Desalination and Water Treatment
www.deswater.com
1944-3994/1944-3986
doi: 10.1080/19443994.2012.747647

51 (2013) 2474–2479
February

Taylor & Francis
Taylor & Francis Group

Performance aspects of *Paracoccus pantotrophus* treating urban solid waste leachate

Vijaya Singh[a,*], Atul K. Mittal[b]

[a]*Environmental Engineering Laboratory, Department of Civil Engineering, Indian Institute of Technology Delhi, New Delhi 110016, India*
Tel. +91 11 2659 6443; Fax: +91 11 2658 117; email: vijaya.singhin@gmail.com
[b]*Department of Civil Engineering, Indian Institute of Technology Delhi, New Delhi 110016, India*

Received 30 November 2011; Accepted 21 August 2012

ABSTRACT

Municipal landfill leachate remains one of the most challenging substrates to be biologically processed. This is the first study to report the behaviour of a mixotrophic bacterium *Paracoccus pantotrophus* which can simultaneously remove carbon and nitrogen. Both synthetic media and leachate have been used as substrate. Different leachate fractions, i.e. 0, 10, 20, 30, 40 and 50% were investigated. Total initial COD was 1,000 mg/L, which was reduced to 106–204 mg/L as leachate fraction varied from 0 to 50%. Removal of NH_4^+ nitrogen was 20–38%. Inhibitory effects of the leachate on the microbial growth and substrate utilization were observed from the maximum microbial growth and sustenance of the biomass. Sustenance of the biomass which was estimated using duration of the stationary phase varied from 124 to 25 h as leachate fraction varied from 0 to 50%. COD levels in all the reactors containing leachate started to increase after 150 h. Such increase continually increased with higher leachate fractions. It indicates the cell lyses and the release of secondary metabolites in the system. These data could be profitably used in a better understanding of the overall process kinetics.

Keywords: Batch reactor; Landfill leachate; Microbial growth; *Paracoccus pantotrophus*

1. Introduction

Leachate is considered to be a complex and highly toxic polluting material generated by the landfills. Leachate are highly contaminated and have much higher concentrations of both organic matter and nitrogen content compared with domestic sewage, besides having other organic and metallic pollutants. In the absence of adequate treatment measures, leachate may cause contamination of groundwater and surface water, with associated deleterious impacts on aquatic ecosystems. Therefore, it becomes necessary to treat leachate before their discharge. Mainly, removal of nitrogen and organic material based on COD, BOD and ammonia is the usual prerequisite before discharging the leachate into natural waters.

Paracoccus pantotrophus (previously known as *Thiosphaera pantotropha*), a heterotrophic nitrifier and aerobic denitrifier, is reported to simultaneously remove carbon and nitrogen from the wastewater. The bacterium has a respiratory metabolism and can use oxygen, nitrate, nitrite or nitrogen oxide as the terminal electron acceptor making it capable of converting most

*Corresponding author.

of its oxidation products into gaseous nitrogen products. *P. pantotrophus* is among the dominant mixotrophs, which can grow autotrophically, heterotrophically and mixotrophically under both aerobic and anaerobic conditions. *P. pantotrophus* was initially isolated by Robertson and Kuenen [1] during studies on a desulphurizing, denitrifying effluent treatment system. This isolate was found capable of oxidizing reduced sulphur compounds and hydrogen while fixing carbon dioxide. In view of its ability to oxidize reduced sulphur compounds and because it is a chain-forming coccus, the isolate was given the generic name *Thiosphaera* and the species name *pantotropha* in recognition of its wide range of potential substrates. However, a comprehensive study by Rainey et al. [2] resulted in re-designation of the organism as *P. pantotrophus*.

P. pantotrophus is a nonmotile, Gram-negative coccus ($0.7 \times 0.9\,\mu m$), which is frequently seen in pairs or long chains. It can grow over a pH range of 6.5–10.5, with an optimum at 8.0; the temperature range permitting growth lies between 15 and 42˚C with an optimum at 37˚C. It was grown and preserved on agar plates by Robertson in a medium prescribed by Taylor and Hoare [3] for *Thiobacillus denitrificans*, but with phenol red omitted. The medium [4] contained the following (in g/L): Na_2HPO_4, 7.9; KH_2PO_4, 1.5; NH_4Cl, 0.3; and $MgSO_4{\cdot}7H_2O$, 0.1; and 2 ml trace metal solution containing the following (in g/L): EDTA, 50; $ZnSO_4$, 2.2; $CaCl_2$, 5.5; $MnCl_2{\cdot}4H_2O$, 5.06; $FeSO_4{\cdot}7H_2O$, 5.0; $(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$, 1.1; $CuSO_4{\cdot}5H_2O$, 1.57; and $CoCl_2{\cdot}6H_2O$, 1.61. For the present study, the bacterial strain *P. pantotrophus* (DSM 2944) was obtained from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH, Braunschweig, Germany. The isolate was cultivated in DSM medium 356 (Table 1).

Due to the high organic and nitrogen content in municipal landfill leachate and the ability of *P. pantotrophus* to simultaneously remove carbon and nitrogen, the study was taken up to investigate treatment of municipal landfill leachate using *P. pantotrophus*. This paper discuss some studies on growth and behaviour of *P. pantotrophus* in leachate mixed synthetic wastewater.

Table 1
DSM medium 356

Components	Amount
$Na_2HPO_4 \cdot 2H_2O$	7.9 g
KH_2PO_4	1.5 g
NH_4Cl	0.3 g
$MgSO_4 \cdot 7H_2O$	0.1 g
Trace element solution SL-10	1.0 ml
Yeast extract	1.0 g
Distilled water	1,000.0 ml

2. Materials and methods

2.1. Microbe, media and growth

P. pantotrophus was cultivated in DSM medium 356 (Table 1), and the trace metal solution was comprised of $FeCl_2$ $4H_2O$, 1.5 g/L; $ZnCl_2$, 70 mg/L; $MnCl_2$ $4H_2O$, 100.0 mg/L; H_3BO_3, 6.0 mg/L; $CoCl_2$ $6H_2O$, 190.0 mg/L; $CuCl_2$ $2H_2O$, 2.0 mg/L; $NiCl_2$ $6H_2O$, 24.0 mg/L; Na_2MoO_4 $2H_2O$, 36.0 mg/L; and HCl (25%; 7.7 M), 10.0 mL/L. Media were sterilized by autoclaving for 20 min at 120˚C. All the components of media and trace element solution were autoclaved separately to avoid precipitation and caremelization. pH was adjusted to 7.5–8.0. Medium for Petri dishes was solidified by the addition of 2% (w/v) Agar to the DSM medium 356. The organism was grown at 37˚C in 250 ml conical flasks containing 50 ml growth media and shaken at 150 rpm on a rotary shaker. The growth test results were crosschecked with the results from the standard biochemical test arrays API 20 E (BioMérieux, Marcy l'Etoile, France) and Himedia test kit number KB008 and KB011.

2.1. Composition of synthetic media for batch experiments

The synthetic mineral media (Table 2) used in various batch experiments were prepared as described by Gupta and Gupta [5]. Two media concentrations were used for acclimatization studies. The first medium containing 1,000 mg/L COD and 110 mg/L NH_4^+-N had two variations in carbon source. The first medium (1000AG) had a combination of sodium acetate and glucose while the second medium (1000A) had only sodium acetate as the carbon source. The third medium (500A) had 500 mg/L COD and 55 mg/L NH_4^+-N. 500A had only sodium acetate as the carbon

Table 2
Composition of synthetic media used for acclimatization studies

Compound	Concentration (mg/L)		
	Medium 1000AG	Medium 1000A	Medium 500A
NH_4Cl	450	450	225
Glucose	625	–	–
$CH_3COONa{\cdot}3H_2O$	708	2,100	1,050
Na_2HPO_4	300	300	150
KH_2PO_4	150	150	75
$MgSO_4$	100	100	50

Table 3
Characteristics of synthetic media and leachate[a]

Parameters	Value			
	Medium 1000AG	Medium 1000A	Medium 500A	Leachate
COD	1,000	1,000	500	12,500
NH_4^+-N	110	110	55	1,500
C/N	3.32	3.32	3.32	6.75
pH	7.4	7.4	7.2	8.1
Alkalinity (as $CaCO_3$)	300	600	400	13,200

[a]All values are in mg/L except pH.

source. The composition of different media is given in Table 2, while the average characteristics of these media are given in Table 3.

2.2. Characteristics of leachate used in the experiments

The leachate used in the batch experiments was collected from Okhla landfill, Delhi. Samples were analysed for COD, ammonia nitrogen, pH, and alkalinity. Table 3 presents the characteristics of the Okhla leachate used in this study.

2.3. Shaking of batch cultures

For the first phase of experiments, the synthetic mineral media (500 mL) described in Table 2 were taken in 1-L shaking flasks. 5% (v/v) preculture of *P. pantotrophus* was inoculated into the media, incubated at 37˚C and shaken at 150 rpm for 267 h.

To evaluate bacterial acclimatization in leachate, medium 1000AG (Table 2) was used as the basal medium for all the second- phase batch experiments. Different concentrations of leachate ranging from 10 to 50% (on COD basis) were added to medium 1000AG, thereby keeping the initial COD in all the reactors constant. Leachate-mixed basal mineral media (500 mL) were taken in 1-L shaking flasks, and the pre-cultures of *P. pantotrophus* were inoculated at 5% (v/v) each, incubated at 37˚C, and shaken at 150 rpm for 300 h.

2.4. Sampling and analysis

Samples (2 mL) were withdrawn periodically for optical density (OD_{430} nm), pH measurement and chemical analysis. The optical densities of withdrawn samples were measured at 430 nm and 10 mm path length using a spectrophotometer (LaMotte Smartspectro). COD was measured by closed reflux method, and ammonia nitrogen and total nitrogen by Kjeldahl's distillation method as per standard methods [6]. Nitrate nitrogen was measured by a nitrate electrode (Thermo-Orion), pH by a pH meter and alkalinity by titrating against a standard H_2SO_4 solution.

3. Results and discussion

3.1. Optimum synthetic mineral medium for growth of *P. pantotrophus*

In order to investigate the effect of different types of carbon sources on the microbial growth and substrate utilization, three batch reactors were operated. The media used in this study were mineral media 1000AG, 1000A and 500A. Under similar growth conditions, *P. pantotrophus* grew better in media 1000AG and 1000A than in medium 500A (Fig. 1). It reached the maximum growth (OD_{430} 1.14) in medium 1000AG after 58 h, the OD_{430} in medium 1000A was 1.15 after 71 h, whereas, the OD_{430} in medium 500A was only 0.67 after 48 h. Though maximum growth in media 1000Ag and 1000A was nearly same, the time taken to reach the maximum was lesser for 1000AG. Also, better sustenance of microbial growth in medium 1000AG as indicated by higher OD_{430} signifies better growth of *P. pantotrophus* in this medium compared to both 1000A and 500A. Similarly, an increase in the maxi-

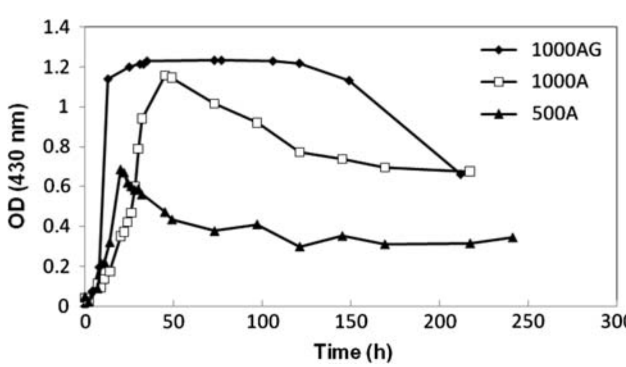

Fig. 1. Growth curves for *P. pantotrophus* grown in synthetic mineral media.

Table 4
Effect of leachate loading on the removal of nitrogen and COD

Reactor	Fraction of COD from leachate in	Alkalinity (mg/L as $CaCO_3$)		NH_4^+-N (mg/L)		TKN (mg/L)		Peak OD_{430} (average)	Duration of stationary	COD[a]	
	The feed (%)	Initial	Final	Initial	Removal	Initial	Removal		Phase (h)	Initial	Remaining
A	0	310	180	112.0	19.4	115.36	17.36	1.19	132	1,000	106
B	10	320	210	123.2	29.4	131.04	52.64	1.05	131	1,000	115
C	20	360	270	134.4	35.84	148.96	59.36	0.97	98	1,000	121
D	30	394	290	151.2	52.24	159.04	30.24	0.96	58	1,000	153
E	40	455	320	173.6	61.6	192.64	33.04	0.84	42	1,000	166
F	50	498	377	179.2	67.2	213.00	19.8	0.74	25	1,000	204

[a]Maximum substrate removal observed at 149 h. Initial COD 1,000 mg/L.

mum specific growth rate was observed when a culture was exposed to mixtures of carbon sources in comparison with growth with either of these substrates as single carbon sources [7–9]. Thus, medium 1000AG, which contained both glucose and acetate as carbon source, was found to be the best medium. So, it was selected as the basal medium for further batch studies.

3.2. Effect of leachate concentration on growth of *P. pantotrophus*

During the batch studies of *P. pantotrophus*, glucose and sodium acetate were used as carbon source, while NH_4-Cl was used as nitrogen source in the basal medium. Six batch reactors were operated in this phase, including one as control, which contained only synthetic mineral medium 1000AG (basal medium). Leachate was mixed (on COD basis) in other five reactors to contribute increasing COD as 10, 20, 30, 40 and 50% of the total COD of the reactor. It is observed that substrate concentration continually decreased in all reactors till 150 h. However, the COD values were different and presented good relation with the fraction of leachate present in the reactor. COD values varied from 106 to 221 mg/L as fraction of COD from the leachate increased from 0–50% (Table 4). The microbial growth, which was measured in terms of the optical density (OD_{430}) at 430 nm, decreased with an increase in the leachate content (Table 4). As shown in Fig. 2, OD_{430} increased during initial 30 h of incubation. This indicates that at the beginning of incubation, *P. pantotrophus* has the ability to utilize a growing portion of the carbon content in the leachate-mixed medium to gain energy for growth. It was a course of assimilation for the heterotrophic bacterium. In the control reactor (Fig. 2(A)) after 74 h of incubation, COD concentration remained more or less stable while the OD_{430} remained in the stationary phase till it reached the declining phase after 7 days (169 h). The sustainability of biomass was investigated using the measured length of the stationary phase. Stationary phase for different reactors is listed in Table 4. It was highest (124 h) for blank, whereas it decreased with an increase in leachate concentration in other reactors except for 20% leachate.

Fig. 3(A) presents variation in COD removal with respect to leachate concentration. It presents good linear relationship (coefficient of correlation, 0.93). In all batch reactors, maximum COD removal was observed near 148–149 h, after which it increased again, possibly due to cell lyses. On the basis of this minimum value, maximum COD removal (%) was determined (Table 4). This suggests that leachate, being complex and highly polluted with toxic compounds present in it, might have inhibited microbial activity. Thus, leachate proved to be a rate-limiting substrate and with its increase in the medium, COD removal decreased.

In this study, removal of the nitrogen was another important parameter. Leachate concentration in different reactors was varied on the basis of the COD contribution from the leachate. However, nitrogen loading also got changed due to different leachate fractions in different reactors. Resulting nitrogen loadings (Table 4) varied from 179.3 mg/L at 50% leachate to 112.0 mg/L in the control reactor without any leachate. Ammonia N removal was different, and it varied continually with the nitrogen loading (Fig. 3(B)). Their correlation is linear and good (coefficient of correlation, 0.94). Similar result was reported by Gupta and Gupta [5] while treating high-strength domestic wastewater by *P. pantotrophus* (then *Thiosphaera pantotropha*) in a rotating biological contactor.

The horizontal portion of the microbial growth curves (Fig. 2) represents the stationary phase. It is

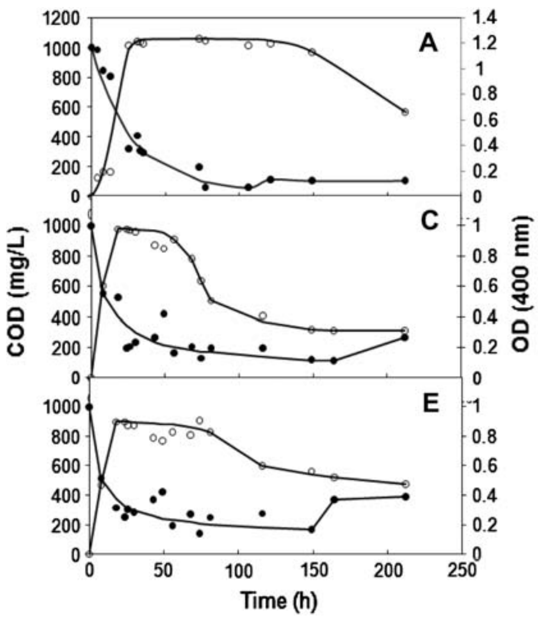

Fig. 2. Variation in substrate utilization, COD (•) and microbial growth (OD_{430}) (○) in the culture medium during the batch studies of *P. pantotrophus* at different leachate COD fractions. (A) Control, (B) 10%, (C) 20%, (D) 30%, (E) 40% and (F) 50%.

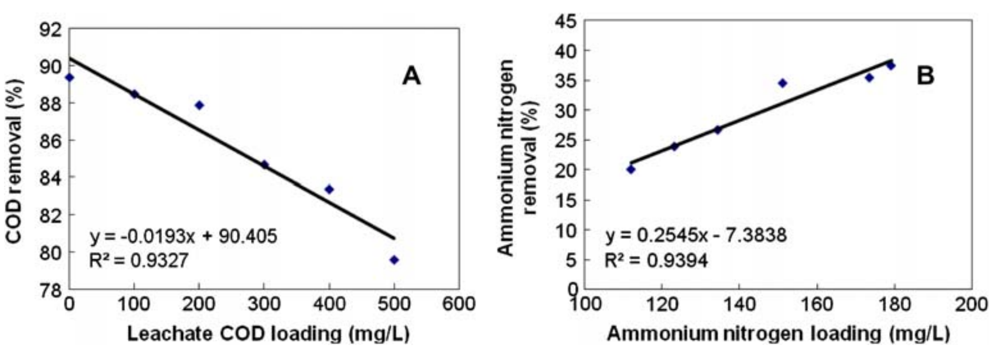

Fig. 3. Effect of leachate loading on the removal of (A) COD and (B) ammonium nitrogen.

evident from these figures that for different reactors, the duration of stationary phase is different for different leachate concentrations (Fig. 2). These values have been listed in Table 4, and variation in the duration of the stationary phase has been plotted with respect to leachate concentration in Fig. 4. It is observed that there is a continual decrease in the duration of the stationary phase (Fig. 4). There is an initial plateau, which is followed by a steep slope. It can be inferred that at higher leachate concentrations, its toxicity becomes inhibitory to the growth and sustenance of the biomass concentration. There is another interesting observation in the pattern of the substrate utilization. It is observed that after 150 h, there was onset of a hump in the substrate utilization curve (Fig. 2). This hump becomes more prominent as the leachate concentration increases. The presence of the substrate hump reflects an increase in substrate levels from the point of the lowest substrate levels. Probably, there have been cell lyses at this point and releases of metabolites that have added COD to the reactors. Higher leachate concentrations would result in more cell lyses; thus, there is likely to be a more prominent substrate hump.

In general, ammonia nitrogen can be removed only by assimilation into biomass or by nitrification. Here, a substantial removal in alkalinity (Table 4) indicates its consumption during heterotrophic nitrification, resulting in the utilization of NH_4^+-N. At the same time removal of TKN (Table 4) suggests a simulta-

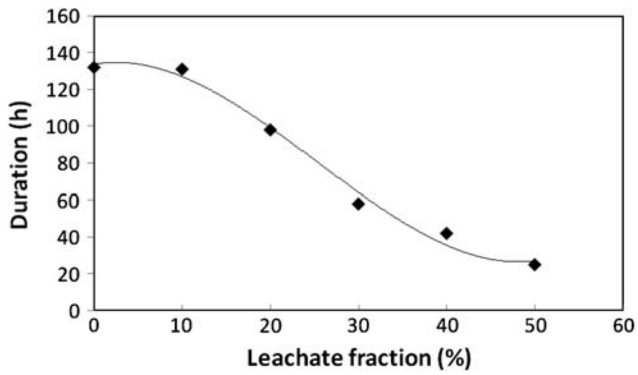

Fig. 4. Effect of leachate concentration on the sustenance of microbial biomass measured in terms of the duration of the stationary phase of growth.

neous aerobic denitrification taking place, as it indicates reduction in total nitrogen. However, a decrease in TKN removal with an increase in leachate concentration also suggests that microbes are not able to utilize organic content from increasing concentrations of leachate. A decrease in both COD removal (Fig. 3(B)) and average peak OD_{430} (Table 4) with an increase in leachate concentration also supports this possibility.

4. Conclusions

Results of the batch studies indicate that *P. pantotrophus* could utilize substrate from a leachate-mixed synthetic mineral medium. There was good removal of COD, which varied from 79.6 to 89.4%. Ammonia removal was 20–37.5% NH_4^+-N. Increased leachate concentration resulted in a linear decrease in microbial growth and COD removal capacity. Besides, there has been a continual decrease in the sustenance of the biomass coupled with corresponding lower maximum optical densities with increased leachate concentration. This shows that higher leachate concentration has inhibitory effects on the substrate utilization.

Generally, COD of leachate varies between 8,000 and 72,000 mg/L and total nitrogen varies between 1,000 and 3,000 mg/L. Average ratio of COD (84%) to nitrogen (29%) removal in the leachate is approximated as 3.0. Reported ratio of COD to nitrogen in leachate is above 8. Though removal of nitrogen is 3.0 times less than the removal of the COD, in view of the high COD/nitrogen ratio in leachate, probably, there is good potential to explore this system further to treat leachate. The inhibitory effects of the leachate toxicity could be minimized by using diluted leachate. Probably, domestic sewage could be used for dilution purposes. The present study is based on batch reactor configuration; however, different types of those reactor configurations, which allow tapering of inhibitory effects of the leachate, viz., fixed film systems, could be explored further.

Acknowledgements

This research study was supported by All India Council of Technical Education (AICTE).

References

[1] L.A. Robertson, J.G. Kuenen, *Thiosphaera pantotropha* gen nov sp nov, a facultatively anaerobic. Facultatively autotrophic sulphur bacterium, J. Gen. Microbiol. 129 (1983) 2847–2855.
[2] F.A. Rainey, D.P. Kelly, E. Stackebrandt, J. Burkhardt, A. Hiraishi, Y. Katayama, A.P. Wood, A re-evaluation of the taxonomy of *Paracoccus denitrificans* and a proposal for the creation of *Paracoccus pantotrophus* comb. nov., Int. J. Syst. Bacteriol. 49 (1999) 645–651.
[3] B.F. Taylor, D.S. Hoare, New facultative Thiobucillus and a reevaluation of the heterotrophic potential of *Thiobacillus norvellus*, J. Bacteriol. 100 (1969) 487–497.
[4] W. Vishniac, M. Santer, The Thiobacilli, Bacteriol. Rev. 21 (1957) 195–213.
[5] A.B. Gupta, S.K. Gupta, Simultaneous carbon and nitrogen removal from high strength domestic wastewater in an aerobic RBC biofilm, Water Res. 35 (2001) 1714–1722.
[6] APHA–AWWA–WPCF. Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, DC, 1998.
[7] M.J. Harte, F.C. Webb, Utilization of mixed sugars in continuous fermentation II, Biotechnol. Bioeng. 9 (1967) 205–221.
[8] A.P. Wood, D.P. Kelly, Heterotrophic growth of Thiobacillus A2 on sugars and organic acids, Arch. Microbiol. 113 (1977) 257–264.
[9] U. Brinkmann, W. Babel, Simultaneous utilisation of heterotrophic substrates by *Hansenula polymorpha* results in enhanced growth, Appl. Microbiol. Biotechnol. 37 (1992) 98–103.