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Removal of dissolved nutrients from wastewater using a microalgal biofilter *Line Christensen, Suvina Sooknandan, Jens Jørgen Lønsmann Iversen* Department of Biochemistry and Molecular Biology, University of Southern Denmark, Odense, DK-5230, Denmark. E-mail: lhm@bmb.sdu.dk (L. Christensen)

A microalgal biofilter can be used for treatment of wastewater from landbased fish farms in order to remove excess amounts of dissolved nutrients such as nitrate, ammonium and phosphate. A bubble column bioreactor has been developed for cultivation and characterization of microalgae. This type of bioreactor is equipped with a control system that enables online determination of the photosynthetic quotient and optimization of light intensity. Furthermore the bioreactor has a dualsparging system simultaneously allowing adequate mixing and high gas-liquid mass transfer coefficients. Different species of microalgae have been cultivated in batch and fed batch cultures to characterize growth and ability to take up the different dissolved nutrients. The specific growth rate and substrate uptake rate have been determined to compare and select the algal species most suited for use in a biofilter. Additionally the composition of lipid, protein and carbohydrates has been measured to determine the nutritional quality of the algae when used as animal feed.

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Application of nitrogen-removal bioreactor capable of simultaneous nitrification and denitrification to wastewater treatment in power plants *Masahiko Morita, Hiroaki Uemoto, Atsushi Watanabe* Environmental Science Research Laboratory, Central Research Institute of Electric Power Industry (CRIEPI), 1646 Abiko, Abiko-shi, Chiba 270-1194, Japan. E-mail: masahiko@criepi.denken.or.jp (M. Morita)

At present, biological nitrogen-removal is mostly carried out through several complicated steps. To simplify the present systems for nitrogen-removal, we have investigated a new nitrogen-removal bioreactor using packed gel envelopes capable of simultaneous nitrification and denitrification. The envelope consists of two plate polymeric gels with a spacer in between. Ammonia oxidizer, *Nitrosomonas europaea* and denitrifier, *Paracoccus denitrificans* are co-immobilized in the plate gels. When the envelopes are exposed to wastewater containing ammonia, the immobilized *N. europaea* oxidizes ammonia to nitrite in the outer aerobic surfaces of envelopes. At the same time, as ethanol solution is injected into the internal anaerobic spaces of envelopes, the immobilized *P. denitrificans* reduces the nitrite to nitrogen gas using the ethanol solution as an electron donor for denitrification. In this way, the envelopes can remove ammonia from wastewater in a single step. We have already reported advantages of our bioreactor in laboratory-scale experiments. In this study, we show our large-scale bioreactor (water volume 1.8 m³) could treat three kinds of wastewater derived from coal power plants. Ammonia-containing wastewater that occurred regularly in a coal power plant was continuously treated with the bioreactor using thirty envelopes for over a year. The bioreactor could remove more than 90% of total nitrogen at hydraulic retention time (HRT) of 24 h. At HRT of 4 h, the bioreactor accomplished a maximum rate (the transformation of NH₄⁺ to N₂) of 6.0 g N/day m² of the envelopes' surface. The performance was equivalent to that obtained in the laboratory-scale experiments. Furthermore, our bioreactor showed similar nitrogen-

removal performances when it treated nitrate-containing wastewater occurring regularly and condensed ammonia-containing wastewater occurring at irregular intervals in coal power plants. These results show that our bioreactor can treat various wastewater containing nitrogen in coal power plants. Thus, our concept is effective to simplify the large-scale systems in coal power plants and the other plants.

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Removal of metals from waste incinerator bottom ash by acidophilic microbes *M.J. Moura, J. Sousa, M. Costa-Ferreira* INETI/DB/UBB-Ed. F, Azinhaga dos Lameiros á Estrada do Paço do Lumiar, P-1649-038 Lisboa, Portugal. E-mail: joao.moura@ibqta.ineti.pt (M.J. Moura)

In order to establish an environmentally friendly process for the treatment of metal containing waste, in a Portuguese refinery a process involving sulphur oxidizing acidophilic microbes is being considered. Bioleaching of metal containing bottom ash, from fluidised bed incineration of sludge resulting from the refinery water treatment station, was performed using a sulphur oxidising acidophilic culture isolated from an acid pool resulting from the weathering of sulphur piles from the Claus plant. This sample served as inoculum for liquid medium cultures with 1% sterile sulphur flowers as source of energy. Application of Monod kinetics to adapted culture growth of free cells presented a value of $\mu = 0.124 \text{ day}^{-1}$. Yield of sulphur conversion to sulfate after 17 days was $\eta = 78\%$. In the presence of bottom ash from the incineration of refinery sludges $\mu = 0.141 \text{ day}^{-1}$ and the yield of sulphur conversion was $\eta = 67.5\%$. A $\eta_{\text{Fe}} = 90\%$ removal of iron is obtained from the treated ash. X-ray fluorescence spectroscopy of the solid residue revealed a total removal of metals namely, V, Cu, Ni, Zn and most of the Fe after 15 days of bioleaching.

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Precipitation of Cr(III), Ni(II) and Zn(II) in solution by sulphate reducing bacteria *G. Cabrera, J.M. Gómez, D. Cantero* Biological and Enzymatic Reactors Research Group, Department of Chemical Engineering, Food Technology and Environmental Technologies, Faculty of Sciences, University of Cadiz (UCA), 11510 Puerto Real, Cádiz, Spain. E-mail: gema.cabrera@uca.es (G. Cabrera)

The present of heavy metals in the environment is a serious problem. They are commonly present in effluents from mining and industrial activities. Usually, chemical conventional methods are very expensive and they have limitations when heavy metals are in low concentrations. At the moment the interest increases for processes that involve microorganisms as alternative method. Some effluents present heavy metal sulphates which are soluble compounds. Sulphate-reducing bacteria (SRB), under anaerobic conditions, oxidize simple organic compounds (such as acetic acid and lactic acid) by utilizing sulphate as an electron acceptor and generate hydrogen sulphide. Hydrogen sulphide reacts with heavy metal ions to form insoluble metal sulphides that can be easily separated from a solution. The purpose of this work was to evaluate the ability of SRB to reduce Cr(III), Ni(II) and Zn(II) in artificial contaminated solution. *Desulfovibrio vulgaris* and *Desulfovibrio* sp. strains has been tested in this study. Batch cultures was carried out in 50 ml sealed bottles with different concentrations of studied metals (1–20 mg/L), with 10% of inoculum bacterial and adapted to Postgate's medium C. A gaseous nitrogen current was employed to purge oxygen and obtain anaerobic conditions. The assays were incubated statically

at 30 °C during 14 days. Bacterial population was determined by counting in a Neubauer chamber with optical microscope. Sulfate concentration was measured by turbidity method and metal concentrations in the filtered supernatant were measured by ICP-AES. The first part of study consists of determine the maximum concentration of each metal at which *D. vulgaris* and *Desulfovibrio* sp. grow in similar way than control culture (without metal). Both cultures tolerate: Cr(III) 15 ppm, Ni(II) 8.5 ppm, Zn(II) 20 ppm. The maximum precipitation percentages were approximately: 25% (15 ppm Cr(III)), 96% (8.5 ppm Ni(II)) and 99% (for *D. vulgaris*—10 ppm Zn(II) and *Desulfovibrio* sp.—15 ppm de Zn(II)). Time to reach the highest precipitation was minor for mixed culture (*Desulfovibrio* sp.) y all the cases. The next part was focused to study the precipitation percentage when metals are present in combination in the same metal levels (Cr(III)–Ni(II), Cr(III)–Zn(II), Ni(II)–Zn(II) and Cr(III)–Ni(II)–Zn(II)). The combination of metals does not affect significantly the bacterial growth and precipitation percentage of metals. This fact supposes an importance advantage so metals are commonly found together in the environment. Future experiments are focused in development of this process in continuous operation mode.

EB60

Kinetic modelling of the fungal biosolubilisation of coal *B.O. Oboirien*¹, *G. Searby*¹, *D. Cowan*², *S.T.L. Harrison*¹: ¹Bioprocess Engineering Research Unit, Department of Chemical Engineering, University of Cape Town, Rondebosch, 7701, South Africa; ²Department of Biotechnology, University of the Western Cape, Belville, South Africa

Biosolubilisation and depolymerisation of coal has potential to produce a clean energy source or high value organic products from low rank coals such as lignite or sub-bituminous coal. These complex soluble phenolic compounds are of value as starting materials for biotransformation to value-added compounds such as antioxidants and flavourants. The bioprocess is carried out at ambient temperature and pressure and is perceived to be environmental benign. In the evaluation of coal solubilisation an important quantity for the assessment of process feasibility is the yield, i.e. the determination of the mass of product obtained per unit mass of coal solubilised. To date, results for coal biosolubilisation reported in the literature are qualitative or at best semi-quantitative, indicating trends with operating variables. The process kinetics has not been determined rigorously because measurement of fungal growth during coal solubilisation is hindered by the presence of the solid coal substrate. Knowledge of the profile of biomass growth is required for the rigorous determination of the kinetic parameters necessary for process design and optimisation. In this paper, the use of an indirect method for the estimation of the growth and metabolism of fungal biomass by measuring CO₂ evolution and O₂ consumption using an off-gas analyser is reported in the study of fungal coal solubilisation. Coal determined rigorously because measurement of fungal growth during coal solubilisation is hindered by the presence of the solid coal substrate. Knowledge of the profile of biomass growth is required for the rigorous determination of the kinetic parameters necessary for process design and optimisation. Biosolubilisation was carried out in a stirred tank slurry bioreactor with working volume of 1.0l. Complete suspension of the coal particles of 650–800 µm mean diameter was achieved at

an agitation rate of 560 rpm. Growth yield coefficients based on coal and oxygen as well as maintenance coefficients were calculated from growth of the fungus under the same conditions using a non-coal carbon source such as glucose. These data were used to determine the stoichiometric coefficients for biomass growth, enabling the biomass production rate to be quantified in terms of CO₂ production rate and O₂ consumption rate.

EB61

A DNA-chip platform for parallel detection of microorganisms related to biofilm in industrial systems and drinking water systems *Pernille Skouboe*, *Dorte Lauritsen*, *Kim Holmstrøm* Bioneer A/S, Kogle Allé 2, DK-2970 Hørsholm, Denmark. E-mail: psk@bioneer.dk (P. Skouboe)

An oligonucleotide microarray for simultaneous detection and identification of pathogenic bacteria related to technical water systems as well as drinking water has been developed. The approach is based on the use of a tandem hybridization technique with two ribosomal 16S rDNA-PCR products, 1000 bp and 500 bp long, generated from two consensus PCR reactions using conserved ribosomal primers end-labeled with Cy3 and Cy5, respectively. The tandem hybridization technique implies an internally quality control for discrimination between target and non-target signals. The current prototype of the DNA-chip platform includes 20 oligonucleotide probes representing 11 different genera (and subgroups of species), e.g. *Legionella*, *Mycobacterium*, *Aeromonas*, *Campylobacter*, *Vibrio* and *Enterococcus*. The platform has been used for detection and identification of species from pure cultures, and initial experiments with water samples from industrial systems have been performed. The potential as well as the limitations of using a DNA-chip based detection format in its present form will be documented. Particularly, its potential application as a rapid method for initial screening of environmental or food samples will be addresses. The aim is to reduce and optimize the number of samples required for traditional microbiological identification tests.

EB62

Isolation and characterization of microorganisms for the biological inactivation of fumonisins *W.-D. Moll*¹, *M. Täubel*¹, *E. Vekiru*², *A. Frank*², *A.P. Loibner*³, *R. Braun*³, *G. Schatzmayr*¹: ¹Biomin GmbH, Industriestraße 21, 3130 Herzogenburg, Austria; ²University of Natural Resources and Applied Life Sciences, Center for Analytical Chemistry, Vienna; ³Department for Agrobiotechnology, Institute for Environmental Biotechnology, Konrad Lorenz Straße 20, 3430 Tulln, Austria

In the course of a project for the development of a novel kind of a mycotoxin inactivating feed additive, the aim of this study was to isolate and characterize microorganisms with the specific ability to enzymatically break down and detoxify fumonisins, a group of structurally related fungal toxins, with fumonisin B₁ (FB₁) being the most abundant and – with respect to toxicology – also the most important representative of this group. These toxins are produced as secondary metabolites by some *Fusarium* species such as *Fusarium verticillioides* and *F. proliferatum* and are naturally occurring contaminants of cereal grains worldwide. They are found especially in maize and maize based products, and are known to be hazardous to human as well as to animal health. A natural feed additive, based on microorganisms and/or enzymes, should ensure the detoxification of