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Application of artificial neural network model for the development of optimized complex medium for phenol degradation using *Pseudomonas pictorum* (NICM 2074)

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Abstract Biodegradation of phenol using *Pseudomonas pictorum* (NICM 2074) a potential biodegradant of phenol was investigated for its degrading potential under different operating conditions. The neural network input parameter set consisted of the same set of four levels of maltose (0.025, 0.05, 0.075 g/l), phosphate (3, 12.5, 22 g/l), pH (7, 8, 9) and temperature (30°C, 32°C, 34°C) on phenol degradation was investigated and a Artificial Neural Network (ANN) model was developed to predict the extent of degradation. The learning, recall and generalization characteristic of neural networks was studied using phenol degradation system data. The efficiency of the model generated by the ANN, was tested and compared with the results obtained from an established second order polynomial multiple regression analysis (MRA). Further, the two models (ANN and MRA) were used to predict the percentage of degradation of phenol for blind test data. Performance of both the models were validated in the cases of training and test data, ANN was recommended based on the following higher coefficient of determination R^2; lower standard error of residuals and lower mean absolute percentage deviation.

Keywords Biodegradation · *Pseudomonas pictorum* (NICM 2074) · Artificial Neural Network (ANN) · Multiple Regression Analysis (MRA)

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Introduction

Increasing the need for good quality water as well as recovery and reuse of waste water has acquired much importance in recent years. Phenol and their derivatives are often found in industrial effluents such as oil refineries, coke oven plants, steel plants, metallurgical operations, pharmaceuticals, paint, varnish industries and textile industries (Mahadeva et al. 1997; George et al. 1993). The persistence of phenols in the aquatic and terrestrial environment can be harmful to humans. Studies have shown phenol to be carcinogenic and to cause taste and odor problem in drinking water (WHO 1989). Phenolic compounds ranges from 6–200 mg l^{-1}, while their permissible limit is only 3 mg l^{-1} in the receiving water bodies (Manivasakam 1984). These compounds are toxic even at low levels and pose a threat to the biosphere because of their recalcitrant nature. A variety of physico-chemical

methods are available for removal of phenol and its derivatives from waste waters focuses on adsorption, ozonation, chlorination, reduction, precipitation, solvent extraction, coagulation.

Chemical and biological treatment are employed successfully for various industrial waste water. Biodegradation is an effective and alternative to the conventional disposal method and is a new technology which emphasizes the detoxification and destruction of the pollutants by acclimatized microorganisms (Baker and Herson 1994; Hardman et al. 1993). Biodegradation of phenolics by certain anaerobic as well as aerobic bacteria and fungi have long ago been reported (Abramowicz 1990; Vera et al. 2003). Biodegradation of aromatic compounds is by the hydroxylation of the ring by oxygen-requiring enzyme systems. Effluent containing aromatic compounds would hasten the process of biodegradation due to lack of oxidative enzymes. In addition, they posses advantages over other bacteria and green algae by their tropic independent for nitrogen as well as carbon sources (Truu et al. 2003; Safonova et al. 2004). It is thus important to discover bioremediating bacteria with the ability to degrade these toxic pollutants. The genus *Rhodococcus*, which is comprised of filamentous *Actinomycetes* of the family *Nocardiaceae,* possesses outstanding metabolic processes and nutritional versatility. The utilization of a wide range of aromatic compounds, including phenol and chlorinated benzenes as carbon sources by a number of *Rhodococcus* species has been described previously (Duncan and Paul 2004; Angela and Todd 2005; Marc and James 2005; Alessandro et al. 2005). Adsorption and biodegradation are two main mechanisms exercised in such units. The results pertaining to adsorption isotherm and kinetics have been discussed elsewhere. There is a general perception that the phenols being toxic are not amenable to biological degradation. Despite the fact that some phenolytic microorganisms exist in nature, these microorganisms show different rates of metabolism in terms of their ability to degrade these compounds (Illaria et al. 2003; Arinjay et al. 2005).

The physico-chemical remedial strategies to clean up sites contaminated by these compounds are not cost effective or adequate (Villaverde et al. 1997; Tawtiki et al. 2000). Therefore, research is increasingly being focused on biological methods for the degradation and elimination of these pollutants (Akay et al. 2005). Sites contaminated by these compounds need urgent remedial solutions. The search which has revealed a diverse range of bacteria that can utilize these xenobiotics as substrates, often mineralizing them or converting them into benign products, and in the process helping to clean up the environment effectively (Rakesh Kumar et al. 2005). Such a strategy will provide the ground for successful interventions into environmental processes and ultimately lead to optimized strategies for tapping of microbial diversity for efficient and effective bioremediation of xenobiotics (Pandey and Jain 2002). In spite of phenol's toxic properties, a number of microorganisms can utilize phenol under aerobic condition as sole energy sources. However, little is known about the biodegradation of phenol at high initial concentration larger than 1700 mg l^{-1} by microorganisms (Tsuey et al. 2004; Jiang et al. 2005).

Kinetic studies are otherwise limited by the aqueous solubility of the substrate, which results in very short degradation times at very low substrate concentrations close to the analytical detection limit, and therefore poor estimates of the intrinsic kinetic parameters (Myung et al. 1993; Kuo et al. 2003). A good understanding of the microbial degradation kinetics of hydrophobic compounds such as biphenyl may provide valuable information for engineered bioremediation processes of other poorly soluble contaminants such as PAHs and PCBs (Alvarez et al. 1991; Tesema 2005; Bruno 2005). Knowledge of the relationship between the microbial growth and the substrate parameters is the first step towards the development of a kinetic model (Giorgia and Marco 2005). One of the most suitable mathematical expressions is the Monod model: The following assumptions were made in the Monod kinetic model: the organic molecule is water soluble and non-toxic, the substrate (S) is limiting and other organic and inorganic growth factors are present in excess of the organism's need. Monod model is based on the

case of a single bacterial species (Alexander 1999; Shingleton et al. 2001; Mark et al. 2002; Mario et al. 2003).

This laboratory study was conducted using *Pseudomonas pictorum* on effect of parameters like maltose, phosphate, pH and temperature on the degradation of phenol. An attempt has been made to apply an Artificial Neural Network (ANN) model to predict the biodegradation of phenol from waste water using different parameter like maltose, phosphates temperature and pH to optimize level a degrading phenolic effluents. Neural Network in process modeling as well as control of chemical and biochemical process (Yamuna Rani and Ramachandra Rao 1999). Neural Network to handle general nonlinear relationships has led to their extensive use in different applications. Introduced the use of Neural Network computational algorithms for dynamic modeling of bioprocesses (Yamuna Rani and Ramachandra Rao 1999; Thibault et al. 1990). An efficiency of ANN model was demonstrated by companying Multiple Regulation Analysis (MRA) model (Box and Behnken 1960; Ravi et al. 1995; Bhat et al. 1990; Hopffield 1992). The model expected to predict the different sources (Maltose, Phosphate, Temperature and pH), with minimum error, there by obtaining the laboratory analysis completely degradation of phenol.

Modeling of the experiments

Artificial Neural Network (ANN)

Artificial Neural Network (ANN) have received considerable interanging during last few years largely due to their wide range of applications and case with which they can handle problems in precisely noisy and highly complex nonlinear data (Ravi et al. 1995; Mathalai Balan et al. 1999). The ANN methodology has become an alternative to modeling of some physical and nonphysical systems with scientific (or) mathematical basis. Once the neural networks are trained it can be applied to make predictions for new input conditions. A large number of ANN have been proposed and used in recent year (Rumelhert et al. 1986). However, most of the commonly used ANNs for process modeling are layered feed forward neural networks, also called multi layer perception with back propagation learning algorithms (Rumelhert et al. 1986). A variety of application of ANNs (Prevost et al. 1994; Venkatasubramanian et al. 1990; Bhat et al. 1990; Polland et al. 1992) trained by back propagation have long ago been reported in the literature. A learning rule is used to train the given data by adjustments of connecting weights by applying suitable algorithms. The optimal weights are determined by training the neural nets. The minimization of the mean square error between the actual output of a multilayer feed forward perceptions and desired output is the main technique used in the back propagation algorithm (Murty et al. 1997).

Data has been reprocessed before training the ANN using back propagation algorithm. The steps were used in preprocessing that data: (i) eliminating correlated input variables, (ii) Transformation, (iii) Scale and Biasing. The variable like, maltose, phosphate, temperature, pHs is highly independent they have been used as such without changing the dimensionality of the model. Further the domain range of the input and output is neither too large nor too small. So no transformation has been used. The even distribution of the date points rules out the option of biasing. The data has been scaled down between 0 and 1 by normalizing them with their respective maximum values. Only 90% of the data set was used for training the ANN. The rest was used for a blind test. Fig. 1 depicts the multilayer feed forward neural network architecture used in this study. The circles represent neurons arranged in three layers, input, hidden and output. Each connection has a weight associated with it. The input, hidden and output units carry out the calculations as shown in Fig. 2 (Venkatasubramanian et al. 1990). First a weighted sum of the inputs is taken and then the output is calculated using a non-decreasing and differentiable transfer functions as shown in Fig. 3. Usually a sigmoid function $f(y)$ is used:

$$f(y) = \frac{1}{1 + e^{-Y}} \quad (1)$$

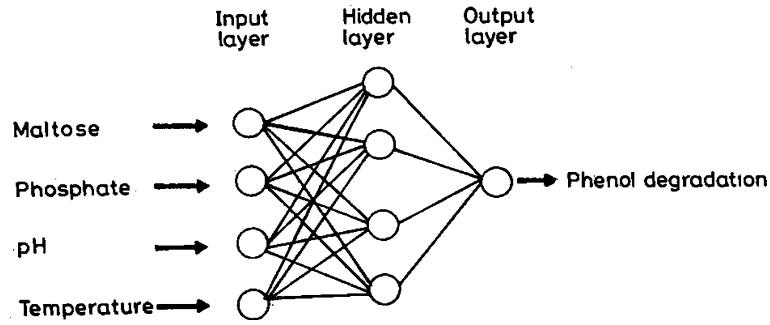

Fig. 1 Neural architecture of the phenol degradation system

The form of this function is shown in Fig. 3. The Networks shown in Fig. 1 learns by making changes in the weights associated with each connection in accordance with the learning rule. The back propagation algorithm (Rumelhert et al. 1986) used in the present study is the most investigated supervised learning algorithm. This algorithm has been used in a lot of applications, written character recognition, data analysis, speech recognitions, game playing programs, properties prediction (Rumelhert et al. 1986; Andre et al. 1993; Sejnowski and Rosenberg 1987; Tesauro and Sejnowski 1989; Ming and Joi 1974; Qian and Senjnowski 1988) and so on. The algorithm uses a learning rule called the Generalized Delta Rule (GDR) which is a generalization of the steepest decent learning algorithm presented by Widrow (Widrow and Hoff 1960; Mathalai Balan et al. 1999). The normalized values of phenol degradation (X^*) can be predicted using the following set of equations;

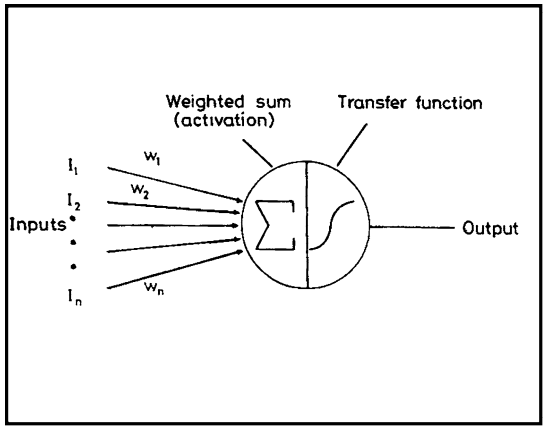

Fig. 2 Processing element (Neuron)

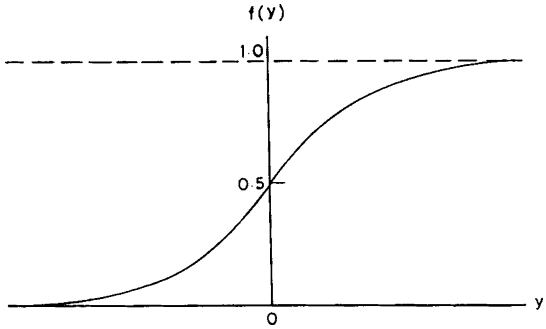

Fig. 3 Sigmoid function

$$Y_j = W_{j1} f(\text{Maltose}^* + W_{j2} f(\text{Phosphate}^*) + W_{j3} f(\text{Temperature}^*) + W_{j4} f(pH^*) \text{with } j = 1, 2, 3, 4 \quad (2)$$

where maltose*, phosphate*, temperature*, pH* are the normalized value of Maltose, Phosphate, Temperature, pH, Y_j—is the weighted sum of inputs to the hidden layer, f is the sigmoid function discussed above;

$$f(Y_j) = \frac{1}{1 + e^{-Yj}} \quad (3)$$

Now the output from the hidden layer is passed on the next layer.

$$Y_{\text{output}} = \sum_{j=1}^{4} W_{0j} f(Y_j) \quad (4)$$

Therefore $X^* = f(Y_{\text{ouput}})$
where Y_{output} is the weighted sum of the inputs from the hidden layer to the output neuron. The training set and blind test data points were then feed to the ANN model and all the output values were calculated according to the above equations.

Multi regression analysis (MRA)

A MRA is done to obtain the coefficients and the equation can be used to predict the responses. The design of experiments chosen for this study was Box and Behnken (1960), a fractional factorial design for four independent variables. In a system involving four independent variables X_1, X_2, X_3 and X_4 the mathematical relationship for the response Y on these variables can be approximated by the quadratic (second degree) polynomial Equation (Box and Behnken 1960; Cochran and Cox 1968; Annadurai et al. 1996).

$$\begin{aligned} Y = {} & b_0 + b_1X_1 + b_2X_2 + b_3X_3 + b_4X_4 + b_{11}X_1^2 \\ & + b_{22}X_2^2 + b_{33}X_3^2 + b_{44}X_4^2 \\ & + b_{12}X_1X_2 + b_{13}X_1X_3 + b_{14}X_1X_4 \\ & + b_{23}X_2X_3 + b_{24}X_2X_4 + b_{34}X_3X_4 \end{aligned} \quad (5)$$

where Y is the predicted response; b_0 is the constant, b_1, b_2, b_3 and b_4 is the linear coefficient, b_{12}, b_{13}, b_{14}, b_{23}, b_{24} and b_{34} is the cross product coefficients, b_{11}, b_{22}, b_{33} and b_{44} is the quadratic coefficients. In the model given in Eq. (5), interactions higher than first order have been neglected. This design is performed because relatively few experimental combinations of the variables are adequate to estimate potentially complex response functions. A total of 29 experiments were necessary to estimate the 15 coefficients of the model. Using multiple quadratic regression analysis (Design Expert: 5.7.0.1, State Ease—Inc. Minneapolis, MN) the set of coefficients for degradation of phenol on *P. Pictorum* (NICM 2074) can be estimated.

Materials and methods

Phenol, 4-amino antipyrine and all other chemicals used were from Himedia Laboratories Pvt. Limited Mumbai, India.

Design experiments

P*seudomonas pictorum* (2074) was obtained from culture collection (NCL) Pune, India. The microorganism was maintained standard nutrient medium. The mineral medium composition is as follows phenol 0.600 g/l, K_2HPO_4; 1.5 g/l; KH_2PO_4 0.5 g/l; $(NH_4)_2$ SO_4; 0.5 g/l, NaCI; 0.5 g/l, $MgSO_4$; 0.5 g/l $CaC1_2$; 0.002 g/l; $FeSO_4$; 0.002 g/l was used for the batch studies. The present study aims at the effect of maltose (0.025, 0.05, 0.075 g/l), phosphate (3, 12.5, 22 g/l), pH (7, 8, 9) and temperature (30°C, 32°C, 34°C) the levels of variables as shown in Table 1. Experimental design as shown in Table 1 was drawn up to study the effect of maltose, phosphate, temperature and pH. The experimental studies were carried out in conical flasks containing minimal medium and inoculated with *Pseudomonas pictorum* at 30°C and 180 rpm in lab-line orbit environ shaker for 48 h. After 48 h estimation of phenol degradation, phenol present was estimated using standard method of spectrophotometer analysis (Beckman Du40 model) 4-amino antipyrine was used as the coloring reagent (λ_{max}—500 nm (Clessceri and Trossel 1989).

Results

Biological treatment using *Pseudomonas pictorum* (NICM 2074) was the most effective method for degrading phenol from variety of industrial effluents. Fundamental studies on the kinetics of microbial biodegradation of phenol under controlled conditions were not stressed enough. The mechanisms responsible for the successful degradation of hazardous compounds are not understood completely and only a few degradation pathways have been unraveled. So there is a need for more studies at the bench scale examining the effects of multiple nutrients on biodegradation of phenol. The previous studies revealed that the phenol degradation by *pseudomonas* sp.is likely to be suitable for quick degradation of chlorophenols, resorcinol (Chitra and Gowri 1996). Biodegradion is a well established and powerful technique for treating domestic and industrial effluents. For low volumes of waste waters, phenol degradation technique using *Pseudomonas* sp. has been adopted.

The mathematical models which explain the effect of various nutrients on the biodegradation of phenol mainly rely on its correlation coefficient, RMS error (%) and average absolute error (%).

Table 1 The actual design of experiments

Experimental No.	Maltose (g/l) X_1	Phosphate (g/l) X_2	pH X_3	Temperature (°C) X_4
1	0.025 (–)	3 (–)	8 (0)	32 (0)
2	0.075 (+)	3 (–)	8 (0)	32 (0)
3	0.025 (–)	22 (+)	8 (0)	32 (0)
4	0.075 (+)	22 (+)	8 (0)	32 (0)
5	0.050 (0)	12.5 (0)	7 (–)	30 (–)
6	0.050 (0)	12.5 (0)	9 (+)	30 (–)
7	0.050 (0)	12.5 (0)	7 (–)	34 (+)
8	0.050 (0)	12.5 (0)	8 (0)	34 (+)
9	0.025 (–)	12.5 (0)	8 (0)	30 (–)
10	0.075 (+)	12.5 (0)	8 (0)	30 (–)
11	0.025 (–)	12.5 (0)	8 (0)	34 (+)
12	0.075 (+)	12.5 (0)	7 (–)	34 (+)
13	0.050 (0)	3 (–)	7 (–)	32 (0)
14	0.050 (0)	22 (+)	9 (+)	32 (0)
15	0.050 (0)	3 (–)	9 (+)	32 (0)
16	0.050 (0)	22 (+)	7 (–)	32 (0)
17	0.025 (–)	12.5 (0)	7 (–)	32 (0)
18	0.075 (+)	12.5 (0)	9 (+)	32 (0)
19	0.025 (–)	12.5 (0)	9 (+)	32 (0)
20	0.075 (+)	12.5 (0)	8 (0)	32 (0)
21	0.050 (0)	3 (–)	8 (0)	30 (–)
22	0.050 (0)	22 (+)	8 (0)	30 (–)
23	0.050 (0)	3 (–)	8 (0)	34 (+)
24	0.050 (0)	12.5 (0)	8 (0)	34 (+)
25	0.050 (0)	12.5 (0)	8 (0)	32 (0)
26	0.050 (0)	12.5 (0)	8 (0)	32 (0)
27	0.050 (0)	12.5 (0)	8 (0)	32 (0)
28	0.050 (0)	12.5 (0)	8 (0)	32 (0)
29	0.050 (0)	12.5 (0)	8 (0)	32 (0)

The total numbers of experimental runs were 29 and are shown in Table 1. 24 experimental data were used to train the network and to fit the MRA model. Twenty-four experimental data were used to train the network and to fit the ANN and MRA model. The remaining 3 data points (10% of data) unused were used to predict the percentage of phenol degradation. The error associated with this blind set is characteristic of the networks ability to generalize. All calculations were performed using Turbo C^{++} and Lotus 1-2-3. Table 2, 3 and 4 suggest that both ANN and MRA perform equally well and the difference in correlation coefficient for ANN model is shown in Fig. 4.

The maximum degradation of phenol was found to be 90.3%. The corresponding maltose and phosphate concentration required were 0.05 g/l and 12.5 g/l respectively. Higher concentration of maltose and phosphate however had no effect on phenol degradation. At these levels mineralization of medium was rapid and the *P. pictorum* (NICM 2074) grew extensively, but the rate and extent of degradation were low and the bacterial population never increased in media with very low and very high concentration of phosphate ions due to feed back inhibition. The same pattern of degradation was obtained (Vela and James 1978) with *Pseudomonas* sp. and *Cotynebacterium* sp. by growing in media containing pentachlorophenol (Inniss 1976).

Discussion

Box–Behnken design experiment was used with independent variables to obtain the combination of values that optimizes the response, which allows the design of a minimal number of experimental runs. Regression results give an R^2-value of 0.8842 and 0.8683 and an F-value (Fisher's F-test) of 7.63 using optimization of phenol degradation. The lack of fit is also significant in this case because the probability values (P-value)—($P > F$) are less than 0.0001. A small probability value indicates that adding the quadratic terms has improved the regression model. It is also seen from analyzed results that equation used here fits the experimental data with a standard deviation (Root MSE/Dep mean) of

Table 2 Weights and coefficients of ANN and MRA models

Coefficient of MRA	Weights of ANN	
	Input layer	Hidden layer
$b_o = 86.13$	$W_{11} = 0.845$	$W_{10} = 5.69$
$b_1 = -0.76$	$W_{12} = -12.32$	$W_{20} = 1.36$
$b_2 = 3.56$	$W_{13} = -0.12$	$W_{30} = 0–99$
$b_3 = 1.49$	$W_{14} = -8.10$	$W_{40} = -5.87$
$b_4 = -1.52$	$W_{21} = -2.33$	
$b_{11} = -7.64$	$W_{22} = 2.72$	
$b_{22} = -3.74$	$W_{23} = 10.33$	
$b_{33} = 1–09$	$W_{24} = -0.97$	
$b_{44} = 0.59$	$W_{31} = 5.224$	
$b_{12} = -3.75$	$W_{32} = 4.59$	
$b_{13} = 3.71$	$W_{33} = -0.27$	
$b_{14} = -2.87$	$W_{34} = 5.07$	
$b_{23} = -3.47$	$W_{41} = -7.11$	
$b_{24} = 1.27$	$W_{42} = 4.08$	
$b_{34} = 0.23$	$W_{43} = -2.79$	
	$W_{44} = -3.01$	

Table 3 Output of ANN and MRA model for the training set

Experimental No.	Maltose (g/l)	Phosphate (g/l)	Temperature (°C)	pH	Phenol degradation	ANN output	MRA output
1	0.075	3.0	32	8	73.20	74.28	73.90
2	0.025	22.0	32	8	83.60	82.75	82.90
3	0.075	22.0	32	8	74.00	76.80	73.50
4	0.050	12.5	30	7	88.10	85.20	90.20
5	0.050	12.5	30	9	90.30	90.48	92.00
6	0.050	12.5	34	7	84.67	85.05	85.30
7	0.050	12.5	34	9	87.80	88.43	77.50
8	0.025	12.5	30	8	79.20	80.23	84.00
9	0.075	12.5	30	8	82.80	80.49	83.10
10	0.025	12.5	34	8	82.30	81.02	73.10
11	0.075	12.5	34	8	74.40	75.84	73.00
12	0.050	3.0	32	7	74.20	73.05	89.10
13	0.050	22.0	32	7	87.30	86.94	84.50
14	0.050	3.0	32	9	87.80	86.53	85.00
15	0.050	22.0	32	9	87.00	88.52	82.10
16	0.025	12.5	32	7	83.20	83.52	78.10
17	0.025	12.5	32	9	75.40	75.43	81.10
18	0.050	3.0	30	8	82.10	83.31	85.10
19	0.050	22.0	30	8	86.50	87.96	76.90
20	0.050	3.0	34	8	76.66	77.54	86.50
21	0.050	22.0	34	8	85.50	87.65	86.50
22	0.050	12.5	32	8	85.60	87.25	86.50
23	0.050	12.5	32	8	85.60	87.25	86.50
24	0.050	12.5	32	8	87.25	87.50	86.50
	RMS error (%)					1.4302	1.5792
	Average absolute error (%)					1.706	1.775
	Coefficient of correlation					0.97	0.90

3.63% and 85.14%, respectively. The regression equation obtained after analysis of variance gives the level of degradation under different conditions. ANN is better and is recommended due to its low RMS error and average absolute error (1.438%, 1.71%) than that of MRA (1.57%, 1.77%) while testing with the data used for training. Also ANN shows further less absolute average error (2.14%), while testing with the blind set. Minimal medium with maltose, phosphate, temperature and pH influenced degradation of phenol was found to be 90.3% and the corresponding optimum level was maltose: 0.05g/l, Phosphate: 12.5g/l, pH: 7.0 and temperature: 30°C.

The internal environment of all living cells in believed to be approximately neutral. Most of the organisms cannot tolerate pH levels below 4.0 or above 9.0 (Kim and Armstrong 1981). The permeated substances can upset the internal pH balance since the bacterial activity decreases as the pH deviates from neutral conditions. Biolog-

Table 4 Output of ANN and MRA model for blind test data

Experimental No.	Maltose (g/l)	Phosphate (g/l)	Temperature (°C)	pH	Phenol degradation	ANN output	MRA output
1	0.025	3.0	32	8	67.8	67.55	68.10
2	0.075	12.5	32	7	77.2	74.57	73.50
3	0.075	12.5	32	9	82.4	82.30	84.00
	Average absolute error (%).					2.14	3.16

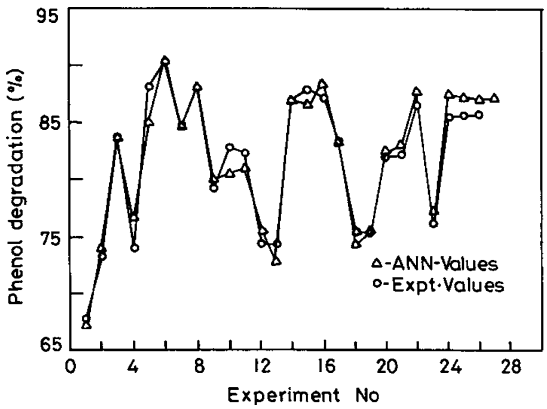

Fig. 4 Graph between ANN values and experimental value

ical waste water treatment process can operate at low temperature provided sufficient time is allowed for these organisms to degrade the organic wastes. Microbiological degradation of phenol in industrial waste water is affected by temperature is an unexpected manner. The efficiency of treatment by microbiological activity on phenol and other contaminants was significantly good (Novak 1974; Vela and James 1978; Vela and Rainey 1976; Inniss 1976; Kushner 1978). The bacterial activity rapidly reduces at temperatures below or above the optimum temperature range, whereas the bacterial activity is not much affected by temperature change within the optimum temperature range.

Conclusion

Degradation of phenol by the *Pseudomonas pictorum* (NICM 2074) with the addition of varying levels of maltose, phosphate, pH and temperature was modeled using ANN and MRA design experiment of the experimental data collected. ANN model with 4 neuron in input and hidden layer and one neuron in output layer performed better than the MRA model owing to its lesser RMS error (%) and average absolute error (%) associated with the predicted values. Temperature and pH also play a key role in determining the rate constant of biological degradation.

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