pH, stirring velocity, sludge concentration and particle size. The kinetic data allowed rate constants, reaction orders and the activation energy to be determined. These data were then analyzed with the help of the fluid particle shrinking-core kinetic model. It showed that the most important reaction resistance was the diffusion of the *in situ* generated renewable base (OH^-) in the sludge particle. The same modeling approach also allowed examination of the electron reduction mechanism. The most influential reaction engineering parameters were found to be pH > temperature > stirring rate.

Phosphate was recovered in up to very high yields of 95% using optimized process parameters.[3] Initially, the scale-up process took about 3 months and with adapted processing the remobilization was completed within a day's time; and this at the scale-up level. The ortho-phosphate containing supernatant catholyte was decanted and phosphorous precipitated as struvite (Fig. 3). This fertilizer was purer than the stringent Swiss legislation requires.[4] The novel bioelectric process generates beside this also a chemical base if needed and is proposed as an alternative for the less sustainable chlorination process used in industry. Another side product is phosphate-free digested sewage sludge useful as biofuel in cement production. A further side product is biohydrogen, and finally potassium is accumulated in the cathode.

Conclusions

The microbial electrolysis cell enables fast and quantitative phosphate recovery for iron phosphate contained in wet digested sewage sludge. It is possible to produce bioelectricity while generating the fertilizer using microbial fuel cell conditions. Scale-up showed that microbial electrolysis cell conditions enable fast processing. The product is not only a fertilizer but also reagent grade phosphate, chemical base, hydrogen and phosphate free sludge as biofuel can be obtained. The most significant result was that the fertilizer contained very low quantities of lead, mercury, cadmium, chromium and other toxic metals fulfilling stringent legislative requirements for recycling fertilizers.

[1] F. Fischer, C. Bastian, M. Happe, E. Mabillard, N. Schmidt, *Biores. Technol*., **2011**, *102*, 5824.

[2] D. Cordell., J.-O. Drangert, S. White, *Global Environ. Change*, **2009**, *19*, 292.

[3] F. Fischer, G. Zufferey, M. Sugnaux, M. Happe, *Environ. Sci.: Processes Impacts* **2015**, *17*, 90; Also published in the '*Themed collection': 2016 Environmental Science: Processes & Impacts 2015 Most Downloaded Articles*.

[4] M. Happe, M. Sugnaux, C.P. Cachelin, M. Stauffer, G. Zufferey, T. Kahoun, P.-A. Salamin, T. Egli, C. Comninellis, A.-F. Grogg, F. Fischer, *Biores. Technol.* **2016**, *200*, 435.

A Novel Approach to Assess the Bioactive and Cytotoxic Potential of Novel Biomaterials: The Agar Diffusion Scratch Assay

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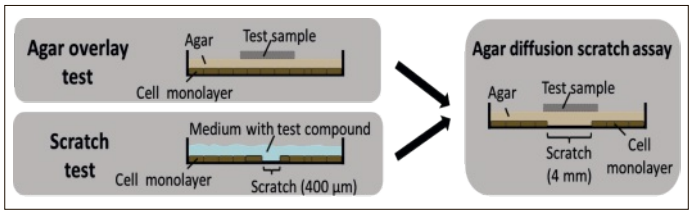

Fig. 1. The recombination of the agar overlay test (ISO10993-5) and the wound healing assay (scratch test) gives new information whether a biomaterial or a chemical solution embedded in an agar test sample is promoting or reducing cell activity (cell migration).

Many physiological processes, such as tissue repair and regeneration, as well as immune system responses rely on cell migration. *In vitro*, cell migration can be affected by numerous different alterations in cell physiology, *e.g.* gene expression, signalling, and/or a modified interaction with the extracellular matrix. Therefore, using cell migration as a bioactivity indicator allows cell performance to be evaluated in a quantitative, qualitative, and time-dependent manner.

One of the most prominent tests is the scratch assay, also known as the wound healing assay. Shortly, a gap, called scratch or artificial wound, is generated by removing a lane of cells within a confluent cell monolayer. Cells on the edge of this gap will migrate into the cell-free space until cell–cell contacts limit further migration. Depending on the compound concentration in the culture medium, the rate of migration might differ, which is evaluated by comparing microscopic pictures. Two key limitations of this test are known: Firstly, its inability to establish a chemical gradient of the test compound within the cell culture dish (impossible to detect a concentration-effect relationship). Secondly, the disperse migration pattern of the cells makes it rather difficult to quantify accurately the migrated distance.

In contrast, the agar overlay test, a method part of the guideline ISO 10993-5 allows cytotoxicity to be measured by indirect contact and gives a dose response. In this assay a sub-confluent cell culture, *e.g.* mouse fibroblasts, is overlaid by a thin layer of agar. Thereafter the test material is placed centrally on top of the agar and as a result, cells are exposed to a concentration gradient of the released compound due to the radial diffusion in the agar. However, this test allows only a qualitative assessment of cytotoxicity, which is done by grading the size of the zone of dead cells around the sample by means of cell morphology and/or by a selective staining of living and/or dead cells. The limits of this test are the following: Firstly, only acute cytotoxic effects of released compounds can be detected. Secondly, released compounds which affect cell functionality without being cytotoxic cannot be identified.

Here we present a novel assay termed agar diffusion scratch assay, which combines the advantages of the scratch assay and the agar diffusion test, and additionally rules out the above-mentioned key limitations (Fig. 1).[1] In addition, it remains a simple, low-cost assay and may be used for a broad range of applications like biocompatibility and cytotoxicity testing as well for more specific fields like cancer and angiogenesis research.

[1] M. Pusnik, M. Imeri, G. Deppierraz, A. Bruinink, M. Zinn, *Sci. Rep.* **2016**, *6*, 20854.

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