Journal of Environmental Management 226 (2018) 448–456

Contents lists available at ScienceDirect

Journal of Environmental Management

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

Research article

Application of hybrid anaerobic reactor: Treatment of increasing cyanide containing effluents and microbial composition identification

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ARTICLE INFO

Keywords:
High strength cyanide effluents
Hybrid anaerobic reactor (HAR)
DGGE
Q-PCR
Statistical analysis

ABSTRACT

The study endeavors the anaerobic treatment of cyanide-containing effluents using the hybrid anaerobic reactor, with self-immobilized granules under high up-flow velocities. Comparison of one-year time-course analyses of HARs treating high strength effluents containing cyanide and control indicates the importance of wastewater characteristics in development and maintenance of microbiome. Efforts were directed towards associating process performance with microbial dynamics. Presence of cyanide results in the accumulation of intermediates paralleled with a drop in abundance of sensitive aceticlastic methanogens. HAR appear to have better resilience than other identified digesters because of shielding effects and enhanced granule-wastewater contact. The predominance of *Methanobacteriales* in the presence of cyanide can be linked to its tolerance. It was found that methane yield is positively correlated with abundance of aceticlastic guilds (R = 0.830, CI = 0.01). Tolerant bacterial groups were also identified. The study advances our knowledge related to less energy intensive technology with the focus on the development of efficient HAR.

1. Introduction

Concentration of cyanide in the environment has increased manifold in recent times. This can be attributed to increasing industrialization and urbanization. Owing to cyanide's high toxicity and great environmental impact, its effects have been widely studied. Cyanide enters water bodies primarily through metal finishing industries, iron and steel mills, runoffs, pesticides, electroplating industries, automobile parts manufacturing units, coal coking industries, etc. (Dash et al., 2009). The general limit of cyanide found in effluents from various industries is 10 mg/L (Novak et al., 2013). However, some electroplating and metal finishing plants store wastes, which may contain 10,000–30,000 mg/L of cyanide, for years. Due to high toxicity of cyanide, environmental protection agencies have imposed limiting standards for discharge of cyanide-containing wastewaters. The US Environmental Protection Agency (USEPA) constrains total cyanide in drinking water and aquatic-biota water to 200 ppb and 50 ppb respectively (Dash et al., 2009). In India, Central Pollution Control Board (CPCB) has set 0.2 mg/L as the minimal national standard (MINAS) limit for cyanide in effluents (Dash et al., 2009).

Several attempts have been made in the context of cyanide removal. The most commonly used methods are alkaline chlorination, biological oxidation process, copper-catalyzed hydrogen peroxide oxidation, ozonation and electrolytic decomposition (Chen et al., 2014). All these processes utilize hazardous chemicals which themselves warrant an extra level of management. Moreover, these processes are expensive and hence, most of them are used in developed nations to meet their discharge standards. For developing nations, a rather inexpensive and feasible treatment technology is required. For this purpose, biological methods seem to be the most cost-effective option. Biological processes efficiently convert cyanide into less toxic products, under both anaerobic and aerobic conditions. Many aerobic pathways have been studied and successfully implemented by researchers (Fallon, 1992; Onukwugha and Ibeje, 2013; Zaher et al., 2006). However, the aerobic route is energy demanding, which makes it economically infeasible. Moreover, methods based on an anoxic pathway are less effective in removing cyanide, as compared to both aerobic and anaerobic methods. Consequently, anaerobic technology provides an attractive option for cyanide removal.

Under anaerobic conditions, cyanide can be bio-degraded only via hydrolytic and reductive processes (Fallon, 1992). However, reductive pathways require nitrogenase enzyme for production of methane and release ammonia as the end products. Nitrogenase is rarely found in living organisms, limiting the occurrence of the reductive pathway for cyanide removal (Gupta et al., 2010). Thus, the hydrolytic pathway is the most commonly occurring process which can be carried out by five

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https://doi.org/10.1016/j.jenvman.2018.08.023
Received 30 December 2017; Received in revised form 1 August 2018; Accepted 6 August 2018
0301-4797/

different enzymes (i) cyanide hydratase, (ii) nitrile hydratase, (iii) thiocyanate hydrolase, (iv) nitrilase, and (v) cyanidase (Gupta et al., 2010). Anaerobic removal has become popular due to economic and environmental advantages. The foremost limitation in success of this approach is slower growth rate of sensitive anaerobes which gets further retarded in the presence of toxic/inhibitory pollutants (Parkin and Owen, 1986; Stronach et al., 1986). In two previous decades; literature reported few studies based on the anaerobic treatment of cyanide-containing effluents. A report on fixed bed methanogenic reactor treating cassava root wastewaters was published, which required six months of start-up phase to treat 10 ppm of cyanide. However, after biofilm establishment, the limit elevated up to 150 ppm (Siller and Winter, 1998). Another study demonstrated efficient COD removal in cyanide polluted water, beside possible acclimatization of biomass and cyanide removal (Gijzen et al., 2000). Investigations related to the metabolic pathways utilized during both aerobic and anaerobic technologies have also been reported by Ebbs (2004). Many related publications are available but, the understanding relating microbial structure to the presence of increasing cyanide, along with the reactor performances is missing (Annachhatre and Amornkaew, 2001; Chakraborty and Veeramani, 2006). A high rate UASB reactor, treating starch wastewater containing cyanide was reported (with an upper limit of 25 ppm of cyanide) without any disclosure about microbial guilds involved (Annachhatre and Amornkaew, 2001). In 2009, Dash et al. reported a detailed review on cyanide removal but failed to provide any information relating to anaerobic microbiome and the presence cyanide and removal efficiency of reactors. Later on, Naveen et al. (2011) identified various microorganisms capable of cyanide removal without providing any evidence related to reactor operation and microbiology. Recently, Novak et al. (2013) investigated biomass obtained from UASB treating brewery effluents containing cyanide, in the suspended system. For the first time, microbial analyses for both bacterial and archaeal constituents were reported. In 2014, Naresh K. Sharma and Philip further demonstrated the positive effects of acclimatizing anaerobic biomass with cyanide while treating coke oven effluents and investigated the dominant bacterial groups.

Adapting new and upgrading existing technologies is important for sustainable development. One such system developed at Department of Biochemical Engineering and Biotechnology, IIT Delhi is a novel hybrid anaerobic reactor (HAR). It uses self-immobilized microbial granules under completely fluidized conditions. Unlike, anaerobic fluidized bed reactor (AFBR) no carrier particle is used. Also, up-flow velocity is maintained above minimum fluidization velocity (> 4 m/h), unlike UASB system where it is > 1 m/h (Saravanan and Sreekrishnan, 2008). Thus, combining positive characteristics of both UASB as well as AFBR, individually the two have been successful in anaerobic wastewater treatment (Barros et al., 2010; Lettinga et al., 1980). HAR presents with pluses of both attached cell system and suspended cell system. Higher up-flow velocities result in enhanced sludge-wastewater contact resulting in the improvement of substrate transport efficiency. HAR advances technology with reduced reactor size without compromising mass transfer efficiency, similar to suspended cell systems. In consequence, it was possible to use HAR for low strength industrial effluents generated in Mangolpuri industrial cluster, Delhi, India (Kumar et al., 2008). Additionally, by providing favorable micro-environment inside the granules, it makes the system resilient to environmental changes. Importantly, granule development is related to the distribution of microbial groups which is dependent on the substrate used to grow them. This decides the structure of granules formed, i.e. layered or homogeneous (Saravanan and Sreekrishnan, 2008). Therefore, upon exposure to industrial effluents, these are affected due to detrimental effects on their granular structure related to the microbial composition.

The present work was taken up to understand the effects of increasing cyanide on the granulated biomass in HAR and its performance. For this high strength – high cyanide containing simulated effluent was used. Previously related experiments were carried out on the suspended cell systems and provided with real insights highlighting the importance of acclimatization and enrichment while treating industrial effluents (Chatterjee et al., 2012; Gupta et al., 2016a). However, as the resilience of high rate digesters is considered higher than the suspended cell systems, the present investigation furthered its study using HAR. Beginning with 10 ppm followed by a stepwise increase up to 70 ppm of cyanide, HAR performance was studied at various toxin concentration levels. Still, anaerobic cyanide degradation is not well comprehended, including the microbial communities involved (Novak et al., 2013). Therefore, the study extended its research on interconnections between microbiome compositions with reactor performance. By using molecular techniques different tolerant as well as sensitive groups were targeted, as their identification will benefit the efforts involved in technology advancement. Enriching and favoring such guilds will build up resistance against failure due to toxicity. It included comparison with identical control set-up to appreciate the changes involved due to cyanide exposure. The possibility of field application of HAR for anaerobic treatment of cyanide-containing effluents was also attended.

2. Materials and methods

2.1. Reactor design and experimental set-up

Two identical laboratory scale HARs (R_{CN} and R_{CO}) were glass fabricated, having a working capacity of 1.2 L. Influent was provided from the bottom of the reactor using a peristaltic pump. Recirculation pump was regulated to deliver superficial velocity between 4 and 5 m/h. All the connections were made using silicon tubes, and open ends were water sealed. For the start-up, both the HARs were seeded with granulated sludge and reactors were operated in the continuous mode for 180 days, until mature granules developed. This granulated sludge was obtained from the HAR operating in the laboratory since three months, which was seeded with anaerobic sludge obtained from Okhla wastewater treatment plant, New-Delhi and cow dung. After the formation of mature granules (1–5 mm in diameter size), R_{CN} was used to treat cyanide containing effluents while R_{CO} acted as control set-up. All the experiments were carried out in a walk in a temperature-controlled room, maintained at 37 °C. For both the reactors, hydraulic retention time (HRT) was maintained at 5 days, with organic loading rate (OLR) of 2 Kg COD/m^3/day.

1% glucose containing wastewater was used as the base medium with COD of 10,000 ppm (Gupta et al., 2016b). At one particular cyanide concentration level, R_{CN} was operated for five reactor volumes, i.e. 25 days. Cyanide was added from the 180th day of reactor startup, starting from 10 ppm to 70 ppm during the operation (Table 1).

Cyanide was added to the influent wastewater fed to R_{CN}, from the cyanide stock solution (100 ppm). It was prepared using cyanide standard solution ($K_2[Zn(CN)_4]$) of 1000 ppm obtained from Merck, India in double distilled water.

Table 1
Detailed understanding of cyanide addition in R_{CN} and naming of the samples obtained on the respective days, in both the reactors.

Day of reactor operation	Reactor treating Cyanide containing wastewaters (R_{CN})		Control reactor (R_{CO})
	Cyanide concentration to which reactor was exposed on the respective days (ppm)	Sample Name	Sample Name
180th	0	CN1	CO1
205th	10	CN2	CO2
230th	20	CN3	CO3
255th	30	CN4	CO4
280th	40	CN5	CO5
305th	50	CN6	CO6
330th	60	CN7	CO7
355th	70	CN8	CO8