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Influence of Exogenous Quorum Sensing Signaling Compounds on the Hydrogen Sulfide Production in Activated Sludge Bioreactors

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Abstract: Wastewater collection and treatment systems often generate offensive odors, which generally come from anaerobic decomposition. Most of the odors generated are sulfur-based compounds, the predominant compound often being hydrogen sulfide (H_2S). One of the possible solutions of such problem is odor control bioreactors or controlling the microbial biodiversity of the activated or its metabolic pathways. The current work aimed to investigate the possible role of exogenous quorum sensing signaling molecules on the hydrogen sulfide production through addition of different *N*-acylhomoserine lactones (AHLs) to the activated sludge bioreactors. The results indicated that the H_2S production was increased 4, 8 and 12 fold by treatment with C4AHL, 3oxo-C6AHL and C6AHL, respectively. Furthermore, the time needed for the activated sludge to convert to black color (due to formation of ferrous sulfide (FeS) and Pyrite (FeS_2) as a result of reaction of the produced H_2S with iron salt in the sludge) was about 72% of the control bioreactor in case of C4AHL and 3oxo-C6AHL while C6AHL turned black much faster (15% of the time of the control). However, the H_2S production was reduced to 62.5% of that of the control bioreactor in case of C12 AHL treatment. These results suggest that the H_2S production could be controlled by interfering with the QS mechanism in the microbial community of the activated sludge and the possibility of controlling of the offensive odor produced in the wastewater treatment station through interfering with the quorum sensing mechanisms of the microbial community of the activated sludge

Key words: Activated Sludge · Wastewater · Quorum Sensing · *N*-acylhomoserine lactones (AHLs)

INTRODUCTION

The activated sludge process is the most used biological wastewater treatment method in the world [1]. The activated sludge process is a wastewater treatment method in which the carbonaceous organic matter of wastewater provides an energy source for the production of new cells for a mixed population of microorganisms in an aquatic aerobic environment [2]. The microbes convert carbon into to characteristic microbial biomass called flocs (a large aggregate of adherent, or floc-forming, microorganisms, such as bacteria) and oxidized end products that include carbon dioxide and water [3]. In addition, a limited number of microorganisms may exist in activated sludge that obtains energy by oxidizing ammonia nitrogen to nitrate nitrogen in the process known as nitrification [3]. Bacteria constitute the majority of microorganisms present in activated sludge. Bacteria that require organic compounds for their supply of carbon and energy (heterotrophic bacteria) predominate, whereas bacteria that use inorganic compounds for cell growth (autotrophic bacteria) occur in proportion to concentrations of carbon and nitrogen [4]. Both aerobic and anaerobic bacteria may exist in the activated sludge, but the preponderance of species is facultative, able to live in either the presence of or lack of dissolved oxygen [4]. The success of the activated-sludge process is dependent upon establishing a mixed community of microorganisms that will remove and consume organic waste material, that will aggregate and adhere in a process known as bioflocculation and that will settle in such a manner as to produce a concentrated sludge [3].

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However, the production of hydrogen sulfide gas with its offensive odor in the anaerobic digestion of the sludge is considered as one of most important problem in wastewater treatment station [5]. It has been reported that many microbial metabolic pathways are under control of quorum sensing mechanism. Bacterial populations co-ordinately regulate gene expression by producing diffusible signal molecules. These signals, known as auto-inducers or, more recently, "quormones", accumulate extracellularly and interact specifically with a receptor protein to effect changes not related to their own metabolism [6, 7]. These diffusible signals frequently act to induce gene expression in response to bacterial cell density, a process often referred to as "quorum sensing" or cell-to-cell communication [7, 8]. An important class of quormones is the family of *N*-acylhomoserine lactones (AHLs) used by Gram-negative bacteria [9]. Variation in the *N*-acyl chain length and the oxidation state of AHLs allows for bacterial strain specificity in the signalling process and subsequent synchronisation of gene expression [10]. Quorum sensing within bacterial populations can promote pathogenesis, symbiosis, cellular dissemination or dispersal, DNA transfer, metabolism and microbial biofilm development [11]. The main goal of the current work was to investigate the influence of exogenous quorum sensing molecules on the anaerobic production of hydrogen sulfide in activated sludge bioreactor

MATERIALS AND METHODS

Materials: The following homoserine compounds were purchased from Sigma-Aldrich chemicals Co. (MO. USA): N-Butyryl-DL-homoserine lactone "BHL" (C4 AHL), N-Butyryl-DL-homocysteine thiolactone (AHTL), N-Hexanoyl-DL-homoserine lactone "HHL" (C6 AHL), N-(β-Ketocaproyl)-DL-homoserine lactone "OHHL" (3oxo-C6 AHL), N-Heptanoyl-DL-homoserine lactone (C7 AHL), N-Octanoyl-DL-homoserine lactone "OHL" (C8 AHL), N-Decanoyl-DL-homoserine lactone "DHL" (C10 AHL), N-Dodecanoyl-DL-homoserine lactone" dDHL" (C12 AHL) and N-Tetradecanoyl-DL-homoserine lactone "tDHL" (C14 AHL).

Activated Sludge Bioreactors: This study was carried out using laboratory scale reactors with a working volume of 2 L as previously reported with some modification [12, 13]. For aerobic digestion: 1.5 L of domestic wastewater was transferred to the bioreactor and inoculated with activated sludge obtained from a domestic wastewater treatment plant (Riyadh, Saudi Arabia). The Mixed Liquor Suspended Solids (MLSS) of the activated sludge was approximately 3000 mg/L. The pH was maintained at about pH 7.5 using 0.25 M NaOH and 0.25 M HCl. The solution was mixed using suitable mixer (IKA, RW20DZM, Germany) and sterile oxygen was supplied using air pump to give oxygen content of 2 mg/l. The digestion was done at room temperature for 19 h. The anaerobic digestion started at the end of the aerobic digestion where 12 ml of the mixed liquor from the aerobic bioreactor was transferred to 30 ml capacity glass tubes and mixed with 3 ml of raw wastewater and were tightly closed and incubated for 7 days. At the end of the incubation period, the produced H_2S was pulled and measured using Dräger MiniWarn (Germany) and color of the sludge was visually determined.

Effect of QS molecules on the H_2S production: To investigate the influence of different quorum sensing (QS) molecules on the H_2S production, stock solutions (1 mM) of different QS compounds were prepared in acetic acid-acidified ethyl acetate (0.01% vol/vol) (BDH), (HPLC grade) except C14AHL, which was prepared in dichloromethane (BDH) (HPLC grade). The stock QS solution was sterilized by filtration using a 0.22 μm-pore-size filter membrane (Millipore) and added to the aerobic bioreactors to give a final concentration of 10 μM [14, 15]. A total of twenty bioreactors were used including control bioreactor (with no QS molecules) in duplicates and nine bioreactors for different QS molecules in duplicates (18 bioreactors).

Activated Sludge Mass and Their Residuals in Liquid Phase: The mass of the activated sludge was determined using mixed liquor suspended (MLSS) method [16]. The activated sludge sample was mixed and 10 to 20 ml was filtered through Whatman filter paper (Whatman GF/C, Pore Size 1.2 μm. UK) .The filter papers with solid materials were then kept for dryness in oven at 105°C till the weight become constant and the dry weight was calculated

Sludge Rising Test: At the end of the aerobic digestion, 10 ml was transferred to glass tube and left vertically for four hours at room temperature and observed visually. Raising of the of the biomass is an indication of anaerobic metabolism and production of N_2 and N_2O as endproducts which causes decrease of the flocs density due to accumulation of the gases [17].

RESULTS AND DISCUSSION

More than 80% of biological wastewater plants are based on the principle of activated sludge process, in which suspended bacteria oxidise the carbonaceous and nitrogen compounds to produce an effluent that is in accordance with legal standards and that corresponds to a minimal environmental impact [18]. Wastewater collection and treatment systems often generate offensive odors, which generally come from anaerobic decomposition. Most of the odors generated are sulfur-based compounds, the predominant compound often being hydrogen sulfide (H_2S). Hydrogen sulfide (H_2S) is extremely toxic; it can cause injury to the central nervous system even at low doses, is toxic to microorganisms and is corrosive to concrete and steel [19, 20].

The current work was aimed to investigate the possible role of different QS molecules in H_2S production by addition of exogenous QS molecules to lab scale aerobic bioreactor using activated sludge followed by anaerobic digestion.

The results presented in Figure 2 shows the conversion of the sludge to black color due to the formation of ferrous sulfide (FeS) and Pyrite (FeS_2) as a result of reaction of the produced H_2S with iron salt in the sludge. Raising of the of the biomass (Figure 3) is an indication of anaerobic metabolism and production of N_2 and N_2O as endproducts which causes decrease of the flocs density due to accumulation of the gases [17]. The results of the effect of addition of different QS on the H_2S production are shown in Table 1. It was found that the H_2S production increased 4, 8 and 12 fold in the anaerobic bioreactors which used activated sludge from the bioreactors treated with C4AHL, 3oxo-C6AHL and C6AHL, respectively. Furthermore, the time needed for the sludge to convert to black color was about 72% of that of the control bioreactor in case C4AHL and 3oxo-C6AHL while C6AHL turned black much faster (15% of the time of the control). However, the H_2S production was reduced to 62.5% of that of the control bioreactor in case of C12 AHL treatment. In recent years, it has become clear that bacterial cells can communicate with each other via small diffusible signal molecules, a process commonly termed 'quorum sensing' [21]. By far the most intensively investigated family of intercellular signal molecules are the *N*-acylhomoserine lactones (AHLs), which differ in the length and degree of saturation of the acyl chain (4-14 carbons; presence or absence of a double bond) and substituent at the 3-position of their *N*-acyl side chains

Fig. 1: The activated sludge bioreactor used in this study. The pH was maintained at about pH 7.5 using 0.25 M NaOH and 0.25 M HCl. The solution was mixed using suitable mixer and sterile oxygen was supplied using air pump to give oxygen content of 2 mg/l. The aerobic digestion was done at room temperature for 19 h.

Fig. 2: Conversion of the activated sludge to black color under anaerobic due to formation of ferrous sulfide (FeS) and Pyrite (FeS_2). Left tube: Control. The produced H_2S was pulled and measured using Dräger MiniWarn.

Table 1: Effect of different QS molecules on the production of hydrogen sulphide. H2S was measured after 7 days of anaerobic incubation. All results are means of three readings

QS molecule	H_2S concentration (mg\mg MLSS)	Time required for conversion of the sludge to black color (days)	Denitrification
Control Reactors	0.8	7	+
"C4 AHL" Reactors	3.0	5	+
"3oxo-C6 AHL "Reactors	7.0	5	+
"C6 AHL" Reactors	10.0	1	+
" AHTL "Reactor	0.8	7	+
"C7 AHL" Reactors	0.8	7	+
"C8 AHL" Reactors	0.8	7	+
"C10 AHL" Reactors	0.8	7	+
"C12 AHL" Reactors	0.8	Color still brown	+
"C14 AHL" Reactors	0.5	Color still brown	-

Fig. 3: Sludge rising test. At the end of the aerobic digestion, 10 ml was transferred to glass tube and left vertically for four hours at room temperature and observed visually. Raising of the of the biomass is an indication of anaerobic metabolism and production of N_2 and N_2O as endproducts which causes decrease of the flocs density due to accumulation of the gases.

(no substituent, keto or hydroxyl). The Autoinducers belonging to *N*-acylhomoserine lactones (AHLs) family are the most common QS molecules and it has been reported to be the most common one in Proteobacteria which usually dominate the microbial community in the activated sludge [21-23]. There are many Proteobacteria, particularly Delta group, produce H_2S under anaerobic conditions using organic compounds or hydrogen as electron donor and sulfur or sulfate as electron receptors e.g. sulfate reducing bacteria using sulfate to produce H_2S while *Desulfuromonas* reduce sulfur to H_2S. Other facultative aerobic Proteobacteria heterotrophic can reduce sulfur to H_2S including *Salmonella, Proteus*, *Pseudomonas* and *Campylobacter* [24]. Therefore the increase of H_2S production in C4AHL, 3oxo-C6AHL and C6AHL and the reduction in case of C12 AHL suggested a possible role of QS mechanism in controlling microbial production of H2S in wastewater treatment system and the possibility of controlling the produces offensive odor in the wastewater treatment station through interfering with the quorum sensing mechanism of the microbial community of the activated sludge.

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