Performance and mechanism of a novel hydrolytic bacteria pretreatment to boost waste activated sludge disintegration and volatile fatty acids production during acidogenic fermentation

Eid S. Gaballah [a,b], Lei Gao [a] , Emad A. Shalaby [c], Biao Yang [a], Mostafa Sobhi [d] , Mahmoud M. Ali [e,f] , Mohamed Samer [g], Chongpeng Tang [h], Guangcan Zhu [a]

a *School of Energy and Environment, Southeast University, Nanjing, 210096, China*
b *Agricultural Engineering Department, Faculty of Agriculture, Fayoum University, Fayoum, 63514, Egypt*
c *Department of Biochemistry, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt*
d *Agricultural and Bio-systems Engineering Department, Faculty of Agriculture, Alexandria University, Alexandria, 21526, Egypt*
e *College of Water Resources and Civil Engineering, China Agricultural University, Beijing, 100083, China*
f *Agricultural Engineering Research Institute (AEnRI), Agricultural Research Center (ARC), Giza, 12611, Egypt*
g *Department of Agricultural Engineering, Faculty of Agriculture, Cairo University, El-Gammaa Street, 12613, Giza, Egypt*
h *China CAMCE Environmental Technology Co., Ltd, Beijing, 100080, China*

Abstract

In this study, an innovative mixed hydrolytic bacteria culture (HB) (the main dominant bacterial species: *Lactobacillus acetotolerans*), as an environmentally friendly pretreatment technique, was developed to enhance the volatile fatty acids (VFAs) production from waste-activated sludge (WAS). The highest VFAs production of 517 and 518 mg/g VSS were achieved with HB 8% and HB 8%-35 ◦C pretreatments, which were almost 3.6 folds compared to the control (143 mg/g VSS), respectively. The mechanism analysis revealed that HB boosted the bioavailability of organics released from WAS and significantly accelerated sludge solubilization. Protease and α-glucosidase enzymatic activity were improved and associated with hydrolysis and acidogenesis. Furthermore,

the microbial community analysis showed that HB pretreatment significantly increased the hydrolytic and acidifying bacteria proportions (e.g., *Veillonella, Macellibacteroides* sp., *Clostridium_sensu_stricto_1* and *Bacteroides* sp., etc.). This study provides a promising, low-cost, and eco-friendly approach for recovering resources from WAS and transforming them into high-value products.

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[a] School of Energy and Environment, Southeast University, Nanjing, 210096, China
[b] Agricultural Engineering Department, Faculty of Agriculture, Fayoum University, Fayoum, 63514, Egypt
[c] Department of Biochemistry, Faculty of Agriculture, Cairo University, Giza, 12613, Egypt
[d] Agricultural and Bio-systems Engineering Department, Faculty of Agriculture, Alexandria University, Alexandria, 21526, Egypt
[e] College of Water Resources and Civil Engineering, China Agricultural University, Beijing, 100083, China
[f] Agricultural Engineering Research Institute (AEnRI), Agricultural Research Center (ARC), Giza, 12611, Egypt
[g] Department of Agricultural Engineering, Faculty of Agriculture, Cairo University, El-Gamaa Street, 12613, Giza, Egypt
[h] China CAMCE Environmental Technology Co., Ltd, Beijing, 100080, China

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ABSTRACT

In this study, an innovative mixed hydrolytic bacteria culture (HB) (the main dominant bacterial species: *Lactobacillus acetotolerans*), as an environmentally friendly pretreatment technique, was developed to enhance the volatile fatty acids (VFAs) production from waste-activated sludge (WAS). The highest VFAs production of 517 and 518 mg/g VSS were achieved with HB 8% and HB 8%-35 °C pretreatments, which were almost 3.6 folds compared to the control (143 mg/g VSS), respectively. The mechanism analysis revealed that HB boosted the bioavailability of organics released from WAS and significantly accelerated sludge solubilization. Protease and α-glucosidase enzymatic activity were improved and associated with hydrolysis and acidogenesis. Furthermore, the microbial community analysis showed that HB pretreatment significantly increased the hydrolytic and acidifying bacteria proportions (e.g., *Veillonella*, *Macellibacteroides* sp., *Clostridium_sensu_stricto_1* and *Bacteroides* sp., etc.). This study provides a promising, low-cost, and eco-friendly approach for recovering resources from WAS and transforming them into high-value products.

1. Introduction

Exploring environmentally friendly and cost-effective disposal methods of waste-activated sludge (WAS) has attracted considerable attention from the public due to rapid urbanization and industrialization (Fang et al., 2021). Reports have shown that China's annual production of WAS exceeds 65 million tons (Wang et al., 2024). Generally, WAS contains a significant amount of water, perishable materials, harmful pollutants, and disease-causing organisms. Additionally, it emits an unpleasant odor. Therefore, improper disposal of WAS poses further risks to human health and the long-term sustainability of the environment (Guo et al., 2021). Fortunately, the resource potential of WAS has received significant attention owing to its abundant organic resources (Zhang et al., 2024).

So far, anaerobic fermentation has been regarded as a promising approach for the disposal of WAS. Additionally, anaerobic fermentation can efficiently transform valuable organic resources, such as proteins and carbohydrates, into high-value products like volatile fatty acids (VFAs) (Zhu et al., 2023). VFAs are valuable products that play a crucial role as intermediate products in acidogenic fermentation (AF). The hydrolysis reaction is the primary rate-limiting step in the formation of VFAs. This process occurs by a sequence of biochemical reactions mediated by several enzymes, including a complex microbial structure and competitive metabolic pathways (Shi et al., 2022). The hydrolytic bacteria break down intricate organic compounds like proteins, carbohydrates, and fats into smaller organic compounds (e.g., amino acids, monosaccharides, and fatty acids) (Nguyen et al., 2019). Following hydrolysis, acidogenic bacteria utilize the hydrolysis products and store

* Corresponding author.
E-mail address: gc-zhu@seu.edu.cn (G. Zhu).

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