Ecotoxicology and Environmental Safety 162 (2018) 296–303

Contents lists available at ScienceDirect

Ecotoxicology and Environmental Safety

journal homepage: www.elsevier.com/locate/ecoenv

Comprehensive assessment of microbial aggregation characteristics of activated sludge bioreactors using fuzzy clustering analysis

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ARTICLE INFO

Keywords:
Activated sludge
Comprehensive assessment
Environmental stress
Fuzzy clustering analysis

ABSTRACT

Understanding microbial aggregation dynamics in response to the often violent environmental fluctuations is important for activated sludge wastewater biotreatment practice, yet remains poorly understood. We investigated microbial aggregation process of an activated sludge reactor in response to various operating conditions of resource limitations, disinfectant and pH stresses, and quantified aggregation characteristics by employing a fuzzy clustering analysis (FCA) method. The results revealed that the FCA provided a means for comprehensive assessment of microbial aggregation dynamics of the bioreactor relying solely on simple parameter estimation. Proper disinfectant stress (of NaClO 1.00% or 2.00%) is a promising strategy to improve the comprehensive performance of microbial aggregation and sludge settleability. Nitrogen– (of C/N ratio > 40) and dissolved oxygen–limitations (of DO < 0.2 mg/L) had medium influence on the comprehensive performance of the activated sludge system, while little impacts for acidic and alkaline conditions. These quantitative estimations offer insights into the underlying bio–physicochemical processes of an activated sludge bioreactor in response to practical fluctuations that is often beyond typical assessment practice. In addition, it may represent a step towards uncoupling the complex biophysical interactions that is essential for optimized designing and proper engineering practice of biological wastewater treatment reactors.

1. Introduction

Notwithstanding numerous evidences showing that resource–limitations, chemical toxicity, and other environmental stresses stimulate bioflocculation, understanding of the comprehensive bio–physicochemical processes of activated sludge bioreactors in response to the often violent operational fluctuations remains poorly understood (Chen et al., 2014; Jarvis et al., 2005; Wang et al., 2017). In practice, enhanced microbial aggregation and improved sludge settleability are keys for such biotreatment systems (Chen et al., 2017; Martins et al., 2011), that otherwise often cause effluent deterioration due to poor bioflocculation and thereby, declined treatment efficiency and even failure of the system (Dong et al., 2017; Han et al., 2018; Zheng et al., 2011). Microbial aggregate (or floc) is a building block of activated sludge, and plays important functions in wastewater treatment systems that often experience violent operating fluctuations (Sarayu and Sandhya, 2012; Yang et al., 2017). Formation of microbial aggregates is a special case where free–living cells gather to form stable contiguous and multicellular aggregates at certain environmental conditions, and thus stabilize their functioning (Yang et al., 2017; Zheng and Yeung, 2003). Such aggregates may continuously grow upon coagulation and successful collisions, with association of microbially excreted extracellular polymeric substances (EPS), forming larger size aggregates till reaching a critical level whereby aggregation balances out the breaking process of microbial aggregates (Ding et al., 2017; Jarvis et al., 2005; Yuan and Farnood, 2010).

Evidence showed that resources limitations, chemical toxicity, hydraulic stress, and other environmental stresses could considerably influence microbial aggregate formation (Han et al., 2012). For instance, microbes were found to experience intensive aggregation in resources limiting environments, where the commonly accumulated microbially excreted EPS often function as bridging agent facilitating bioflocculation and adhesion (Badireddy et al., 2010; Feng et al., 2018). In contrast, inhabiting resources rich environments, microbes were found to

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https://doi.org/10.1016/j.ecoenv.2018.06.096
Received 12 April 2018; Received in revised form 14 June 2018; Accepted 30 June 2018

display higher concentration of cell surface functional groups and reduced proteins excretion, and thereby yielding suppressed aggregation (Eboigbodin et al., 2007). Chen et al. (2014) found that resources availability may also influence early-stage microbial aggregation process via energy–dependent cell motility regulation. In addition, Liao et al. (2011) observed enhanced bioflocculation yet declined settleability of microbial flocs upon increased dissolved oxygen (DO) concentration, reflecting crucial functioning of DO on microbial aggregation. Other than such physicochemical driving forces, Dulekgurgen et al. (2008) reported that high shear stresses would also enhance EPS production of activated sludge microbes and thereby facilitate cells aggregation. Despite of these numerous advances, understanding of the comprehensive bio–physicochemical processes of common activated sludge wastewater treatment systems in response to the complex operational fluctuations remains challenging (Ding et al., 2017; Feng et al., 2018; Yuan and Farnood, 2010). We employed a fuzzy clustering analysis (FCA) method to quantify how operational environmental factors (e.g., resources limitations, disinfectant and pH stresses) and their fluctuations conspire to regulate microbial aggregate formation and yielded aggregate characteristics of a model activated sludge bioreactor. Application of the FCA would yield some of quantitative estimations on the degree of the multiple operational factors influencing microbial aggregation performance of the activated sludge bioreactor. The comprehensive estimation should narrow the gap between systems practical engineering and traditional singular parameter assessments, that represents an important step towards uncoupling the complex biophysical processes of such biotreatment systems that is essential for optimized designing and practical functioning.

2. Material and methods

2.1. Synthetic wastewater

A synthetic wastewater was used for sludge cultivation, consisting of sucrose (1000.0 mg/L, or 1122.8 COD mg/L), NH_4Cl (160.8 mg/L, or 42.1 NH_4–N mg/L) with its value set according to Yoo et al. (1999), KH_2PO_4 (58.0 mg/L) and $NaHCO_3$ (200.0 mg/L). Additional microelements were applied for sludge cultivation, including (unit in μg/L) $CaCl_2{\cdot}6H_2O$ 130.0, $FeCl_3$ 1000.0, H_3BO_3 6.0; $ZnCl_2$ 70.0; $CuCl_2$ 2.0; $MnSO_4{\cdot}H_2O$ 100.0; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$ 206.0; $AlCl_3$ 50.0; $CoCl_2$ 238.0 and $NiCl_2$ 24.0. Sodium bicarbonate solution was applied as a buffering system. Identical synthetic wastewater was used throughout the study except for scenarios with variable carbon source conditions.

2.2. The batch experimental system and preparation of seeding sludge

The aerobic batch reactors used for the study have identically effective volume of 2.0 L. Activated sludge was taken from a biological unit (oxidation ditch) of a local municipal wastewater treatment plant in Hefei, China. The activated sludge was pre–cultured with synthetic wastewater in a 20.0 L sequential batch reactor at room temperature, and the DO and pH values were maintained at 4.0 mg/L and 7.0, respectively. Prior batch experiment, the seeding sludge sample was aerated for 24 h at high air flow to deplete organics, which yielded mean aggregate size of 0.0098 ± 0.0015 mm^2 (n = 20) and the mixed liquor volatile suspended solids (MLVSS) concentration of 3500.0 mg/L.

2.3. Experimental setup

To estimate the effects of common environmental stresses on comprehensive performance of microbial aggregation process and yielded aggregate properties, four sets of experiments were conducted at various pH values, carbon and nitrogen concentrations, DO and chlorination levels, respectively, with details modified according to Chen et al. (2014).

Carbon and nitrogen variation experiment: pre–treated activated sludge (200.0 mL for each reactor) was inoculated into six sets of aerobic batch reactors in parallel at room temperature, yielding a total medium volume of 1.0 L. If not specified, all experiment scenarios consist of the same amount of initial sludge inoculum and total medium volume, and were conducted identically at room temperature of (25 ± 2) °C throughout the study. The experiment medium consists of a fixed nitrogen level of 160.8 mg/L NH_4Cl (if not specified the same value applied throughout the study), and with different carbon source (sucrose) levels and thereof, yielding an initial C/N ratio of the experiment medium of 5.0, 10.0, 20.0, 30.0, 40.0 and 50.0, respectively (thus resulting in carbon– or nitrogen–limiting conditions). The dissolved oxygen level was set to 4.0 mg/L, and pH value of the experimental system was initially set at 7.0 with sodium bicarbonate solution applied as a buffering medium.

Dissolved oxygen variation experiment: identically pre–treated activated sludge was introduced into five sets of aerobic reactors in parallel. The dissolved oxygen levels of the five sets of reactors were set to 0.2, 2.0, 4.0, 6.0 and 8.0 mg/L, respectively. The C/N ratio of the medium was initially set at 20.0, with pH set-up identical to carbon and nitrogen variation experiment.

NaClO variation experiment: identical activated sludge was introduced into six sets of aerobic reactors in parallel. NaClO concentrations (%, v/v) of the six sets of reactors were initially set at 0.01, 0.10, 0.20, 0.50, 1.00, and 2.00, respectively. Dissolved oxygen was set to 4.0 mg/L, and the C/N ratio was initially set at 20.0, with pH set–up identical to carbon and nitrogen variation experiment.

Fluctuation pH experiment: identical activated sludge was introduced into five sets of aerobic reactors in parallel. The pH values of the five sets of reactors were initially set to 3.0, 5.0, 7.0, 9.0 and 11.0, respectively. Dissolved oxygen level and initial C/N ratio were set identically to those of NaClO variation scenario.

2.4. Quantitative analysis of microbial aggregate size and sludge properties

Fifty μL aliquots of mixed culture containing microbial aggregates were collected from each reactor at 0, 3, 6, 9, 12, and 24 h, respectively, for analysis. Microbial aggregate size was estimated using an optical microscope system (Olympus BX41, Japan), with its size represented by the projected area of an aggregate (Chen et al., 2014). For image acquisition of microbial aggregates, 10 randomly selected slides were examined for each sample. Microbial aggregate forming rate was estimated according to least squares methodology by weighing the relationship between microbial aggregate size and time. The DO level was controlled through an air compressor equipped with flow meter through porous diffuser stones, which was calibrated by a DO probe (MO128, Mettler-Toledo Gmbh, Switzerland). At the end of each experiment, 100.0 mL of mixed cultures were collected from the reactors for estimating the settled sludge volume (SV) according to the standard methods (APHA, 2005). Prior to experiment, the mixed liquor suspended solids (MLSS) and MLVSS of the seeding sludge were determined (triple measurements, with each measurement used 100.0 mL of the seeding sludge) according to the standard methods (APHA, 2005).

2.5. Scanning electron microscope visualization

Activated sludge samples were dehydrated using a graded series of ethanol solutions (v/v) of 30.0%, 50.0%, 70.0%, 90.0% and 100.0%, respectively, in a freeze drier, with each step lasting for 15 min. Afterwards, identical treatment processes were applied to the samples except using a graded series of isoamyl acetate solutions of 30.0%, 50.0%, 70.0%, 90.0% and 100.0%, respectively. Dried samples were coated with gold by a diode sputtering system and observed with scanning electron microscope (Su8020, Hitachi).