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Deposition of manure nutrients in a novel mycoalgae biofilm for Nutrient management

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A B S T R A C T

With the increase in intensive crop and livestock production, excess application of P as fertilizer and manure contributes to the build-up of soil P levels causing eutrophication. A novel biofilm-based technology was developed to recover and reposition the nutrients in manure, producing biofilm fertilizer and the treated water with better nutrient composition. Anaerobically digested and pretreated manure was used as a medium to grow the surface-attached composite biofilm, which constitutes the selected polyphosphate accumulating fungi and nitrogen accumulating fresh water microalgae for efficient recovery of nutrients on a matrix for better biomass harvesting. Under the tested conditions in lab-scale with the pretreated digested manure, the removal efficiency of the nutrients by attached mycoalgae biofilm was 76.74% P and 76.40% N with COD removal of 65.75%. To increase the nutrient content of the biomass and for enhancing the cell growth the wastewater generated in corn ethanol process (thin stillage) was added as an external nutrient at different ratios in the digested manure. The cell growth, nutrient removal efficiency, lipid content of the biomass, COD removal and reducing sugar content at different medium conditions were evaluated. The nutrient-rich solid biofilm can be harvested by scraping off the biofilm from the matrix and the nutrient lean liquid can be discharged or further used for agriculture. The microbial biofilm assimilates the organic and inorganic components in manure and converts them into cellular constituents together with N-P-K resulting in deposition of manure nutrients in biofilm, which can be directly used as a bio-fertilizer.

1. Introduction

Nutrient management, especially nitrogen and phosphorus conservation and reuse from agricultural/industrial wastewaters is gaining significance due to the environmental concerns and the increasing value of the resources. The Clean Water Act (U.S. EPA, 1994) lists nitrogen (N) and phosphorus (P) as potential pollutants of impaired water bodies. Movement of nitrate–nitrogen (NO^{3-}) and soluble reactive phosphate ($H2PO^{4-}$, HPO_4^{2-}, and PO_4^{3-}) from agricultural sites may lead to excessive algal and aquatic plant growth in surface waters, resulting in accelerated eutrophication. With the excessive dairy waste application in agricultural land, the soil-P in surface water runoff moving from dairy waste application fields to streams, rivers or other drainages cause a serious threat to the fresh water causing eutrophication. The alternative to an excessive land application of manure, growing algae or plants in wastewaters, which could recover excess P and could possibly be recycled poses a better strategy in nutrient management. Management practices that target excess nutrients from animal manure include oxidation ponds, facultative lagoons, constructed wetlands, storage ponds and land spreading, and composting (NRCS, 1999).

Land application of liquid manure has been a more sustainable practice to provide an alternative source for nutrients in order to increase the agricultural crop production (Fares et al., 2008) and to improve the chemical, physical, and biological properties of the soil. P and other nutrients for the plants are achieved through the effective recycling of organic materials including crop residues and livestock manures. There are multiple barriers to the land application of manure for P utilization and uptake by plants. First, land application of animal manure is limited by its composition. For example, nutrients such as N and P are present in swine manure in N: P ratios ranging from 1:1 to 2:1 (Munch and Barr, 2001), while the N: P ratios needed by crops are between 3:1 to 15:1. Therefore, when manure is applied to supply the crops demand for N, then the over-application of P is evident. Second, recent developments in corn ethanol production are transforming the feed industry for wide applications of corn ethanol coproducts in

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animal diets (Rodrigues Reis and Bo Hu, 2017). These new feeding materials are causing an increase in P excretion in animal manure (Rodrigues Reis and Bo Hu, 2017). Finally, the application is limited to the site close to the livestock, due to the low nutrient content (less than 1% of P in dry volatile solids, and the solid content of swine manure around 6%) and subsequent high transportation cost. With the increasing size of livestock farms, especially in the areas where animal farming is highly concentrated, tremendous amounts of surplus manure must be discharged while the land in the surrounding area is oversaturated with P. The land application of manure, is considered an important contributor of pollutants entering surface waters. Movement of nitrate–nitrogen (NO^{3-}) and soluble reactive phosphate ($H2PO^{4-}$, HPO_4^{2-}, and PO_4^{3-}) from agricultural sites may lead to excessive algal and aquatic plant growth in surface waters, resulting in accelerated eutrophication. This practice will not only result in under-application of N in most cases but also will require more land to apply the same volume of manure. With the excessive dairy waste application in agricultural land, the soil-P in surface water runoff moving from dairy waste application fields to streams, rivers or other drainages cause a serious threat to the fresh water causing eutrophication. Therefore, there are great needs to develop treatment and application practices that can improve the quality of the manure applied in the soil. The recovery of phosphorus and nutrients from manures is critical, especially as we strive for a more sustainable food production system. A cost-effective recovery of phosphorus would allow the livestock industry to successfully implement more phosphorus-based nutrient management plans on their current land base and increase the value of the waste stream for sustainable development in organic farming.

There are only a few technologies available currently to recover nitrogen and phosphorous simultaneously from different wastewaters (Wang et al., 2017), and among them, microbial-based technologies have shown potential (Olguin, 2003; Ruiz-Marin et al., 2010). Microbial-based nutrient recovery from digested manure (DM) especially with algae has been in research recently, but the bottleneck is the algae recovery after growth (Singh et al., 2011).Growing algae or plants in wastewaters, which could recover excess P and could possibly be recycled poses a better strategy in nutrient management. Management practices that target excess nutrients from animal manure include oxidation ponds, facultative lagoons, constructed wetlands, storage ponds and land spreading, and composting (NRCS, 1999). Digested effluent has relatively lower carbon levels compared to typical agricultural, municipal, and industrial wastewater and the nitrogen is mainly in the form of ammonium. Therefore, for bio-recovery process, dilution of manure and supplementation with additional carbon source becomes essential.

The thin stillage (TS) is a profuse remaining of the corn ethanol process which has a slurry of corn solids and water left over after cornstarch has been fermented and distilled (Rodrigues Reis and Bo Hu, 2017). The whole stillage is separated into distiller's grains and thin stillage, which is later concentrated through evaporation and added to the distiller's grains. Even though TS is rich in nutrients like crude proteins, glycerol, and fats, which is a viable medium for bio-production (Rodrigues Reis and Bo Hu, 2017), it requires energy-intensive processing (evaporation) to be treated and mixed with Distiller's dried grains with solubles. In this present work, the nutrient recovery efficiency of the “mycoalgae biofilm” in digested manure and with additional nutrients from thin stillage was tested. The biofilm growth, biomass lipid concentration, COD and reducing sugar concentration were analyzed at different medium conditions. The research on mycoalgae biofilm-based technology consists of selected polyphosphate accumulating fungi and nitrogen removing algae, which attaches to the support matrix (Aravindan Rajendran and Hu, 2016a, 2016b).

2. Materials and methods

2.1. Microorganisms and culture maintenance

Chlorella vulgaris 2714, a unicellular green microalga was selected as the model algae strain for the mycoalgae biofilm formation. The strain was obtained from the Culture Collection of Algae at the University of Texas (UTEX). The fungal strain *Mucor circinelloides* UMN-B34 used in this work, for co-culturing with algae, was isolated from the soybean, soybean hull, and soil samples surrounding the soybean roots collected from croplands in Rosemount, MN (Yang et al., 2014).

The microalgae were cultivated and maintained in medium (A) that contained (g/ L): glucose 2, KNO_3 1, KH_2PO_4 0.075, K_2HPO_4 0.1, $MgSO_4.2H_2O$ 0.5, $Ca(NO_3)_2.4H_2O$ 0.0625, $FeSO_4.7H_2O$ 0.01, yeast extract 0.5, and trace metal solution 1 ml/ L. The trace metal solution contained (mg/ L): H_3BO_3 2.86, $Na_2MoO_4.2H_2O$ 0.39, $ZnSO_4.7H_2O$ 0.22, $MnCl_2.4H_2O$ 1.81, $CuSO_4.5H_2O$ 0.079, and $Cu(NO_3)_2.6H_2O$ 0.049. The stock culture of algae was maintained on the agar slants at 25–27 °C (Media A + 1.5% agar) under white fluorescent light illumination and periodically revived. The fungal spores were preserved in 60% glycerol solution at −80 °C. The glycerol stocks were aseptically streaked on potato-dextrose agar plates and incubated at 37 °C. The spore solution was prepared using sterile water and stored at 4 ° C for inoculation. For inoculating the algae cells and fungal spores, cell counts were done using a 0.1 mm deep Neubauer improved hemocytometer (Hausser Scientific, USA) under a microscope (National DC5–163 digital using 40× magnitude).

2.2. Digested manure and thin stillage

Digested manure was collected from a 1000-acre dairy farm located in Minnesota with anaerobic digester to treat the manure from around 1000 cows. Thin stillage (TS) was collected from a dry grind corn ethanol plant in the mid-west region. The samples were stored at −20 °C before use.

2.3. Culture medium and Erlenmeyer shake flask cultures

To test the effect of pretreated dairy manure and manure-based medium supplemented with TS at different ratios on the biomass growth and nutrient recovery by a novel mycoalgae biofilm was studied. The experiments were conducted in 250 mL Erlenmeyer flasks with 100 mL of the culture medium (different ratios of Manure: TS – 100:0, 75:25, 50:50, 25:75) and a submerged supporting matrix for biofilm formation. The medium was adjusted to an initial pH of 6.8 using 2 mol/L HCl or 1 mol/L NaOH, and heat sterilized (250 °F, 15 psi for 20 min) along with a matrix (4×4 cm). The matrix is a biodegradable polymer-cotton composite with 50% yarn and 50% polypropylene material that is woven into a matrix. The possibility for coexistence of *C. vulgaris* with various fungal cultures, different matrices for attachment and process conditions were tested previously to identify the best strain combination and process conditions (Aravindan Rajendran and Bo Hu, 2016a, 2016b). The strain combination *Mucor circinelloides* UMN-B34 – *C. vulgaris* was selected for this study based on the algae attachment efficiency and better biomass production in a stable matrix. The culture medium was inoculated with the co-cultures of fungal spores and algae cells at a ratio of 1:300 (Initial algae count: 2.50×10^9 cells) or axenic cultures of fungal spores unless otherwise specified and incubated in an orbital shaker at 150 rpm and 26 °C in the presence of light (light intensity of continuous illumination was set to approximately 60–75 μmol/m^2/s) for the entire cultivation period of about 18 days.

Samples from the flasks were withdrawn aseptically at different process periods (0, 6, 12 18 d) and were analyzed for nutrients (total phosphorus, total nitrogen and reducing sugar), biomass composition, solid content in the biofilm, cell counts of suspended algae, lipid