Topic: Innovative microalgae treatment processes

An innovative symbiotic microalgae-IFAS process for nutrient removal and photo-oxygenation: Multiscale investigations using microelectrodes and next-generation molecular tools

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Given that sustainable energy production and advanced wastewater treatment are two major challenges faced by modern society, microalgae make a desirable wastewater treatment alternative. A combination treatment of wastewater, utilizing the interaction of algae and bacteria, can offer lower energy consumption using photosynthetic aeration while also providing algal biomass for biofuel production. A consortium of algae and bacteria is known to be effective in removing nitrogen (N) and phosphorus (P) from wastewater; however, the concept has largely been limited to suspended processes and fixed filmed (biofilm) processes have not fully been explored. Here we demonstrate the successful algal-bacterial biofilm where an algal biofilm present on top of the bacterial biofilm can assist in photo-aeration for nitrification. The algal-driven direct denitrification without additional carbon sources was also observed.

In this work, three 4L lab scale sequencing batch reactors (SBR) were operated in the sequence of fill (10 min), anaerobic reaction (80 min), aerobic reaction (300 min), anoxic reaction (150 min), aerobic reaction (60 min), settling (120 min), and decanting (30 min) over 130 days. 40% of the bulk working volume was filled with AnoxKaldnes (K1) media (specific surface area of 500 m^2/m^3) in two of the reactors (microalgae-based integrated fixed film activated sludge (IFAS) and IFAS control) and the third reactor contained no media and used as a control. Activated sludge from a regional wastewater treatment plant was used to seed the reactors while *Chlorella vulgaris* (UTEX) was used as representative microalgae. All three reactors were fed with synthetic wastewater containing 300 mg COD/L, 30 mg TN/L and 10 mg P/L with 12 hrs of hydraulic retention time (HRT) and 30 days of SRT (suspended solids basis). The reactors were initially operated for 60 days without microalgae to develop a nitrifying biofilm on the IFAS media. After confirming nitrifying biofilm growth from ammonia removal, the microalgae-IFAS and suspended control reactors were inoculated with *C. vulgaris*. Throughout the operation, the biofilm formation and the interaction between green algae and bacteria in a biofilm set were investigated using microelectrodes and next-generation molecular tools. A unique microelectrode technique was used to show interactions between the heterogeneous layers of the biofilms and to determine net specific consumption rates of dissolved oxygen (DO) and ammonia. Bulk analyses (pH, NH_4, TP, COD, TSS, VSS, and chlorophyll α) were also conducted to monitor treatment efficiencies and biomass growth. The total RNA and DNA were extracted from biofilms, and microbial community structures were analyzed with Illumina MiSeq PE250 approach by targeting 16S rRNA and 16S rDNA. After mechanical aeration was turned off and light was used to photo oxygenate the microalgae-IFAS and suspended reactors, Chl. α concentration increased the mg Chl. α/g biomass ratio to 20.3 and

13.0 mg/g in the microalgae-IFAS and suspended reactors, respectively. Nutrient removal was stabilized after 36 days with ammonia removal reaching 100% and 57% for the microalgae-IFAS and suspended reactors, respectively. Microalgae-IFAS and suspended reactors were able to remove 51 and 49% of P, respectively. Furthermore, the algae bacteria consortia showed excellent settling with an SVI ranging from 74 to 111 and less than 1 mg/L Chl. α in the effluent. Microelectrode investigations clearly demonstrated algal biofilm photo-aeration as an alternative to costly mechanical aeration. DO concentrations in bulk solutions reached 1.3 mg O_2/L and 0.6 mg O_2/L in the microalgae-IFAS and suspended reactors under light only, respectively. DO concentration microprofiles showed oxygen concentrations reached 6.8 mg O_2/L at the surface of the microalgal biofilm and 0.9 mg O_2/L at the substratum which means the underlying nitrifying biofilm has ample supply of oxygen for nitrification. pH and ammonia microprofiles also showed a decrease in pH and ammonia at the bottom layers of the biofilm, indicating well-defined nitrification. *Candidatus Accumulibacter* known having the capability of phosphorus removal in wastewater was highly enriched within the microalgae-IFAS biofilms. Interestingly, *C. Accumulibacter* was predominant in the RNA libraries (about 50%) whereas its population substantially decreased to about 10% in the DNA libraries, indicating that *C. Accumulibacter* is metabolically active on the algae-bacteria consortium biofilms. In addition, microbial community analyses confirmed the presence of nitrifying bacteria such as *Nitrosomonas* and *Nitrospria* within the biofilm. Overall, this research is the first to show that the cohabitation of microalgal and bacterial biofilm enables an efficient symbiotic process for reducing the energy requirements for N and P removal from wastewater.