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Aerobic biodegradationof Triethylamine (TEA) by*Pseudomonas aerougenosa* in designed synthetic and industrial wastewaters: Optimization through conventional and statistical approach

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ABSTRACT

A new bacterial strain named as AGR/IICT/4 was isolated from agas phase biofilter treating triethylamine (TEA) and it was used for understandingthe removal pattern of TEA in designed synthetic and industrial wastewater. The strain was identified as *Pseudomonas aeruginosa* based on biochemical and 16SrRNA gene sequence analysis.Parameters affecting biodegradation of TEA were selected based on conventional approach as well as statistical analysis (full factorial design and central composite design model). It was observed that initial TEA concentration, temperature and pH are the key controllingfactorsand *Pseudomonasaeruginosa,* could completely degrade 300mg/Lof TEA to ammonia in 60hrat a pH of 7.5 and temperature of 31°C. Thestrain could also effectively degrade diethyl amine, ethylamineand amine to ammonia as final product, which were identifiedas intermediates in aqueous medium. Maximummono oxygenase activity of 315.29 U/mg was observed under optimized conditions.

1. Introduction

Amines are abundantly used in industry for multiple purposes (Rappert& Muller, 2005a,b). Dimethylamine (DMA) is mainly used as an accelerator in rubber vulcanization processes, during the tanning of leather, production of detergents and pesticides (Shirkot et al., 1994). Trimethylamine (TMA) is frequently found in effluents of fishmeal manufacturing processes (Rappert&Muller, 2005a; GangagniRao et al., 2012).TMA is also produced by microbial activities from choline, bataine or trimethylamineN-oxide present in marine fish (King, 1984;Barrett and Kwan, 1985; Lin and Hurng, 1989; Moune et al., 1999). Diethylamine (DEA) is used in the rubber industry, resin, colouring materials and pharmaceutical products (Van Agteren et al.,1998). Triethylamine (TEA) is extensively used in industries as a catalyst for polymerization reactions, corrosion inhibition and as intermediate in the production of various chemicals & pesticides, desalination of seawater, hair curing products and coal casting operations (Aimin et al., 1999; Belin et al., 1983; Cai et al., 2011). Hence, DMA,TMA, DEA and TEA are the major pollutants in the liquid effluents and gaseous emissions of aforesaid industrial segments (Pandey et al., 2010;Van Agteren et al.,1998; Chang et al., 2004;Rappert& Muller, 2005a). Wastewater and gases consisting of these amines instigates problems of environmental pollution and adverse effects on aquatic ecosystem (Aimin et al., 1999). These amines are malodorous, causes adverse effects on human health such as irritation to the dermal, ocular, respiratory systems, DNA damage and teratogenic effect on embryos (Aimin et al., 1999; Belin et al.,1983; Cai et al., 2011;Niu et al., 2014). Amines are considered as a possible carcinogen and mutagen (Aimin et al., 1999; Belin et al.,1983; Cai et al., 2011; Lundstrom and Racicot,1983; Guest and Varma, 1992). Therefore, it is necessary to remove amines from wastewater and gases emanated from the abovementionedindustrial clusters before discharging into the atmosphere. Moreover in the recent past regulatory bodies are stringent in enforcing laws to combat the discharge of these effluents and gases to the environment (CPCB, 2013; EPA, 1990). Accordingly, effective processes are highly essential for the treatment and disposal of liquid and gaseous effluents containing these compounds. It was realized that biological methods of treatment are emerging as an effective and inexpensive alternative to conventional physicochemical treatment systems for treatingeffluents containing amines (Frings et al., 1994). DMA can be biologically oxidized toammoniacal nitrogen through methylamine (MA) as an intermediate under aerobic conditions (Rappert & Muller, 2005a,b; Ho et al., 2008). DMA-degrading microorganisms from diverse bacterial genera have been isolated and characterized (Rappert & Muller, 2005a; Ho et al.,2008;Van Agteren et al.,1998). Microbial degradation of TMA under aerobic conditions has been intensively studied (Ohara et al., 1990; Lobo et al., 1997; Kimet al., 2001) and many microorganisms that can aerobically degrade TMA have been isolated. DEA and DMA degraded by Hypho microbiumand Pseudomonas strains up to 5 g/l (Suresh et al., 2010). Besides bacteria, some yeast such as Candida utilis and Hansenula polymorpha can also degrade DEA; these strains use the compound as a nitrogen source (Pietsch et al.,2001). Biotechnological process for the

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removal of TEA from gas phase (Gandu et al., 2012) as well as liquid phase (Frings et al., 1994) is still under developmental stage. Calamari et al. (1980) are the first to report aerobic biological degradation of TEA in aqueous phase from the initial concentration level of 50 mg/l. Subsequently, it was reported by Wang et al.(2009) that mixed bacterial cultures completely removed TEA when the initial concentration was below 200 mg/l. Rappert& Muller, (2005a,b)isolated two pure bacterial strains, Pseudomonas citronellolis RA1 and Mycobacterium dienhoferi RA2, which could use TEA as the sole carbon and energy source for growth but up to the concentration of 50 mg/l in 4 days. In was reported by Cai et al. (2011) that Arthrobacter protophormiae R4 could completely degrade100 mg/l TEA within 32hrthroughbiodegradationhypothetical pathway proposed for amines Van Agteren et al. (1998) and Rappert & Muller, (2005a,b). Recently TEA bio degradation pathway experimentally proved by Cai et al. (2011) with Arthrobacterprotophormiae R4. It was realized that research work on the removal of TEA from wastewaters is very limited and it needs to be fortified with additional knowledge base for improved understanding of TEA removal from wastewaters. In the framework of industry adopting energy intensive conventional processes for the treatment of wastewaters containing TEA, additional research would be a step forward for the possible employment of aerobic bioprocess. Hence, in the present study, TEA degradation in designed synthetic and industrial wastewaters were carried out with isolated culture of *pseudomonas aeruginosa* at elevated concentrations with simultaneous analysis of metabolic products.

2. Materials and Methods

2.1 Microbial seed and Enrichment

Microbial seed (5 g) sourcewas from gas phase bio filter treating TEA. This seed source was added to 100 ml of designed synthetic wastewater (DSW) containing 100 mg/L TEA, (KH_2PO_4: 0.1 g, K_2HPO_4: 0.25 g, $(NH_4)_2SO_4$: 0.2 g, $MgSO_4$: 0.02 g, H_2O: 100 mL) and incubated on a rotary shaker at 30 °C at 120 rpm for 4 days at a pH of 7.0 (Gandu et al., 2013). Subsequently, pH of the media was adjusted to 7.0 and 5 ml of this culture was then sub cultured into fresh DSW. This procedure was repeated for enrichment of the culture for 10 generations (Gandu et al., 2013).

2.2 Isolation and Identification

Enriched cultures capable of degrading TEA were diluted by serial dilution and spread on to DSW agar containing different concentrations of TEA ranging from 50-400 mg/L and tested for TEA degrading potential. Amongst these, thestrain (AGR/IICT/4) showed highest TEA degrading potential. This isolated pure stock culture was preserved with glycerol stocks at -20 °C and working cultures were maintained on nutrient agar at 4 °C. Subsequently subcultures were made every 2 months. The culture was identified based on its morphological, physiological and biochemical properties with standard methods (Buchanan et al., 1984; Seneath et al., 1986) and 16s rRNA sequencing method (Gandu et al., 2013; Ho et al.,2008; Ching-pinget al., 2008; Doddapaneni et al., 2007). In 16s rRNA sequencing method, genomic DNA was extracted and the 16S rRNA gene was amplified using the polymerase chain reaction as described previously (Ho et al.,2008). The nucleotide sequences coding for the 16S rRNA of strain AGR/IICT/4 were sequenced by Bio Serve biotechnologies (India) Pvt. Ltd. This sequence was established using BLASTN facility and was also tested for possible chimera formation with the CHECK CHIMERA program and further aligned with the closest matches found in the GenBank database with the CLUSTALW function of molecular evolutionary genetics analysis (MEGA) package. Neighbor joining phylogenetic tree was constructed with MEGA version 4.0. A bootstrap analysis with 500 replicates was carried out to check the robustness of the tree (Cai et al., 2011; Doddapaneni et al., 2007). This sequence was submitted to NCBI-GenBank for accession number.

2.3 Experimental set up with DSW

In the biodegradation experiments, the newly isolated strainwas pre-cultured t in DSW at 30 °C and 120 rpm of shaking for 72 hr. The culture was added into a 120 mL of serum bottle with 100 mL ofDSW containing 100 mg/LTEA to perform the biodegradationexperiments under the conditions of 30 °C, pH 7.0 and120 rpm. The serum bottles were sealed with a rubber stopper toavoid the volatilization of TEA.

In order to further investigate the TEA biodegradation capability of this strain, the effect of four operating parameters, such as inoculum size, initial TEA concentration, pH value and thetemperature on the removal of TEA was analysed by conventional and statistical approach. All experiments carried out in triplicate for reproducibility.

2.4 Experimental with industrial wastewater (IW)

The IW samples were collected from the polymermanufacturing plant at Hyderabad, India. The samples were collected from the outlet of the holding tank where the wastewater is stored before it is sent to the effluent treatment plant (ETP).Batch experiments were conducted using 100 mL of IW in 120 ml sterile serum bottles in a rotary shaker at 120 rpm. The bottles were sealed with teflon-coated silicone septa and aluminium crimp caps to avoid the volatilization of TEA during the experiments. The pH and temperature of the IW were fixed at 7.5 and 31°C respectively based on the optimized studies with DSW and all experiments were carried out in triplicate.

2.5 Process parameters optimization by conventional approach for improvement of removal efficiency (RE)of TEA

In order to study the optimal processparametersrequirement for enhanced removal of TEA, the DSWmentioned in previous section was chosen as the basal medium. The process parameters such as TEA concentration, fermentation time, inoculum size, pH and temperature sources were optimized by single variables optimizationmethodology. In each batch experiment, one of the parameter was altered while keeping others constant in this approach. The course of biodegradation time was monitored from 1 to 72 hr to achieve the optimum incubation time for the removal of TEA. The influence of TEA concentration was evaluated by different concentration of TEAin the range of 50 to 400mg/L with an increment of 50mg/L.The influence of inoculum level on removal of TEA was evaluated by different concentration of inoculum in the range of 2 to 10 % v/v with an increment of 2 %. The effect of temperature on biodegradation of TEA was studied by varying the same in between 10°C to 50°C. Theeffect of pH of the DSWon the removal pattern of TEA was also studied by varying the pH from 5.0 -9.0.

2.6 Process parameters optimization by statistical approach for improvement of RE of TEA

The RE obtained in the conventional approach was fine tuned and optimized further by employing a statistical approach (STAVEX 5.2 software). In the experimental plan, 2-Level fractional-factorial design, 2-level full-factorial design and central-composite design (CCD) with confirmatory experiments were applied. Sixteen sets of experiments were performed as per 2-level fractional and full factorial design.Four important parametersviz;Initial concentration (300-400 mg/L with increment of 10 mg/L), pH (6.5 -7.5 with increment of 0.1), temperature (25 °C- 35 °C with increment of 1 °C) and inoculum size(3-5% with increment of 1%)were selected as factors for carrying out the above 16 experiments.The time period of biodegradation was monitored from 1 to 72 hrto achieve the optimum incubation time for the removal of TEA. Based on the above,CCDwas run and further 15 sets of experiments were carried out.Finally CCD evaluated itwith confirmatory experiments.STAVEX 5.2 software was used for regression and graphical analysis of the data. The significance of the regression coefficients was determined by transformation analysis.The proportion of variance explained by the polynomial models was given by the multiple coefficient of determination, R^2.In order to confirm the maximum RE of TEA predicted by the model, new set of experiments were performed using the optimal conditions as indicated in the statistical approach.

2.7 Enzyme activity

The enzyme activity of the cells was estimated using the following procedure used previously by Cai et al.(2011).AGR/IICT/4 isolated microbial cell culture was recovered by cold centrifugation at 8900 x g for 10 min. The cells were washed and re suspended in 1ml phosphate buffer solution (100Mm, pH7.0) and it was disrupted using an ultra sonicator. The extract was centrifuged at 10,000 x g for 15min.The supernatant was used for monooxygenase enzyme activity assayswith aspectrophotometer by measuring the oxidation ofNicotinamide adenine dinucleotide phosphate (NADPH) at 340 nm and 30°C and all the experiments were carried out in triplicate.

2.7 Analytical methods

The concentration of TEA in the liquid phase (DSW and IW) was estimated using Gas chromatography (GC)(Cai et al., 2011). The metabolites (DEA,EA, Amine) in the samples were identified by standard electron ionization- mass spectrometer (EI-MS)(Finnigan TSQ Quantum Ultra AM (Thermal, USA)) (Cai et al., 2011). The mechanism involved ionization by electro spray with a positive polarity. The sample was scanned in the normal mass range, from 40 m/z (mass to charge ratio)

to160 m/z. NH^{+4}, $NO_2^-$and NO_3^- were analysed by using a UV–visible (Perkin Elmer lambda 25) spectrophotometer as per standard methods (APHA, 1998). pH was determined with pH meter (Elicopvt ltd) and temperature was measured with mercury thermometer.IW was characterized for COD, suspended solids and turbidity as per standard methods (APHA, 1998).

3. Results and Discussion

3.1 Strain identification

The isolate was identified on the basis of morphological, physiological characteristics, biochemical tests and 16S rRNA sequencing. The isolate was found to be gram-negative rod shaped bacteria and was positive for oxidase, gelatine, nitrate, motility, urea, citrate, catalase, methyl red tests and negative for galactose, maltose, sucrose, mannitol, lactose tests. The 16S rRNA sequence and their phylogeny are presented in Fig.1. As per the in-silico analysis and rRNA gene database, all the species showed 95-99% homology to isolate AGR/IICT/4, indicating the probability of isolate as *Pseudomonas aeruginosa*. Hence, isolate AGR/IICT/4 was submitted to Genbank (NCBI)& its accession number is HF679288.

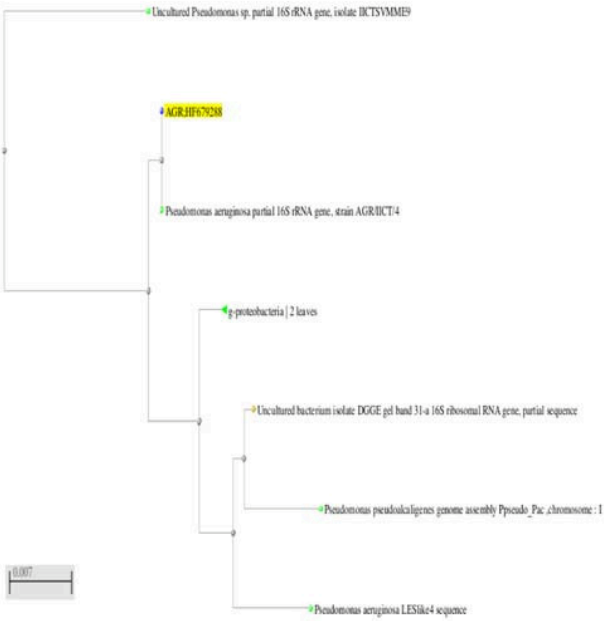

Fig.1 Phylogenetic tree based on the 16S rRNA gene sequences of strain AGR/IICT/4 and related species using the neighbor-joining approach

3.2 Biodegradation studies of TEA by conventional single variables approach

The newly isolated bacterial strain *Pseudomonas aeruginosa* was examined for its ability to degrade TEA under different conditions. The effect of initial concentration of TEA on degradation was determined by varying the originalconcentration at fixed pH value of 7.0, temperature of 30°C and time duration of 60 hr. The RE with respect to the initial concentrationwas shown graphically in Fig. 2.It could be understood from Fig.2 that for initial TEA concentration of 50 mg/l, 100 mg/l, 150 mg/l, 200 mg/l, 250 mg/l, 300 mg/l, 350 mg/l and 400 mg/l, RE was 99.0%, 98.5%, 96.1%, 93.4%, 91.5%, 87.7%, 65.3% and 45.6% respectively. It could be derived from the results that maximum RE of 87% could be obtained when the initial concentration was below 300 mg/L. However, RE fell down to 45.6%, when the initial concentration was increased beyond 300mg/L. Therefore, it could be establishedfrom this trend that high initial TEA concentration beyond 300 mg/L might be inhibiting the microbial organism due to overloading.Hence,300 mg/L was selected as initial concentration of TEA for further studies.Previously, it was reported that (Cai et al.,2011) Arthrobacterprotophormiae R4 could completely degrade100 mg/L TEA with in 32hr, whereas *Pseudomonas aeruginosa* isolated in the present study could completely mineralize higher concentration of 300 mg/L of TEA in 60 hr.

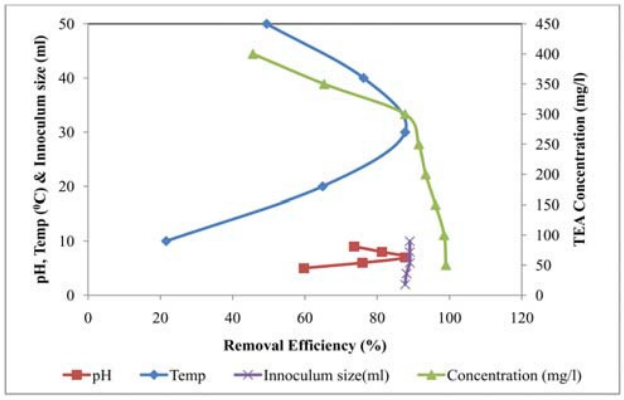

Fig.2 Removal efficiency Vs.TEA concentration, pH, Temperature and Innoculum size

The effect of pH on RE by the strain *Pseudomonas aeruginosa* was investigated at initial TEA concentration of 300 mg/L and temperature of 30°Cby varying the pH from 5.0 to 9.0. The resultsobtained from this study are shown in Fig.2. It could be observed from Fig.2 that RE was increasing gradually from 59.7 to 87.7% with increase in pH from 5.0 to 7.0, and subsequently, it decreased to 73.6% while pH was increased from 7.0 to 9.0. It could be observed from the graph that highest RE of 87.7% was attained at pH 7.0. This trend showed that the biodegradation of TEA is strongly affected by buffering capacity of the system because of the inhibitory activity of intracellular monooxygenaseand the optimum pH of 7.0 obtained in our study confirms the results in the previous study (Cai et al.,2011).

Temperature is another key parameter that affects the biodegradation of TEA. Hence, the relationship between RE and the reaction temperature of DSW was also investigated at fixed initial concentration of 300 mg/L and pH of 7.0 by varying the temperaturefrom 10 to 50°C. The results obtained are presented graphically in Fig.2. The results revealed fact that (Fig.2), RE of TEA was increasing from 21.6 to 87.7% when the temperature was raised from 10-30 °C, where as it was decreased to 49.4% when the temperature was further rose from 30-50°C. It is worth mentioning here that 30°C marks a turning point for the microbial consortia for exhibiting optimal performance since at the temperature of 30°C, highest RE of 87.7 % (Fig. 2) was observed. This might be due to the fact that repression of the enzyme activities might be taking place at high and low temperature ranges (Cai et al., 2011).

The effect of inoculum size on RE of TEA by *Pseudomonas aeruginosa* was also investigated in this study. Inoculum size was varied from 2-10 mL, with increment of 2 mL at constant initial concentration of 300 mg/L, temperature of 30 °C and pH of 7.0. The results revealed the fact that highest RE of 89% of TEA was obtained in 100 mLDSWwhen the inoculum size was 6mL (Fig.2).

3.3 Biodegradation studies of TEA by statistical approach

3.3.1 2-level full factorial design

In order to optimize the range of experiments 2level fractional and full factorialwas applied. The range and levels of the process variables under study are; initial concentration (300-400mg/L with increment of 10 mg/L), pH (6.5 -7.5 with increment of 0.1), temperature (25°C- 35°C with increment of 1 °C) and inoculums size (3-5% with increment of 1%). The actual design of experiments is given in Table 1 for each run, it was observed from this experiment that atinitial concentration of 300 mg/L, pH of 7.5 and temperature of 35^{0}C;removal efficiency of 94.31 % was obtained.Therefore, it could be noted from the level 2 optimization studies that initial concentration of TEA was only effecting at RE and size of the inoculumwas an arbitrary parameter.

3.3.2 Central composite design (CCD)

CCD design was selected from the above observations for further improvement of RE of TEA. In this onlythreefactors were selected (Initial concentration, pH and temperature) omitting inoculum size based on 2 level full factorial designs. CCD of 15 experimental runs were carried out and the results are shown in Table.2. The summary of results along with

Table 1: 2 level full factorial design with observed values for RE of TEA

Run	Initial concentration	pH	Temperature	Inocula size	Removal efficiency
	mg/l		^{0}C	%	%
1	300	6.5	25	3	89
2	400	7.5	25	3	44
3	400	6.5	35	3	44
4	300	7.5	35	3	95
5	400	6.5	25	5	40
6	300	7.5	25	5	93
7	300	6.5	35	5	95
8	400	7.5	35	5	46
9	400	6.5	25	3	40
10	300	7.5	25	3	94
11	300	6.5	35	3	90
12	400	7.5	35	3	46
13	300	6.5	25	5	85
14	400	7.5	25	5	49
15	400	6.5	35	5	46
16	300	7.5	35	5	95

Table 2: CCD design with observed values for RE of TEA

Run	Initial concentration	pH	Temperature	Removal efficiency
	%		^{o}C	%
1	300	6.5	25	87
2	400	6.5	25	40
3	300	7.5	25	94
4	400	7.5	25	44
5	300	6.5	35	90
6	400	6.5	35	42
7	300	7.5	35	96
8	400	7.5	35	48
9	350	7	30	62
10	310	7	30	96
11	390	7	30	42
12	350	6.6	30	60
13	350	7.4	30	63
14	350	7	26	59
15	350	7	34	60

contour plots is shown Fig.3. The CCD showed that initial concentration of 300 mg/L, temperature of 31^{o}C and pH of 7.5 werefound to be optimum as theregression analysis observed to be at R^2=0.9835 (corrected goodness of fit: very good). Subsequently, CCD design with confirmatory experimentswas carried out and results are tabulated in Table 3a. It was further confirmedfrom the CCDthat initial concentration of 300 mg/l, temperature of 31°C and pH of 7.5 were found to be optimum (Table 3b).

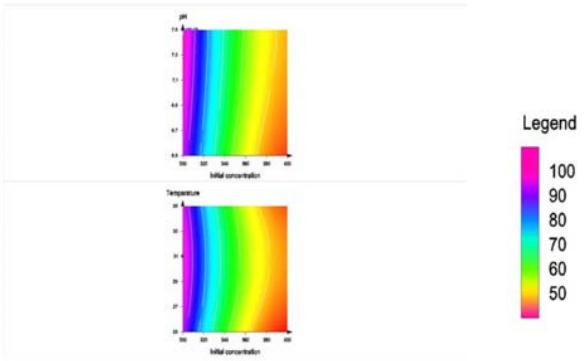

Fig.3 Contour plots showing the mutual effect of factors on RE of TEA; a) TEA concentration Vs. pH; b) TEA concentration Vs. Temperature

Table 3: 3a CCD design summary for RE of TEA; 3b CCD confirmatory design summary for RE of TEA

Table.3a	
Response	**Removal efficiency**
Factor/Optimum	
Initial concentration(mg/l)	300
pH	7.5
Temperature(^{o}C)	31
Optimization direction	max
(lower bound)	90.43
Optimum value	**100.44**
(upper bound)	110.44
Corr. goodness of fit	**very good**

Table.3b	
Response	**Removal efficiency**
Factor/Optimum	
Initial concentration(mg/l)	300
pH	7.5
Temperature(^{o}C)	31
Optimization direction	max
(lower bound)	90.43
Optimum value	**100.44**
(upper bound)	110.44
Confirmatory experiment	99 ok
Corr. goodness of fit	**very good**

3.4 Product analysis

In order to recognize the formation of metabolites formed during the degradation of TEA, Initial and final TEA, DEA, EA concentrations in DSW under optimized conditions were analysed by GC and EI-MS. Besides above, NH_4^+, $NO_2^-$and NO_3^-were also analyzed using a UV-visible spectrophotometer. The metabolites were identified as diethyl amine and ethylamine from EI-MS studies (Fig.4) and NH_4^+ from UV-visible spectrometric studies. The results showed that initially EA is degraded to ammonia and acetaldehyde and subsequently it wascompletely mineralized.

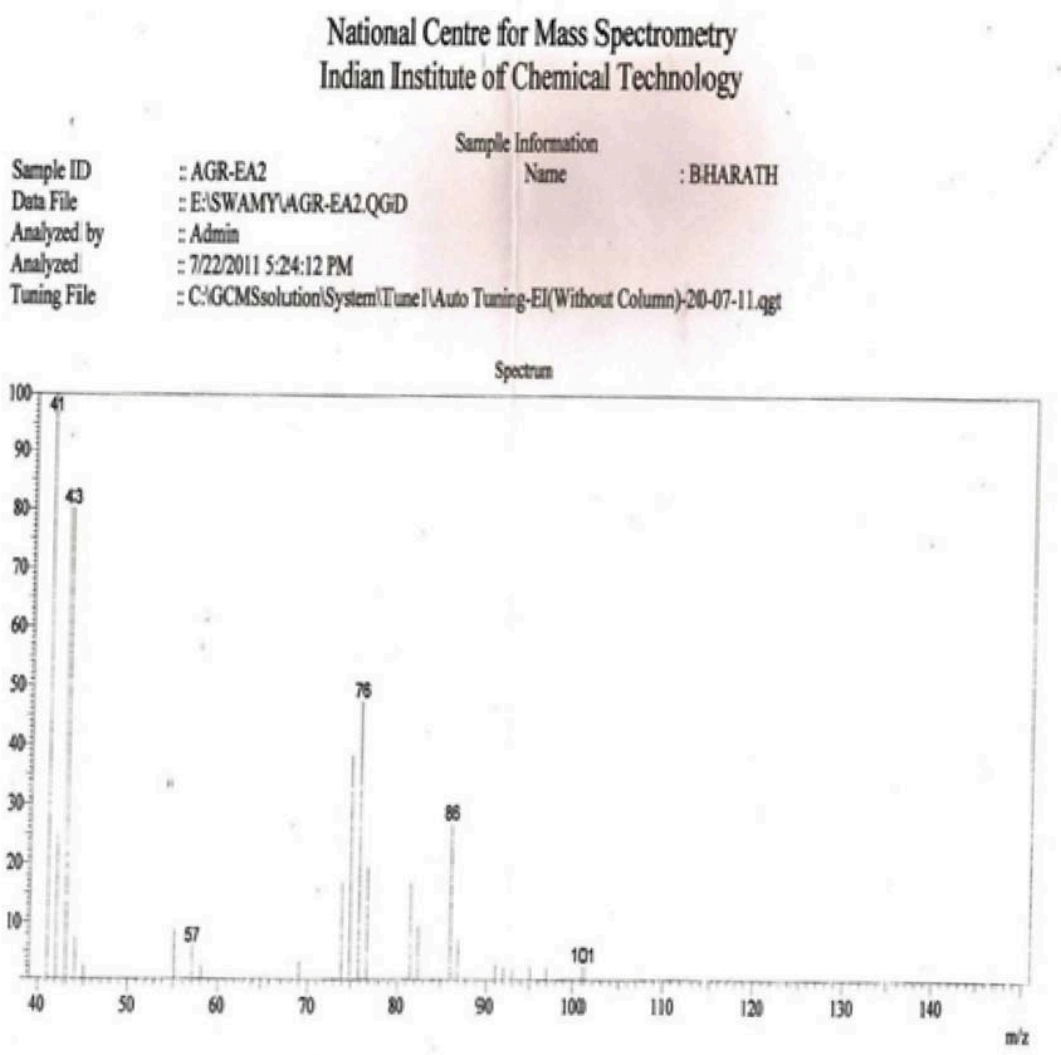

Fig.4 Analysis of TEA metabolic product by EI-MS

3.5 Enzyme activity

Results obtained with respect to monooxygenase activity were tabulated in Table 4. The monooxygenase activities were high at optimized conditions of pH, temperature, initial concentration and inoculum size of 7.5, 31°C, 300mg/L and 6mL respectively. The enzyme activity was estimated in each optimized condition separately.Monooxygenase activity resembled the activity of *Pseudomonas aeruginosa*as per RE capacity. It was observed that the enzyme activity of Pseudomonas*aeruginosa* was highest at 315.29 U/mg whenTEA initial concentration was 300mg/L, pH: 7.5, Temperature: 31°C and Inoculum size: 6mL (Table 4).

Table 4: Monooxygenase enzyme activity of TEA under various optimized conditions

Optimized Conditions	Enzyme activity(U/mg)
TEA initial concentration:300mg/l+ pH:7.0 + Temperature:30°C	284.12
TEA initial concentration:300mg/l+ pH:7.0 + Temperature:30°C+Inoculum size:6 ml	295.61
TEA initial concentration:300mg/l+ pH:7.5 + Temperature:35°C+Inoculum size:6 ml	301.57
TEA initial concentration:300mg/l+ pH:7.5 + Temperature:31°C+Inoculum size:6 ml	315.29

3.6 Biodegradation of TEA in IW

The inlet characteristics of IW were tabulated and shown Table 5. It could be realized from the characteristics that IW was having TEA in the range of 300 mg/L (average value) apart from COD of about 20,000mg/L (average value). Batch experiments were carried out with the IW as such (without dilution) at an inlet TEA and COD concentration of 300 mg/L and 20,000 mg/L respectively.The optimized residence time (60^{th} hr) and pH (7.5) obtained in case DSW was followedfor IW also. However, samples were collected andanalysed at the time intervals of 40^{th} hr and 50^{th} hr also apart from analysing the samples at end of the experiment (60^{th}hr). It was observed that RE in the range of 90% for TEA and 60% for COD was obtained in 60 hr (Table.5). It was worth noting that the results obtained for IWwere in tune with optimal process parameters obtained for DSW. Therefore, *Pseudomonas* aeruginosa was effective in removing TEA from industrial wastewater under optimised conditions evaluated through CCD.

Table 5: Physico-chemical characteristics of the pre-treated effluent

Parameters	Units	Before treatment	After treatment
Temperature	^{O}C	35	31
pH	-	13	7.5
COD	mg/l	>20,000	<8000
SS	mg/l	3500	1300
Turbidity	NTU	750	124
TEA	mg/l	250	1

4. Conclusions

In the present study, a new bacterial strain AGR/IICT/4 was isolated and identified as genus *Pseudomonas aeruginosa*according to its 16S rRNA gene sequence analysis.Experimental conditions were optimized by statistical approach of full factorialdesign and CCD in addition to conventional design approach.Aerobic biodegradation of TEA to the tune of 90% was obtained by*Pseudomonas aeruginosa*under optimized conditionsin DSWand same results were observed in IW also. Monooxygen aseactivityincreased from 284.12 U/mg to 315.29 U/mgand metabolic products were identified as DEA, EA and NH_4^+.

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6. References

1. Aimin, H., Lixin, C., Jie, C., Spiess, F.J., Steven, L.S., Timothy, N.O., Steve, O.H., James, D.F., 1999. Photocatalytic degradation of triethylamine on titanium oxide thin films. J. Catal. 188, 40–47.
2. APHA, 1998, Standard Methods for the Examination of Water and Wastewater, 20^{th}ed. APHA, AWWA, WEF, Washington, D.C.
3. Barrett, E.L., and Kwan, H.S., 1985. Bacterial reduction of trimethylamine oxide. Annu. Rev. Microbiol. 39, 131-149.
4. Belin, L., Wass, U., Audunsson, G., Mathiasson, L., 1983. Amines/ : possible causative agents in the development of bronchial hyperreactivity in workers manufacturing polyurethanes from isocyanates. Br. J. Ind. Med. 40, 251-257.
5. Buchanan, R.E., Gibbons, N.E., 1984. Bergey's Manual of Determinative Bacteriology,Williams and Wilkins Co., Baltimore.
6. Cai, T.M., Chen, L.W., Ren, Q., Cai, S., Zhang, J., 2011. The biodegradation path way of tri ethyl amine and its bio degradation by immobilized Arthrobacterprotophormiae cells. J. Hazard. Mater. 186 (1), 59-66.
7. Calamari, D., Da Gasso, R., Galassi, S., Provini, A., Vigh, M., 1980. Biodegradation and toxicity of selected amines on aquatic organisms. Chemosphere 9, 753-762.
8. Chang, C.T., Chen, B.Y., Shiu, I.S., Jeng, F.T., 2004. Biofiltration of trimethylamine-containing waste gas by entrapped mixed microbial cells. Chemosphere 55 (5), 751-756.
9. CPCB,India,2013.http://www.cpcb.nic.in/divisionsofheadoffice/pci-ssi/MATERIAL_SAFETY-DATABASE/MSDS2008/652.pdf,2014.
10. Doddapaneni, K.K., Tatineni, R., Vellanki, R.N., Gandu, B., Panyala, N.R., Chakali, B., Mangamoori, L.N., 2007. Purification and characterization of two novel extra cellular proteases from Serratia rubidaea. Process Biochem. 42, 1229–1236. doi:10.1016/j.procbio.2007.05.019
11. EPA, 1990.http://www.epa.gov/ttnatw01/orig189.html,2013.
12. Frings, J., Wondrak, C., Schink, B., 1994. Fermentative degradation of triethanolamine by a homoacetogenic bacterium, Arch. Microbiol. 162,103-107.
13. Gandu, B., Sandhya, K., GangagniRao, A., Swamy,Y.V., 2013. Gas phase bio-filter for the removal of triethylamine (TEA) from air: Microbial diversity analysis with reference to design parameters. Bioresource technol. 139, 155–160.
14. Gandu, B., Sandya,K., GangagniRao, A., Swamy, Y.V.,2012. Removal of Air Containing Tri Ethyl Amine (TEA) Using Vapor Phase Biofilter Packed With Wood Chips and Rice Husk. Bonfring International Journal of Industrial Engineering and Management Science. 2,17-20.
15. GangagniRao, A., Gandu, B., Swamy, Y.V., 2012.Mass Transfer Dynamics of Ammonia in High Rate Biomethanation of Poultry Litter Leachate, Bioresour. Technol.109, 234-238.
16. Guest, I., Varma, D.R., 1992. Teratogenic and macromolecular synthesis inhibitory effects of trimethylamine on mouse embryos in culture. J. Toxicol. Environ. Health 36, 27–41.
17. Ho, K.L., Chung, Y.C., Tseng, C.P., 2008. Continuous deodorization and bacterial community analysis of a biofilter treating nitrogen-containing gases from swine waste storage pits. Bioresour. Technol. 99 (2), 2757–2765.
18. Kim, S.G., Bae, H.S., Lee, S.T., 2001. A Novel denitrifying bacterial isolated that degrades trimethylamine both aerobically and anaerobically via two different pathways. Arch. Microbiol. 176, 271-277.
19. King, G.M., 1984. Metabolism of trimethylamine, choline, and glycine betaine by sulfate-reducing and methanogenic bacteria in marine sediments. Appl. Environ. Microbiol. 48, 719–725.
20. Lin, J.K., Hurng, D.C., 1989. Potentiation of ferrous sulphate and ascorbate on the microbial transformation of endogenous trimeth- ylamine N-oxide to trimethylamine and dimethylamine in squid extracts. Food Chem. Toxicol. 27 (9), 613–618.
21. Lobo, N., Rebelo, A., Partida´rio, P.J., Roseiro, J.C., 1997. A bubble column continueous fermentation system for trimethylamine con- version by Aminobacteraminovorans. Enzyme Microb. Technol. 21, 191–195.
22. Lundstrom, R.C., Racicot, L.D., 1983. Decomposition in foods: Gas chromatographic determination of dimethylamine and trimethyla- mine in seafoods. J. Assoc. Off. Anal. Chem. 66 (5), 1158-1163.
23. Moune´, S., Manac'h, N., Hirschler, A., Caumette, P., Willison, J.C., Matheron, R., 1999. Haloanaerobactersalinarius sp. nov., a novel halophilic fermentative bacterium that reduces glycine-betaine to trimethylamine with hydrogen or serine as electron donors; emendation of the genus Haloanaerobacter. Int. J. Syst. Bacteriol. 49, 103–112.
24. Niu, Z.G., Xu, S.Y., Gong, Q.C., 2014. Health risk assessment of odors emitted from urban wastewater pump stations in Tianjin, China. Environ SciPollut Res Int. 21,10349-60.
25. Ohara, M., Katayama, Y., Tsuzaki, M., Nakamoto, S., Kuraishi, H., 1990. Paracoccuskocurii sp. nov., a tetramethylammonium-assimilating bacterium. Int. J. Syst. Bacteriol. 40, 292-296.
26. Pandey, R.A., Giri, B.S., Mudliar, S.N., Deshmukh, S.C., Banerjee, S., 2010. Treatment of waste gas containing low concentration of dimethyl sulphide (DMS) in a bench-scale bio-filter. Bioresour. Technol. 101, 2185-2190.
27. Pietsch, J., Sacher, F., Schmidt, W., Brauch, H.J., 2001. Polar nitrogen compounds and their behavior in the drinking water treatment process. Water Res. 35 (15), 3537-3544.
28. Rappert, S., & Muller, R., 2005a. Odor compounds in waste gas emissions from agricultural operations and food industries. Waste Manage, 25, 887-907.
29. Rappert, S., & Muller, R., 2005b. Microbial degradation of selected odorous substances. Waste Manage, 25, 940-954.
30. Seneath, P.H.A., Mair, N.S., Sharpe, E.M., Holt, J.G., 1986. Bergey's manual of systematic bacteriology, 9th ed.,Williams and Wilkins, Baltimore.
31. Shirkot, C.K., Shirkot, P., Gupta, K.G., 1994. Isolation from soil and growth characteristics of the tetramethylthiuramdisulfide (TMTD) degrading strain of Pseudomonas aeruginosa.. J. Environ. Sci. Health, 29, 605–614.
32. Suresh, K., Dubey, A., Shukla, K., Pranjali, V., Singh, R.S., Upadhyay, S.N., 2010. Bio- filtration of trichloroethylene using diazotrophic bacterial community. Bioresour. Technol. 101, 2126–2133.
33. Van Agteren, M.H., Keuning, S., Janssen, D.B., 1998. Handbook on Biodegradation and Biological Treatment of Hazardous Organic Compounds. Kluwer Academic Publishers, London.
34. Wang, C.C., Lu, K.L., Chen, X.Y., 2007. Removal of triethylamine from synthetic waste water by acclimated mixed bacteria cultures. Int. Biodeterior. Biodegrad. 59, 202-205.