ijCEPr

International Journal of Chemical, Environmental and Pharmaceutical Research

Vol. 2, No.1, 44-48
January-April, 2011

Utilisation of Thiocyanate (SCN^-) by a Metabolically Active Bacterial Consortium as the Sole Source of Cellular Nitrogen

Yogesh B. Patil
Department of Energy and Environment, Symbiosis Institute of International Business (SIIB)
[A Constituent of Symbiosis International Institute (SIU)], G. No. 174/1, Rajiv Gandhi Infotech Park, Hinjewadi, Pune – 411 057, Maharashtra, India.
E-mail: dr_ybpatil@rediffmail.com

Article History:
Received:26 April 2011
Accepted:28 April 2011

ABSTRACT

Thiocyanate (SCN^-), a toxic chemical species from cyanide family, consists of both carbon (C) and nitrogen (N) in equimolar ratio and is being emanated through liquid effluents by several industrial processes. Since bioremediation technologies for waste treatment are gaining enormous importance in the recent times; microbial treatment of SCN^- is therefore being researched worldwide. However, utilisation of SCN^- by microbes as a suitable growth substrate (C and/or N source) is poorly understood and presents the problem in waste treatment systems. A heterotrophic bacterial consortium comprising of three *Pseudomonas* sp. isolated from activated sludge and having potentials for environmental clean-up, was capable of utilising thiocyanate (SCN^-) from aqueous solutions as the sole source of cellular nitrogen (N) in the presence of carbon (C) source viz. glucose. The consortium ceased to grow and degrade SCN^- when supplemented with C and N sources alone. Diauxie pattern was observed when the consortium culture was supplied with two N sources (NH_4Cl and SCN^-) in the presence of glucose as a source of carbon (C). NH_4Cl was the preferred growth substrate utilised by the bacterial consortium followed by SCN^-.

Keywords: Biodegradation, Bacterial consortium, Diauxie, Nitrogen source, Thiocyanate.

INTRODUCTION

Industrial processes like metal extraction, dyeing, photo-finishing, thiourea, pesticide production and electroplating industries produce large quantities of thiocyanate (SCN^-) bearing effluents [6]. The concentration of SCN^- in these effluents is in the range of 5 to 100 mg/L. Since SCN^- is toxic to all living cells [14], it is imperative for the industries to detoxify the effluents prior to their discharge in environment. Several physical-chemical technologies have been reported for the SCN^- removal [4]; the most widely being used is chlorination. However, conventional methods are beset with problems that are environmentally hazardous and fail to bring SCN^- level within safe limits. Moreover, SCN^- content in the waste inhibits the degradation of other pollutants (like cyanide and metal-cyanides) present in the waste, and therefore, has detrimental impact on aquatic flora and fauna. It is, thus, necessary to develop an alternative treatment process capable of achieving high degradation efficiency at low-cost. Bioremediation technologies using microorganism for detoxification of waste chemical is an eco-friendly alternative. Considerable literature is available on the removal of toxic C-1 compounds like free cyanide and metal cyanides by metabolically active [5,9] and metabolically inactive (passive) microorganisms [8]. In the recent times Thakur and Patil (2009) had reported the removal of SCN^- from solutions using low-cost waste biomass [13]. Although several reports are available on microbial SCN^- degradation [4,11,12], utilisation of it by microbes as a suitable growth substrate (C and/or N source) is poorly understood. Lack of scientific knowledge in this regard may pose problems in the biological treatment systems. The present paper highlights some key laboratory experiments that confirm the degradation of SCN^- by a heterotrophic bacterial consortium utilising it as the sole source of cellular N in the presence of external carbon (C).

MATERIALS AND METHODS

A heterotrophic bacterial consortium comprising of three *Pseudomonas* species and capable of utilising SCN^- was isolated from activated sludge by an enrichment culture technique [11]. The consortium was grown for 24 - 48 h in M-9 minimal salts medium – MSM [7] with 1 ml/L micronutrient solution [3], which contained SCN^- (50 mg/L ≅ 1 mM) and glucose (10 mM) as the sole N and C & energy sources, respectively. The medium was totally free of synthetic organics like peptone, beef extract and yeast extract. Bacterial cell suspension (0.1 ml) containing 10^8 cells/ml was used as an inoculum. Batch culture experiments on SCN^- biodegradation were performed under aseptic

and aerated conditions in 250 ml Erlenmeyer flasks with 100 ml M-9 MSM. The medium was supplemented with C and N sources with various permutations and combinations as shown in Table 1.

Table-1: Experimental manipulation of carbon and nitrogen sources in various proportions for bacterial growth

Combinations	Carbon Source	Nitrogen Source	Overall C/N Molar Ratio in M-9 MSM
A	Potassium thiocyanate i.e. KSCN (50 mg/L ≅ 1 mM)	KSCN (50 mg/L)	1
B	Glucose (10 mM)	KSCN (50 mg/L)	11
C	KSCN (50 mg/L)	Ammonium chloride i.e. NH_4Cl (1 mM)	0.5
D	Glucose (10 mM)	KSCN (50 mg/L) + NH_4Cl (1 mM)	5.5

The pH of medium before starting the experiment was adjusted to 7.0. All the flasks were incubated at 30°C in a rotary shaker incubator (Remi, CIS-24 BL) at 150 rpm for 48-72 h. Suitable controls were run simultaneously along with experimental flasks to detect air stripping or auto-oxidation of thiocyanate, if any. Experiments were repeated twice to confirm the results. Analytical grade chemicals were used in all the experiments. Reagents were prepared in RO (Sartorius, Arium-61315) water (conductivity <5 µS) and refrigerated (4°C). SCN^- content was measured spectrophotometrically (Spectronic-20D) as per the Standard Methods [1]. pH was determined using pH meter (Elico, Ll-120). Bacterial population was checked microscopically (Metzer, 778A) using Neubauer's chamber (Fein-Optik, Blankenburg) and by total viable count (TVC) procedure.

RESULTS AND DISCUSSION

The data in Fig. 1 shows the growth of bacterial consortium when SCN^- was supplemented in the medium as both C and N source. The results clearly indicated that the consortium failed to utilise SCN^- as either C or N source. The SCN^- concentration and bacterial population remained constant throughout the experimental period of 45 h. The data in Fig. 2 depicts that the bacterial consortium was capable of utilising SCN^- (with an efficiency of > 99%) as the sole source of cellular N in the presence external C source like glucose, wherein increase in bacterial cell density from 10^5 to $>10^8$ cells/ml was observed with simultaneous decrease in SCN^- level from 50 to < 0.1 mg/L. In uninoculated control, the SCN^- level remained unaltered (Fig. 2). SCN^- when supplemented with NH_4Cl, inhibited growth of bacterial consortium (Fig. 3). Fig. 4 shows diauxic growth (diauxie) pattern of the bacterial consortium when SCN^- was supplied along with C and N sources (Glucose and NH_4Cl). It was observed that when SCN^- and NH_4Cl were supplied, the consortium did not utilise both N sources simultaneously. The consortium initially utilised NH_4Cl and then SCN^- as N source. The growth of consortium in the first 25-30 h and the unchanged levels of SCN^- in the same period confirmed utilisation of NH_4Cl during first phase and later the SCN^-. This resulted in a biphasic (two phase) growth pattern known as diauxie / diauxic growth (Fig. 4).

The prime objective of the present work was to elucidate the potentials of isolated bacterial consortium for its utilisation of SCN^- from aqueous solution as (i) both C and N source or (ii) only C source or (iii) only N source for growth. Revealing this fact is important in biological treatment of wastewater because if toxic chemical like SCN^- is used by the consortium as both C and N source, then at practical scale, external supplementation of nutrients wouldn't be required, which in author's opinion, would be beneficial from the economic point of view to the user industries. This holds true even for the wastewaters containing other toxic compounds like cyanide and metal-cyanides. However, in the present study, the bacterial consortium failed to utilise SCN^- as both C and N source (C/N molar ratio=1). This fact clearly indicates that toxic SCN^- compound presents problem to the consortium for growth utilising it as suitable substrate (Fig. 1). Thus, a sufficiently high concentration of SCN^- to support appreciable growth might prove to be too toxic to allow growth to occur. Since the concentration of N required for a given amount of growth is less than the requirement for C, it might be easier for bacterial consortium to utilise SCN^- as the source of N in the presence of a separate source of C and energy. Therefore, microorganisms capable of degrading SCN^- as the source of N were isolated by an enrichment technique [11]. The consortium in the present study clearly showed (Fig. 2) the utilisation of SCN^- as the sole N source in the presence of external C source like glucose (C/N molar ratio=11). Therefore, from the process development point of view, it is essential to supplement some cheaper

source of C like molasses, which is readily available in developing country like India at cheaper rate. In the earlier studies, Patil (1999) had successfully demonstrated the use of molasses as the source of C to develop a microbial technology for metal cyanides biodegradation/removal from wastes utilising it as the sole source of N [10]. Uninoculated controls run simultaneously along with the experiments did not show any decrease in SCN^- levels (Fig. 2) confirmed that biodegradation of SCN^- was the predominant reaction taking place by the bacterial consortium. There are a few reports, which describe microbial SCN^- degradation utilising it as the sole N source [4, 12]. When SCN^- was supplied as the sole C source in the presence of external N (viz. NH_4Cl), the consortium ceased to grow keeping the 50 mg/L of SCN^- amount unaltered (Fig. 3). This might be due to the higher amount of available N (2 mM) compared to the C (1 mM) source (C/N molar ratio=0.5). Obviously, the consortium culture will find it more difficult to obtain the energy from low amount of C and utilise the toxic SCN^-. Diauxic (Biphasic) growth pattern was observed (Fig. 4) when two N salts (i.e. SCN^- and NH_4Cl) along with one C source (glucose) were supplied to the consortium. NH_4Cl was the preferred growth substrate utilised by the bacterial consortium followed by SCN^- degradation suggests that SCN^- utilisation by consortium is inducible. Diauxie pattern in *Escherichia coli* (and many other microorganisms) in the presence of two C sources viz. glucose and lactose is a well known example [2]. However, there are no reports on the diauxie pattern when two N sources like SCN^- (a toxic compound) and NH_4Cl are supplemented and therefore, this paper may be considered as the first report. In the present study, the diauxic growth experiments (Fig. 4) showed rapid removal/decrease of toxic SCN^- from the medium within 25 h. This was because in the first phase of diauxie, the consortium grew to a substantial level (cell density from initial 10^5 to final $>10^8$ cells/ml) utilising glucose and NH_4Cl. After the exhaustion of NH_4Cl from the medium, the consortium in the second phase (after a lag of 10-12 h) degraded toxic SCN^- rapidly utilising it as the sole N source in the presence of glucose (10 mM) as C. It has not escaped through author's notice that high initial cell density leading to rapid SCN^- biodegradation in diauxie experiment, immediately suggests its possible application in wastewater treatment reactor vessels capable of retaining high microbial biomass by way of immobilisation, which is the key to hasten biodegradation of SCN^-.

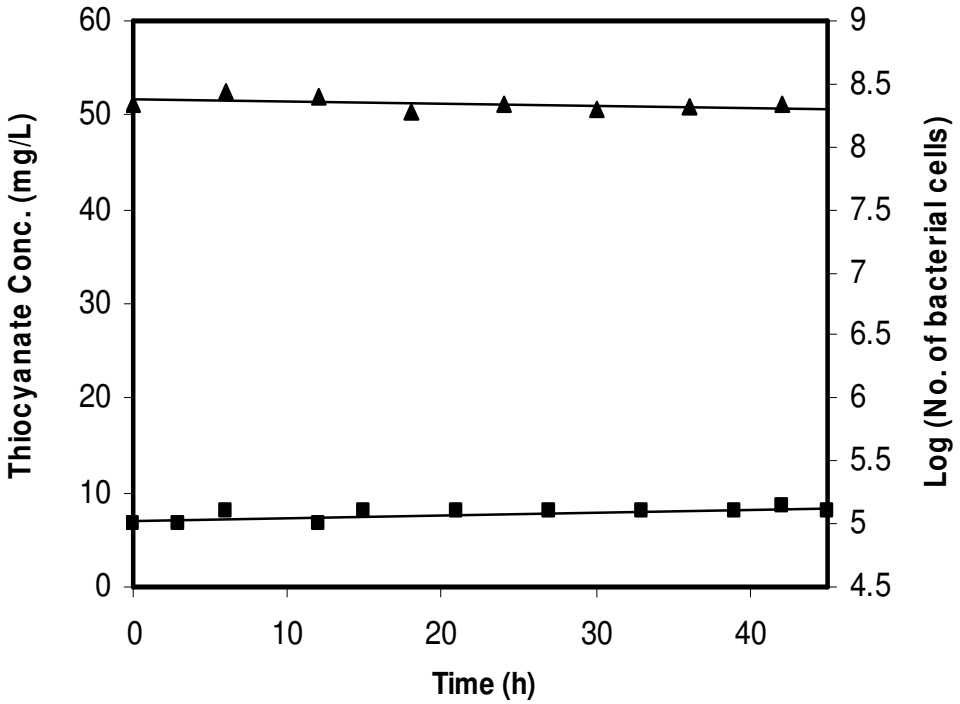

Fig.-1: Supplementation of SCN^- as both C and N source. Cessation of bacterial growth (■) and unaltered SCN^- concentration (▲)

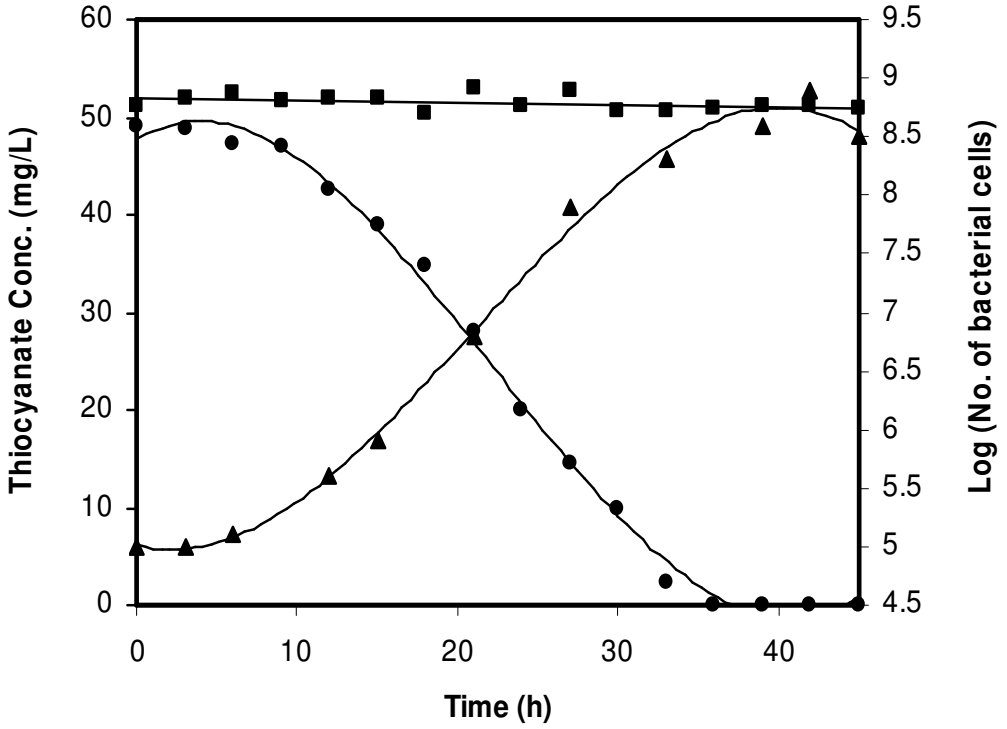

Fig.-2: Utilisation/Degradation of SCN^- by bacterial consortium as the sole source of N in presence of external C source. Growth of bacterial consortium (▲) with simultaneous decrease in SCN^- concentration (●); SCN^- concentration in the absence of consortium (■)

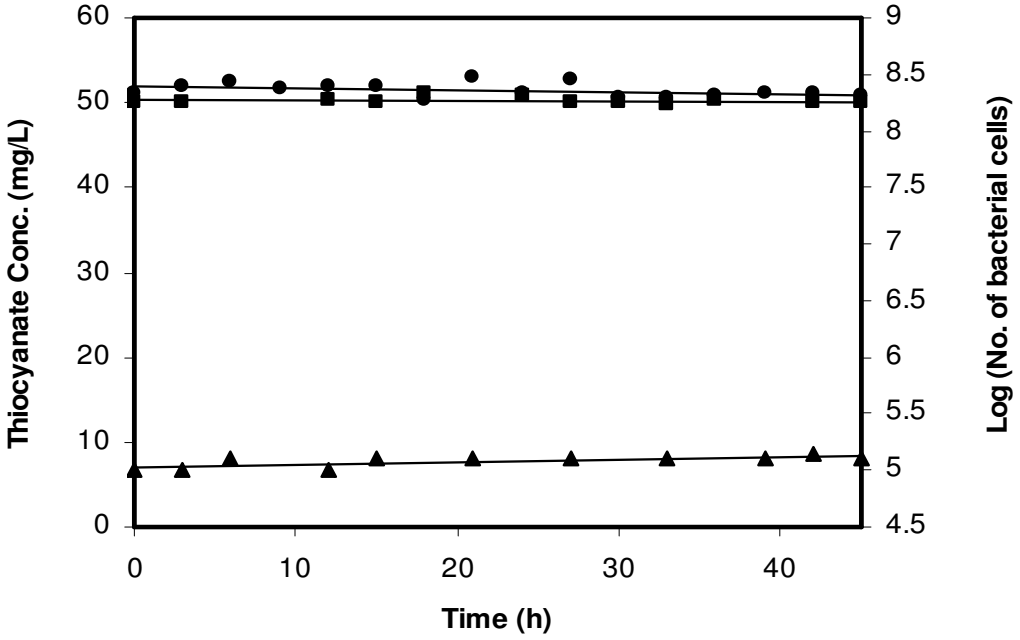

Fig.-3: SCN^- as the sole C source with external supplementation of N (NH_4Cl). Growth of bacterial consortium (▲); SCN^- concentration in presence (●) and absence of consortium (■)

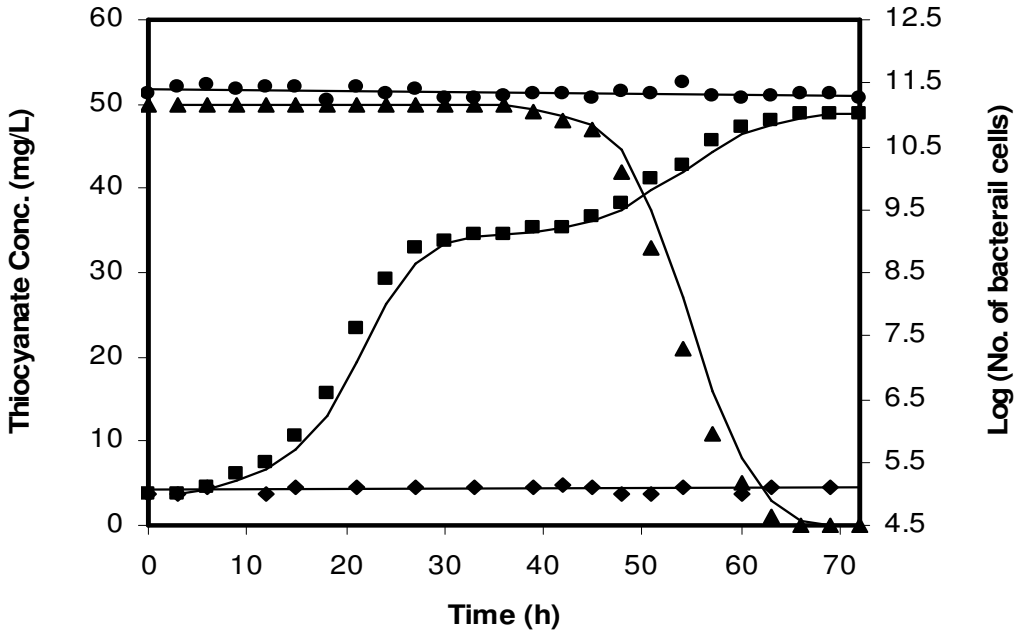

Fig.-4: Diauxic growth pattern exhibited by bacterial consortium in the presence two N sources (SCN^- and NH_4Cl) in presence of glucose as C source. Growth of consortium (■) and SCN^- degradation (▲) in the presence of two N sources; SCN^- concentration in absence of consortium (●); Cessation of bacterial growth in absence of either N or C source (♦)

CONCLUSION

Foregoing experimental results and discussion conclude that the bacterial consortium isolated from activated sludge is capable of utilising/degrading SCN^- as the sole source of cellular N from aqueous solutions in the presence of external C source.

ACKNOWLEDGEMENT

This research was supported by University Grants Commission (UGC), WRO, Pune through a grant to YBP.

REFERENCES

1. APHA, AWWA, WPCF, Standard Methods for the Examination of Water and Wastewater, Washington, DC, 18th Edn. (1998).
2. Atlas, R.M., Principles of Microbiology, McGraw-Hill, New York, (1997).
3. Bauchop, T., Elsden, S.R., General Microbiology, **23** (1960) 457.
4. Bipinraj, N.K., Joshi, N.R., Paknikar, K.M. Biohydrometallurgy: A Sustainable Technology in Evolution, *In: International Biohydrometallurgy Symposium,* Athens, (2003) 491.
5. Gurbuz, F., Ciftci, H., Akcil, A., Karahan, A.G. Hydrometallurgy, **72** (2004) 167.
6. Hughes, M.N. General Chemistry, In: Newmann, A.A., (Ed.), Academic Press, London (1975).
7. Millar, J.H., Experiments in Molecular Genetics, Cold Spring Harbour, NY: Cold Spring Harbour Laboratory (1972).
8. Patil, Y.B., Paknikar, K.M., Biotechnology Letters, **21** (1999) 913.
9. Patil, Y.B., Paknikar, K.M., Process Biochemistry, **35** (2000) 1139.
10. Patil, Y.B., Ph.D. Thesis, University of Pune, Pune, India (1999).
11. Patil, Yogesh B., Research Journal of Chemistry and Environment, **12(1)** (2008) 69.
12. Sorokin, Dimitry Y., Tourova, Tatyana P., Lysenko, Anatoly M., Kuenan, Gigs J., Applied and Environmental Microbiology, **67(2)** (2001) 528.
13. Thakur, Ravindra Y., Patil, Yogesh B., South Asian Journal of Management Research, **1(2)** (2009) 85.
14. Westley, J. Cyanide and Sulfane Sulfur. In: Vennesland, B., (ed.), Academic Press, London (1981) 201.

[IJCEPR-163/2011]