Enhanced growth-driven stepwise inducible expression system development in haloalkaliphilic desulfurizing bacteria *Thioalkalivibrio versutus*

Moustafa M. Sharshar, Tingzhen Mu, Maohua Yang, Jianmin XING*

State Key Laboratory of Biochemical Engineering, Institute of Process Engineering, Chinese Academy of Sciences (CAS), Beijing 100190, P.R. China University of Chinese Academy of Sciences, Beijing 100049, P.R. China Email: jmxing@ipe.ac.cn

Highly toxic and flammable H_2S gas has become an environmental threat. *Thioalkalivibrio versutus* is an autotroph that can transform reduced sulfur species, such as sulfide S^{2-} and thiosulfate $S_2O_3^{2-}$ to sulfur, in the form of extracellular globules via oxidation. Haloalkaliphilic autotrophs, like the bio-desulfurizing *T. versutus*, grow weakly. Weak growth makes any trial for developing potent genetic tools required for genetic engineering far from achieved. In this study, the fed-batch strategy improved *T. versutus* growth by 1.6 fold in maximal growth rate, 9-fold in O.D600 values and about 3-fold in biomass and protein productions. The strategy also increased the favorable desulfurization product, sulfur, by 2.7 fold in percent yield and 1.5-fold in diameter. A tight iron-inducible expression system for *T. versutus* was successfully developed. The system was derived from fed-batch cultivation coupled with new design, build, test and validate (DPTV) approach. The inducible system was validated by toxin expression. Fed-batch culturing coupled with DBTV approach successfully led to the first tight inducible system construction in *T. versutus* for improving bio-desulfurization processes by future metabolic engineering. The selected inducible system heterologously expressed the MazF toxin at high O.D₆₀₀ value as a validation step. It lets the strain grow much faster than the control. The Fed-batch cultivation coupled with DPTV approach could be applied to other autotrophs. Fund project : The National Science Foundation of China (No. 21878307 and 31872633)

Jianmin Xing

Professor

Institute of Process Engineering, Chinese Academy of Sciences, China University of Chinese Academy of Sciences, China

Education:

PhD, 1995 – 1998 Major Biochemical Engineering, Institute of Process
Engineering, CAS.
MSc, 1989 – 1992 Major Microbial Engineering, Shandong University.

BSc, 1985 – 1989 Major Microbial Engineering, Shandong University.



Professional Career:

1998 – 2000 Postdoctoral fellow, Institute of Zoology, Chinese Academy of Sciences, China.
2000 – 2005 Associate Professor, Institute of Process Engineering, Chinese Academy of Sciences.
2005.7 – 2006.1 Visiting Scientist, Karlsruhe Research Center, Germany.
2009.10 – 2009.12 Visiting Scientist, University of California, USA
2006 – Present Professor, Institute of Process Engineering, Chinese Academy of Sciences, China.

Research Interests:

Genome editing and Metabolic Engineering of Microbes Biodesulfurization of Natural Gas, Bio-Gas, and Waste Gases with Haloalkaliphilic Microbes Bioaugmentation and Biotreatment of Industrial Wastewater

Selected publications

- 1. Xing et al., *Bioresource Technology*, 2019, accepted.
- 2. Xing et al., Bioresource Technology, 2018, 268: 45-51.
- 3. Xing et al., Bioresource Technology, 2018, 266: 26-33.
- 4. Xing et al., Bioresource Technology, 2018, 265: 443-449.
- 5. Xing et al., Bioresource Technology, 2016, 214: 653–659.