TECHNICAL BRIEF

Metaproteome analysis of sewage sludge from membrane bioreactors

Ramona Kuhn1,2,3, Dirk Benndorf4, Erdmann Rapp5, Udo Reichl4,5, Luigi Leonardo Palese2 and Alfieri Pollice1

1 IRSA CNR, Water Research Institute, Bari, Italy
2 Department of Medical Biochemistry, Biology and Physics, University of Bari, Policlinico, Bari, Italy
3 BTU Cottbus, Department of Biotechnology of Wastewater Treatment, Cottbus, Germany
4 Otto von Guericke University, Bioprocess Engineering, Magdeburg, Germany
5 Max Planck Institute for Dynamics of Complex Technical Systems, Bioprocess Engineering, Magdeburg, Germany

Microbial dynamics and enzymatic activities of activated sludge processes are not completely understood yet. A better understanding about the biology is indispensable for further process optimization. Since proteins play a key role as catalysts in sludge processes, a protocol for protein extraction and analysis by 2-D PAGE was established. It is based on phenol extraction of alkaline extracts and on a subsequent precipitation with ammonium sulphate. 2-D protein patterns obtained from different sludges collected from membrane bioreactors showed – besides common spots – significant differences. Selected proteins were identified with nano-HPLC-ESI-MS/MS. All membrane biological reactor (MBR) sludge samples investigated in this study contained elastase 3A, which implies that this human serine protease is a significant constituent of municipal wastewater. Although the identification of proteins from ammonia-oxidizing bacterium *Nitrosomonas europaea* was expected, the detection of a protein with homology to the marine bacterium *Saprospira grandis* in MBR1 was surprising.

Keywords:
De novo sequencing / Membrane bioreactor / Metaproteomics / Microbial communities / Microbiology / Protein extraction

The performance of microbial communities in natural and technical habitats is strongly influenced by interactions between microbes. A great number of studies describe structures and functions of microbial communities by applying molecular tools based on genomics and transcriptomics, DNA and 16S rRNA, respectively [1, 2]. However, proteome analysis of pure cultures revealed great differences between gene expression and protein concentration. To estimate microbial activity more precisely, proteomic tools are increasingly applied to samples from microbial communities [3–6]. Major limitations of the metaproteomic approach are the contamination of sample with non-proteinaceous compounds, the complexity of microbial communities and the limited availability of metagenome data for protein identification [7]. Based on a previously published protocol [8], we describe here an optimized protein extraction procedure for activated sludge from membrane bioreactors (MBR) [9] and the identification of proteins by database search as well as by de novo sequencing.

Five different MBR located in Bari (Italy) and Berlin (Germany) were investigated throughout this study. These MBR were all fed with local municipal wastewater, but operated under different configurations and applications (Table 1).

For protein extraction, 100 mL sludge were taken from each MBR and centrifuged for 10 min at 5000 × g at 4 °C.