

Metaproteomics applied to activated sludge for industrial wastewater treatment revealed the dominance of *Hyphomicrobium zavarzinii*

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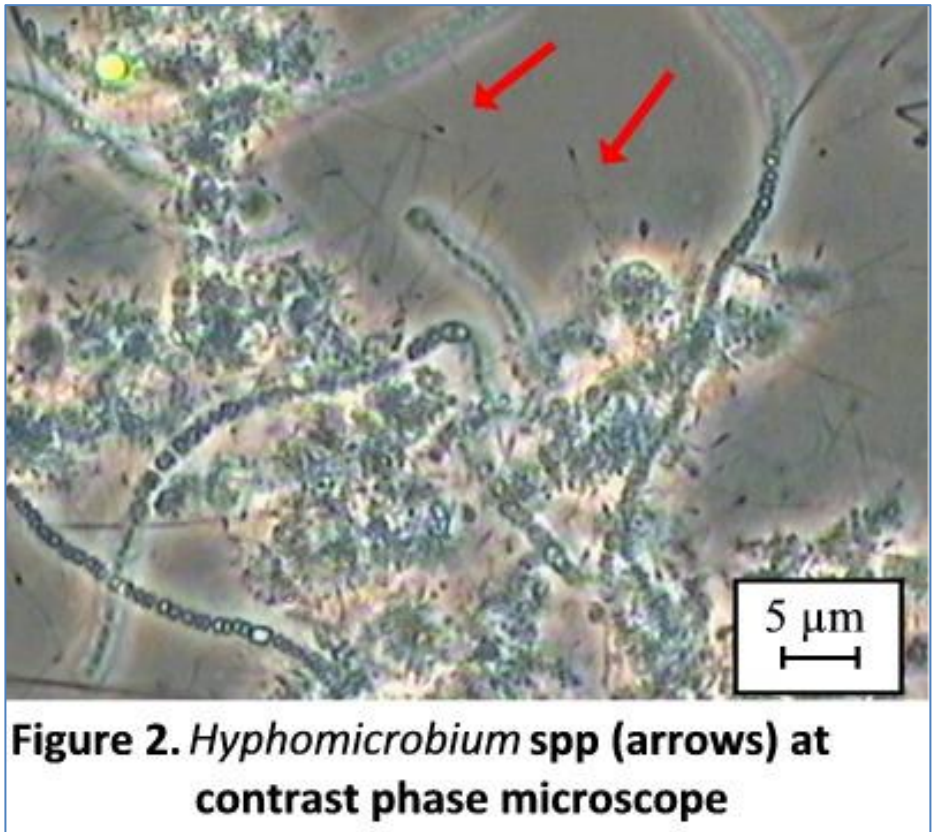
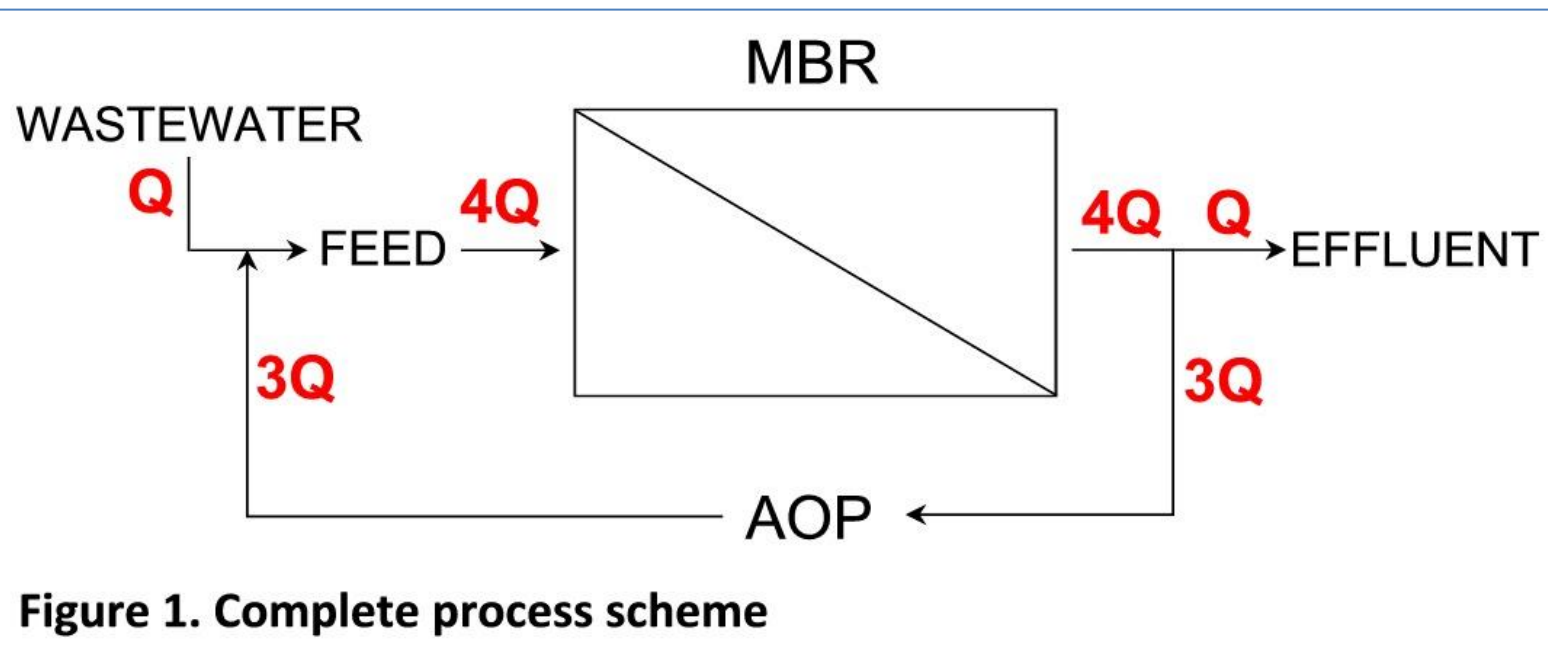
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Introduction

In biological wastewater treatments microbial populations often cooperate in degradation of pollutants present in wastewater representing the biomass of the so-called activated sludge. In this work the metabolic behavior of the biomass of a lab-scale plant treating industrial pharmaceutical wastewater was investigated. Figure 1 shows the complete treatment process which included a membrane biological reactor (MBR) coupled with an advanced oxidation process (AOP) for partial breakdown of non-biodegradable molecules [1]. Proteins from biomass samples collected pre- and post-AOP application were investigated by two-dimensional gel electrophoresis (2DE) and MS then identified by database search. Results showed methanol dehydrogenase (MDH) belonging to *Hyphomicrobium zavarzinii* as the most constantly expressed protein in the studied consortium. Other constant identified proteins belonging to *Hyphomicrobium* spp. (figure 2) revealed a predominant methylotrophic metabolism, particularly *H. zavarzinii* appeared as key actor in the studied microbial community.



Methods

Proteins were extracted as described elsewhere [2] and resolved by 2DE: first dimension was carried out through isoelectric focusing (IEF) by 18 cm IPG strips pH 4-7 (GE Healthcare), the second one through SDS-PAGE [3]. Delta2D™ software (Decodon, Greifswald, Germany) was used for image analysis. Spots of interest were manually excised, subjected to tryptic digestion and analyzed by MALDI TOF MS/MS (Ultraflextreme, Bruker Daltonics, Massachusetts, U.S.). Proteins were identified by searching peptide spectra with MASCOT (<http://www.matrixscience.com>) against NCBI.

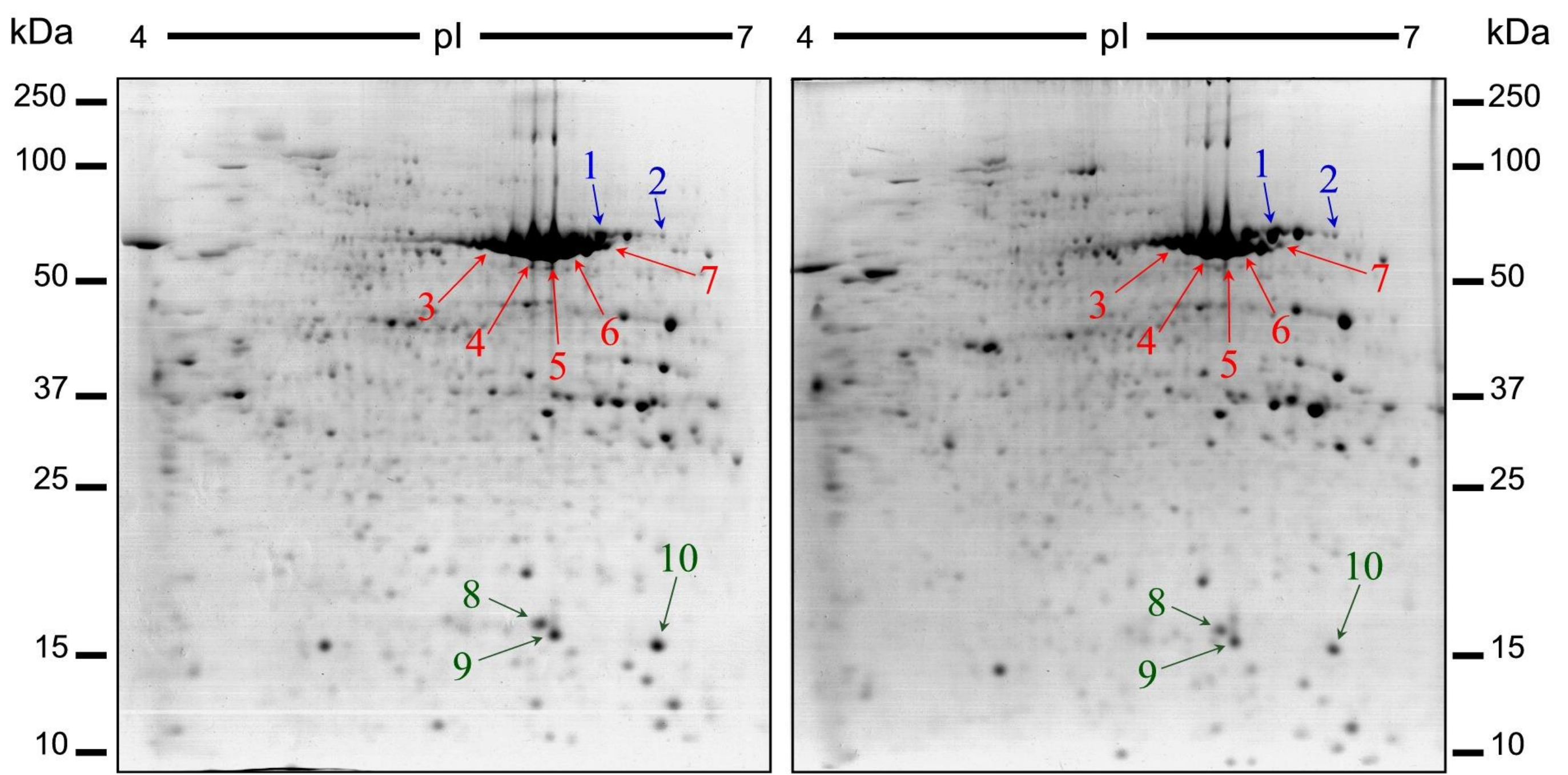


Figure 3. 2D gels with main identified spots. Pre- and post-AOP gels are respectively on the left and on the right.

Results

The main part of spots resulted constantly abundant from image analysis of 2DE. From overall 144 spots, 74 were analyzed by MS, and someone have been excluded for low Mowse score (less than 100) or insufficient number of peptides (less than 2 peptides). In conclusion 40 spots were reliably identified by MALDI TOF MS/MS: 36 constantly abundant spots and 4 spots with changing abundance. Methanol dehydrogenase (MDH) resulted the more expressed protein, ethanol dehydrogenase (EDH) and aldehyde-activating protein (AAP) were found constant too (figure 3 and table 1). All these proteins are involved in methylotrophic metabolism. Metaproteome analysis showed that 31 database hits of identified proteins were taxonomically affiliated in the genus *Hyphomicrobium*: 17 particularly to *H. zavarzinii*, 9 to *H. nitratorans* and 4 to *H. denitrificans*. Interestingly AAP presents the same domain of formaldehyde-activating enzyme (FAE) belonging to *Methylobacterium extorquens* AM1 (KEGG entry W911_13190), and Chistoserdova and coworkers showed the strong relationship between MDH and FAE using methanol as source of energy and carbon [4].

Conclusions

The high and constant expressions of MDH and EDH proposed the methylotroph *H. zavarzinii* as principal player in the biomass. The identification of AAP of *H. nitratorans* leads to the hypothesis of a metabolic cooperation with MDH of *H. zavarzinii* in the oxidation pathway of methanol. Further studies should be addressed to different samples of wastewater treatment, and could open new perspectives and scenarios towards novel possible applications of activated sludge biological consortia.

References

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