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Disruption of cell to cell communication in the aeration unit of a cannibal process: Sludge reduction efficiency and related mechanisms

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ABSTRACT

The Cannibal process is a commercial method for excess sludge reduction in activated sludge plants with small sizes. However, the relatively low efficiency in sludge reduction (up to 50%) for some plants is the main impediment to its more widespread adoption. This study provides a biological method for upgrading Cannibal process. The suggested method includes disruption of cell to cell communication between bacterial communities in aerobic flocs in order to increase endogenous respiration. A quorum quenching (QQ) agent is applied in the aerobic tank in order to increase the free bacteria population, which leads to enhanced microbial predation and a decrease in extracellular polymeric substances. The sludge production yield (Y_{obs}) of three pilots including a control sequencing batch reactor (SBR), a Cannibal system, and a QQ-Cannibal system was 0.5, 0.38, and 0.29, respectively. The results of a respirometery study also indicated that enhanced endogenous respiration caused sludge reduction in the QQ-Cannibal pilot system.

1. Introduction

Activated sludge (AS) process in the most implemented process to treat industrial and municipal wastewater treatment plants (WWTPs). Excess sludge production is the main challenge related to operation AS processes which take up 50–60% of total operation costs in WWTPs to manage and disposal. A modified activated sludge system, consisting of an anaerobic tank in the sludge return pathway to the aeration basin of an activated sludge system, was first introduced in 1991 by Chudoba et al. This system reduced the excess sludge production rate. It has been reported that this system removes the connection between bacterial anabolic and catabolic pathways from its normal state and leads to an increased energy use in the maintenance metabolism in cells [1,2]. Reports have also confirmed that changes in the activated sludge ecology and dominance of slow-growing bacteria (SGB) [3,4] and/or degradation of extracellular polymeric substances (EPS) [5,6] are among the mechanisms of excess sludge reduction in systems that have a side-stream anaerobic reactor in the sludge return pathway. Several advantages of this process such as low capital and operation costs have been asserted in literature for WWTPs in small size range [7]. Relatively low sludge reduction efficiency, up to 50–60%, is one of the bottlenecks to develop this process.

During the past two decades, the quorum sensing (QS) signaling system has attracted the interest of researchers. This system is based on cell density and the concentration of generated signals. Many Gram-negative bacteria use *N*-acyl homoserine lactones (AHLs) as QS signal molecules [8]. AHLs are usually produced by a protein homologous to LuxI and they will in turn bind to LuxR protein (Fig. 1) [9]. Once the concentration of the AHLs reaches a specific threshold, transduction leads to the induction of genes that control a variety of survival functions [10]. It has been reported that bacteria which use the QS system are present in the activated sludge system [11], and their population in the activated sludge may reach up to 70%, whereas the contribution of quorum quenching (QQ) bacteria is typically about 30% [12]. Quorum quenching system, which means degradation or inactivation of AHLs, can be achieved by quencher enzymes. One of the major classes of enzymes that degrade AHLs, through breaking the homoserine ring, is lactonase (Fig. 1) [13,14].

In gram-negative bacteria, the QS system regulates secretion of EPS and formation of biofilms [15,16]. Also, this system regulates pathogenicity factors, and has an important role in regulating the microbial structure of the activated sludge [17]. In recent years, the well established application of QQ system in wastewater treatment processes was regarding to befouling reduction in membrane bioreactor (MBRs) systems [18,19].

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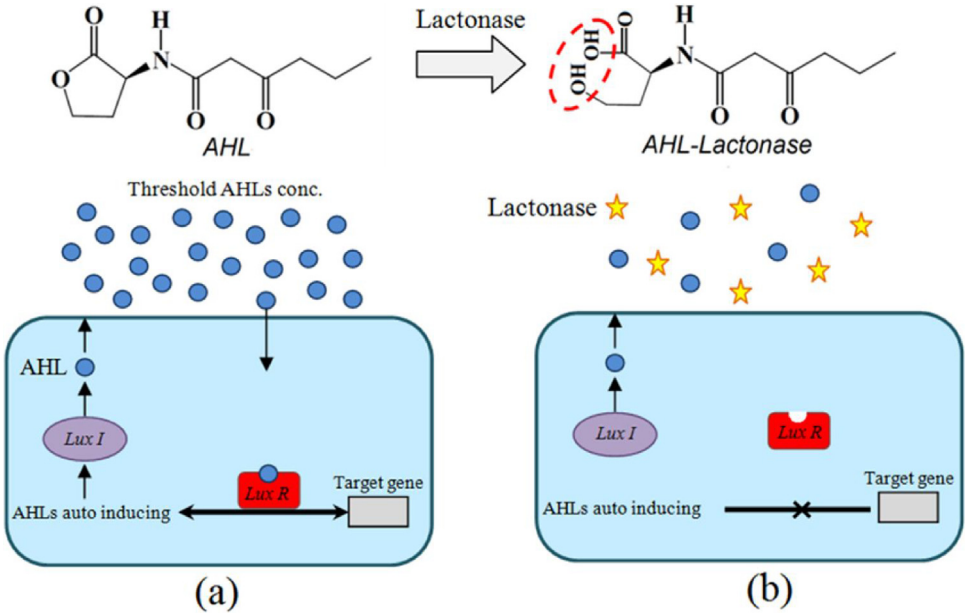

Fig. 1. Schematic regulation of (a) quorum sensing; (b) quorum quenching.

However, two important features regarding to activated sludge have been established in literature which may affect on sludge production yield, consist (i) up to 80% of the sludge volume can be composed of EPS [20]; (ii) intensify of microbial predation mechanism to sludge reduction is depend on free bacteria and fine microbial aggregates abundance which may be influenced by EPS secretion and therefore, QS disruption may promote two mentioned features to sludge reduction.

Alongside investigations related to establishing ASSR performance and involved mechanisms, some attempts have been undertaken to upgrade the sludge reduction efficiency of this system, such as addition of uncoupler chemicals [21], and ultra sonication of anaerobic sludge as a post-treatment [22].

The present research was aimed to suggest a new upgrade based on disruption of QS system in aerobic reactor of SBR-ASSR system as a new application of QQ systems. Therefore, a QQ system was augmented into a Cannibal process and the parameters of sludge production yield (Y_{obs}), EPS concentration, particle size distribution, and unicellular and multicellular microbial populations were investigated.

2. Materials & methods

2.1. Quencher entrapped beads (QEBs)

QEBs were produced based on the method introduced by Kim et al. [18]. In summary, *Rhodococcus* sp. BH4 bacteria were incubated at 28 °C for 24 h after being cultured in LB medium. The suspension was then centrifuged (4000 rpm, 10 min), the supernatant was removed, and a 4% sodium alginate solution was added. The obtained solution was poured drop by drop into calcium chloride solution (3% wt.) and the desired QEBs were formed. After 20 min, the beads were removed and washed several times with distilled water [18].

2.2. Experimental setup and operation

Three lab-scale pilot systems were constructed, including a sequencing batch reactor (SBR) as a control, an SBR + anaerobic side stream reactor as the Cannibal system, and a pilot system similar to the Cannibal reactor containing 600 QEBs, called the QQ-Cannibal system. All these pilots had a working volume of 4 liters and were designed and operated under identical conditions. Sequential SBR cycles consisted of 6.5 h of aeration, 45 min of sedimentation, 15 min of drainage, and half an hour of idle. The sludge interchange with the anaerobic reactor was carried out at the end aeration cycles from the aerobic reactor. The sludge interchange rate was 10% of the total biomass per day, where the biomass was measured as total suspended solid (TSS). The activated sludge seed was obtained from a local activated sludge wastewater treatment plant and was cultivated for three months with the synthetic sewage to achieve a stable state. The mean hydraulic retention time and the influent chemical oxygen demand were 16 h and 400 mg/L, respectively. Table 1 lists the compounds in the synthetic sewage. All of the main required materials in this research were obtained from the Merck Company, Germany.

Table 1
Composition of synthetic wastewater.

Micronutrient solution		Macronutrient solution	
[g/l]	Compound	[mg/l]	Compound
2.73	Citric acid	300 (COD)	Peptone
1.5	$FeCl_3 \cdot 6H_2O$	100 (COD)	Sodium acetate
0.25	H_3BO_3	60	NH_4HCO_3
0.15	$ZnSO_4 \cdot 7H_2O$	57	NH_4Cl
0.12	$MnCl_2 \cdot 4H_2O$	220	$CaCl_2$
0.06	$CuSO_4 \cdot 5H_2O$	394	$NaHCO_3$
0.03	$Na_2MoO_4 \cdot 2H_2O$	34	$KHSO_4$
0.03	$CoCl_2 \cdot 6H_2O$	44	KH_2PO_4
0.03	$NiCl_2 \cdot 6H_2O$	150	$MgSO_4 \cdot 7H_2O$
0.03	KI	1 (ml/l)	Micronutrient solution

2.3. Quorum quenching activity of QEBs

2.3.1. AHLs extraction

A liquid-liquid extraction method based on a specific ratio between organic solvent (ethyl acetate) and liquid sample (effluent) has been used in the present study. A solution with ethyl acetate (20 ml) and the effluent sample (10 ml) (volumetric ratio 2:1) was prepared and vortexed for 2 h. Then, the supernatant (ethyl acetate) transferred to a new tube. The tube was placed under