Chapter 2

Development of a Bioremediation Technology for the Removal of Thiocyanate from Aqueous Industrial Wastes Using Metabolically Active Microorganisms

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Additional information is available at the end of the chapter

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1. Introduction

Contamination of water and soil environment due to the release of toxic and hazardous chemicals as a result of industrialization has taken its toll by causing environmental pollution. If not treated and managed appropriately, toxic and hazardous pollutants may cause severe detrimental (negative), reversible or irreversible, intangible and incapacitating impacts on all forms of living cells. Thiocyanate ($N{\equiv}C{-}S^-$) is one such known hazardous chemical and an important member of cyanide (CN^-) family. It is a simple inorganic and one carbon (C-1) compound. Despite its toxicity, it is introduced into the environment by natural (principally by biological cyanide detoxification processes) as well as industrial processes (Kelly and Baker, 1990; Wood, 1975). Thiocyanate (SCN^-) has some novel properties. It is linear in nature, electronegative polyatomic ion and a good example of pseudohalide; and therefore produced on a grand scale for its use in diverse industrial processes such as dyeing, acrylic fibre production, thiourea production, photo-finishing, herbicide and insecticide production, metal extraction and electroplating industries (Hughes, 1975). SCN^- is also known for its applications in soil sterilization and corrosion inhibition (Beekhuis, 1975). Consequently, these industries emanate large volumes of SCN^- bearing wastewaters. Apart from SCN^-, these effluents might contain other contaminants like heavy metals, cyanide (CN^-), metal-cyanides (M_xCN) and metal-thiocyanates (M_xSCN). Cyanide has the potential to reacts readily with sulphur to produce SCN^- and any industry with cyanide in its waste is a potential source of SCN^- contamination. Steel manufacturing, metal mining and electroplating units are some examples of such industries.

All the species of cyanide family (viz. thiocyanate, cyanide and their metal complexes) have potential to interacting with living cells and strong tendencies to connect to proteins and thereby acts as a non-competitive inhibitor (Westley, 1981). This fact necessitate the industries using and/or emanating SCN^- to adequately detoxify the effluents on priority basis before its discharge in soil and water environment; as it may pose detrimental implications on aquatic life. Moreover, in water scarce situations such untreated and partially treated wastewaters could not be recycled back into the industrial processes. The concentration of SCN^- arising from all the above mentioned sources is normally in the range of 5 - 110 mg/l (Mudder and Whitlock, 1984). Although many statutory agencies across the world have set the statutory limits for cyanide and heavy metal discharge, till date there are no such prescribed limits set or documented for SCN^- discharge. Earlier scientific studies indicate that in general, SCN^- is approximately 7 to 10 times less toxic then free cyanide species. The US-health service cites 0.01 mg/l as guideline and 0.2 mg/l as the permissible limit for cyanide species. In India, the Central Pollution Control Board (CPCB) had set a Minimum National Standard (MINAS) limit for cyanide as 0.2 mg/l. Therefore, the cyanide bearing effluents generated from industries needs suitable treatment to bring down the total cyanide levels below 0.2 mg/l. Taking into consideration the mentioned facts, standards for discharge of SCN^- could readily be deduced to 1 mg/l to be on the safer side. In order to minimize the risk of exposure to the public and aquatic ecosystems, the clean-up of SCN^- contaminated wastewaters is therefore necessary. Patil and Kulkarni (2008) have reported the environmental sensitivity and safety aspects in mining industries in regard to cyanide. Impact of cyanide species on fresh water fish Catla catla have also been reported (Prashanth and Patil, 2008).

Numerous technologies are currently employed to detoxify SCN^- bearing effluents; and the most widely being used is direct alkaline chlorination or addition of hypochlorite. However, this method produces large aggregates of chemical sludge, which does not have any further utilization and is environmentally hazardous to handle (Lanza and Bertazzoli, 2002). As per Indian regulations, such hazardous chemical sludge is transited from the industrial location to a specially designed Treatment, Storage and Disposal Facility (TSDF) thereby increasing the overall energy consumption, transportation cost and air pollution. Secondly, chlorination also fails to bring the concentration of SCN^- (and other CN^- species) within the statutory limits especially when heavy metals are present in the effluents. Thirdly, chlorination increases the total dissolved solids (TDS) content of the treated wastewaters, which makes it unfit for further use. Other physico-chemical processes like hydrogen peroxide oxidation, ozone oxidation, electrolytic decomposition, etc. are highly expensive and are rarely used for the treatment of SCN^-. Thus, there is a pressing need for the development of an alternative treatment process capable of achieving high degradation efficiency at low cost.

Bioremediation (biological treatment system) using metabolically active (live) microorganism is one such effective alternative for the detoxification of toxic chemical wastes. This process has immense potential of treating variety of pollutants (both toxic and non-toxic); has several advantages over conventional methods and therefore being explored by the researchers world-wide. Microorganisms capable of utilizing C-1 compounds like CN^- and

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M_xCNs are well documented and have been studied for long time (Dash et al., 2009; Gurbuz, et al., 2004; Karavaiko et al., 2000; Patil and Paknikar, 2000a; Patil and Paknikar, 2000b; Patil and Paknikar, 2001; Patil et al., 2012). Some research papers on biodegradation of SCN^- have also been reported (Chaudhari and Kodam, 2010; Hung and Pavlostathis, 1998; Patil, 2008a; Van Zyl et al., 2011). Use of metabolically passive (dead or inactive) microorganisms for the removal and recovery of metal-cyanides and SCN^- have also been reported (Gaddi and Patil, 2011; Patil, 2012; Patil and Paknikar, 1999; Thakur and Patil, 2009). Successful efforts to set-up large scale bioremediation technology for the treatment of cyanide, metal-cyanide and SCN^- from mining effluent have been made on commercial scale (Mudder and Whitlock, 1984). However, there are very few reports on the microbial SCN^- degradation from the process development point of view (Patil, 2006; Patil, 2008a; Patil, 2008b; Patil, 2011; Sorokin et al., 2001; Stratford et al., 1994). Moreover, utilization of SCN^- by microbes as a suitable growth substrate (carbon and/or nitrogen source) is poorly understood. Lack of scientific knowledge in this regard may pose problems in the biological treatment systems. The author in the present research chapter focuses on the development of a bioremediation technology for the removal of SCN^- from aqueous industrial wastes using metabolically active microorganisms.

2. Materials and methods

2.1. Analyses, chemicals and glassware

Potassium SCN^- (KSCN) was obtained from Qualigens, Mumbai, India. SCN^- assay was carried out spectrophotometrically (Spectronic, Model-20D, India) using ferric nitrate method at 460 nm as described in Standard Methods (APHA-AWWA-WEF, 1998). Digital pH meter (Elico, Model Ll-120, India) was employed to determine pH of solutions. Bacterial population from culture media, activated sludge and soil were determined microscopically (Metzer, India, METZ-778A) using Neubauer's chamber (Fein-Optik, Blankenburg, GDR) and by total viable count (TVC). Analytical grade chemicals were used for all experiments. Reagents were prepared in glass distilled water and stored under refrigerated conditions (8-10°C).

2.2. Enrichment and isolation of SCN^- degrading bacterial consortium

Enrichment culture and growth of mixed bacterial community (bacterial consortium) was carried out using M-9 minimal salts medium (MSM) (Patil and Paknikar, 2000a). One litre of medium contained $Na_2HPO_4.2H_2O$ - 3.0 g; KH_2PO_4 - 1.5 g; NaCl - 0.25 g; distilled water - 1000 ml and 1 ml/l trace metal solution (Bauchop and Elsden, 1960; Millar, 1972). The medium pH was adjusted to 7.5 using 1 M NaOH/HCl. Glucose (10 mM) was added as the sole source of carbon and energy. SCN^- (50 mg/l) was supplemented to the enrichment medium as the sole source of nitrogen. Enrichment culture for the isolation of SCN^- degrading microorganisms were set-up in aerobic and unsterilized conditions using activated sludge (obtained from secondary treatment of sewage treatment plant) and garden soil. Both the

samples were collected in clean polythene bags and carried to the laboratory. Two cylindrical glass jars (reactors) of 1200 ml capacity each were employed for the enrichment purpose. Working volume of the glass reactor was 1000 ml. 100 ml of activated sludge or 100 g of garden soil was added in 900 ml M-9 MSM containing SCN^- and glucose as the source of nitrogen and carbon, respectively in order to obtain the final concentration of SCN^- and glucose as 50 mg/l and 10 mM, respectively. Enrichment was carried out at the pH 7.5. Both the glass reactors were incubated at room temperature (30±2°C). Air was sparged continuously at the bottom of medium at the rate of 1000±50 ml/min using electrical aerator units. Seven to eight successive transfers of 10% solution enriched with bacterial flora were given periodically in the fresh M-9 MSM containing SCN^- and glucose as mentioned earlier (Patil, 2006; Patil, 2008a).

2.3. Purification and Identification of bacterial cultures

The enrichment cultures as obtained were streaked on nutrient agar medium and M-9 agar medium (containing SCN^- and glucose) plates in aseptic conditions at 35°C for 48-96 h. In all, six diverse types of bacterial colonies (three each from garden soil and activated sludge enrichment) appeared on the plates. The cultures were further purified and then transferred to nutrient agar and M-9 agar slopes. By way of periodic transfers, one set of bacterial consortium was consistently maintained in liquid medium (i.e. M-9 MSM) (Patil 2008a; Patil, 2008b). Further, the isolated bacterial cultures were subjected for microscopic examination (Gram staining and motility) and cultural characteristics on the nutrient agar plates. Bergey's Manual of Systematic Bacteriology (Holt, 1989) was used to identify the SCN^- degrading bacterial cultures up to genus level only.

2.4. SCN^- degradation efficiency of the isolated bacterial cultures

Quantitative studies on SCN^- degradation were performed to determine the efficiency of isolated cultures in their individual capacity and mixed form. Experiments were performed in 250 ml Erlenmeyer flasks containing 100 ml sterile M-9 MSM (pH 7.0) and 50 mg/L potassium thiocyanate (KSCN), which acted as a nitrogen source. 10 mM glucose was used as carbon source. Bacterial cell suspension of 0.1 ml containing 10^8 cells/ml were inoculated into the flasks and were incubated at 30°C in a rotary shaker incubator (Remi, India) at 150 rpm for 48 h. Requisite controls were used and experiments were performed in duplicates and repeated twice. SCN^- contents were determined periodically as mentioned earlier. SCN^- degradation efficiency of individual and mixed bacterial culture was expressed in terms of percent total SCN^- degraded in 48 h. Reaction rate and first order rate constant for SCN^- biodegradation were calculated experimentally using equation 1 and 2, respectively (Sellers, 1999).

$$\Delta C / \Delta t = k\, C \tag{1}$$

$$\ln C_t - \ln C_o = -\, k\, t\, \mu \tag{2}$$

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Where, C = SCN^- concentration (mg/l); t = time (h); k = rate of reaction / first order rate constant (h^{-1}); C_o= initial SCN^- concentration (mg/l) and C_t= SCN^- concentration at time t.

2.5. Utilization of SCN^- as the sole source of cellular nitrogen by bacterial consortium

Batch mode experiments on SCN^- biodegradation were conducted in aseptic conditions with 100 ml M-9 MSM in 250 ml conical flasks. The medium was augmented with carbon and nitrogen sources with different combinations as mentioned: - (i) potassium thiocyanate - KSCN (50 mg/l) as the sole carbon and nitrogen source or (ii) KSCN (50 mg/l) and glucose (10 mM) as the sole nitrogen and carbon, respectively or (iii) KSCN (50 mg/l) and NH_4Cl (1 mM) as the sole carbon and nitrogen, respectively or (iv) KSCN (50 mg/l), NH_4Cl (1 mM) and glucose (10 mM) as the nitrogen, nitrogen and carbon sources, respectively. All experiments were conducted at pH 7.0. Flasks were incubated at 30°C on a rotary shaker incubator (Remi, CIS-24 BL) at 150 rpm for 48-72 h (Patil, 2011).

2.6. Factors influencing SCN^- biodegradation

Series of batch culture experiments were conducted to investigate the influence of various parameters on SCN^- biodegradation. Experimental conditions used were as follows: 150 ml capacity Erlenmeyer flasks with 25 ml M-9 MSM containing SCN^- (50 mg/l) and glucose (10 mM). 1 ml of previously grown culture (for 24 h) having cell density of 10^8 cells/ml was used as inoculum. The flasks were incubated in stationary conditions. Impact of pH (5.0 - 9.0), temperature (20 – 45 °C), initial cell density (10^5 – 10^9 cells/ml) and glucose (1-20 mM) were checked by running different set of experiments, wherein, one parameter was varied keeping the others constant. Periodic analyses were conducted as mentioned earlier.

2.7. Biosorption of SCN^- by bacterial consortium at high cell density

Experiments were performed in 150 ml flasks. 50 ml aliquots of SCN^- (50 mg/l) adjusted to optimum pH (7.0) was contacted with bacterial consortium (10^8 cells/ml). The culture was inactivated by boiling for the period of 10 min prior to contact. The flasks were incubated at 30°C on a rotary shaker (150 rpm) for 24 h. Bacterial cells were separated by centrifugation at 1000 rpm for 10 min and the cell free supernatant was subjected to determine the residual SCN^- concentration.

2.8. Impact of cations and anions on SCN^- biodegradation

Batch experiments were performed under optimized conditions as described earlier. Impact of diverse cations, especially heavy metals (0.1 mM each) on biodegradation of SCN^- was studied. Metals were added as sulfate salts (range 0.1-1 mM). To study the influence of sulfates, additional sulfate was added to the medium as sodium sulfate. Chlorides were added as sodium chloride in the range of 1-10 mM, while cyanide was added as sodium cyanide (0.1-1 mM).

2.9. Degradation of SCN^- from industrial effluent by bacterial consortium

SCN^- effluent was synthetically prepared in the laboratory because of the difficulty in procurement of effluent from industry. This was to test the practical applicability of the microbial process for degradation of SCN^-. Batch experiments were performed as mentioned earlier under optimized conditions (pH 7.0, temperature 30°C and bacterial cell density of 10^8 cells/ml). Thiocyanate served as nitrogen source, while sucrose (COD 500 mg/l) was used as carbon source. Parameters such as pH, SCN^-, COD and soluble metal content were measured at regular intervals for a period of 48 h.

2.10. Treatment of SCN^- waste in a Continuous Treatment System (CTS)

Thiocyanate containing simulated was treated in a continuous treatment system (CTS) as shown in Fig. 1. The CTS comprised of cylindrical glass column (height, 24 cm; diameter, 8 cm and total volume 0.2 L) containing one-litre simulated SCN^- effluent (50 mg/l SCN^-) having COD of 500-600 mg/l. The consortium culture was inoculated at the level of 10^8 cells/ml (final cell density) and the contents of the reactor were stirred by sparging air at the rate of 1000 ml/min. The pH of wastewater supplemented with nutrients was adjusted between 7.0-7.3 (using 1 M $NaOH/H_3PO_4$) and then added from the top of the reactor by manual adjustment at the flow rate of approximate 40-50 ml/h as calculated from mass balance equation. The treated effluent was removed from the bottom at the same flow rate. The CTS was operated at ambient temperature (30±2°C) in continuous mode for over a period of 30 days (720 h) by periodically checking the influent and effluent water characteristics for pH, SCN^-, COD and cell count according to the method prescribed in Standard Methods (APHA- AWWA-WEF, 1998).

3. Results and discussion

3.1. Enrichment and isolation of SCN^- degrading bacterial consortium

Both the enrichment culture (garden soil and activated sludge) elucidated that the time incurred reduced significantly with each subsequent transfer cycle for complete disappearance of SCN^-. Time taken for biodegradation in first, third and fifth cycle was 100, 80 and 70 hours, respectively. After seventh cycle the time taken for biodegradation of >98% SCN^- was stabilized around 40-45 hours. Each subsequent transfer was given in fresh M-9 MSM soon after the SCN^- concentration reached to < 1 mg/l (efficiency ≥98%) in the previous cycle. The bacterial count was consistently $>10^8$ cells/ml during each transfer cycle. pH and total viable count (TVC) of garden soil prior to enrichment was 8.12 and 3.5×10^8 cells/ml, respectively; and for activated sludge it was 7.64 and $>1.2 \times 10^{10}$ cells/ml, respectively.

The main objective of the present work was to isolate bacterial cultures capable of degrading SCN^- from the aqueous industrial wastes. In order to accomplished this objective, activated sludge and garden soil was subjected to the most powerful tool called 'enrichment culture', which is popularly being used world across by the microbiologists to selected desired type

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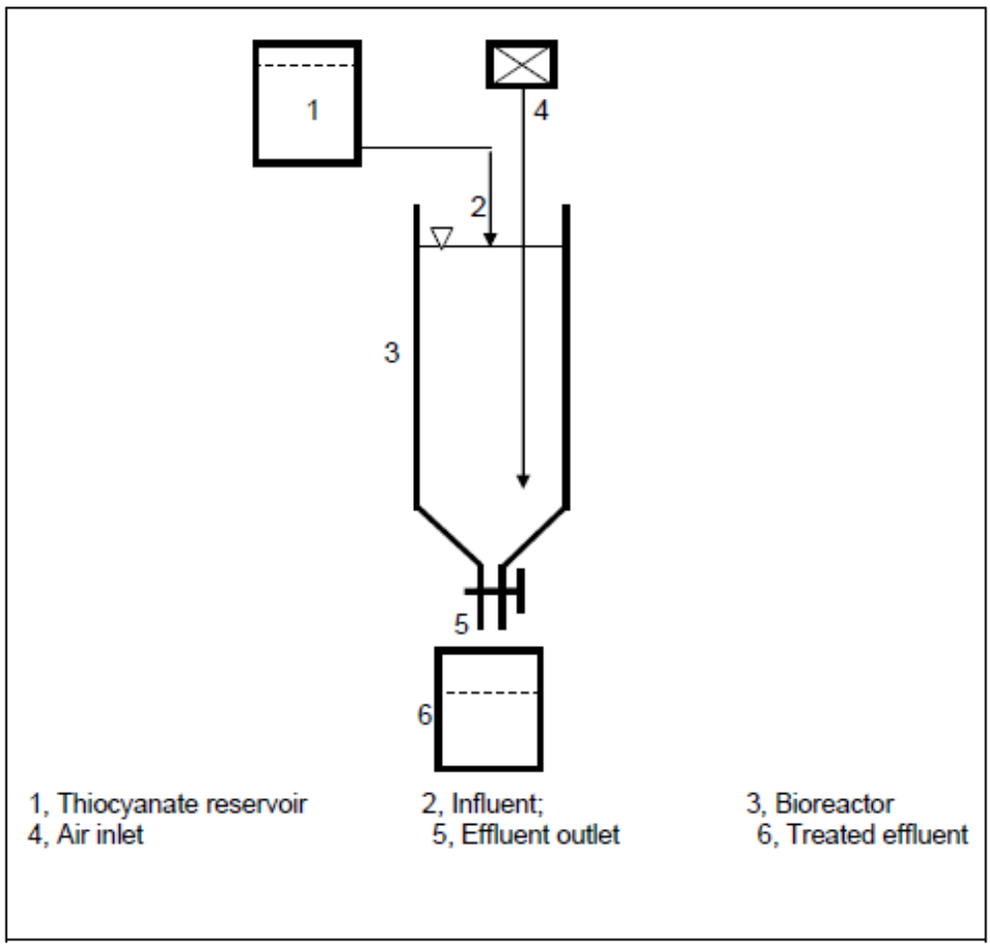

Figure 1. Schematic outline of laboratory scale Continuous Treatment System (CTS) for degradation of thiocyanate

of microorganims. There are reports of successfully utilizing this tool for the isolation of microorganism capable of degrading toxic and hazardous chemicals like SCN^- and M_xCN (Patil, 1999; Patil, 2008a; Sorokin *et al*., 2001). Reduction in time during each subsequent transfer could be explained by the fact that bacterial flora in the enrichment medium got gradually acclimatized to the hazardous chemical environment. High bacterial population (>10^8 cells/ml) in both the procured samples indicated the presence of substantial organic matter content and nutrient availability, thus giving enhanced probability to obtain SCN^- degrading cultures.

3.2. Purification and identification of bacterial cultures

In all, six heterotrophic bacterial cultures (three each from both enrichment cultures) capable of degrading SCN^- were isolated by enrichment technique subsequently followed by streak plate and spread plate technique that were employed for purification of cultures. Microscopic examination showed that all the six bacterial culture were Gram-negative rods and motile. Detailed cultural characteristics were previously reported by Patil (2008a). Based on cultural

and biochemical characteristics, all the six identified bacterial cultures belonged to the genus *Pseudomonas* as and reported by Patil (2008b).

The microbial source employed for enrichment culture for the isolation of thiocyante degrading microorganisms were garden soil and sewage sludge of STP. These sites did not have any past history of cyanide or SCN^- contamination. The prime objective was to test whether SCN^- degrading bacterial cultures could be isolated from such non-cotaminated sites and secondly to conduct a comparative assessment of the cultures isolated from two completely different niche areas. The fact that six SCN^- degrading cultures could be isolated from these samples indicates that SCN^- degradation is an intrinsic property of certain microorganisms and that no prior exposure is required to induce this property. SCN^- degrading ability of various heterotrophic and autotrophic microorganisms have been reported by few authors (Kwon *et al*., 2002; Patil, 2006; Sorokin *et al*., 2001; Stratford *et al*., 1994).

3.3. SCN^- degradation efficiency of the isolated bacterial cultures

Data in Table 1 depicts the wide variation of SCN^- degradation efficiency of the bacterial isolates. However, the bacterial consortium isolated from garden soil and activated sludge showed maximum degradation of SCN^- (>99.9%) in 42 and 36 h giving the SCN^- degradation rate constant (k) of 0.0931 and 0.1086 per h, respectively. In contrast, isolate-2 degraded only 75.9% of SCN^- in 48 h (k = 0.029 per h). It was also observed that the first order rate constant of bacterial consortiums was 2-3 folds higher than their individual isolates.

Source	Bacterial Isolates	% SCN^- degradation		Rate of Reaction (mg/l/h)	First Order Rate constant (per h)
		With culture	Control (without culture)		
Bacterial cultures isolated from garden soil	*Pseudomonas* sp. # 1	78.24	0	0.815	0.0317
	Pseudomonas sp. # 2	75.91	0	0.790	0.0297
	Pseudomonas sp. # 3	82.47	0	0.859	0.0362
	Bacterial consortium (1+2+3)	>99.9 (in 42 h)	0	1.189	0.0931
Bacterial cultures isolated from activated sludge	*Pseudomonas* sp. # 4	92.07	0	0.959	0.0528
	Pseudomonas sp. # 5	87.65	0	0.913	0.0435
	Pseudomonas sp. # 6	89.41	0	0.931	0.0467
	Bacterial consortium (4+5+6)	>99.9 (in 36 h)	0	1.387	0.1086

Table 1. SCN^- degradation efficiency of pure and mixed bacterial cultures (Conditions: pH 7.0; Temperature 30°C; Inoculum size 10^5 cells/ml; SCN^- conc. 50 mg/L; Glucose 10 mM; Agitation speed: 150 rpm; Incubation time: 48 h) (Patil, 2008b)

This experiment was conducted to ascertain the efficacy of bacterial cultures in their individual capacity and in consortium form. And the results clearly revealed that that consortium

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of bacteria were efficient compared to individual (pure) isolates (Table 1). These results confirmed the studies carried out by Patil and Paknikar (2000a) on biodegradation of copper- and zinc-cyanide using bacterial consortium. In this study, the consortium consisted of four bacterial isolates out of which three were *Pseudomonas* sp. and one was *Citrobacter* sp. The wide variation in SCN^- degradation efficiency among the cultures tested in the present study could be a manifestation of the natural diversity. Bacterial consortium isolated from activated sludge was more efficient than the consortium isolated from garden soil. Uninoculated controls did not show any decrease in SCN^- levels confirmed that biodegradation of SCN^- was the predominant reaction taking place during SCN^- degradation by the cultures. Experimental determination of reaction rate and first order rate constants are essentially needed because such data gives valuable information regarding the time requirement for reaction completion and the size of the treatment facilities that must be provided (Patil, 2008b; Sellers, 1999).

3.4. Utilization of SCN^- as the sole source of cellular nitrogen by bacterial consortium

This experiment was carried out only on bacterial consortium isolated from activated sludge because it was more efficient than the consortium isolated from garden soil. Detailed results of this experiment could be obtained from Patil (2011). Overall results are summarized as follows. It was established that when SCN^- was supplemented in M-9 MSM as the sole carbon and nitrogen, the consortium failed to utilize SCN^-. The SCN^- concentration of 50 mg/l remained unchanged throughout the tested period of 48 h. However, it was found that the bacterial consortium was capable of utilizing SCN^- as the sole source of cellular nitrogen in the presence external carbon source like glucose (10 mM) within 40 h with an efficiency of >99.9%. This was also confirmed from the control experiments run simultaneously. In the third combination, SCN^- when supplemented as the sole carbon source in the presence of external nitrogen like ammonium chloride resulted in complete cessation of consortium growth. In the fourth combination, when SCN^- was supplied in MSM along with external carbon (glucose) and nitrogen (ammonium chloride) source, showed an interesting diauxic growth (diauxie) pattern of the bacterial consortium as shown in Fig. 2. The bacterial consortium preferentially utilized ammonium chloride first until its depletion and only then switched over to the utilization of SCN^- as nitrogen source.

All bioremediation processes essentially depends on the availability of principal nutrients in the wastes that could potentially be utilized by the microorganisms as either carbon and/or nitrogen source. Elucidating this is crucial because if SCN^- is utilized by bacterial consortium as both carbon and nitrogen source, then at practical scale external supplementation of nutrients will not be required, thereby benefiting the industries economically those using microbial technologies for the effluents containing SCN^-, cyanide and metal-cyanides. However, in the present study, the SCN^- compound posed toxic problems to the consortium for growth utilizing it as suitable growth substrate. It is well known that concentration of nitrogen required for a given amount of growth is less than the requirement for carbon it might be easier for bacterial consortium to utilize SCN^- as the source of nitrogen in the presence of a separate source of carbon and energy (Patil, 1999). Therefore, enrichment culture was de-

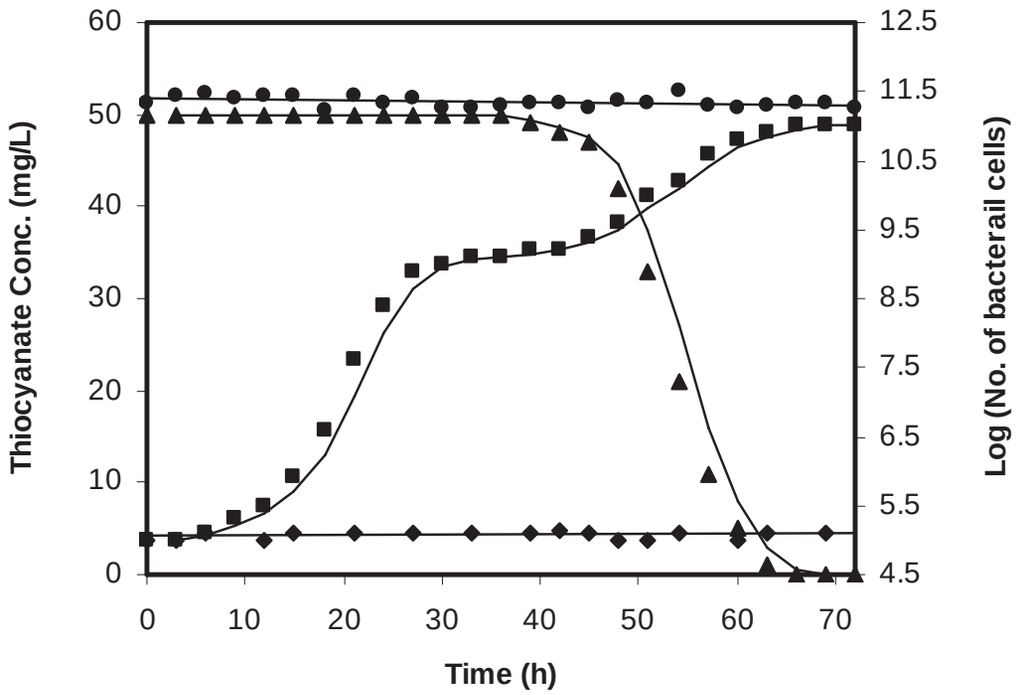

Figure 2. Diauxic growth pattern exhibited by bacterial consortium in the presence two nitrogen sources (viz. SCN^- and ammonium chloride) in the presence external carbon source (glucose). Growth of consortium (■) and SCN^- degradation (▲) in the presence of two N sources; SCN^- concentration in absence of consortium (●); Cessation of bacterial growth in absence of either nitrogen or carbon source (◆) (Patil, 2011)

signed/manipulated for the isolation of microorganisms capable of degrading SCN^- as the source of nitrogen nitrogen (Patil, 2006; Patil, 2008a). The experiments conducted explicitly proved that SCN^- is used by the consortium as nitrogen source in the presence of external carbon viz. glucose, thereby giving the C/N ratio of 10. In view of microbial process development, it is imperative to supplement some cheaper source of carbon like molasses, which is readily available in India at cheaper rate. Patil (1999) had successfully demonstrated the use of molasses as carbon source to develop a microbial technology for metal cyanide biodegradation/removal from wastes utilising it as the sole nitrogen source. There are few reports, which describe microbial SCN^- degradation utilising it as the sole nitrogen source (Bipinraj *et al.* 2003; Patil, 2011; Sorokin *et al.* 2001). The bacterial consortium ceased to grow when SCN^- was supplied as sole carbon source in the presence of external nitrogen. This could be attributed to the higher amount of available nitrogen compared to carbon (C/N ratio 0.5). Obviously the culture would find it more difficult to obtain sufficient amount of energy from low amount of carbon.

Example of diauxie pattern (biphasic growth) in *Escherichia coli* in the presence of two carbon sources (viz. glucose and lactose) is well documented (Atlas, 1997). In the present study, diauxic growth pattern was observed when two nitrogen salts (i.e. SCN^- and ammonium chloride) along with one carbon source (glucose) were supplied to the consortium. Ammonium chloride acted as preferred growth substrate by the consortium followed by SCN^- degra-

dation. This suggests that SCN^- utilization by consortium culture is inducible. It was also revealed that biodegradation of SCN^- took place rapidly (within 25 h) in second phase of growth after the exhaustion of ammonium chloride from medium in the first phase. The biomass that built-up in the first phase of growth was readily available in the medium for SCN^- degradation in the second phase, which ultimately led to the rapid biodegradation of SCN^-. This result immediately suggests its possible application in bioreactor designing that will retain large microbial biomass. Immobilization of the biomass in bioreactor using inert material will certainly hasten the process of biodegradation of toxic SCN^-.

Further, it could be also observed from the experiments that decrease of SCN^- concentration in the MSM was concomitant with the increase in bacterial population. The fact that the final cell density obtained was considerably high (>10^8cells/ml) indicated the use of well acclimatised SCN^- tolerant culture, having high SCN^- removal efficiency and therefore has immense potential of using the microbial technology on industrial scale. The treatment of wastewater involves a number of chemical and biological reactions and conversions. The rate at which these reactions and conversions occur decides the size of the treatment facilities that must be provided (Tchobanoglous and Burton, 1997). The study also showed that SCN^- degradation by the bacterial consortium isolated from activated sludge was comparatively more efficient than the consortium isolated from garden soil. This might be due to the acclimation/tolerance of sewage microorganisms to a variety of hazardous and non-hazardous waste contaminants/components naturally existing in it, thereby making them more tolerant and efficient degraders as compared to the microbial flora prevailing in garden soil.

3.4. Factors influencing SCN^- biodegradation

Factors influencing SCN^- biodegradation was restricted to the bacterial consortium isolated from activated sludge because of its high degradation efficiency compared to the consortium isolated from garden soil as mentioned earlier. Table 2 shows that degradation of SCN^- was significantly influenced by the various factors tested. Optimum pH and temperature for maximum SCN^- biodegradation (>99.9%) was found to be 7.0 and 30°C, respectively. Under the optimized conditions of pH and temperature, the initial cell density had a substantial influence on the biodegradation efficiency of SCN^-. With initial cell density of 10^8 cells/ml, SCN^- degradation process completed within 24 h with >99.9% efficiency. As regard to carbon source, the consortium culture exhibited maximum biodegradation efficiency only above glucose concentration of 5 mM.

In general, the SCN^- containing effluents released from various industries have pH in neutral to alkaline range. Our study showed that growth of SCN^- degrading bacterial consortium occurred in wide range of pH (6.0-9.0), while the optimum being 7.0. From practical applicability point of view very little or no pH adjustment would be required for the effluents containing SCN^-. Experiments also showed the unchanged pH of the solution after SCN^- biodegradation. This may be perhaps due to the formation of ammonia as one of the by-products of SCN^- degradation, which neutralized the accumulated carboxylic acids in the medium. These results corroborate with the studies carried out by other researchers on the

Parameter	Range selected	% SCN^- biodegradation	Parameter	Range selected	% SCN^- biodegradation
pH	Control*	0	Cell Density (cells/ml)	Control	0
	5	35.3		10^5	19.5
	5.5	43.0		10^6	47.1
	6	75.3		10^7	62.8
	6.5	84.6		10^8	>99.9
	7	>99.9		10^9	>99.9
	7.5	89.2			
	8	87.6	Glucose (mM)	Control	0
	8.5	84.5		0.1	6.2
	9	84.2		1	24.7
	9.5	56.9		5	>99.9
Temperature	Control	0		10	>99.9
	10	16.3		20	>99.9
	20	51.8			
	30	>99.9			
	37	36.3			
	45	0			

* Control indicates flask without culture; All the values are the average of two readings

Table 2. Degradation of SCN^- by a bacterial consortium isolated from activated sludge as a function of pH, temperature, initial cell density and glucose

biodegradation/biodetoxification free cyanide (Babu *et al*., 1993) and metal-cyanides (Patil and Paknikar, 2000b). The bacterial consortium used in the study was neutrophilic and mesophilic. Optimum temperature for thiocyanate degradation by bacterial consortium was 30°C. This is very important from the view point of actual applicability of the bioremediation process in a tropical country like India having ambient temperature ranging from 20-40°C. Results on the impact of inoculum size clearly showed that SCN^- degradation increased with the increase in inoculum size. Therefore, from the point of view of process development, it is essential to use a reactor capable of retaining high microbial biomass that will hasten the degradation of SCN^-. Results on the influence of glucose requirement for SCN^-degradation could possibly be explained on the basis of nutrient availability. Even though the available nitrogen in the form of SCN^- was ample the externally supplied glucose at concentrations < 5 mM limited the biodegradation process. However, at adequate glucose concentration of ≥ 5 mM complete degradation could take place. These findings confirm the utilisation of SCN^- as the sole source of nitrogen. In the previous studies carried out by Strat-

ford *et al.* and Wood *et al.* glucose was supplemented at the concentration of 10 and 25 mM for the degradation of 3 mM (≈ 174 mg/l) and 2.5 mM (≈ 145 mg/l) of SCN^-, respectively (Stratford *et al.*, 1994; Wood *et al.*, 1998). However, these reports did not mention optimisation of this parameter, which needs to be worked out for economizing the process. In another study carried out by Patil (1999) on the biodegradation of various metal-cyanides (copper-, nickel-, zinc- and silver-cyanide), glucose was required in the range of 1-5 mM (≈ COD 100 – 500 mg/l) (Patil, 1999). Scanty information is available on the biochemical pathway involved in SCN^- biodegradation. For heterotrophic bacterium, Stratford *et al.* has proposed the conversion of SCN^- to carbon dioxide and ammonia via cyanate by an inducible enzyme; while the sulphur moiety gets hydrolysed to sulphide, which further gets oxidised to tetrathionates via formation of thiosulphate (Stratford *et al.*, 1994). It might be possible that bacterial cultures isolated in the present study also have similar SCN^- removal or tolerant mechanism.

3.5. Biosorption of SCN^- by bacterial consortium at high cell density

It can be seen from Table 3 that the bacterial consortium had low biosorption efficiency (~7-14%) at the pH values tested (6.5 to 7.5). In fact it is possible that biosorbed SCN^- also could subsequently be biodegraded by the live culture used in the biodegradation process. These observations confirmed that biodegradation of SCN^- was the predominant reaction taking place during detoxification of SCN^- by the consortium culture isolated from activated sludge.

pH	SCN^- concentration (mg/l)		% Sorption
	Initial	Final	
6.5	53.29	48.22	9.51
7.0 (optimum pH)	50.94	45.97	13.68
7.5	51.03	47.50	6.91

*Optimum pH

Table 3. Biosorption of SCN^- by consortium culture

3.6. Impact of cations and anions on SCN^- biodegradation

Apart from SCN^- various metal cations and anions are normally present in the various industrial effluents. Therefore, the influence of some of the commonly occurring cations such as copper, cadmium, iron, lead, nickel, zinc and anions such as sulfates, chlorides and cyanide on SCN^- biodegradation was checked.

Table 4 shows the effect of various cations such as copper, nickel, zinc, cadmium, iron and lead on biodegradation of SCN^- in thiocyanate-metal system. It can be seen that biodegradation of thiocyanate was not affected in the presence of copper, nickel and zinc (degradation >90%). In presence of lead and cadmium the biodegradation efficiency was

reduced by approximately 30 and 45%, respectively. Chromium and iron significantly affected the degradation of SCN^- by >80%. Anions such as sulfates and chlorides (1000 μM and 10000 μM, respectively), and cyanide (0.1-1 mM) however, did not had much impact on SCN^- degradation.

Thiocyanate + cations/anions	% Thiocyanate biodegradation
Thiocyanate (Control without culture)	0
Thiocyanate (Control with culture)	>99
Thiocyanate + Copper	95.2
Thiocyanate + Nickel	>99
Thiocyanate + Zinc	90.4
Thiocyanate + Cadmium	56.2
Thiocyanate + Iron	17.9
Thiocyanate + Lead	69.5
Thiocyanate + Chromate	11.8
Thiocyanate + Sulfate	74.0
Thiocyanate + Chlorides	90
Thiocyanate + Cyanide	>98

Table 4. Effect of cations and anions onμSCN^- biodegradation

Biodegradation of the SCN^- was adversely affected in the presence of metals such as iron and chromium. In case of free cyanide, it is known that cyanide ion has a great tendency to act as a ligand and can thus be found associated with metal-complexes (Pohlandt *et al*., 1983). Cyanide complexes with different metals have widely varying stabilities depending on metal oxidation states (Cotton and Wilkinson, 1972), but can be broadly classified as weakly complexed cyanides and strongly complexed cyanides. The former group includes complexes with copper, cadmium, lead, nickel and zinc (Chapman, 1992) while the latter group consists of very stable hexacyanoferrates and chromium-cyanide (Lordi *et al*., 1980). Since the chemistry of free cyanide and SCN^- almost being similar, the heavy metals are capable of forming ligands with thiocyanate to form metal-thiocyanate complexes. The high stability of iron-thiocyanate and chromium- SCN^- might be the reason for poor degradation efficiency observed in presence of these ions.

3.7. Degradation of SCN^- from industrial effluent by bacterial consortium

The SCN^- containing effluent simulated in laboratory could be effectively treated by the bacterial consortium with a degradation efficiency exceeding >99.9%. The time incurred for the complete biodegradation of SCN^- from waste waters was less than 24 h. Table 5 shows the parameters such as pH, total cyanide, COD and metal content before and after biodegrada-

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tion. COD removal was more than 80%. It was also observed that the level of soluble copper, zinc, silver and nickel was reduced to less than 5 mg/l. There was no significant change in the pH after biodegradation.

Parameter	Simulated Industrial Effluent concentration (mg/l)			% Removal efficiency
	Before biodegradation	After biodegradation	Uninoculated control	
Color	Colourless	Colourless	Colourless	-
Turbidity	Clear	clear	Clear	-
pH	7.3	7.7	7.4	-
Thiocyanate	51.5	< 0.1	52.4	>99
Copper	12.5	1.92	13.1	84.6
Nickel	8.1	0.55	8.0	93.2
Zinc	18.3	2.17	16.9	88.1
Iron	3.9	0.1	4.1	97.4
Sulfates	55	57	62	-
Chlorides	42	45	50	-
Cyanide	5.2	0.13	4.7	97.5
COD	< 550	97	600	82.3

All the values given in the table are in mg/l, except pH

Table 5. Composition and biodegradation of simulated industrial effluent

The microbial process for degradation of thiocyanate was found to be highly effective in the detoxification of simulated industrial effluents. The levels of total thiocyanate, COD and metals could be brought down below the statutory limits as per Indian Standards (IS: 2490-1981). The treatment of effluent required more time as compared to the synthetic solutions. It is known that the applicability of any such process to real effluents is always complicated by the fact that effluents contain a variety of other contaminants which might interfere with or prolong the detoxification process. However, it must be emphasized that the microbial process described was highly efficient, safe and environment-friendly. In addition, the process had the potential of becoming economically attractive if scaled-up to a sufficient level, especially as a continuous operation. Therefore, it was decided to further develop the process in continuous mode and evaluate its performance.

3.8. Treatment of SCN^- waste in a Continuous Treatment System (CTS)

Studies in CTS showed that SCN^- level in the treated effluent was consistently below 0.1 mg/l for over a period of 30 days. The HRT of CTS was constant during the treatment period around 20 h. A closer monitoring of the CTS revealed that further reduction in the HRT was

not possible because the bacterial cells could not be retained in the system. The COD removal efficiency after treatment was >75% for thiocyanate effluent (Table 6).

Parameter	Influent	Effluent	Bureau of Indian Standards (BIS)
pH	7.0 - 7.3	7.5 - 7.7	5.5 - 9.0
Thiocyanate	51.67 ± 2.1	0.03± 0.01	NA
COD	596 ± 103	147 ± 41	250

All the figures in the table are expressed in mg/l, except pH; *HRT of the system was ~20 h. Figures represent average values of 30 readings taken each at 24 h interval

Table 6. Treatment of metal-cyanide waste waters in CTS

The results of CTS showed that SCN^- was degraded efficiently by the bacterial consortium with the minimum hydraulic retention time (HRT) of approximately 20 h. However, there was no reduction in the HRT of CTS further. The main reason for this was the continuous loss of active biomass from the reactor, which makes it unattractive from process economics point of view. This necessitates the immobilization of the bacterial consortium in the reactor. In principle, it is possible to retain active biomass in CTS if the culture used has an inherent property of producing wall growth. Further, biomass retention is also possible by changing the reactor design, introducing inert support material or changing nutrient supplementation, etc. However, the consortium culture used in the present studies did not show wall growth. Also, in our studies during optimization of process parameters it was conclusively proved that degradation efficiency increased with the increase in cell number, which in turn hastens the degradation of SCN^-. Thus, the above results emphasize the fact that the bioremediation process developed during the course of present work is highly efficient and completely safe. After further scale-up the bacterial process developed could have the following advantages: (i) no sludge generation; (ii) no expensive chemical additives required; (iii) very little or no pH adjustment required; (iv) the process would be easy to operate and maintain. Thus, the bacterial process developed could have the potential of becoming an economical and reliable alternative to the conventional processes employed for the treatment of SCN^- bearing industrial effluents on a commercial scale.

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