MICROBIOLOGY SOCIETY

FOCUSED MEETING
2017

Microbial Resources for Agricultural and Food Security

& All Island Phosphorus Sustainability Workshop

Poster Abstract Book

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Focused Meeting 2017: Microbial Resources for Agricultural and Food Security

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Poster number: P01

Ash2®Phos - Clean commercial products from sludge ash

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Background

EasyMining Sweden AB has developed a process for phosphorus recovery from sludge ash named Ash2®Phos. The process is based on wet chemical processing and is suitable for processing ash of mono-incinerated sewage sludge, as well as ash from co-incineration of sewage sludge and waste according to a specific concept.

Methods

The process starts by dissolving the sludge ash in acid. The next step is removal of recoverable elements from the obtained solution. Phosphorus, iron and aluminium are removed from the solution by a unique precipitation step. Thereafter, the solution is neutralized and treated for removal of heavy metals. The recoverable elements are separated into three intermediate products: calcium phosphate, ferric hydroxide and aluminium hydroxide. The final step is conversion of intermediate products into final products. Several options exists depending on the form of the desired end-products.

Results

The Ash2®Phos process was tested in pilot trails for treatment of both ash of mono-incinerated sewage sludge and co-incinerated sewage sludge. The recovered final products are of high commercial quality. Phosphorus is first recovered in form of intermediate calcium phosphate which is thereafter converted into a final product such as ammonium phosphate. Recovered iron and aluminium are converted into coagulants for wastewater treatment by reaction with suitable acid.

Conclusion

We believe that technological development will lead to sustainable phosphorus recycling in society, characterized by efficient processes and high quality products.

Poster number: P02

Investigation into IASBR system efficiency for treatment of dairy processing wastewater

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Ireland has seen unprecedented growth in milk production in the last 2 years, which is expected to reach a 50% increase by 2020. This increase, along with the strict water discharge standards set about by the EPA, has instigated a need for new and innovative technologies for the treatment of dairy wastewater. The intermittently aerated sequencing batch reactor (IASBR) system is one such technology as it biologically treats wastewater more efficiently, in terms of cost and energy demand, in comparison to the technologies currently being employed in the Irish dairy industry. A pilot scale IASBR system is on site at Aurivo Daries, Ballaghderreen from which an auto-sampler takes samples of treated effluent daily and refrigerates. These samples are then collected once weekly and analysed here in the environmental engineering lab at NUI Galway. Preliminary testing at lab and pilot scale has shown that the IASBR system has the capacity to remove nitrogen, phosphorus and chemical oxygen demand (COD) in wastewater to an acceptable level (as set about by the EPA) for discharge from a wastewater treatment plant (WWTP). The primary scope of this research is to investigate the potential for the IASBR technology as a viable option within the Irish dairy industry and optimise operational parameters. This system will be tested over a period to prove that it can continuously remove nutrients and COD from dairy processing wastewater. As this system can biologically remove phosphorus, something many WWTPs cannot do it negates the need for expensive chemicals such as aluminium sulphate. Initial testing has shown the IASBR system has great potential for application within the dairy industry.

Poster number: P03

Response of soil microbiota to phosphate and sulfate fertilization in grassland columns

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Phosphorus (P) and sulfur (S) are vital for living organisms and are widely used as fertilizers in agro-ecosystems. However, mineable reserves of P are finite and atmospheric supply of S has drastically reduced leading to plant nutrient deficiencies. In this study, soil columns with *Lolium perenne* were setup in a greenhouse and fertilized with 0 (control), 5 (low), 10 (medium) and 20 (high) kg/ha inorganic phosphate P and sulfate S respectively alongside a full complement of other nutrients. Grass dry matter yield did not change with P fertilization while medium and high S fertilization resulted in higher grass growth. *L. perenne* rhizosphere of the highest P treatment had significantly lower abundance of bacterial *phoD* genes, bacterial- and fungal-feeding nematodes, mycorrhizal colonization rates, and certain P-solubilizing bacterial families compared to the control. High S fertilization significantly reduced the abundance of bacterial-feeding nematodes, mycorrhizal colonization rates, and sulfatase activity but not the abundance of aromatic sulfonate-utilizing bacteria and other nematode groups. All P and S treatments significantly shifted the bacterial and fungal community structures compared to the control. Overall, our findings suggest that i) a positive effect of soil P fertilization on grass growth was largely cancelled out by its negative effect on the soil microbiota; ii) the soil microbiota response to S-fertilization was less pronounced than for P and thus an S-based fertilizer increase in plant growth could be achieved. Future research is needed to better predict the impact of soil microbiota on plant growth under fertilization events.

Poster number: P04

The importance of organo-sulfur mobilizing bacteria in rhizo- and hyphosphere to supply plant hosts with the essential macro nutrient sulfur

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Many agricultural soils are nowadays at risk of sulfur limitation as plants almost entirely depend on inorganic sulfate for uptake. About 95% of the soil sulfur is bound organically, which can be released by a variety of soil microbes. To improve agricultural fertility, inorganic sulfur may be added to soils to reduce short-term sulfur limits. However, this fertilizer application may disfavour organo-sulfur mobilizing soil microbes, resulting in shifts in the bacterial community structure and potentially reduced ability to mobilize organo-sulfur in the future. A series of studies have demonstrated that bacterial mobilization of sulfur from sulfonates may be a key process in plant sulfur supply. Bacteria, capable of desulfurization of sulfonate, the major organo-sulfur source in soils, have been found to be abundant in various rhizospheres of crops, grasses and in the hyphosphere of mycorrhizal hyphae. Long-term wheat monocultures with and without sulfate fertilization have shown to contain a distinct sulfonate desulfurizing bacterial community. Further studies on grasslands have confirmed this distinctive development of bacterial community structures when sulfate or sulfonate was added as a sulfur source to the soil. Distinct sulfonate desulfurizing bacteria were also detected on the hyphae of arbuscular mycorrhiza, where they putatively attach to the fungal hyphae via a T3SS. Introduction of recently immobilized isotopically labelled sulfate revealed that among the direct transfer of mobilized organo-sulfur through the mycorrhiza, other release and transport mechanisms must be important. Future studies are needed to explore the mobilization of bound sulfur further to improve plant growth sustainably.

Poster number: P05

Phytate solubilisation in Paenibacillus polymyxa is controlled by the catabolite control protein CcpA

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Maintaining global food security is a major challenge of the 21st century. One limiting factor for crop yield is the solubility and thus bioavailability of phosphorus. Fertilisers are environmentally problematic and derived from finite resources. Phytate is an abundant, inaccessible natural soil phosphorus source. Bacterial root symbionts such as Paenibacillus polymyxa can release phosphorus from phytate, improving its bioavailability. Therefore, developing these bacteria as biofertilisers provides an excellent opportunity to optimise phosphorus usage from natural sources.

Here we present the first systematic genetic analysis of phosphorus solubilisation by Paenibacillus polymyxa involving a random mutant library screen, phosphorus solubilisation, promoter activity assays and RNA sequencing.
We found that disruption of the gene encoding catabolite control protein A, ΔccpA, caused a 50% decrease in the ability of Paenibacillus polymyxa to solubilise phytate compared to the wild type. Surprisingly, promoter activity assays showed a 9-fold increased expression of phytase, the enzyme that solubilises phytate, in the ΔccpA compared to the wild type. We therefore performed RNA sequencing in wild type and ΔccpA strains in the presence of organic phosphorus (phytate) or inorganic phosphorus (tricalcium phosphate) to elucidate the genetic programme underpinning the decreased phosphorus solubilisation in the ΔccpA and identified a total 70 genes that are differentially expressed in organic versus inorganic phosphorus that are also responsive to ccpA.

In conclusion, our data suggests that phytase is not the key factor in phytate solubilisation, but other mechanisms such as organic acid production might be involved.

Poster number: P06

Influence of pH, electrical conductivity and soil organic matter on the number of soil culturable bacteria and fungi following liming and fertilisation

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pH, electrical conductivity (EC) and soil organic matter (SOM) have important roles in physical-chemical and biological processes in soils. This study evaluated the influence of liming and fertilisation on soil pH, EC and SOM and how they affect the numbers of culturable microorganisms. The experiment was conducted in a grassland dominated by perennial ryegrass (*Lolium perenne* L.). A randomized block design was established with three replicates. Treatments were four anaerobic digestates, cattle slurry, ammonium nitrate, and no fertilisation. Culturable bacterial and fungal numbers (colony forming units (CFUs)) were quantified using selective planting methods. ANOVA analysis demonstrated that among physical-chemical characteristics evaluated, only soil pH differed significantly ($P<0.05$) between before (pH= 5.32) and after (pH= 7.12) liming/fertilisation. The numbers of culturable bacteria and fungi significantly increased ($P<0.05$) from prior (1.65×10^6 and 1.51×10^4, respectively) to after liming/fertilisation (3.81×10^6 and 2.30×10^5, respectively). The only significant correlation found between pH, EC and SOM and numbers of culturable bacteria and fungi was between pH and the number of culturable fungi ($r=0.81$). The relation of numbers of culturable fungi in function of pH presented best fit in a cubic regression ($y = -0.2588x^3 + 4.8489x^2 - 29.363x + 62.01$, $R^2=0.75$). Fungal growth generally correlates negatively or weakly with pH (Rousk *et al.* 2009; 2010). Bacterial and fungal numbers in these samples will be further analysed using molecular techniques, aiming to verify these relations in the whole microbiological community.

Poster number: P07

Phosphorus recovery from municipal wastewater: potential market for recovered phosphorus in an Irish context

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The depletion of global phosphorus deposits, widening supply-demand gap and security of supply risks have economic, social and environmental implications especially in relation to food production and security for a growing population. Of the approximately 22 million tonnes of phosphorus added to the world economy annually from mined fossil phosphate resources, approximately 80-90% is used as fertiliser in agriculture, 5-7% for animal feed additives and the remainder in various other applications. Phosphorus cannot be produced synthetically and has no substitute in food production. Almost all of the phosphorus consumed in food by the global population enters the wastewater sector. Municipal wastewater therefore represents a major point source to recover phosphorus and re-establish a circular economy. Numerous technologies have been developed for recovering phosphorus from municipal wastewater. This study examined the potential market for recycled phosphorus products. Phosphorus recovered from municipal waste may be used directly as a fertiliser, processed further to produce fertiliser or used as a raw material substitute for mined phosphate rock. The recovered phosphorus must match the needs and requirements of potential users. Challenges identified for the market entry of recovered phosphorus include quality, price and regulatory barriers. The potential market for recovered phosphorus in Ireland is presently limited. Possibilities include the development of a niche market for recovered phosphate as the slow-release fertiliser, struvite and/or other uses identified in consultation with the fertiliser industry. Productive use of recovered phosphorus will require co-ordination between relevant players, including wastewater treatment plants, potential users, industry and regulators.

Poster number: P08

The application of microbial consortium constructed by functional microbiome technology for preventing absorption of Cadmium by crop plants

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Toxic metals such as cadmium do not undergo microbial degradation and tend to persist in soils following release. The benefits of preventing crop plant heavy metal uptake in combination with essential metal inclusion can be attained by the application of multifunctional microbial consortia.

Recent initiatives implemented by the Chinese government call for the reclamation of 90% of polluted arable land in the country by 2020. The application of lime to the soil to reduce the bioavailability of the pollutant cadmium in these regions has impacted the availability of zinc, resulting in a negative impact on plant growth. Possessing high mobility and bioavailability, cadmium is a severe public risk in China due its highly prolific occurrence in ground soils. Meanwhile zinc deficiency in soils is a limiting factor in crop growth, impacting food availability and quality in affected regions. The formulation of methods to address this issue are potentially lucrative from an economic standpoint and will improve regional food safety considerably.

This collaborative research study between MicroGen biotech and IT Carlow will examine cadmium and zinc levels in agricultural soil in Jiangsu province, Danyang and Ningbo City in China. Previous application of Microgen biotech's functional microbiome technology has resulted in the reduction of polycyclic hydrocarbons soil concentrations from 40,000 ppm to > 100 pm with 4000 tonnes of soil processed. This project will utilize this patented technology to produce commercially viable solutions to the unique challenges presented by cadmium pollution of Chinese soils.

Poster number: P09

Drinking water treatment residuals as a mitigative option for reducing incidental P loss from biosolid incorporated soils

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Al-based water treatment residuals (Al-WTRs) may reduce Phosphorus (P) availability in high P impacted soils. However, their impact on incidental P losses remains unclear. An integrated experimental programme investigated this issue at bench top and intermediate (metre) scales.

Batch adsorption studies show P adsorption capacity (Langmuir 'P_{max}') of Al-WTRs for lowland catchments proved 76% higher ($P<0.001$) than for upland areas (n=4) (P_{max} range: 25-135g kg^{-1}). Results suggest negative correlation between P_{max} and total Carbon content of WTRs ($R^2=0.86$, $P<0.01$), indicating that high organic C fractions in more peaty upland Al-WTRs may inhibit P adsorption compared to lowland Al-WTRs.

Intermediate scale rainfall simulations evaluated P delivery in overland flow (OLF) by (a) varying incorporation rates of Al-WTR (0-65Mg ha^{-1}) and biosolid ($\lesssim$449 kg P ha^{-1}) (n=36), and (b) using three difference application methods of Al-WTR on biosolid-amended soil (299 kg P ha^{-1}) (n=18). Repeated rainfall events (i= 33-36mm hr^{-1}) conducted on 0.3-m^2 x 0.25-m deep runoff boxes, 2-16-days post application showed WTR incorporated soils had no significant effect on P runoff. However, surface applied WTR (65 Mg ha^{-1}) resulted in no OLF, 2 and 13 days post application. Leached P losses proved significantly higher ($P<0.01$) compared to incorporated WTR soils (7.43 - 1.58mg TP l^{-1} on day 2, and 3.28 -0.37mg TP l^{-1} on day 13).

Overall Al-WTR increased soil infiltration capacity, which was primarily responsible for variations in incidental P losses observed. Results demonstrate the aggregate stability of WTR limits infiltration excess runoff, indirectly preventing incidental P losses through OLF.

Poster number: P10

Effects of co-application of drinking water treatment residuals and biosolids on the performance of willow (*Salix* spp)

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Co-application of Al-based water treatment residuals (WTRs) with biosolid, may induce Phosphorus (P) deficiency and/or Aluminium (Al) phytoxicity in Willow (*Salix* spp.) grown for biomass. Greenhouse pot studies were conducted to (i) quantify the effect of varying WTR application rate (0-100Mg WTR ha^{-1}) on four commercial varieties of *Salix* spp (n=120). (ii) Determine the effect of co-applied biosolid (0-40Mg ha^{-1}) and WTR on soil Olsen-P in relation to plant P concentration (n=120). (iii) Compare the effect of co-applied WTR:biosolid ratios (0:100% & 8:40% vice versa) on biomass yield (n=128).

Results show yield increased, and plant P concentration decreased significantly ($P<0.05$) for all genotypes treated with ≥10Mg WTR ha^{-1}. Yield was lowest ($P<0.05$) and Al uptake highest ($P<0.05$) for Tora (S. *viminalis* x S. *schwerinii*) compared with other genotypes. No Al phytoxicity was observed.

Co-applied WTR and biosolid had no significant effect on biomass yield. However, P uptake was significantly reduced by 100Mg WTR ha^{-1} when co-applied with 0, 10, or 20 Mg biosolid ha^{-1} ($P<0.05$). At 40Mg biosolid ha^{-1}, no reduction in P uptake was observed despite a 30% reduction in soil Olsen-P ($P<0.05$) achieved at 100Mg WTR ha^{-1}.

Yields proved 51% higher ($P<0.05$) for 40:8% WTR:biosolid ratios when compared to 8:40% WTR:biosolid, despite similar plant P concentrations. Willows grown in 100% WTR produced second highest yield, whilst all plants treated with 100% biosolid died.

Overall, results suggest WTR has no negative agronomic effects on Willow with potential to reduce bioavailable P; however, more research is needed under field conditions.

Poster number: P11

Double-stranded DNA viruses "in control" of their own evolution

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The Red Queen hypothesis posits that antagonistic coevolution between interacting species results in recurrent natural selection via constant cycles of adaptation and counter-adaptation. Such interactions are at their most profound between bacteria and their phages. Studies of viral evolution provide an unparalleled insight into the truly remarkable elasticity of living entities. Whilst studies into the effects of phage evolution in relation to changes in host range are abundant, as are those directed towards understanding quasispecies phenomena exhibited by RNA and ssDNA viruses, the study of the molecular processes at play in dsDNA representatives are lacking. Here, we present a quasispecies-like phenomenon operating within a group of T7likeviruses known as the phiKMVlikeviruses. This group of phages shows a ubiquitous distribution in the environment and are utilised in a number of therapeutic preparations targeting the nosocomial pathogen Pseudomonas aeruginosa. Through deep NGS analysis, representatives of the phiKMVlikeviruses were shown to possess between 15 and 88 variable sites with substitution frequencies of 1 - 47.2%. The majority of such polymorphisms are found within genes implicated in host infectivity and show extremely variable levels of co-occurrence, resulting in the existence of a highly heterogeneous population reminiscent of a viral quasispecies. Predicted error prone DNA polymerase activity via the absence of a thioredoxin binding domain in the phiKMVlikeviruses is likely to play a role in the generation of variant rich populations. This may form a basis of a “bet hedging” strategy for circumventing host resistant phenotypes via rapid selection according to Red Queen dynamics.

Poster number: P12

Molecularly Imprinted Polymers for the Removal of MCPA from Water

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The aim of this project is to develop a molecularly imprinted polymer (MIP) capable of removing 2-methyl-4-chlorophenoxyacetic acid (MCPA) from water. MCPA is a commonly used phenoxy herbicide for the selective control of perineal and annual weeds.

MCPA has been widely overused leading to a number of problems due to its high solubility in water and lack of binding with soil. MCPA can cause huge damage to aquatic environments and may pose a threat to human health through the contamination of potential sources of drinking water. Due to these concerns WHO set a maximum value for MCPA in drinking water at 2 μg L^{-1}. This value is even lower in Northern Ireland, where MCPA is regarded as the greatest herbicide pollution problem, with a maximum limit set at 0.5 μg L^{-1}.

Current methods for MCPA removal from water are not removing MCPA at a suitable level or are too expensive to implement on a wide scale. MIPs could provide an efficient and cost-effective method for the removal of MCPA from water. Two MIPs have been developed to address this problem, one using the commercial monomer 2-(diethylamino)ethyl methacrylate and another using a synthetic urea-based vinyl monomer.

It has been shown through equilibrium rebinding and solid phase extraction experiments that the urea-based vinyl monomer-containing polymer was far superior, both in terms of its ability to bind MCPA in aqueous environments and its selectivity for MCPA. This polymer could offer an efficient and cost-effective alternative to current methods of MCPA removal from water.

Poster number: P13

Use of a natural polymer for the removal and recovery of phosphorus from wastewater

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Phosphorus is a finite resource that is geographically concentrated and an essential part in supporting global food production. When used excessively it is the main contributor to eutrophication as it enters bodies of water through runoff or as wastewater. Recent years have seen an increase in the awareness of the need for phosphorus recycling, however due to the increased cost for recovery when compared to mining, phosphorus is typically captured and not recovered for reuse.

Our team has developed a biopolymer based sorbent material, and trialled it for the removal and recovery of phosphate from water. Initial trials involving filtration of aqueous phosphate solutions through a filter packed with the sorbent demonstrated a 70% reduction in phosphorus concentration and a facile recovery cycle consisting of a simple acid wash.

When a synthetic wastewater was passed through the same filter, 12% of the phosphorus was removed, while 7 – 10% of the other three anions present, chloride, sulfate and nitrate, was also removed. When repeated with actual effluent wastewater, a similar removal of 10% of total phosphate was observed.

In these preliminary tests, the tested materials did not reach the required levels of phosphate removal, however we are currently optimising the polymer composition and filter construction to create a material capable of recovery both phosphate and other pollutants of interest from wastewater systems, with a view to enable recovery of phosphate, sulfate and nitrate could for direct use in the production of fertilizers.

Poster number: P14

Phosphorus Recovery from Municipal Wastewater: Potential Recovery Technologies for an Irish Context

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Phosphorus (P) is essential for life. P fertilisers, from phosphate rock are indispensable to feed the world's population. Of the ~ 22 Mt P of phosphate rock mined/yr, ~ 90% is used as fertiliser in agriculture. P cannot be produced synthetically and has no substitute in food production. Phosphate rock is finite and non-renewable, with supply vulnerable to future scarcity, volatile pricing and geopolitical tensions. With the necessity of P for food security, there is a need to promote efficient use of P and its recovery and reuse. Most of the 3 Mt of P consumed in food per year by the global population enters the wastewater streams. Municipal wastewaters therefore represent a potential source to recover P. A number of technologies have been developed for the recovery of P from municipal wastewater. The main objective of the current study is to evaluate these technologies with the aim of assessing their feasibility in an Irish context. Recovery technologies based on crystallization and precipitation of P are the most technologically advanced but are limited to WWTPs with EBPR (which is not present at any of the major Irish WWTPs). Other technologies (acid leaching or thermochemical processes) on sludge/sludge ash can recover greater amounts of P (48-99% of influent phosphorus v's 10% for precipitation technologies). If introduced these technologies would focus on WWTPs with greater than 100,000 p.e., however these technologies are still in technical development and require significant investment. To the best of our knowledge no current Irish site recovers phosphorus.

Poster number: P15

Effect of model root exudate on denitrifier community dynamics and activity at different WFPS levels

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Denitrification represents the main emission source of the potent greenhouse gas nitrous oxide (N_2O) in agricultural soils. Plants have been shown to increase denitrification rates in their rhizospheric zone with this effect mainly attributed to rhizodeposition of carbon sources. However, the extent to which this release affects denitrifier community dynamics and associated denitrification rates remains obscure. A controlled microcosm experiment was designed to explore this interaction in terms of flux and community dynamics. Different quantities of artificial root exudates (0 to 3 mg C day^{-1}) were added daily to soil at three water regimes (50, 70 and 90% WFPS). After 7 days, denitrification rates and N_2O emissions were measured and community dynamics were assessed using molecular methods. Significant denitrification activity and N2O emissions were observed only at 90% WFPS, which increased with C input. Molecular analyses showed the response and sensitivity of the *nirK*, *nirS*, *nosZ*-I and *nosZ*-II communities to C addition are distinct and highly dynamic. Different responses were observed at the three WFPS levels tested, with *nosZ*-I communities in the rhizosphere responding strongly at 50% WFPS while *nirS* community dynamics were affected most at 90%. Our study demonstrated artificial exudate addition stimulates changes in the denitrifier community size and structure even in conditions unfavourable for denitrification and can impact denitrification rates in favourable ones. Final N_2O emissions, however, are determined by the *nosZ*-containing community ability to convert N_2O to N_2.This demonstrates that effects of root exudates on denitrifier communities are complex and these changes could impact soil processes.

Poster number: P16

In Situ Spectral Monitoring of Biological Phosphorus Removal from Wastewater

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Enhanced biological phosphorus removal (EBPR) is a wastewater treatment process that produces a good quality sludge from which phosphorus may be recovered and reused. EBPR is, however, a complex process that is challenging to control and optimise. It critically relies on maintaining a subpopulation within a mixed microbial community but is yet subject to daily variation in influent composition and environmental conditions. EBPR process optimisation and stabilisation through development of efficient control strategies and early detection of abnormal situations is an acknowledged need. We propose that *in situ* spectral monitoring of EBPR bioreactors can contribute to quasi real-time process control.

In vitro results showed that single cell Raman spectroscopy can characterise key intracellular EBPR process metabolites such as polyphosphate, polyhydroxyalkanoates and glycogen. Furthermore, biological lipids, carbohydrates and proteins also have characteristic Raman signals. These spectral signals, monitored at a population level, could constitute useful markers of process performance and 'health'. Early results have encouraged us that acquiring process Raman spectra *in situ*, is readily complemented by capture of additional spectra. Currently, we are investigating a multi-spectral approach combining Raman, FTIR and fluorescence, to give a "broadband" snapshot of system state.

Poster number: P17

Biological phosphorus removal during low-temperature anaerobic digestion

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Phosphorus (P) is essential to life and is incorporated in many biomolecules, such as DNA, RNA and ATP. It is, however, also a major pollutant of water systems. The mining process by which phosphate is sourced for fertilizer is unsustainable and the recovery of P from resources, such wastewaters, is necessary for the sustainability of agriculture, and the global food market. This project investigated the potential for a novel anaerobic digestion-based microbiological biological approach for the removal and recovery of P from industrial wastewaters. Two laboratory-scale (2.5 L) anaerobic reactors were seeded with 20 g volatile solids L-1 of anaerobic granular sludge. R1 treated a synthetic dairy wastewater whilst R2 treated a wastewater composed of the methanogenic substrates of acetate and methanol. The total chemical oxygen demand (COD) removal of both R1 and R2 was consistently high, up to 94% and 98% for R1 and R2, respectively. Operating conditions (including organic loading rate and hydraulic retention time) were altered to attempt to stimulate P uptake. While no extended period of phosphate uptake was observed in in microbial biofilms in R1 during treatment of complex wastewater, a 2 week period of phosphate uptake was recorded in R2 during treatment of the methanogenic wastewater. Up to 53% P removal was observed during the period. P removal ceased following the an increase in the applied influent COD, which was followed by an extended period of phosphate release. Ongoing work is seeking to establish the triggers for P uptake and removal towards practical applications.

Poster number: P18

Metagenomic analyses of aeration rate impacts on a low temperature, intermittently aerated sequencing batch reactor (IASBR) treating dairy processing wastewater

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The European dairy industry is experiencing a period of rapid growth following the abolition of European milk quotas in 2015, with a 50% increase in Irish milk production predicted by 2020. Several environmental challenges accompany this development with considerable attention being focused on effective management of the increased wastewater outputs within the industry. Such wastewaters require on-site remediation prior to release into receiving water bodies and there is considerable interest at present in the development of robust, cost-effective, microbial-based technologies to sustainably achieve this goal. Intermittently aerated sequencing batch reactors (IASBRs) represent a novel approach that combines multiple anaerobic/aerobic operational cycles to achieve COD, nitrogen and phosphorus remediation in a single reactor. In this study an IASBR system used to treat synthetic dairy processing wastewater was subject to 16S rRNA based metagenomic profiling. Aeration rate conditions were varied between 0.8, 0.6 and 0.4 Litres per minute to determine the impacts on IASBR microbial community structure andorthophosphate (PO_4-P) and ammonium (NH_4-N) removal efficiencies. Aeration rates of 0.6 LPM resulted in >90% removal efficiencies for both orthophosphate and ammonia and the family Comamonadaceae were observed to stably dominated the IASBR reactor biomass community profile, >80% relative abundance. Results show the potential of IASBR application to dairy processing wastewater remediation and suggests members of Comamonadaceaemay contribute significantly to biological nutrient removal processes.

Poster number: P19

Extreme Halophilic Microorganisms as a source of Antibiotic Chemical Diversity for Medical Device applications

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With the advent of the post-antibiotic era, the disturbingly low number of novel antibiotics classes, and high prevalence of multidrug-resistant pathogens is almost apocalyptic. New approaches are necessary to improve the efficiency of the discovery process. This study aims to establish the potential of ancient, extremely halophilic microorganisms, isolated from ancient (Triassic) halite evaporites (Kilroot salt mine), as a source of novel therapeutically relevant antibiotics.
Different isolation techniques involving pre-treatments, selective media, the addition of inhibitory agents, and variation of salinity and incubation temperatures were employed. Following 2 weeks of incubation, the organic extracts of isolates were screened for their antimicrobial, and quorum sensing inhibitory properties. The activities of extracts were further quantified with MIC and MBC determinations against susceptible pathogens. Initial purification of one extract was attempted using TLC-overlays and automated flash chromatography. Following 16S rRNA sequencing revealed that many of the isolates were Halorubrum sp. While no isolates demonstrated antifungal activity, many isolates demonstrated antibacterial activity.
The organic extract of Haloferax sp was further investigated. The crude extracts had MICs and MBCs of 8mg/ml against S. aureus, MRSA and K.pneumoniae strains and the anti-biofilm potential on MRSA was tested using the Minimum Biofilm Eradication Concentration (MBEC) assay. Initial characterisation of the extract suggests the compound is heat tolerant, proteinaceous, and potentially a halocin. Screening demonstrates that halophilic microorganisms are good producers of antimicrobials. Whilst high purity of these compounds has yet to be achieved, they remain a promising reservoir possessing both broad antimicrobial bioactivities.

Poster number: P20

Stability analysis in a high-rate AD reactor for biomethane production treating raw dairy waste water

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Biogas production from wastewater is commonly carried out in reactors under mesophilic conditions (30-38°C). Industrial wastewaters such as dairy are typically discharged at lower temperature, meaning that generally need to be heated up. The pilot-scale AD unit employed in this trial was a hybrid up-flow granular sludge based system incorporating a top section anaerobic filter. The reactor was inoculated with anaerobic granular sludge from a previous industrial anaerobic reactor treating starch processing wastewater. The reactor operated at hydraulic retention times (HRTs) of 36, 24 and 18 hours. The wastewater treated was raw having received no pre-treatment for fat, oil and grease (FOG) removal. During the trial different parameters were analysed in the inlet and the outlet line, soluble chemical oxygen demand (COD), proteins, ammonia, FOGs and accumulative methane production. Over the three phase reactor trial performances measured by soluble COD removal of 51, 62 and 69%. Protein in the inlet ranged between 105-405mg·l^{-1}. The ammonia concentration was below inhibition concentration. FOGs average was 114mg·l^{-1}, with 100mg·l^{-1} suggested as maximum concentration permitted, although the reactor received higher concentrations on four occasions, the last one corresponded to 1,100mg·l^{-1}, after which the reactor failed to recover. The highest methane production of 332.7l_{CH4}·(m^3 of wastewater)$^{-1}$ occurred during the 18 hour phase. However the profile of methane production fluctuated mainly due to FOGs content. In spite of varying yields in methane production, anaerobic treatment of dairy wastewater was demonstrated to be feasible. The issued presented by FOGs could be resolved through pre-treatment for FOGs removal.

Poster number: P21

Soil Health in Northern Ireland's grassland, arable and silvopastoral soils

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'Soil Health' has many definitions but it is generally accepted that a healthy soil is one that has the ability to act as a living system to sustain, in the long term, its most important functions. In NI agricultural systems, primary soil functions include biomass production, the regulation of nutrients and water in soils and the maintenance and enhancement of soil carbon stocks. The recent SALMS Report, 2016, shows that 98% of soils in NI are inadequately analysed every year and 82% of soils are below optimum fertility level. There is an urgent need to obtain baseline data on the health status of soils in NI and to use this information to empower farmers to make management decisions to improve soil health. An important research challenge remains how to 'define' and 'measure' soil health. The project will address the priority area of soil and growing medium management by undertaking research to measure a range of key chemical, physical and biological parameters in grassland, silvopastoral and arable soils in both experimental plots and on-farm. There will be particular focus on the impact of organic manures and slurries and on the practice of reseeding in grassland soils. Aspects of soil microbiology will be explored and related to key chemical and physical parameters in grassland and arable sites across NI under a range of management histories. The project aims to provide guidelines for policy makers, farmers and growers about what soil health characteristics should be promoted and sustained in the long-term.

Poster number: P22

Effect of early life diet supplementation on the rumen microbiome of lambs

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Microorganisms in the foregut of ruminant animals are critical for digestion of fibrous materials that make up the host diet. In addition, rumen microbiome structure has been correlated with trends in animal productivity, feed conversion efficiency and health. Diet influences microbial community structure in the rumen and could be used to manipulate the structure, creating microbiomes desirable for animal productivity and health. For example, Rowntree *et al,* (2010) found that inclusion of linseed oil in the diet of ruminants led to reduced enteric methanogenesis which has both economic and environmental benefits.
This study examined the effect of dietary supplementation in early life on the rumen microbiome of lambs to determine if altered microbiomes persisted once administration of the supplement ceased. New born lambs were randomly assigned to one of three treatment groups (n=9). The control group received standard lamb meal throughout the study, the second group received lamb meal supplemented with 4% linseed oil throughout the study and the final group received the supplement during pre-weaning only. At 16 weeks, VFA concentrations and microbial communities in the rumen were analysed, and results compared between treatment groups.
Dietary supplementation had no negative effect on animal health, daily intake or weight. Lambs fed linseed oil throughout the study had different bacterial ($p=0.029$) and archaeal ($p= 0.005$) community structures in their rumen. Lambs fed the supplement pre-weaning only also had an altered rumen microbiome compared to the control group. This indicates that the microbiome remained altered even after supplementation had ceased.

Poster number: P23

The effect of long term phosphorus fertilization and above ground management on the soil microbiome

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Application of fertilizers to soil is used by the agricultural sector to enhance crop productivity and support livestock. However, fertilizer application is often costly, inefficient, and can be environmentally damaging. Phosphorus (P) fertilization is particularly challenging; it is a finite resource and is readily adsorbed onto soil particles which restricts uptake by plants. Microbial activities within soil are essential for P release from organic matter, but require sufficient carbon (C) and energy to do so. This study compared the long-term impact of three inorganic P application rates across two grassland field sites that varied in terms of their carbon (C) input. Biological, physical and chemical soil properties were measured along with microbial diversity. When C input was not limited, total soil P, available P, microbial biomass C and P increased with P application rate. However, when C input was limited, total soil P was reduced. Available P, microbial biomass C and P were also lower in the site with reduced C input and remained relatively constant regardless of P application rate. Analysis of bacterial, archaeal and fungal assemblages identified a clear separation of communities with reduced C input. Communities were seen to cluster based on P treatment but only when C was not limiting. This information will facilitate the development of more efficient and appropriate P fertilizer regimes.

Poster number: P24

Enhanced hydrolysis and medium chain carboxylate accumulation during anaerobic biodegradation of food waste

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A high-yield medium-chain carboxylate (caproate) production strategy is described, using anaerobic food waste (FW) fermentation without external electron donor supplementation. Using a leach-bed reactor (LBR) configuration, efficient hydrolysis was achieved, as indicated by a maximum soluble chemical oxygen demand (sCOD) accumulation within 2 days. Caproate production (21.86 ± 0.57 g COD l^{-1}) was obtained under the optimised conditions, which included high leachate recirculation regime and a low volatile fatty acid concentration in the starting liquid. Caproate concentrations as high as 23.91 g COD l^{-1} (11 g l^{-1}) were achieved, at a maximum rate of 3 g l^{-1} d^{-1}, in batch trials using LBR leachate supplemented with H_2. This highly efficient caproate production was mainly attributed to lactate oxidation. High-throughput sequencing analysis of the active microbial communities profiling from cDNA revealed that *Clostridium* and *Peptoniphilus* were likely both involved in butyrate formation whereas *Lactobacillus reuteri* were likely involved in caproate production. The findings of this study have strong application potential, specifically in the design of a process that will allow for the continuous and sustainable production of caproate, not only from food waste but also from other low-cost wastes with high carbohydrate content.

Poster number: P25

Bacterial microcompartment-directed polyphosphate kinase promotes stable polyphosphate accumulation in E. coli

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Processes for the biological removal of phosphate from wastewater rely on temporary manipulation of bacterial polyphosphate levels by phased environmental stimuli. In E. coli polyphosphate levels are controlled via the polyphosphate-synthesizing enzyme polyphosphate kinase (PPK1) and exopolyphosphatases (PPX and GPPA), and are temporarily enhanced by PPK1 overexpression and reduced by PPX overexpression. We hypothesised that partitioning PPK1 from cytoplasmic exopolyphosphatases would increase and stabilise E. coli polyphosphate levels. Partitioning was achieved by co-expression of E. coli PPK1 fused with a microcompartment-targeting sequence and an artificial operon of Citrobacter freundii bacterial microcompartment genes. Encapsulation of targeted PPK1 resulted in persistent phosphate uptake and stably increased cellular polyphosphate levels throughout cell growth and into the stationary phase, while PPK1 overexpression alone produced temporary polyphosphate increase and phosphate uptake. Targeted PPK1 increased polyphosphate in microcompartments 8-fold compared with non-targeted PPK1. Co-expression of PPX polyphosphatase with targeted PPK1 had little effect on elevated cellular polyphosphate levels because microcompartments retained polyphosphate. Co-expression of PPX with non-targeted PPK1 reduced cellular polyphosphate levels. Thus, subcellular compartmentalisation of a polymerising enzyme sequesters metabolic products from competing catabolism by preventing catabolic enzyme access. Specific application of this process to polyphosphate is of potential application for biological phosphate removal.