1. Introduction

The need to treat and reuse wastewater generated by urban and industrial areas for industrial and agricultural purposes has become urgent due to environmental crises resulting from wastewater discharge and the depletion of freshwater resources (1). The use of biological processes is highly prevalent in wastewater treatment and recycling, with activated sludge processes being particularly popular (2, 3). These processes involve the utilization of microorganisms to effectively treat wastewater and facilitate its reuse. To reuse treated wastewater, the levels of total suspended solids (TSS), biochemical oxygen demand (BOD), chemical oxygen demand (COD), nitrate, ammonia, and various other parameters should fall within specified ranges set by standards. These parameters serve as indicators of the water quality and must meet the required criteria for safe and acceptable reuse (4). Step-feed biological nitrogen removal (SFBNR) is a wastewater treatment process that is specifically designed to remove nitrogen compounds, such as ammonia and nitrate, from wastewater (5).

2. Problem Description

Considering the widespread use of the SFBNR process in wastewater treatment plants of Iran, such as Hamadan Wastewater Treatment Plant (WWTP), the objective is to ensure that the discharged effluent contains contents of ammonia, nitrate, TSS, BOD, and COD that are below specific threshold values of 1, 10, 10, 30, and 60 mg/L, respectively. Besides, the turbidity of the effluent must be less than 10 NTU. In many cases, the discharge of industrial wastewaters to municipal sewage networks is associated with the presence of organic compounds in the treatment plant, thereby impairing the quality of the effluent, including increased content of ammonia. As a result, the excessive growth of filamentous bacteria, especially Microthrix parvicella, may happen, which is one of the most common operating problems of treatment plants.
In this study, the decline of the excessive growth of filamentous *M. parvicella* and the removal efficiency of nitrogen compounds on the real scale in Hamadan WWTP were investigated by changing the hydraulic flow distribution (Fig. 1). According to Fig. 1, the WWTP has been designed and built in accordance with mode A to remove simultaneously organic, nitrogen, and phosphorus compounds. However, the flow configuration was changed to mode B in 2017, mode C in 2018, and ultimately to mode D starting in 2019 to assess the mode that exhibited the most optimal performance. The observations indicated that mode D yielded the most favorable outcomes, as it led to a reduction in the overgrowth of filamentous *M. parvicella*, foam formation, and an increase in the removal of nitrogen compounds. To assess a decrease in the growth of *M. parvicella*, Gram and Neisser staining and enumeration of the mentioned filaments were done using the methods developed by Pitt and Jenkins, and the results of the investigation showed a decrease in the number of the mentioned filaments. In addition, the volume indices of sludge and filamentous index were also investigated, which indicated the improvement of sludge settling quality. It was found that with changing the hydraulic flow distribution, the WWTP was capable of decreasing the concentration of ammonia and nitrate to less than 1 and 10 mg/L, respectively.

Noutsopoulos et al also concluded that the plug flow configuration in biological nutrient removal (BNR) systems resulted in fulfilling control of *M. parvicella*. They reported that this configuration provides a selective advantage for floc-forming bacteria to remove RBCOD and therefore limits the growth of *M. parvicella*, which is mainly based on SBCOD and its hydrolysis products (16). In this full-scale study, the change in the configuration of the inlet flow from modes B and C (step-feeding) to mode D is actually a change in the flow pattern to the plug flow mode, which is accompanied by a decrease in the number of filamentous *M. parvicella*, an increase in filamentous Type 0041/0675, and an improvement in the quality of sludge sedimentation. Stephenson and Luker compared a full-scale application of plug-flow and step-feeding configurations and claimed that systems with step-feed flow distribution in nitrification showed more stability. Moreover, in a study conducted by Zhu et al, a high proportion of effluent was transferred to the first step, which showed a high total nitrogen removal efficiency. They explained that when the input ammonia concentration is higher, a relatively large part of the input flow must be transferred to the first stage in order to maintain the effluent ammonia concentration at a low level (2). Considering that the typical input ammonia levels at the studied WWTP consistently range from 30 to 50 mg/L and the requirement is to treat the wastewater to achieve an ammonia concentration below 1 mg/L, opting for mode D in Hamadan WWTP proved to be the most effective approach.

Considering the widespread use of the SFBNR process in wastewater treatment in Iran, it is recommended that operators of WWTPs, water and wastewater company managers, and industrial estate companies enhance the quality of treated wastewater by adjusting hydraulic flow distribution. By reallocating a significant portion of the inflow to the initial stages of the SFBNR system following mode D, notable improvements are observed in sludge settling quality and the reduction of filamentous *Microthrix parvicella* growth. These changes led to better effluent and
sludge quality, including a reduction in ammonia levels to below 1 mg/L and enhanced system stability against organic loading shocks (Fig. 2). Furthermore, the reduction in foam formation resulted in improved effluent quality regarding turbidity and suspended particles. As depicted in Fig. 3, the SVI exhibited a distinct pattern during the years 2016, 2017, 2018, and 2019, in which the system was operated in step-feeding mode. It consistently peaked during the cold seasons, typically averaging between 250 and 300 mL/g. This peak was attributed to the overgrowth of filamentous *M. parvicella*. However, following the alteration of flow distribution and the transition to mode D, the growth of this filamentous organism declined. This reduction in the population of filamentous *M. parvicella* was accompanied by a decline in SVI, which reached its lowest value, dipping below 140 mL/g in this state.

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References