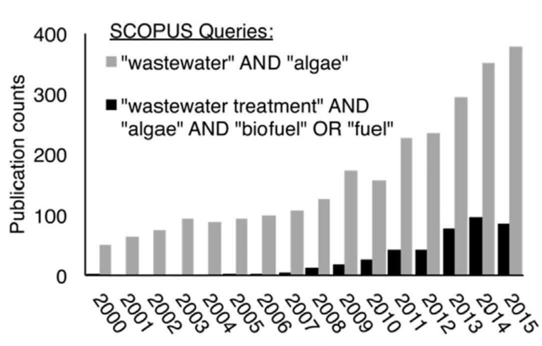

Fig. 1. Publication counts as identified by SCOPUS.

Given the extensive and rapidly developing field of algal cultivation on wastewaters [6] it is worth pausing to attempt to understand the variability in nutrient profiles in the wastewaters employed for cultivation of microalgae, to eventually support a coherent experimental approach that facilitates comparability and reproducibility of results.

2. Methodology

We carried out a review of the peer-reviewed literature with the aim to illustrate the variability in nutrient parameters of a range of wastewaters reportedly employed as a nutrient substrate for cultivation of microalgae. The goal was to identify nutrient parameters for a wide range of wastewaters of various sources as used for algal cultivation for biomass or biofuel production, employing a representative subsample of literature, and not necessarily to comprehensibly summarize the very extensive entire literature available on algae and wastewater treatment research. The units for nutrient concentrations were re-calculated to molar concentrations, a rather better indicator of algal uptake stoichiometry than the too commonly employed mass per mass or mass per volume units. Ideal molar N:P ratios for a few algal species, as described in selected scientific articles, are also presented here as a means to contextualize the known wastewater nutrient ratios (Fig. 2).

3. Results and discussion

3.1. Managing nutrients and algal species

The general N:P ratio of 16:1, initially developed for marine phytoplankton and known as the Redfield ratio [21], is a biological constant inherent to the fundamental protein-to-RNA ratio, across living entities on Earth [22]. A more recent, comprehensive revision of ocean organic particulates reported a global median N:P ratio of 22:1 [23]. Differential metabolism under nutrient deficits [22], variable CO_2 availability [24], will affect the measured N:P ratio. Nutrient deficits may be due to variable nutrient concentrations, but also due to variable chemical speciation profiles of nitrogen or phosphorus in diverse wastewaters. A purely physiological control of the N:P ratio might therefore not be necessarily always true [25]. For example the capacity of algae to store unassimilated nutrients, especially nitrogen [26], will skew the N:P ratio in raw biomass. Empirically, phosphorus content in algae has been shown to vary between 0.3 and 3% and nitrogen content between 3 and 12% [27].

Algal growth has been attempted and evaluated in many types of wastewater (see Supplementary Material) but not many studies (e.g. [7,12–14,28–30].) have investigated the effect of the variability of nutrient concentration on algal growth. Moreover, many studies on algal biomass growth and nutrient removal have used synthetic wastewaters (e.g. [7,10,31,32].) but it is often unclear or unspecified if and how nutrient profiles of such artificial media reflect the nutrient parameters of wastewaters (Fig. 2). Often "synthetic wastewater" is assumed to signify municipal wastewaters but this is not always clearly specified. While the N:P ratio and concentrations of some synthetic wastewaters are similar to primary effluents of municipal wastewater treatments this is not always true (Fig. 2). Common characteristics of various wastewaters, including examples of synthetic wastewaters used for research into algal biomass growth and nutrient removal are summarized in Table 1. Reported concentrations of total nitrogen (TN) and total phosphorus (TP) vary between 0.08 and 491 mmol L^{-1} and 0 to 19.5 mmol L^{-1}, respectively. For most wastewaters more nitrogen than phosphorus is present, which generally corresponds to global cell stoichiometry, albeit