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Differentiated effects of osmoprotectants on anaerobic syntrophic microbial populations at saline conditions and its engineering aspects

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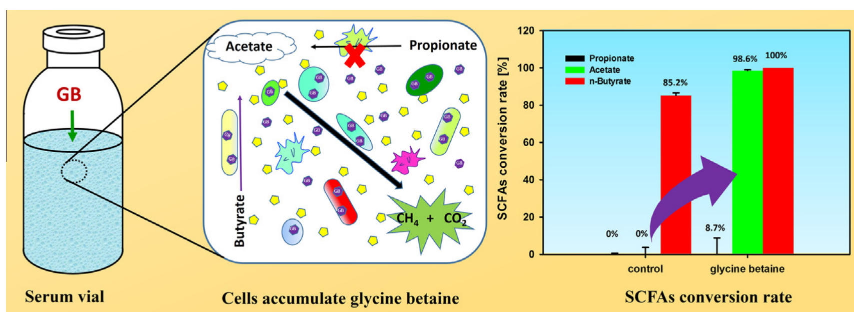
HIGHLIGHTS

- Osmoprotectants enhanced the biomethanization of different substrates.
- GB exerted some differentiated influences on syntrophic microbial populations.
- Osmoprotectant was helpful for the recovery of anaerobes from salt inhibition.
- Osmoprotectant played a prolonged positive role on high saline anaerobic digestion.

GRAPHICAL ABSTRACT



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ABSTRACT

The objective of this study was to evaluate the effects of osmoprotectants on the high salinity anaerobic digestion in engineering and microbial aspects with a special focus on syntrophic microbial populations. The experimental results indicated that glycine betaine (GB) exerted some differentiated influences on anaerobic syntrophic microbial populations at high salinity conditions. It was found that glycine betaine was beneficial for acetic acid producers, aceticlastic methanogens, n-butyrate utilizers, and comparatively not so effective for the propionate producers and utilizers. Moreover, although the addition of GB in advance could not prevent the imbalance of anaerobic process caused by the high salt shock, it was helpful for the anaerobes to recover the methanogenic activity following salt inhibition. Repeated feeding experimental results indicated that the osmoprotectant played a prolonged positive role on high saline anaerobic digestion, which was proved to be closely related to the extracellular osmoprotectants. These findings could provide a deep insight on the microbiological aspect on osmoprotectants enhancing anaerobic digestion, and some operating strategies could be developed based on it.

1. Introduction

A large amount of saline wastewater is generated from many industrial sectors, e.g. seafood processing, textile dyeing, and tanneries [1]. The wastewater contains a high salinity and high organics content. The discharge of the un-treated wastewaters into the environment causes many environmental problems, such as contaminations of soil, surface and groundwater [2]. Due to the tightening regulations, therefore, the economical treatment of saline wastewater is nowadays becoming a challenge task for researchers, regulators and engineers [2].

Many physical and chemical techniques, e.g. advanced oxidation, multiple-effect evaporation (MEE), ion exchange, reversed osmosis (RO), were successfully employed to treat the saline

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wastewaters [2]. However, the highly technical complexity and high operating cost limited their wide applications. Biological process is thought to be more cost-effective method to remove the organics. Compared with aerobic methods, anaerobic digestion is a more attractive biological process because of its advantages of organics removal and biofuel gas recovery. However, the high salt content in the saline wastewaters could cause microbial cell plasmolysis and cell death due to the dramatic increase in osmotic pressure. Soto et al. [3] reported a toxic concentration in the range 14–18 g/L Na^+ provoked a 50% reduction in methanogenic activity. Fang et al. [4] also reported that a 50% inhibition occurred at a sodium concentration of approximately 11 g/L. Therefore, it is difficult to treat the highly saline wastewater by conventional anaerobic processes due to the damaging of microbial activity.

From engineering aspect, the high salt content increases the buoyancy force, which will disturb the settleability of sludge flocs, thus causing the washing out of sludge in conventional biological processes [5]. For UASB system, the high salt content broke the anaerobic sludge granules, thus leading to the failure of the process [6]. In order to increase the retention of sludge biomass, anaerobic filter process configuration was employed to treat the saline wastewater, where the microbial cells could attach on the surface of carriers. Mendez et al. [7] reported that the high COD removals of 73% and 64% were achieved in thermophilic anaerobic filter and mesophilic one, respectively. However, anaerobic filter process is not suitable for the particulate substrates due to the blockage of the carrier-filling zone. A submerged anaerobic membrane reactor (SAMBR) technique could also improve the process performance of the saline wastewater treatment by increasing the sludge retention time (SRT). Vyrides and Stuckey [8] reported that a 99% removal of dissolved organic carbon (DOC) at the salinity of 35 g/L NaCl was achieved in the SAMBR treating the saline sewage. However, it should be noted that technically effectively and economically controlling the membrane fouling is still remaining as a challenge work, especially for the high solid anaerobic digestion. Therefore, some more sophisticated strategies are still needed for biologically processing of saline wastewater.

To achieve a high organics removal, the strategy of acclimation of anaerobic sludge to high salinity concentration was often adopted. Mendez et al. [7] reported that, after a start-up period of 9 months, the high COD removals of 73% (thermophilic anaerobic filters) and 64% (mesophilic anaerobic filters) were achieved. Feijoo et al. [9] also reported that IC_{50} (50% reduction of methanogenic activity) for unadapted sludges were in the range 6–13 g/L Na^+, whereas it was higher than 20 g/L Na^+ with continuous exposure to salt in pilot or industrial reactors. However, an extremely long start-up period was required for biomass acclimation to high saline conditions. In addition, the isolation and bioaugumentation of salt tolerant microorganisms were also proved to be effective for organics removal from saline wastewater [10,11]. However, the fluctuated salinity induced the decreased COD removal [11] or even system failure [10]. Because there was an optimal salinity for each halophilic microorganism, they cannot well adapt to the fluctuated salinity condition. From microbial aspect, some more flexible methods still need to be developed.

It is known that the cytoplasmic membrane of microbial cell is permeable to water but serves as a barrier for most solutes present in the medium and metabolites in the cytoplasm. Under hyperosmotic conditions, a rapid efflux of water and loss of turgor occur, which cause the death due to the plasmolyse of cell [12]. Two basic mechanisms for microorganisms to survive osmotic stress have been identified [13,14]. One is the 'salt in' strategy, which was frequently adopted by extreme halophilic archaea and halotolerant bacteria, in which inorganic ions are accumulated to balance the external osmotic pressure. The unique potassium pump systems in their cell membranes, which facilitate K^+ ion transport, and thus make K^+ as a good candidate osmolyte in cells [15]. Other divalent ions such as Mg^{2+}, Ca^{2+} also showed an enhancement on the salt tolerance for microorganisms. For instance, Ahring et al. [16] reported that the concentrations of Na^+ resulted in completely inhibition of growth for *Methanosarcina thermophila* TM-1 were 0.35, 0.52 and 0.6 M with the supplementation of Mg^{2+} in the concentration of 0.05, 5 and 50 mM, respectively. However, this strategy requires a unique intracellular machineries to counter the high salt concentrations, and limits microbial growth up to certain salinities [13].

Another is 'compatible solutes' strategy, a mechanism used by the majority of the living cells, in which osmotically active compatible solutes are accumulated to balance the external osmotic pressure. Compatible solutes are usually neutral molecules that can be accumulated by cells to molar level and be compatible with metabolic processes and cellular structures [17,18]. These solutes can be synthesized by the cell, or accumulated from the medium [19]. Osmoprotectants are defined as exogenously provided organic solutes that enhance bacterial growth in media of high osmolality [14]. For most species, taking up compatible solutes from the medium is energetically favorable than synthesis [15]. This strategy can accelerate the initial response on an osmotic upshock [12]. Vyrides and Stuckey [20] reported a significantly increased acclimation potential with the addition of glycine betaine in the batch assays with glucose. Our previous study also primarily proved the effectiveness of osmoprotectants on anaerobic digestion of food waste [21]. Yerkes et al. [22] also found that glycine betaine successfully alleviated the symptoms of sodium toxicity in batch anaerobic reactor and CSTR, fluidized bed reactor, UASB reactor. In addition, most osmoprotectants, such as glycine betaine, choline, widely exist and are rich in many plants, like sugarbeet, or could be produced economically by chemical synthesis [23]. Therefore, the method of osmoprotectants enhancing anaerobic digestion of saline wastewater is considered as a feasible and advanced strategy. However, Vallero et al. [24] reported that the compatible solutes were not effective to alleviate the acute sodium toxicity on methanogenesis of methanol with sulfate reducing granular sludge. Those reports that the osmoprotectants could exerted different effects on diverse microbial consortia, and more research is needed.

Anaerobic digestion is an engineered biochemical process that converts organic matters to biogas (a mixture of methane and carbon dioxide) by the concerted actions of syntrophic microbial populations, e.g. hydrolytic fermentative, syntrophic acetogenic (SAB) and methanogenic archaea [25]. Each step is mediated by different enzymes and consortia of microorganisms which differ widely in terms of physiology, nutritional needs, growth kinetics and sensitivity to environmental conditions [25]. Since different microorganisms play their unique roles in each step, the inhibition of any microbial activity will lead to the failure of the whole process. Theoretically, osmoprotectant enhancing biological process is closely related to the properties of microbial cells. The different membrane structures of the fermentative bacteria, acidogenic and acetogenic bacteria, methanogenic archaea deem the different sensitivity to high salinity conditions. So far, most researches on salt inhibition and its enhancement by osmoprotectants focused on overall performance of a whole anaerobic process or particularly on methanogenic stage. Therefore, little information is available regarding the impact of salt and osmoprotectants on different syntrophic groups.

Although a great potential of osmoprotectants enhancing the high salinity anaerobic digestion was present, some engineering aspects need to be considered, e.g. highly fluctuated salinity, the recovery of salt inhibited process. For example, in some cases, the increase of salinity is predictable such as the routine increase in certain periods of the year. However, in some industries the