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Biodegradation of wastewater organic contaminants using *Serratia* sp. ISTVKR1 isolated from sewage sludge

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ABSTRACT

Wastewater was collected from various stages and sewage sludge was collected from sludge bed of Vasant Kunj sewage treatment plant (VK STP) in the post-monsoon season. The treatment plant is based on extended aeration activated sludge (EAAS) process. Preliminary analysis of the wastewater and sewage sludge samples revealed 11.5 fold reduction in chemical oxygen demand (COD) of wastewater after treatment while GC–MS analysis showed the presence of bisphenol P (RT = 41.63), certain pharmaceuticals, pesticides, and industrial compounds in effluent and sewage sludge, thus indicating the need for further treatments before their discharge into the environment. One indigenous bacterial strain isolated from sewage sludge of VK STP and identified as *Serratia* sp. ISTVKR1 was used for biodegradation of wastewater contaminants. Preliminary analysis of bacterial treatment culture showed almost 12 fold COD removal after 240 h of treatment while the GC–MS analysis revealed removal of complex organic compounds like phosphoric acid triphenyl ester (RT = 30.017) and 4H-1-benzopyran-4-one, 2-(3,4-dimethoxyphenyl)-3,5-dihydroxy-7-methoxy- (RT = 45.36) and formation of simpler compounds like alcohols and aliphatic hydrocarbons such as 1-heptacosanol (RT = 32.66), docosane (RT = 34.18) and hexadecane (RT = 29.621). Results of the study indicate the efficient removal of wastewater organic contaminants by the isolated *Serratia* sp. ISTVKR1.

1. Introduction

In the past few decades, there has been an increasing concern for potential adverse environmental effects as a result of the production, use, and disposal of various industrial, agricultural, medical and household chemicals.

Municipal wastewaters and sewage sludge are an important sink as well as the source of such chemicals into the environment [1–3]. The composition of municipal wastewaters is very complex and it contains abundant mixture of simple to more complex molecules, which are recalcitrant to biodegradation [4]. Many trace organic pollutants, such as nonylphenol, alkylphenols, bisphenols, and phthalates are often not effectively removed by the traditional treatment processes and have been discharged into the water bodies causing adverse health effects on aquatic and terrestrial biota as a result of bioaccumulation, biomagnifications, and endocrine disruption among others [5,6].

In developing countries like India, the discharge of untreated and inefficiently treated wastewaters in the environment is a common practice and one of the major reasons for surface and groundwater pollution due to a large disparity between generation and treatment of wastewaters. Commonly, a single facility is employed to treat sewage for diverse kinds of waste products which in most of the cases are not efficient enough to treat all the contaminants including some xenobiotic compounds present in the effluent and sludge, thus resulting in the release of these chemicals directly into the environment.

Apart from the nature of chemical contaminants, the processes employed for treatment also affects the degradation of contaminants entering a wastewater treatment plant (WWTP) [7]. Activated sludge process (AS) is widely used in municipal and industrial wastewater treatment plants and is capable of effectively removing suspended solids (SS), and nutrients from sewage. Extended aeration activated sludge (EAAS) process being one of the modifications of the AS process, usually employs an 'extended aeration period' for aerobic digestion of the generated biological sludge [8]. Although, the EAAS process has advantages like low sludge

Abbreviations: EAAS, extended aeration activated sludge; WWTP, wastewater treatment plant; VK STP, Vasant Kunj sewage treatment plant; I, influent; AT, aeration tank; FST, final settling tank; E, effluent; S, sludge; EC, electrical conductivity; TDS, total dissolved solids; RP, redox potential; DCM, dichloromethane.

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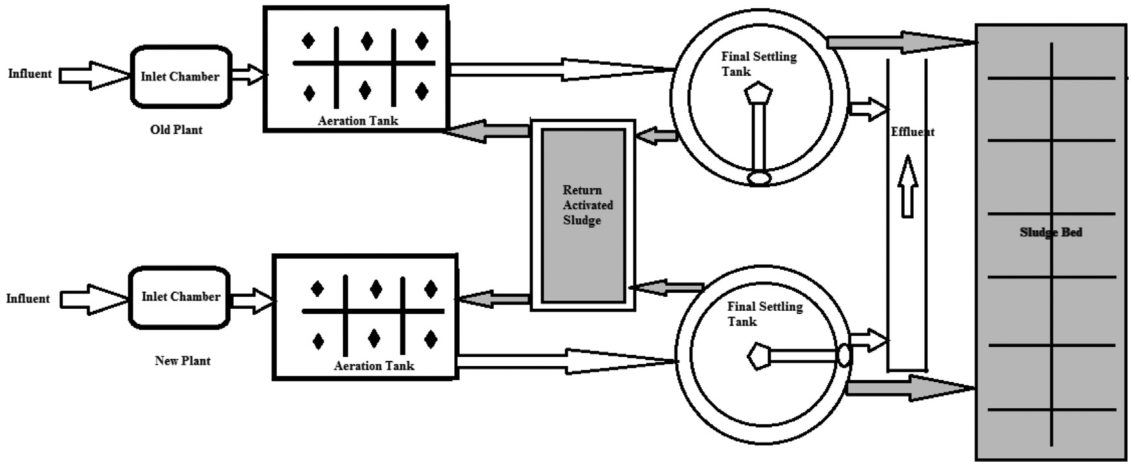

Fig. 1. Schematic diagram of VK STP representing old and new plant units.

generation and low ammonia effluent, yet high retention time (HRT), low organic loading rate and active biomass, limits the use of this process for industrial wastewater treatment [9]. The process also has low nutrient removal efficiency [10], which is mostly related to the accumulation of some of the biodegradable and recalcitrant organic compounds in the effluent and sewage sludge [8,11].

Enhanced treatment involving physico-chemical processes like ion exchange, ultrafiltration, reverse osmosis, chemical precipitation and electrochemical technologies although effective have high operating cost, limited versatility, plausible formation of secondary hazardous byproducts, incomplete purification and also generate large amounts of sludge [12]. However, certain advanced methods, such as biodegradation using potent microorganisms, can prove effective for further treatment of persistent contaminants found in municipal and industrial wastewaters and sewage sludge. The use of microbes for biodegradation of xenobiotics is an efficient, relatively cost-effective and environment friendly tool for the treatment of wastewaters. Several microorganisms such as algae [13–15] have been explored for their potency to degrade and detoxify complex contaminated wastewaters.

Research on bacterial biodegradation of contaminants has intensified in the past few years not only because of the ability of bacteria to degrade, transform or accumulate a wide range of compounds including aromatic compounds [16], halogenated congeners [17], pharmaceuticals [18] and hydrocarbons [19] but also due to the opportunity of decoding the biodegradation pathways operating within bacteria owing to the advancements in metagenomic approaches. Bacteria isolated from sludge have been studied for biodegradation of various contaminants [20,21]. Previous studies have shown the ability of *Serratia* sp. to degrade several compounds such as organophosphorus pesticides [22], beta-cypermethrin [23], methyl parathion and *p*-nitrophenol [24], chitin [25], DDT [26] and RDX [27]. Measurement of physico-chemical parameters such as pH, temperature, electrical conductivity (EC), total dissolved solids (TDS), redox potential (RP) and COD which are an indirect indicator of mineralization and solubilization of compounds in wastewater samples during bacterial treatment have also been previously used to assess the efficiency of the biodegradation process [28,29].

The present investigation intended to analyze the organic contaminants present in wastewater and sewage sludge of a sewage treatment plant. The study simultaneously aimed to isolate, screen and identify potent biodegrading bacterial strain form sewage sludge and study biodegradation of wastewater contaminants using the selected bacterial strain.

2. Materials and methods

2.1. Chemicals and reagents

All chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA) or Merck (Darmstadt, Germany) unless otherwise stated and were used without further purification. All organic solvents and solid chemicals were of analytical reagent grade.

2.2. Study area and sampling

Vasant Kunj sewage treatment plant (VK STP) which is located at Vasant Kunj, (28°31′29.3″N latitude and 77°10′03.5″E longitude), New Delhi, India, was selected as the sampling site. VK STP (Fig. 1) was established in 1988 and functions on EAAS process for the treatment of municipal wastewater. Therefore, primary settling tank, sludge digester and gas holders are not present in this treatment plant. The plant has a total capacity of 5.2 million gallons per day (MGD) with an actual flow of 4.16 MGD. It has 2 units of capacity 3.0 MGD (new) and 2.2 MGD (old), respectively, with an actual flow 2.60 MGD and 1.45 MGD, percent utilization of 66.7% and 54.5% and hydraulic retention time (HRT) of 36.6 and 44.7 h, respectively. Raw sewage or influent comes through the inlet and flows through a bar screen and grit chamber for preliminary screening after which the wastewater is pumped to the aeration tank (AT) for secondary treatment followed by the liquid–solid separation in the final settling tank (FST) before the discharge of final effluent through a canal to Sanjay Van and Hauz Khas lake. The settled solid part (sewage sludge) is placed on sludge beds for drying and is sold to farmers for use as manure [30].

Grab sampling was done from the new plant unit in the month of September, 2013, in the post monsoon season. Wastewater samples were collected from inlet (influent, I), aeration tank (AT), final settling tank (FST) and outlet (effluent, E) while sewage sludge samples were taken from the sludge bed (S). Wastewater samples from I, AT, FST and E and sewage sludge samples from S were collected in triplicates from three randomly selected points at each of the treatment stages and then pooled together in air-tight glass containers. Once in lab, all samples were stored at 4 °C. Sludge sample was air dried, powdered using mixer grinder, sieved using 0.2 mm stainless steel sieve and stored at 4 °C. All glassware were pre-soaked in 1 M HNO_3 for 24 h and then rinsed with distilled water. All samples were typically extracted within a week of collection.