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# Enhanced flocculation of two bioflocculation-producing bacteria by secretion of *Philodina erythrophthalma*

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ABSTRACT

Bdelloid rotifer are reported to play a promoting role in microbial aggregation and floc formation in activated sludge systems; however, the mechanisms involved in this process are unclear. This study explores the effect of a rotifer secretion (RS) from the species *Philodina erythrophthalma* on the flocculation and growth of two bioflocculation-producing bacteria isolated from activated sludge. Results show that although the secretion has weak bioflocculability in itself, it can significantly enhance the flocculability of bioflocculation-producing bacteria and promote formation of microbial aggregation and floc. The possible mechanism is that the RS causes an increase in the bacteria densities and extracellular polymeric substance contents. The improvement of flocculability using RS shows an S-curve changing tendency with collection time, and corresponds with the first-order model with secretion dosage. Chemical composition analysis shows that low contents of non-protein organic nitrogen and polysaccharides are found in the RS, which implies that RS acts more like a growth-promoting substance or infochemical than as a nutrient in the promotion of bacterial growth. In conclusion, the findings provide a novel and potential strategy for promoting sludge floc formation using the infochemical secreted by this rotifer.



## 1. Introduction

The activated sludge process has been the central process used in wastewater treatment for the past 100 years (van Loosdrecht and Brdjanovic, 2014). The core constituent involved in the process is activated sludge, which is a complex mixture of inert solids from sewage combined with a microbial population (van Loosdrecht and Brdjanovic, 2014). In the system, it is essential that microbial aggregation and flocculation occurs within good settling sludge for its effective functioning of wastewater treatment (Bossier and Verstraete, 1996); poor sludge aggregation leads to an increase in effluent turbidity (Liu et al., 2010).

Some researchers have reported that protozoa and metazoan can trigger microbial aggregation by direct predation within activated sludge systems (Bossier and Verstraete, 1996; Lapinski and Tunnacliffe, 2003; Pajdak-Stós et al., 2010), and it has been suggested that colony formation and aggregation of microbes is a predator-induced defense that reduces predation risk (Yang et al., 2009). Bacterial cells living in aggregates can physically protect against predation, because protozoa and metazoan are not able to reach the bacteria which were tucked away in the inner part of a floc. However, there is a lack of current information pertaining to the indirect effect of the microfauna (such as substance secreted by the protozoa or metazoan) on bacterial aggregation within activated sludge systems.

The infochemical substance released by a predator (e.g. zooplankton, protozoa) can induce coaggregation (colony formation) and morphology modification in its prey (e.g. plankton, bacteria) in a natural ecological system. Von Elert and Franck (1999) showed that an infochemical released by the grazer *Daphnia magna* induces the formation of many-celled coenobia in uni-cellularly growing green alga *Scenedesmus acutus*. In addition, bacteria can sense diffusible chemical products secreted by protozoan predators and respond by forming inedible filaments or microcolonies (Blom et al., 2010a; Corno and Jurgens, 2006; Jousset, 2012). Cyanobacterium can detect grazer presence, even without direct contact, due to the presence of chemical signals; it then modifies its morphology to enable full expression of a defence reaction (Fiałkowska and

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Pajdak-Stós, 2014). Ha et al. (2004) reported that the infochemicals released from herbivore zooplankton (*Daphnia magna* and *Moina macrocopa*) can act as signals for triggers that induce colony development of two algal species. Furthermore, Blom et al. (2010b) reported that chemical cues discharged by flagellate grazing induce floc formation of *Sphingobium* sp. Strain Z007. However, to date, there is scarce literature data relating to the ability of bacteria to sense the presence of a predator (e.g., metazoan) through infochemicals within a wastewater treatment system, and how they respond by modifying their morphology or growth characteristics.

Rotifer are ubiquitous in most water treatment systems (wastewater and drinking water plants) (Ding et al., 2003). Research shows that rotifer can improve effluent clarify by grazing suspended biomass and particulate matter (Lapinski and Tunnacliffe, 2003; Li et al., 2013); and that they control activated sludge bulking by foraging on filamentous bacteria in biological wastewater treatment plants (Drzewicki et al., 2015; Fiałkowska and Pajdak-Stós, 2008; Kocerba-Soroka et al., 2013). However, little is understood about the effect of the substances secreted by rotifer on microbial aggregation and growth (Lapinski and Tunnacliffe, 2003).

Previous studies have shown that rotifer secretion (RS) plays an important role in its own growth and defence. Ohmori et al. (2011) showed that a rotifer can secrete a growth-promoting substance to promote its own exponential growth. In addition, it has been determined that colony formation and attachment of rotifer are triggered by an adhesive gluey substance secreted by its pedal gland (Lapinski and Tunnacliffe, 2003), and that the gelatinous materials secreted by rotifer enable them to protect themselves against dryness (Tunnacliffe and Lapinski, 2003). Hochberg et al. (2015) found that defensive compounds secreted by rotifers made them unpalatable, thereby reducing the predation risk. It is thus of considerable interest to determine how RS affects growth and aggregation of bacteria (prey) in the water treatment system.

Most of bdelloid rotifer are a group of small, filter-feeding, colonial metazoan. Their name relates to the fact that most species have a corona of cilia around the mouth opening, which beats sequentially to generate a vortex of water. Lapinski and Tunnacliffe (2003) speculated that some substance secreted by the pedal gland of bdelloid rotifer promotes sludge floc formation by functioning as a bioflocculant, but this study lacks direct data. However, because of the small amount of substance actually secreted, it is considered that this secretive substance may have a negligible effect as a bioflocculant. Therefore, the hypothesis of this study is that the promotion of microbial aggregation and floc formation is related to the infochemical contained within the excrement of bdelloid rotifer. In the present study, *Philodina erythrophthalma*, a species of bdelloid rotifer, is collected from a wastewater treatment plant and used to produce the RS.

Chemical flocculants, including aluminium sulphate, ferric chloride and polyacrylamide (PAM), are effective in promoting the aggregation and flocculation of activated sludge within wastewater treatment systems (Lian et al., 2008). However, their use has caused a number of serious environmental and health problems in relation to toxicity because they are non-readily degradable (Guo and Ma, 2015; Lian et al., 2008; Salehizadeh and Yan, 2014). In this respect, the use of microbial bioflocculant has been attracting increasing attention. It is secreted by microorganisms during active secretion and cell-lysis and is an environmentally-friendly material that has harmless and biodegradable characteristics (Guo and Ma, 2015). However, the relatively high price of raw materials and the low yield of flocculants limits the practical application of bioflocculants, and thus, with the aim of commercialization, an immense amount of effort has been put into enhancing the yield rate of microbial bioflocculant and reducing its production cost (Salehizadeh and Yan, 2014). In this respect, it is considered that RS may be a potential and novel pathway for use in improving bioflocculant production through affecting the growth or morphology of bioflocculation-producing bacteria. In the present study, two bioflocculation-producing bacteria, *Brevundimonas vesicularis* LW13 and *Bacillus cereus* LW19, are applied as representative microorganisms.

The objectives of this study are: 1) to investigate the influence of RS on bioflocculation of two bacteria; 2) to further study the effect of RS collection time and dosage on bioflocculation of bacteria; 3) to explore the possible mechanism involved in the effect of RS on bacterial bioflocculation using inversion and scanning electron microscopy, and analyses of the extracellular polymeric substances (EPS) content and chemical composition of the secretive substance. It is considered that this study will contribute toward gaining an in-depth understanding of the function of bdelloid rotifer within an activated sludge system.

## 2. Materials and method

### 2.1. Screening and culture of two bacteria with flocculability

Two flocculation-producing bacteria strains, *Brevundimonas vesicularis* LW13 and *Bacillus cereus* LW19, were isolated from activated sludge at the Quyang wastewater treatment plant in Shanghai, China. The two bacteria were screened and identified according to the method presented in Dermlim et al. (1999), and their growth medium was prepared according to the method presented in Bala Subramanian et al. (2010). The initial pH of media was adjusted to 7.0, and glucose and $MgSO_4$ were separately sterilised and mixed with other ingredients before inoculation. After the isolated bacterial strains were inoculated, the substrate was cultured in an orbital shaker at 160 rpm for 24 h at 30 °C. After incubation, the medium was centrifuged at 10000 rpm for 10 min at 4 °C; the residue was then collected and suspended by sterilised physiological saline, which maintained optical density of the bacterial suspension (BS) in the range of 0.6 and 0.8 for the following experiments.

### 2.2. Culturing of bdelloid rotifer and collection of secretion

*P. erythrophthalma* filter-feeds on suspended solid like bacteria and small particles, and is observed often within activated sludge systems. It was isolated from activated sludge at the Quyang wastewater treatment plant and maintained in a continuous passage culture at 30 °C on a diet of flour grain that had been crushed by an ultrasonic cell crusher (Ningbo Xinzhi Biological Technology CO., LTD, China). The basic medium for the rotifer was prepared according to that presented in our previous experiment (unpublished), which used flour as food.

Before initiating production of secretion, the cultured rotifer were collected in a nylon mesh (50 μm pore size), and rinsed five times using 1 L sterile deionised water to remove the impurities attached to their body. Using the nylon mesh, the rotifers were then transferred to a sterile Erlenmeyer flask containing deionised water and maintained at a density of 100 ind. $L^{-1}$, before being cultured in an orbital shaker at 30 °C and 120 rpm. After incubation for 24 h, the rotifers were removed from the flask by the mesh. Subsequently, the deionised water containing RS was filter-sterilised using a membrane filter (0.22 μm pore size), and was used in the following experiments, as RS.

### 2.3. Bioflocculability test

Bioflocculability was determined using kaolin clay suspension with minor modification (Bala Subramanian et al., 2010). Kaolin