High-altitude-acclimated activated sludge exhibits reduced resistance to mercury stress: Insights from nitrogen conversion, oxidative stress and multi-omics analysis

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Abstract

The high mercury (Hg) content in high-altitude wastewater treatment systems reduces treatment efficiency. Activated sludge acclimated under these conditions exhibits distinct microbial characteristics compared with those under low-altitude conditions, potentially influencing its response to Hg stress. In this study, the nitrogen conversion and oxidative stress responses of low-pressure-acclimated activated sludge (65 and 72 kPa) under short-term Hg (II) stress were investigated using metagenomic sequencing, enzyme activity assays, and metabolomic analysis. The results showed that nitrification and denitrification in low-pressure sludge were more significantly inhibited under Hg (II) stress, with a greater decline in functional gene expression and key enzyme activities than those in sludge acclimated under normal pressure (100 kPa). This phenomenon was attributed to disrupted carbon metabolism, impaired electron transport chain (ETC) function, and a weakened antioxidant defense system. Low-pressure conditions facilitated acetate metabolism and altered the abundance of ETC related genes. This change affected electron flow and increased the possibility of electron leakage under Hg (II) stress, ultimately leading to increased reactive oxygen species production and exacerbated oxidative stress in sludge. The metabolomic analysis further revealed that low-pressure sludge exhibited more pronounced lipid peroxidation, tricarboxylic acid cycle disturbances, and purine metabolism dysregulation after Hg (II) exposure. These changes intensified oxidative stress and reduced microbial resistance to pollutant stress. Additionally, gene expression analysis showed reduced Hg reduction (*merA*) and increased Hg methylation (*hgcA*) gene expression. These findings reveal the high sensitivity of activated sludge acclimated under low-pressure conditions to Hg (II) stress, highlighting the

different pollutant resistance characteristics of wastewater treatment systems in high altitude regions.

Gao, L., Chen, Y., Lu, Y., Li, S., Yang, Z., Zhu, G., & **Gaballah, E. S. (2025)**. High-altitude-acclimated activated sludge exhibits reduced resistance to mercury stress: Insights from nitrogen conversion, oxidative stress and multi-omics analysis. *Chemical Engineering Journal*, 164228. https://doi.org/10.1016/j.cej.2025.164228

Chemical Engineering Journal 517 (2025) 164228

Contents lists available at ScienceDirect

Chemical Engineering Journal

journal homepage: www.elsevier.com/locate/cej

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ARTICLE INFO

Keywords:
High-altitude regions
Mercury (Hg)
Metagenomic analysis
Metabolomic analysis
Activated sludge

ABSTRACT

The high mercury (Hg) content in high-altitude wastewater treatment systems reduces treatment efficiency. Activated sludge acclimated under these conditions exhibits distinct microbial characteristics compared with those under low-altitude conditions, potentially influencing its response to Hg stress. In this study, the nitrogen conversion and oxidative stress responses of low-pressure-acclimated activated sludge (65 and 72 kPa) under short-term Hg(II) stress were investigated using metagenomic sequencing, enzyme activity assays, and metabolomic analysis. The results showed that nitrification and denitrification in low-pressure sludge were more significantly inhibited under Hg(II) stress, with a greater decline in functional gene expression and key enzyme activities than those in sludge acclimated under normal pressure (100 kPa). This phenomenon was attributed to disrupted carbon metabolism, impaired electron transport chain (ETC) function, and a weakened antioxidant defense system. Low-pressure conditions facilitated acetate metabolism and altered the abundance of ETC-related genes. This change affected electron flow and increased the possibility of electron leakage under Hg (II) stress, ultimately leading to increased reactive oxygen species production and exacerbated oxidative stress in sludge. The metabolomic analysis further revealed that low-pressure sludge exhibited more pronounced lipid peroxidation, tricarboxylic acid cycle disturbances, and purine metabolism dysregulation after Hg(II) exposure. These changes intensified oxidative stress and reduced microbial resistance to pollutant stress. Additionally, gene expression analysis showed reduced Hg reduction (*merA*) and increased Hg methylation (*hgcA*) gene expression. These findings reveal the high sensitivity of activated sludge acclimated under low-pressure conditions to Hg(II) stress, highlighting the different pollutant resistance characteristics of wastewater treatment systems in high-altitude regions.

Abbreviations: A/A/O, Anaerobic/Anoxic/Aerobic; A/O, Anoxic/Aerobic; AMO, Ammonia Monooxygenase; AOB, ammonia-oxidizing bacteria; CAT, Catalase; DEMs, Differentially Expressed Metabolites; DO, Dissolved Oxygen; ETC, Electron Transport Chain; ETSA, Electron Transport System Activity; GSH, Glutathione; H_2O_2, Hydrogen Peroxide; Hg, Mercury; HRT, Hydraulic Retention Time; KEGG, Kyoto Encyclopedia of Genes and Genomes; LDH, Lactate Dehydrogenase; MDH, Malate Dehydrogenase; MeHg, Methylmercury; N, Nitrogen; N_2O-N, Nitrous Oxide Nitrogen; NAR, Nitrate Reductase; NH_4^+-N, Ammonia Nitrogen; NIR, Nitrite Reductase; NO_2^--N, Nitrite Nitrogen; NO_3^--N, Nitrate Nitrogen; NOR, Nitric Oxide Reductase; NOS, Nitrous Oxide Reductase; NXR, Nitrite Oxidoreductase; O_2, Oxygen; $\cdot O_2^-$, Superoxide Ions; ROS, Reactive Oxygen Species; RT-qPCR, Reverse Transcriptase Quantitative Polymerase Chain Reaction; SBR, Sequencing Batch Reactor; SCS, Succinyl-CoA Synthetase; SDH, Succinate Dehydrogenase; SEM, Scanning Electron Microscope; SOD, Superoxide Dismutase; SRT, Sludge Retention Time; TCA, Tricarboxylic Acid; TN, Total Nitrogen; VIP, Variable Importance In Projection; WWTPs, Wastewater Treatment Plants; XO, Xanthine Oxidase.

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https://doi.org/10.1016/j.cej.2025.164228
Received 7 March 2025; Received in revised form 15 May 2025; Accepted 27 May 2025
Available online 27 May 2025