Microalgae biorefinery symbiosis: screening, production, and process analytical technology — DTU Orbit (24/08/2019)

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Microalgae treatment of municipal wastewater (WW) has been the focal point of microalgal biotechnology research for several decades. However, this technology did not have a competitive advantage over other WW treatment technologies, which could be implemented in smaller areal footprints. In the past few decades, microalgal WW treatment has made a resurgence with the idea of using biomass from microalgal WW treatment, as a source of lipids for conversion into biodiesel. However, the savings from the treatment of nutrients and organic matter, as well as biodiesel production, are still not competitive with the price of crude oil. In recent years, microalgal research continued with the prospect of a microalgae biorefinery, where microalgal byproducts and coproducts are extracted to valorize the entire microalgal production, in which the sum of the parts of the microalgae is greater than the whole microalgae. However, in large part, the microalgae biorefinery does not comply with the treatment of nutrient-rich municipal WWs, due to regulatory concerns. Only recently, it was realized that bioindustrial WWs are viable and conceivably regulatory compliant nutrient rich waste streams, capable of sustaining microalgal growth, as much as municipal WWs. The concept of an “industrial symbiosis” has also emerged in the past several decades, in which networks of industries cooperate to use waste sources from neighboring industries, in industrial parks, to create added value. The intersection of the microalgae biorefinery and industrial symbiosis, in a microalgae biorefinery symbiosis (MBS), may be the next generation scheme to valorize the microalgal production and promote industrial and global sustainability. Moreover, technological advances in screening, outdoor photobioreactor (PBR) design, macromolecular monitoring and process automation must all be addressed to execute the complex bioprocesses needed to valorize an MBS successfully.

In order to properly identify viable MBS partnerships with industry, microalgal species capable of producing an array of valuable products must first be screened on these potential bioindustrial WW streams for their growth potential. During screening, microalgae may have a preference or aversion for a given bioindustrial WW media, based on the types and ratios of nitrogen (ammonium, nitrate, or urea) in the WW. Furthermore, identifying algae capable of withstanding fluctuations between these nitrogen forms in dynamic WWs, is an important criterion for productivity. However, when screening microalgae on WWs containing different nitrogen sources and concentrations, assimilation of different nitrogen sources can result in starkly different physicochemical changes, specifically pH changes. In many microalgae, ammonium is the preferred nitrogen source, because it can passively transport into the cell and is directly assimilated into amino acids, without relying on light-mediated enzymatic processes to be reduced. However, when microalgae assimilate ammonium, the pH of the system can drop sharply, inhibiting growth after that; however, these pH changes do not directly reflect the microalgae’s affinity to grow on ammonium. By growing batch cultivations of microalgae in 24-well microplates, a microplate reader can be used to measure relative fluorescence of chlorophyll in vivo, during balanced growth, before these pH changes occur. This technique can be used to preempt the effects of pH changes on growth and reflects the true preference or aversion of microalgae to a particular nitrogen source or a WW media. Additionally, along with being spatially high-throughput in a 24-well microplate—where 24 batch reactions can be conducted simultaneously in a small footprint—the early and low detection of growth rates is also more temporally high-throughput than any other screening method. This method can also be used to quickly screen for robust and adaptable microalgae, capable of acclimatizing to different nitrogen sources and fluctuating media as well as to screen for the upper and lower tolerances of the microalgae to various concentrations of the WW. The latter must also be addressed when screening dynamic WW capable of large fluctuations.

Over the years, there have been very few demonstrations of outdoor microalgal growth in enclosed PBRs; demonstrations, which are essential for understanding the feasibility of an MBS as a whole. From microplates to large-scales—six orders of magnitude larger—the industrially important screened microalgae Chlorella sorokiniana was grown on bioindustrial WW, inside a novel, solar tracking, 4000 L, airlift PBR. Despite cold temperatures and low irradiance, the microalgae reached a growth rate of 0.48 day⁻¹, in the four-day period immediately following inoculation of bioindustrial WW containing ammonium, as a primary nitrogen source. After that, after ammonium was depleted and the media was augmented with nitrate, a long lag phase persisted, before undergoing the predominant production phase with a specific growth rate (SGR) of 0.15 day⁻¹ over an 18-day period. It was evident that the transition from ammonium to nitrate metabolism can severely stunt microalgal growth in the outdoor PBR under low temperature and irradiance. More importantly, the delay in growth did not appear to be due to deleterious effects of the contents of bioindustrial WW media, since rapid growth was observed early in the experiment on the unaugmented WW. Moreover, it was demonstrated that microalgae could continue to grow in adverse environmental conditions at large-scales.

The success of the in vivo fluorescence microplate assay and the complexity of these outdoor reactions demonstrate the value of pursuing real-time data of microalgae in vivo at large-scales. The complex and dynamic nature of large-scale outdoor microalgal reactions, when grown on dynamic WW media, encourages the need for on-line, real-time monitoring to improve automation models of PBRs. In outdoor conditions with fluctuating light and temperature, there are several factors that can change the growth of microalgae, at time-scales less than a minute and as low as microseconds, which may not be accounted for in microalgal productivity models. Similarly, fluctuations of WW media are not accounted for in these models, especially in outdoor conditions. However, recent advances in hardware and software can significantly improve microalgal bioprocess models and automation, by manipulating large, time-resolute data sets, so-called “big data,” which can be acquired through high-selectivity vibrational spectroscopy, such as mid-infrared (MIR), near-infrared (NIR), or Raman vibrational spectroscopies. These large, real-time data sets can now be used to create adaptive models from artificial intelligence/machine learning tools or “black-box” models, to automate large-scale, outdoor PBRs treating WW.

With microalgae, now entering into a new paradigm of food, feed, pharmaceuticals and functional products, on top of biofuels in a biorefinery, there will be a growing need to maintain product quality, regulate, and mitigate contamination, especially in a symbiosis with WW. Vibrational spectroscopies can be used to monitor several microalgal components
simultaneously, which can be used to aid fractionation of microalgal compounds in a biorefinery, while improving model building for automation and control of product quality and contamination, where quality can be built into the system. The results and research summarized in this thesis demonstrate that the modernization of microalgal research is becoming increasingly necessary and beneficial to microalgae production in an MBS. The focus of this thesis is to bring together lab-scale demonstrations, scaled up knowledge, and a critical outlook of modern technologies capable of making the MBS a reality.

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