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Treatment of Atrazine bearing Wastewater by Mixed Methanogenic Culture

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Abstract

Performance of an anaerobic reactor in treating atrazine bearing wastewater using a mixed anaerobic microbial culture having high acetoclastic methanogen population was evaluated in batch and semi-continuous mode of operation. At an initial atrazine concentration of 5.0 mg/l, atrazine reduction of 30.2% after 5 days and 64% after 50 days were observed in semi continuous and batch mode of operation respectively. Carbonaceous material removal measured as chemical oxygen demand (COD) was more than 90% in both the cases. Initial COD ws 305±7 mg/l. Experimental results showed that atrazine degradation was better in presence of dextrose than that of sodium acetate. Percentage of methane in biogas was more in acetate fed reactor than dextrose fed reactor.

Introduction

Atrazine (2-chloro-4-ethylamino-6-isopropylamino-s-triazine), a chlorinated herbicide has been a major agricultural herbicide in the world for more than 35 years. Over 65% of all corn acreage, in USA, is treated with atrazine. At present atrazine has been used in more than 70 countries and probably it is the most commonly used herbicide in the world. Due to its various toxicity properties, permissible limit of atrazine has been restricted to 2 ppb by world health organization[1] and as per Indian condition there should be no pesticide present in drinking water. Although permissible limit of atrazine has been restricted to ppb level in drinking water, as high as 22 mg/l of atrazine occurrence has been reported[2,3]. It has been reported that the concentration of striazine in wastewater, measured as Total Kjeldahl Nitrogen (TKN), was 810 mg/l N from synthesis unit of chloro-s-triazine herbicides[4]. In another report, per capita consumption of pesticide containing chloride by an Indian is the highest in the world[5].

Removal or detoxification of atrazine can be done by physical, chemical or biological means. Among these processes, biological process is the only process by which atrazine can be mineralized. Wastewater is commonly treated by biological process and anaerobic treatment of wastewater has many advantages over conventional aerobic process. Anaerobic dechlorination of many chlorinated organic substances remained successful, which were earlier considered as recalcitrant in aerobic process[6,7]. Methanogens play quite dominant role in ring fission of converting aromaic ring of benzoate to methane and carbon dioxide. Although methanogens alone are unable to break the aromatic ring, but other anaerobes can break the ring and then methanogens play dominant role in ultimate detoxification of the toxic material[8]. Although there are several reports on the role and performance of mixed methanogens in detoxification of many toxic organic materials like lignin[9], benzoate and other aromatic acids[10], treatment of atrazine bearing wastewater by anaerobic culture enriched with methanogens has not been reported yet. The objective of this work is to study the performance of mixed culture anaerobic bacteria enriched with acetate degrading methanogens in treating atrazine bearing wastewater.

Materials and Methods

All the chemicals used for feeding and analysis were of AR grade. Technical Grade of atrazine was supplied by Rallis India Limited, Mumbai, India. Synthetic wastewater was prepared by adding K_2HPO_4 = 150 mg; KH_2PO_4 = 50 mg; $MgCl_2$. $6H_2O$=300 mg; $CaCl_2.2H_2O$ = 50 mg; NH_4Cl = 200 mg; $FeCl_2$. $4H_2O$=5 mg; $ZnCl_2$ = 0.5 mg; $NiCl_2$. $6H_2O$ = 0.5 mg; $CoCl_2$. $6H_2O$ = 0.5 mg; $MnCl_2.4H_2O$ = 0.5 mg; Yeast extract = 50 mg and Sodium acetate = 300 mg in 1 L of distilled water. Stock solution of atrazine and all other chemicals were prepared in distilled water.

Unless otherwise specified, all the parameters were determined as per the standard method[11]. pH was measured in a digital pH meter (DPH500, SISCO, India). Methane and total gas production was measured by water displacement method. Methane gas was collected by passing toal biogas through 32% (w/v) KOH solution and collecting by water displacement method. Volatile fatty acid (VFA) and alkalinity was measured by liquid-liquid extraction method using Dichloromethane as extractant and measurement was done in UV-visible spectrophotometer. (Model: UV-160A, shimadzu, Japan) using dichloromethane as blank. Maximum absorbance was observed at 228.8 nm. The extraction efficiency was 88-92% in this method. To know the amount of atrazine adsorbed on the reactor sludge, 20 ml of biomass samples

were centrifuged for 10 min, supernatant was decanted, the sludge were washed with distilled water, resuspended and again centrifuged. Atrazine was extracted from the pellet of the sludge in methanol by shaking for 2 hours, centrifuged, then evaporated by dry nitrogen gas, re-dissolved in dichloromethane and analyzed using UV-Visible spectrophotometer. Specific acetoclastic methanogenic activity test was conducted as suggested by Valcke and Vestrate (1983)[13].

Seed sludge for this study was well acclimatized anaerobic mixed microbial culture, which was earlier used for the treatment of atrazine bearing wastewater in cometabolic process where dextrose was used as primary carbon source.

The seed sludge was taken in an aspirator bottle, fed with above composition of wastewater containing sodium acetate as primary carbon source at a concentration equivalent to 300 mg/l of chemical oxygen demand (COD) and operated in semi-continuous mode at an HRT of 5 days for a period of 3 months to increase the population of acetoclastic methanogens. In semi continuous mode of operation, everyday 20% of the treated effluent was replaced by fresh synthetic wastewater. No atrazine was added during this period. After 3 months, the MLSS (mixed liquor volatile suspended solid) concentration in the reactor was adjusted to 2000 mg/l and atrazine at a concentration of 5 mg/l was added to the wastewater. The reactor performance was evaluated in terms of COD reduction, gas production and atrazine reduction. The reactor was designated as reactor M-1. Two other reactors (R-0 and R-1) were operated in parallel with the reactor M-1 with similar feeding and operating except that the primary carbon source was dextrose. Reactor R-0 was operated as control reactor in which no atrazine was added to the feed. MLSS was kept same (2000 mg/l) to that of the reactor M-1. Reactors R-1 and M-1 were fed with wastewater containing 5 mg/l of atrazine. Performance evaluation of reactor M-1 operated in batch mode of operation was also evaluated. All the reactors were operated in a temperature controlled chamber at a temperature of 35±1°C. pH was maintained between 6.8-7.2 using appropriate amount of 1 N $NaHCO_3$ and dilute HCl.

Results and Discussions

Semi continuous mode of operation: The performance of all the three reactors was monitored in terms of COD reduction, methane gas production and atrazine degradation.

Reactor M-1 exhibited a COD reduction of 90.03% within 5days. The COD reduction in reactor R-0 and R-1 were 87.9% and 90.14% respectively. This shows that atrazine did not affect the performance of methanogens upto a concentration of 5 mg/L.

Methane gas production from the reactors was monitored continuously. The methane gas production (volume at 35°C) from the reactors M-1, R-0 and R-1 were 191,195 and 192 ml/g of COD reduction, respectively. This also shows that there was no inhibition of atrazine on the microbes up to a concentration of 5.0 mg/l. Specific acetoclastic methanogenic activity (SMA) tests were conducted to evaluate the methanogenic activity of the sludge from the three reactors. The methanogenic activity of the sludge in reactor M-1 was 142 mlCH_4/g VSS day. This value was almost the same as that obtained for the sludge from reactor R-0 and R-1. SMA of reactor sludge R-0 and R-1 was 138 and 129 mlCH_4/g VSS day respectively. This also shows that, the methanogens were equally active in all the three reactors irrespective of the primary carbon source or the atrazine concentration tried.

The atrazine removal from reactors R-1 and M-1 were monitored periodically. For an initial atrazine concentration of 5 mg/L, reactor M-1 showed a removal efficiency of 30.2% whereas reactor R-1 could degrade 48% of atrazine in 5days. This sows that dextrose stimulate atrazine biodegradation in anaerobic condition better than that of sodium acetate. Adsorption of atrazine was not observed in any of the reactor sludge. Presence of a combination of methanogenic and non methanogenic bacteria in reactor R-1 compared to reactor M-1 might be the cause of better atrazine removal in R-1. Three months operation of reactor providing sodium acetate as sole source of carbon and energy might have been decreased the population of non methanogenic bacteria. As methanogens can not metabolize the aromatic ring[9], roie of non methanogens is quite significant in the degradation of aromatic chlorinated compounds. Several reports also showed that glucose stimulate the metabolism of many chlorinated organic compounds better than other primary carbon sources[14]. Chung et al., (1996)[15] observed that dextrose stimulates the metabolism of atrazine more than organic carbon compounds supplied as primary carbon sources in anaerobic process.

Batch mode of operation: When reactor M-1 was operated in batch mode, 94% of COD removal was observed after 3 days and an atrazine removal of 64% was observed after 50 days. No atrazine was detected in the reactor sludge. This shows that the present system could achieve better atrazine degradation capacity than that observed by Chung et al., (1996)[15]. Chung et al., (1996) (15) observed less than 50% of atrazine removal

from sediment bioreactor after 38 weeks of incubation. Better removal efficiency in the present study might be due to the presence of large anaerobic microbial population.

Conclusion

No inhibition was observed on the anaerobic microorganisms enriched with methanogens in COD removal and methane gas production in presence of 5.0 mg/l of atrazine. Atrazine reduction was observed to be more in presence of dextrose than sodium acetate as primary source of carbon and energy.

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