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# Effect of operational strategies on activated sludge's acclimation to phenol, subsequent aerobic granulation, and accumulation of polyhydoxyalkanoates

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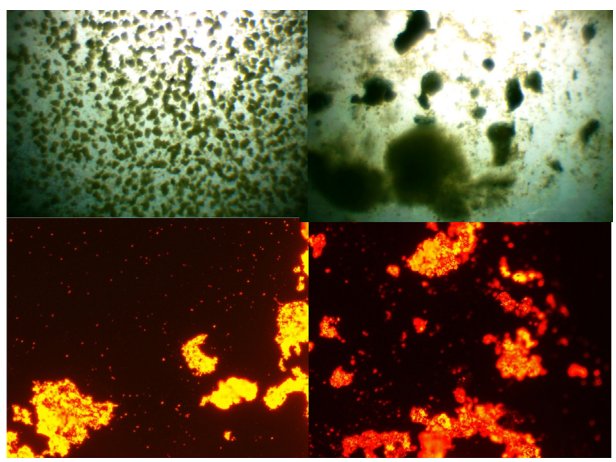
## HIGHLIGHTS

- Activated sludge was acclimated to phenol with 2 different strategies.
- Acclimated sludge later underwent aerobic granulation process.
- Sludge acclimated with phenol only degraded phenol and formed granules faster.
- Sludge acclimated with phenol + acetate formed more stable and robust granules.
- Both sludge exhibited significant PHA accumulation in early granulation stage.

## GRAPHICAL ABSTRACT



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## ABSTRACT

Aerobic granules, a relative novel form of microbial aggregate, are capable of degrading many toxic organic pollutants. Appropriate strategy is needed to acclimate seed sludge to the toxic compounds for successful granulation. In this study, two distinct strategies, i.e. mixed or single carbon sources, were experimented to obtain phenol-acclimated sludge. Their effects on reactor performance, biomass characteristics, microbial population and the granulation process were analyzed. Sludge fed with phenol alone exhibited faster acclimation and earlier appearance of granules, but possibly lower microbial diversity and reactor stability. Using a mixture of acetate and phenol in the acclimation stage, on the other hand, led to a reactor with slower phenol degradation and granulation, but eventual formation of strong and stable aerobic

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granules. In addition, the content of intracellular polyhydoxyakanoates (PHA) was also monitored, and significant accumulation was observed during the pre-granulation stage, where PHA >50% of dry weight was observed in both reactors.



## 1. Introduction

Aerobic granular sludge (AGS) is a relatively new wastewater treatment technology that is receiving increasing research and application interests. AGS is usually formed in aerated column type sequencing batch reactors (SBRs) [1], and characterized by its round shape, compact structure, high density and settling speed [2]. AGS reactors have demonstrated remarkable ability to remove toxic and recalcitrant substances [3], which include phenol [4], *p*-nitro phenol [5], trichloroethylene [6] and halogenated phenols [7], and high concentrations and stressful loadings have been applied [8].

Some of these substances, e.g. phenol and halogenated phenols, though toxic are able to support microbial growth. Their toxicity is generally associated with concentration levels. For example, phenol, a pollutant found in many industrial effluents [9], can be biodegraded efficiently under low concentrations [10]. However it poses severe cytotoxicity at higher levels by affecting the mobility of cell membrane [11], showing typical substrate inhibition effect. Therefore removal of high strength phenol requires special measures. Some researchers adopted cell immobilization, i.e. formation of biofilm or granules, which improved cellular resistance to phenol toxicity [9,12,13]. In addition, whether as attached or free-moving cells, microorganisms can greatly benefit from a proper acclimation procedure to these substrates [10].

To adapt common activated sludge to a toxic but biodegradable substance, at least two strategies can be used. One is to provide it as the sole carbon and energy source, and stepwise increase the concentration. The other is to use a benign substance like glucose as the supplementary substrate, and gradually replace it with the toxic one [14]. These two strategies might have various impacts. Using the first strategy, it is possible that an over dramatic increase could lead to severe inhibition or even total reactor collapse [15]. On the other hand, including a benign substrate in the carbon sources can quickly increase the microbial population during early acclimation stages, providing better resistance against toxicity. However it might also hinder the acclimation process, as the sludge preferentially degrades the benign substrates, thus fails to develop the activity towards the toxic substances. Indeed both beneficial and adverse effects have been reported for the biogenic organic compounds [16].

Phenol degradation by aerobic granules has been studied before, using fully acclimated activated sludge as the seed [4,13]. However, in those studies the acclimation process was conducted rather tentatively, and little is known on its possible impact on the granulation process. Therefore in this study, it is intended to test the two strategies in parallel, to acclimate common activated sludge to phenol for aerobic granulation. The objective is to assess these two strategies, and obtain primary knowledge on their relative effects on the efficiency and extent of phenol degradation, the structure and physiological state of the microbial population, and the relative effects on subsequent granule formation. Successful application of aerobic granule in the removal of toxic substances can benefit from an efficient acclimation process and a stable resultant culture. Therefore this study also aims at providing guidance for further engineering application. In addition, accumulation of PHA has been briefly studied with aerobic granules [17,18]. This study also intends to investigate this phenomenon with a toxic compound as the growth substrate.

## 2. Materials and methods

### 2.1. Reactor setup and operational strategy

The study was carried out in two lab-scale SBRs ($R_1$ and $R_2$) with the height of 157 cm, inner diameter of 6 cm, and a working volume of 3 L. Aeration was provided by air stones placed at the bottom, at a rate of 5 L min$^{-1}$, resulting in a superficial upflow air velocity of approx. 3 cm s$^{-1}$. The whole operation was divided into two phases, i.e. acclimation and granulation phases. During acclimation, $R_1$ was fed with two carbon sources, sodium acetate (NaAC) and phenol, and $R_2$ with phenol only. The concentration of NaAC in $R_1$'s influent was gradually decreased and that of phenol increased, but their combined carbon loading (calculated as g TOC d$^{-1}$) was kept constant. Also kept constant were its cycle time (4 h), settling time (30 min per cycle) and volumetric exchange ratio (50%). In contrast, phenol of increasing concentration was applied in $R_2$, and the cycles were prolonged according to each increase of phenol concentration. Correspondingly, there were less cycles in a day, with longer settling time and higher exchange ratio per cycle. Throughout acclimation, the total settling time per day was kept constant at 180 min, and the hydraulic retention time was 8 h for both reactors. At the end of acclimation, the cycle length, settling time and exchange ratio of $R_2$ was adjusted to the same level as those of $R_1$, and the two reactors were operated identically. The detailed operational strategies and parameters are listed in Table 1.

After 36 days of acclimation, phenol concentration in the reactors when the cycles started rose from 50 mg L$^{-1}$ to 250 mg L$^{-1}$, thus achieving acclimation. Granulation was then initiated by gradually decreasing the settling time from 30 min to 2 min per cycle over a period of 7 weeks. During this phase, the reactors were operated identically, and phenol concentration at cycle beginning was further increased to 500 mg L$^{-1}$ in the last two weeks. The detailed conditions are listed in Table S1.

### 2.2. Seed and feeding medium

The reactors were seeded with activated sludge obtained from a domestic wastewater treatment plant in east Shanghai. Analytical grade chemicals and tap water were used to make the reactor influent, which contained the carbon sources, $NH_4Cl$, $KH_2PO_4$, macronutrients and trace elements, and buffer solution if necessary. Changes in the carbon sources can be seen in Table S2, while constant TOC/N ratio of 10:1 and TOC/P ratio of 15:1 (w/w) were applied throughout the cultivation, and macronutrients were supplied in the influent as (in mg L$^{-1}$): $CaCl_2$, 20; $MgSO_4$, 23.4; and $FeSO_4 \cdot 7H_2O$, 30. 1 mL stock solution of trace elements was added into per liter of reactor influent, whose detailed composition is also listed in Table S2. When pH of the reactor effluent dropped below 6.5, 10 mL 1 M $NaHCO_3$ was added into per liter influent as buffer.