Next Generation Sequencing of Environmental Samples: A Comparison of DNA Extraction Methods

Environmental Engineering

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Abstract

In recent years, technologies available for genome sequencing of bacteria have evolved in terms of affordability, speed, and accuracy. Advantages of genome sequencing such as identification of potential pathogens are beneficial for ensuring public safety from illness and disease. Further, engineered systems such as wastewater treatment facilities can benefit from understanding bacterial community changes under differing influent compositions (e.g. pharmaceuticals, personal care products).

DNA based technologies are dependent on the quality of DNA from extraction methods or commercial kits used for sample preparation. For this study, we selected four common commercial DNA extraction kits for comparison among three different environments on both suspended and attached bacteria communities. Here, we critically analyze MiSeq bacteria community datasets among commercial kits, locations, and community type. This study compared four popular DNA extraction methods: QIAamp DNA Mini Kit, QIAamp DNA Stool Mini Kit, MoBio Soil DNA Kit, and MoBio Water DNA Kit. Water and biofilm samples were collected from three separate locations: lake water samples from Beaver Lake Reservoir (Lowell, Arkansas); wastewater and biofilm samples from the moving bed bioreactor (MBBR) at Waterford Estates package plant (Fayetteville, Arkansas); and wastewater and biofilm samples from the trickling filter reactor (TF) at Massard Wastewater Treatment Plant (Fort Smith, Arkansas). Community profiling was completed using Illumina MiSeq followed with bioinformatics analyses in Mothur. Alpha diversity results from the Simpson index reflected increased diversity in biofilms compared to wastewater column communities. It also presented a statistically significant difference ($P = 0.029$) between microbiota within lake samples and MBBR biofilm samples, MBBR suspended and MBBR biofilm, TF biofilm and TF suspended. There was no significant difference between wastewater biofilm communities (TF and MBBR, $P=0.485$). With examination of the relative abundance of phyla, we observed the occurrence of Gemmatimonadetes. Previously isolated from a freshwater lake in the Gobi Desert, Gemmatimonadetes was recently identified as a new phototrophic bacteria phylum. It was identified in Lake and TF biofilm samples. This novel phylum
identified as a polyphosphate accumulating bacteria has also been isolated from a sequential batch reactor and a fluidized bed reactor for wastewater treatment. All three locations were dominated by proteobacteria and bacteroidetes. Next, we applied Beta diversity analyses to compare differences between community samples. PCoA mapping revealed four distinct clusters: Lake samples, MBBR water samples, TF water samples, and both biofilm type samples. The PCoA reflected minimal differences between extraction methods. Hierarchical clustering showed a clear distinction between each location and sample type, however each extraction method shares the same node with the exception of TF suspended (PowerWater Kit) and Lake (Stool Kit). With this dataset, we conclude there is not one superior method. Certain rare bacteria such as Chloroflexi and Gemmatimonadetes were extracted by all methods, whereas PowerWater Kit did not perform as well with biofilm type samples. It is our recommendation from these results to combine DNA extracts from a minimum of 2 methods when performing high throughput sequencing to greatly reduce the chances of excluding rare phyla.
Plant responses to heat stress: integration of genotype and phenotype with crop models

Environmental Engineering

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Abstract

The next generation of crop models are using genetic information to predict the performance of a genotype to targeted environments. However, to build these gene-based crop models, large amounts of data on the phenotypic responses of different genotypes in many environments are required. In addition, certain processes in crop models, such as flowering, are more easily measured and are readily linked to genes, while others like leaf area and dry weight are more challenging. Here, we present different approaches for collecting large scale phenotypic data that can then be linked to genes. We demonstrate an approach to build these gene-based crop models by integrating sub-models of different biological processes to predict plant growth and development based on genetic information. These models allow flexibility in modifying the sub-models as more genetic information or genetic by environment information is obtained.
Behavior of CuO Nanoparticles as Influenced by Soils, Bacteria, and Wheat Roots

Environmental Engineering

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Abstract

Nanoparticles (NPs) are increasingly used in industry and commercial products, and environmental contamination must be considered. We aimed to evaluate the behavior of CuO NPs, an NP with high antimicrobial potential, in the soil environment. A meta-analysis of journal articles measuring CuO NP solubility showed that accurate measurement of NP solubility requires a variety of separation techniques and measurements. Therefore, we chose two NP separation techniques (high-speed centrifugation and ultrafiltration) and measured CuO NP solubility in soil pore water extractions (SPWs) and the rooting zone of wheat seedlings. We compared the solubility results to results predicted by geochemical models. We also measured plant response of wheat seedlings to determine if SPW chemistry affected CuO NP bioavailability.

SPWs enhanced solubility of CuO NPs compared to 3.34 mM calcium nitrate at equal pH via complexation with dissolved organic matter (DOM), but did not reach predicted geochemically modeled dissolved copper concentrations. Dissolution was inhibited proportional to DOM concentration because of strong coating by organic matter. Certain individual factors, such as fulvic acid, alkalinity, and the presence of bacteria were also shown to increase CuO NP dissolution.

In sand with SPWs, and particularly the rooting zone of wheat, CuO NPs dissolved in proportion to DOM, which was contrary to our previous results but in accordance with geochemical modeling predictions. The solid growth matrix provided greater surface area for coating by DOM, which resulted in less inhibitive coatings on the NPs. Wheat seedling root lengths decreased in wheat exposed to CuO NPs in sand with 3.34 mM calcium nitrate, while wheat root lengths were partially rehabilitated when SPWs were included instead of 3.34 mM calcium nitrate. CuO NPs increased the concentrations of root exudates released by wheat seedlings, including the iron siderophore 2’-deoxymugineic acid,
which is dominated by complexation with copper over iron at the pH in the rooting zone. A soil bacterium, Pseudomonas chlororaphis O6, altered the root exudate output of the roots and thus lowered solubility of the CuO NPs, although the root lengths were unchanged.

Future research should focus on NPs in the solid soil matrix, and whether copper complexed to DOM is more, or less toxic to microbes and plants. Dissolved organic matter may be a suitable stabilizer for CuO NP contamination, but in a solid/liquid matrix it may also increase CuO NP dissolution and lead to toxic outcomes for soil life.
Leveraging Advanced Biological Nutrient Recovery (ABNRTM) in Wastewater for Biomass Production

Environmental Engineering

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Abstract

Clearas Water Recovery’s Advanced Biological Nutrient Recovery (ABNR™) system is a tertiary wastewater treatment technology delivering a simple, sustainable, and modular approach to nutrient recovery, with high quality oxygenated water and nutrient enriched algal biomass as byproducts. ABNR™ harnesses the best aspects of the emerging circular economy in a system not only capable of cleaning industrial and municipal wastewater streams, but actually utilizing that waste for the efficient production of a reliable, non crop-based feedstock valued across various industries. Past algal growth operations were challenged with production efficiencies and sustainable quality of the biomass produced. These challenges are addressed with ABNR™ as a suspended growth treatment platform operating in a continuous and controlled environment, guaranteeing quality improvements over other open-air algae growth processes. Municipal and industrial wastewater operators can now support technology investments by capitalizing on downstream biomass value, serving as the primary resource providers for a growing global bioeconomy.
Soft Soils Sink Logan’s Wastewater Treatment Project until an Emerging Technology Saves the Day

Environmental Engineering

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Abstract

When unforeseen geotechnical challenges threatened Logan City’s plans for a new wastewater treatment facility, the project team had to come up with a way to shrink the footprint without reducing capacity. Logan City was in the early stages of design of a new wastewater treatment facility project required to meet water quality limits for phosphorus and ammonia imposed by the Utah Division of Water Quality (DWQ), when a geotechnical investigation determined that soils at the proposed site were very soft and could settle up to 34 inches. Steel piles are needed to support structures and will be driven 80-90 feet deep. Because of this the project’s cost quickly exceeded the planned project budget.

An evaluation of treatment alternatives conducted previously by Carollo Engineers identified a recommended alternative based on low capital and O&M costs. Due to the cost of the piles and additional foundation, alternatives in the master plan were reconsidered on the basis of footprint. Membrane bioreactors and a new technology for activated sludge processes called BioMag® were evaluated as these two processes have a smaller footprint compared to traditional wastewater treatment technologies. Carollo is recommending the BioMag® technology for the Logan project as reduces footprint without compromising flow capacity, incorporates most of the elements of the previous design, a savings in engineering cost, and reduces project costs back to within five percent of the original budget.

This presentation will review each technology in depth, address pros and cons associated with the project, report the technology Logan City chose to implement, and outline the construction and start-up schedule. Participants will learn of the challenges environmental engineers face in balancing water quality improvements with budget limitations.