研究报告

何志仙,魏旖旎,刘婧晶,袁林江.活性污泥羟氨氧化还原酶粗酶提取及活性测定方法的优化研究[J].环境科学学报,2015,35(12):3797-3804

活性污泥羟氨氧化还原酶粗酶提取及活性测定方法的优化研究

Optimization of crude hydroxylamine oxidoreductase extraction from activated sludge and its activity measurement

关键词：N_2O 生物脱氮 羟氨氧化还原酶 粗酶提取 活性测定

基金项目：国家自然科学基金项目(No.51278406);西安建筑科技大学校青年科技基金资助课题(No.QN1137)

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摘要：污水生物脱氮过程中N_2O的产生与活性污泥中细菌的羟氨氧化还原酶(Hydroxylamine oxidoreductase, HAO)活性有着密切关系.但目前活性污泥中细菌的HAO提取和活性测定方法尚未建立.本文首先探索了在25℃、酶活性反应液电子受体供体配比1:1和终止剂选用2 mol·L^{-1} HCl条件下超声或高压破碎细胞法对HAO粗酶活性的影响,结果表明高压破碎比超声破碎获取的粗酶活性高($p < 0.01$).在此基础上,我们进一步优化了高压破碎下压力大小、破碎次数和裂解液用量的参数.粗酶提取液中DNA含量、酶活力及酶比活力结果进行多因素方差分析表明压力大小(50、110或160 MPa)、破碎次数(1、2或3次)和裂解液用量(2、5或10 mL)均对脱氮活性污泥破碎效果、酶活性和比活力有显著影响($p < 0.01$);综合DNA含量、酶活力及酶比活力结果看,110 MPa压力条件下,加5 mL裂解液破碎2次更适合污水生物处理中HAO的提取和活性测定,既节省时间,又能较好的保持酶活性.

Abstract: The emission of N_2O during biological nitrogen removal is closely related to the activity of hydroxylamine oxidoreductase (HAO) of bacteria in the activated sludge. However, the methods of extraction and activity measurement of HAO have not been established. First, under 25℃ in temperature, electron acceptor and donor ratio of 1:1 in activity measurement reaction system, and of 2 mol·L^{-1} HCl as terminating agent, we compared the effects of ultrasonic disruption, high-pressure homogenization on the activity of crude enzyme obtained for the assessment. Results showed that high-pressure homogenization was more efficient for cell disruption ($p < 0.01$). Furthermore, we optimized important variables with high-pressure homogenization including the pressure, treatment times and dosage of lysate. The results of DNA contents in crude enzyme extraction, tested activities and specific activities suggested that the pressure, treatment times and dosage of lysate have significant effect on the cell disruption, and specific enzyme activity of the sludge ($P < 0.01$). Considering DNA content, enzyme activity and specific enzyme activity, the treatment under pressure of 110 MPa, with addition of 5 mL of lysate and broken twice is recommend for time saving and maintaining enzyme activity.

Key words: nitrous oxide biological nitrogen removal hydroxylamine oxidoreductase crude enzyme extraction activity measurement