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Bioremediation of Cr(VI) contaminated soil/sludge: Experimental studies and development of a management model

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ABSTRACT

Bioremediation studies were carried out for the treatment of Cr(VI) bearing sludge using indigenous microorganisms isolated from a chromium contaminated site. Effects of moisture content, initial substrate and biomass concentrations on the bioremediation process were studied by conducting batch and continuous experiments. The leachability of total chromium and Cr(VI) from remediated soil was evaluated and compared with that of untreated soil. Experimental data was used to determine biokinetic parameters and validate a mathematical model. Single objective and multi-objective management models were developed by embedding the mathematical model describing the process in a simulation–optimization framework. Single objective management models considered either cost minimization or minimization of time for treatment. Genetic Algorithm, available in MATLAB tool box was used for solving the optimization problems. Applicability of proposed management models was demonstrated for the remediation of Cr(VI) bearing sludge in Ranipet, Tamilnadu, India. Multi-objective management model was used to derive the Pareto-optimal front, which describes the trade off between the cost of treatment and the time taken for treatment.

1. Introduction

Chromium contaminated sites exist in India and other countries [1,2]. In the chromium contaminated site at Ranipet in Tamilnadu, identified as one of the most contaminated sites [3], approximately 220,000 tons of solid waste has been lying untreated. Due to high porosity of the soil, leachate containing Cr(VI) has contaminated the soil and aquifer in the nearby areas. Remediation of such contaminated hazardous waste sites poses a number of unique challenges and there is an urgent need to find cost-effective and environmentally friendly techniques.

Various hazardous and industrial wastes are currently managed using physico-chemical processes which transfer the contaminants from one environmental compartment to another, and in addition incur high energy and chemical costs in their operations. Bioremediation is one of the promising technologies which is expected to play an important role in a contaminated site clean up. Bioremediation strategy for remediation of Cr(VI) contaminated soil/aquifer involves detoxification of Cr(VI) by reducing it to Cr(III). Cr(III) forms insoluble $Cr(OH)_3$ in the pH range of 6–9 ($Ksp = 6.7 \times 10^{-13}$), severely restricting its ability to migrate through groundwater [4].

Many microbes were reported to reduce Cr(VI) under aerobic and anaerobic conditions [5–9]. Polti et al. [10] employed *Streptomyces* sp. MC1 (an isolate from sugar cane plantation) for remediation of chromium contaminated soil. Chai et al. [8] studied Cr(VI) remediation by indigenous bacteria in soils contaminated by chromium containing slag from a steel-alloy factory. Rama Krishna and Philip [6] developed a novel *ex situ* treatment technology in which the leached Cr(VI) from the contaminated soil was treated in a bioreactor for Cr(VI) reduction, followed by a biosorption column for Cr(III) removal. Jeyasingh and Philip [5] reported that a bacterial concentration of 15 ± 1.0 mg/g of soil (wet weight) and 50 mg of molasses/g of soil as carbon source were required for the maximum Cr(VI) reduction. Desjardina et al. [11] showed that *Streptomyces thermocarboxydus* could precipitate Cr(VI) as a chromium oxyhydroxide with a γCrOOH like local structure. Douglas et al. [12] reported that addition of molasses and nutrients resulted up to 67% reduction of Cr(VI) (initial concentration, 67 mg/L) in 35 days in an unsaturated batch experiment. Tseng and Benefieldt [13] studied *in situ* bioremediation of Cr(VI) contaminated soil by supplying various electron acceptors such as oxygen, nitrate, sulfate, and iron at low temperatures of 10 °C and demonstrated that addition of sugar enhanced chromium reduction. Quana et al. [14] studied the kinetics of detoxification efficiency of *B. megaterium* for the treatment of hexavalent chromium contaminated slag.

Though many studies have been reported on Cr(VI) contaminated soil remediation, most of them were confined to laboratory

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Table 1
Soil characteristics.

Parameter	Concentration
Soil organic matter	6.7%
Cr(VI)	2.4–3.0 mg/g of soil
Total chromium	9.5–10.5 mg/g of soil
pH	9.8
Sand	57%
Silt	37%
Clay	6%

scale systems. However, it is essential to conduct a pilot scale study and evolve an appropriate management strategy before starting the field remediation. Parameters such as moisture content, initial biomass concentration and electron donor play a significant role in achieving the remediation in shortest possible time, with least cost. Thus there is a need for developing a management model based on pilot scale experimental data. Several mathematical models are available for performance evaluation and management of Cr(VI) contaminated wastewaters and aquifers [15–18]. Shieh and Peralta [15] developed a model combining Genetic Algorithms and simulated annealing with BIOPLUME II for the optimal design of bioremediation systems. Hu et al. [19] have developed a dynamic predictive control system for *in situ* bioremediation process. Although many generic simulation–optimization packages are available for optimal design of aquifer remediation strategies, not many studies have been carried out with particular reference to large scale soil remediation.

The objective of this work was to isolate and enrich Cr(VI) reducing microbes for the remediation of Cr(VI) bearing solid waste and evaluate the performance of the process in a pilot scale system. Also, an attempt was made to develop a mathematical model for simulating the process. The simulation model was used in a simulation–optimization framework for evolving optimal strategy for cost-effective remediation of large scale Cr(VI) contaminated dump sites.

2. Materials and methods

2.1. Soil and solid waste samples

To isolate Cr(VI) reducing microbes, soil samples (150 g each) were collected from seven different locations of the contaminated site at Ranipet, Tamilnadu, India in clean polyethylene bags and preserved in a deep freezer (APNA Scientific Suppliers, Chennai). Composite samples of the Cr(VI) bearing solid waste were taken at a depth of 0.5 m from the surface of the dump in Ranipet, Tamilnadu, India. After air drying, the solid waste samples were crushed and sieved. Soil with an average particle size less than or equal to 300 μm was used for the characterization in the laboratory as per standard procedure [20]. The characteristics thus determined are given in Table 1. The solid waste samples collected from the site were crushed lightly and large boulders were removed before they were used in remediation studies.

2.2. Nutrient and mineral media

The nutrient medium for microbial growth consisted of peptone 5 g, beef extract 1.5 g, yeast extract 1.5 g and sodium chloride 5 g in 1 L of distilled water and the mineral medium consisted of K_2HPO_4—0.03 g, KH_2PO_4—0.05 g, NaCl—0.01 g, NH_4Cl—0.03 g, $MgSO_4$—0.01 g, molasses 5 g, and yeast extract 1 g in 1 L of distilled water. The pH was maintained at 7 ± 0.2 by using HCl or NaOH. Sterilized media were used for all the studies.

2.3. Analytical procedures

2.3.1. Extraction and analysis of Cr(VI) and total chromium

For the extraction of Cr(VI) and total chromium from soil, an alkaline digestion method and nitric acid/sulfuric acid digestion method were used, respectively, as per the Standard Methods [21]. Hexavalent chromium was measured colorimetrically at 540 nm by reaction with diphenyl carbazide in acidic conditions [21]. Cr(III) was analyzed using Atomic Absorption Spectrometer (Perkin Elmer, USA).

2.3.2. Measurement of cell density

Overnight cultures were centrifuged, the cell pellets were washed with saline water thrice, re-suspended in saline water, homogenized and were used as stock solution. Known volumes of these solutions were filtered through 0.45 μm filter paper (Millipore, USA) to find out dry weights of cells. Corresponding absorbance was measured at 600 nm using a UV spectrophotometer (Techcomp, UK). This information was used to prepare a calibration curve. For unknown samples, the absorbance was measured at 600 nm and was converted to dry weight using absorbance versus dry weight calibration curve [22].

2.4. Experimental methods

2.4.1. Enrichment of the Cr(VI) reducing bacterial strains

Bacterial strains were isolated from the soil samples collected in and around the contaminated site. About 1 g of soil sample was added to flask containing 100 mL of nutrient medium, closed using Teflon stoppers, and incubated for 24 h in facultative condition. Then, 1 mL of supernatant from this flask was transferred to 100 mL nutrient broth containing 100 mg/L of Cr(VI). This procedure was repeated by progressively increasing Cr(VI) in the nutrient medium up to 500 mg/L. A loopful from the above mixture was streaked on agar slants, incubated for 24 h and stored in a freezer at 4 °C for further use.

2.4.2. Screening of the enriched cultures

The enriched cultures obtained from seven locations of the contaminated site were evaluated for their Cr(VI) reduction potential. Further studies were conducted using the most promising bacterial strain emerging from the screening test. For the screening test, seven conical flasks with 100 mL of autoclaved nutrient medium, spiked with 50 mg/L of Cr(VI), were inoculated with an equal quantity of pre-grown bacterial cultures isolated from different locations. A control (all conditions were same except that the bacterial cells were not added) was employed to quantify the abiotic reduction of Cr(VI) in all the experiments.

2.4.3. Optimum moisture content in soil reactors

The effect of moisture content on Cr(VI) reduction in soil reactors was studied by varying the moisture content of the reaction mixture. These experiments were conducted under aerobic condition in four reactors containing 25 g of Cr(VI) contaminated soil with initial Cr(VI), molasses and microbial concentration of 2.5, 50 and 15 mg/g of soil, respectively. The moisture content was varied from 20% to 50%, respectively. One reactor with 50% moisture was used as control.

2.4.4. Effect of concentration of substrate (molasses) on Cr(VI) reduction in soil

The effect of substrate (molasses) concentration on Cr(VI) reduction was evaluated using three plastic containers of 500 mL capacity, each with 200 g of hexavalent chromium bearing soil. In the first container, 10 g (50 mg/g of soil) of molasses and 50 mL of mineral medium were added, and this container was used as

control. In the second and third containers, 3 g (15 mg/g of soil) of bacteria, and 50 mL of mineral medium were taken. The concentrations of molasses in the second and third containers were 5 g (25 mg/g of soil) and 10 g (50 mg/g of soil), respectively. The moisture content was maintained at 40% by adding mineral medium at regular intervals. All the reactors were operated under facultative conditions, without any leachate collection system. The soil samples were collected and analyzed for Cr(VI), total chromium, COD and protein.

2.4.5. Effect of concentration of biomass (cell) on Cr(VI) reduction in soil

The effect of biomass concentration on Cr(VI) reduction was evaluated by using four plastic containers of 500 mL capacity each with 200 g of hexavalent chromium bearing soil. Ten grams (50 mg/g of soil) of molasses and 50 mL of mineral medium were added to the first container, which was kept as control. In the second, third and fourth containers, the biomass concentrations were 15, 20 and 40 mg/g of soil, respectively. The concentrations of molasses and the mineral medium in these three containers were same as that in the control reactor. The moisture content was maintained at 40% by adding mineral medium as and when needed. All the reactors were operated under anaerobic conditions without any provision for leachate collection. Soil samples were collected at regular intervals and analyzed for Cr(VI), total chromium, COD and protein.

2.4.6. Cr(VI) reduction in soil reactors

Two plastic containers of 5 L capacity were used for this study. Two hundred grams of contaminated soil, 3 g (15 mg/g of soil) of biomass (cell), 10 g (50 mg/g of soil) of molasses and 50 mL of mineral medium were added to the first container. The second container was used as a control reactor in which all the conditions were same as in the first reactor except that there was no biomass. Sufficient quantity of mineral medium was added periodically to maintain the required moisture content. Both the reactors were operated under facultative anaerobic condition in composting mode, without any provision for leachate collection. Soil samples were collected at regular intervals and analyzed for Cr(VI), COD, protein and total chromium. Once the Cr(VI) concentration in the soil in the reactor reduced to non-detectable limit, equal amount of contaminated soil was added, without any further addition of biomass and the reactor was operated until the Cr(VI) concentration reduced to non-detectable limit again. This process was repeated for a total of five cycles, with a geometric increase in soil mass at the beginning of each cycle. Also, 10 g of molasses was added to the reactor at the beginning of each cycle. The soil samples were collected at regular intervals and analyzed for Cr(VI), total chromium, COD and protein.

2.4.7. Leaching study with remediate and unremediated soil

Soil sample was treated with 0.1N acetic acid (pH = 2.88) at a liquid-to-solid ratio of 20:1 for a period of 18 h. The leachate was filtered and analyzed for total chromium and Cr(VI) as per the toxicity characteristic leaching procedure (TCLP) test prescribed by US Environmental Protection Agency [23]. Leaching studies were also conducted with tap water and distilled water where 20 g of soil was added to 400 mL of respective extraction fluids and the filtered leachates were analyzed for total and hexavalent chromium after 18 h of contact time.

3. Mathematical model

First, a mathematical model was developed for simulating the Cr(VI) reduction, substrate utilization and microbial growth in the batch experiments. This model considers the effect of moisture content on the microbial growth. The governing equations, based on the Monod's model with inhibition [24,1], are presented below

$$\frac{dM}{dt} = M\left(\frac{\mu_{\max}S}{K_s+S}\right)\left(\frac{K_i}{K_i+\mathrm{Cr}_6}\right) \quad (1)$$

$$\frac{dS}{dt} = M\left(\frac{1}{Y_T}\right)\left(\frac{\mu_{\max}S}{K_s+S}\right)\left(\frac{K_i}{K_i+\mathrm{Cr}_6}\right) \quad (2)$$

$$\frac{d\mathrm{Cr}_6}{dt} = M(\eta)\left(\frac{1}{Y_T}\right)\left(\frac{\mu_{\max}S}{K_s+S}\right)\left(\frac{K_i}{K_i+\mathrm{Cr}_6}\right) \quad (3)$$

$$\frac{\mu_{\max}}{\mu_{\max,o}} = a\left[\frac{\theta}{\theta_o}\right]^b \quad \text{for } \theta \le \theta_o \quad (4)$$

$$\frac{\mu_{\max}}{\mu_{\max,o}} = 1 \quad \text{for } \theta > \theta_o$$

where, M is the bacterial biomass concentration in mg/L, S is the concentration of residual substrate (organic matter, OM) in mg/L, Cr_6 is the concentration of hexavalent chromium in mg/L, $\mu_{\max}$ is the maximum specific growth rate, $\mu_{\max,o}$ is the maximum specific growth rate for reference moisture conditions, K_i is the chromium inhibition constant in mg/L, K_s is the half saturation constant in mg/L, η is mg of Cr(VI) reduced/g of substrate utilized, Y_T is the yield coefficient, θ is the moisture content in the soil and θ_o is the reference moisture content.

It may be noted that Eq. (1) is Monod's equation with inhibition for microbial growth. Cr(VI) concentration used in the batch studies was much above the inhibition concentration (5–7 mg/L). Microbial activity in unsaturated soils strongly depends on the moisture content. This is because the substrate/pollutant availability for microbes depends upon the moisture present in the system. In this work, this dependency on moisture content is modeled through Eq. (4), in which a and b are empirical parameters, which need to be estimated from the experimental data. Reference moisture content, θ_o in this study represents that value of moisture content above which variation in moisture content does not have significant effect on microbial activity. Eqs. (1)–(3) are numerically solved using the classical fourth-order Runge-Kutta method.

4. Management model

To remediate a given amount of Cr(VI) contaminated soil/sludge, first a certain quantity of the contaminated soil will be mixed with a certain amount of biomass and molasses. Water will be added to the contaminated soil as and when the moisture content of the soil reduces below the specified limit of 40%. Once the Cr(VI) concentration in the contaminated soil reduces to non-detectable level, again an equal amount of contaminated soil as in the previous cycle will be mixed with already remediated soil. There will not be any addition of fresh biomass. Molasses will also be added in such a way that the molasses concentration becomes equal to the initial molasses concentration in the first cycle. It may be noted here that molasses will also be added to the system whenever molasses concentration reduces to below the detectable limit during any cycle of operation. This process will be continued until all the soil is treated. COD will be added at the beginning of the last cycle ($\mathrm{COD}_{\mathrm{last}}$), in such a way that the residual COD in the treated soil is not more than a specified value. The optimization has to be carried out with respect to the time required for the entire soil to be remediated and the total cost involved in the entire process. This can be achieved by solving the optimization problem using a Genetic Algorithm (GA). A simulation–optimization frame work is utilized for this purpose. In the optimization problem, the decision vector comprises of (i) amount of soil taken initially in the first cycle, W_{initial}; (ii) initial concentration of biomass in the first

cycle, $M_{initial}$; (iii) initial concentration of molasses in the first cycle, $S_{initial}$; (iv) make up concentration of molasses added during any cycle when it falls below detectable limit (as a fraction of initial molasses concentration in the first cycle), f_{inter}; (v) concentration of molasses added during the last cycle, COD_{last}. The objective function of the optimization problem could be either cost minimization (Management Model-I) or minimization of time taken for remediation of entire sludge/contaminated soil (Management Model-II) or both (Management Model-III). Details of only the multi-objective management model (Management Model-III) are presented below for the sake of brevity.

4.1. Multi-objective management model

Management Model-III is a multi-objective management model in which the two objectives are to (i) minimize the total cost involved in the remediation of entire amount of the sludge, and (ii) minimize the total time taken for remediation. Optimization problem for this model can be formulated as given below.

4.1.1. Objective functions

$$\text{Minimize } C = C_m \cdot W_{initial} \cdot M_{initial} + C_s \cdot W_{initial} \cdot S_{initial} + \sum_{i=2}^{N_c-1} C_s \cdot W(i) \cdot S_{inter} + C_s \cdot W_{last} \cdot COD_{last} + \sum_{i=1}^{N_c} C_s \cdot 2 \cdot W(i) \cdot f_{inter} \cdot S_{initial} \cdot \delta(i)$$

Minimize T = time taken for remediation

Subject to following constraints:

$$0 < M_{initial} < M_{max}$$
$$0 < S_{initial} < S_{max}$$
$$0 < W_{initial} < W_{max}$$
$$S_{final} < S_{permissible}$$
$$T = f_1(M_{initial}, S_{initial}, W_{initial}, W_{total}, COD_{last}, \text{bio-kinetic parameters of system})$$
$$C = f_3(M_{initial}, S_{initial}, W_{initial}, W_{total}, F_{inter}, COD_{last}, \text{bio-kinetic parameters of system})$$
$$S_{final} = f_2(M_{initial}, S_{initial}, W_{initial}, W_{total}, COD_{last}, \text{bio-kinetic parameters of system})$$
$$N_c = f_4(W_{initial}, W_{total})$$

In the above equations, S_{final} = molasses concentration in the soil at the end of remediation i.e. at the end of last cycle of operation; T = total time taken for the complete remediation i.e. time taken for completion of all cycles of operation; W_{total} = total amount of contaminated soil to be remediated. M_{max} gives upper bound for the available biomass. S_{max} gives the upper bound for the concentration of molasses at the start of the first cycle of remediation, and W_{max} gives upper bound for the amount of soil to be taken in the first cycle. $S_{permissible}$ = permissible molasses concentration in the soil. This is the permissible COD concentration in the soil such that any leachate from this remediated soil will not have a COD more than 20 mg/L (permissible COD in drinking water). This value may be determined based on the adsorption/desorption equilibrium studies. In the present study, this value is fixed at 0.30 mg/g, based on laboratory experiments. An upper bound on the $M_{initial}$ is imposed because there is a limitation on the availability of Cr(VI) in the aqueous phase based on the desorption characteristics of the soil mass and any concentration of biomass in excess of what is really needed would not be useful as far as Cr(VI) reduction is concerned. Batch experiments with the soil from contaminated site in this study have indicated that this concentration is 25 mg/g. An upper bound is imposed on the $W_{initial}$ because the total amount of biomass available to start the remediation process could be limited, based on the available fermenter capacity. An upper bound is imposed on $S_{initial}$ from the requirements of implementing the Genetic Algorithms for solving the optimization problem. It could be any value. f_1 and f_2 are the functions which give the total time taken for remediation and the final molasses concentration as related to (i) $M_{initial}$, (ii) $S_{initial}$, (iii) $W_{initial}$, (iv) W_{total}, (v) f_{inter}, (vi) COD_{last}, and (vii) biokinetic parameters of the system. The simulation model presented in Section 3 is used for evaluating these functions. S_{inter} is the concentration of molasses added to the soil at the beginning of any intermediate cycle in such a way that the average molasses concentration becomes equal to initial molasses concentration. In the above equations, C_m = unit cost of the microbes, and C_s = unit cost of the molasses. $\delta(i) = 1.0$ in case the molasses concentration goes below the detectable limit during the cycle i and it is equal to zero otherwise. N_c = total number of cycles of operation involved in the remediation, and $W(i)$ = amount of soil during the ith cycle. W_{last} = amount of soil added during the last cycle.

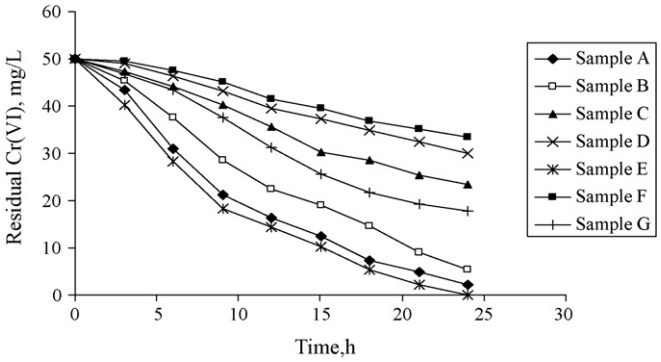

Fig. 1. Kinetics of Cr(VI) reduction by different bacterial strains.

5. Results and discussion

5.1. Screening of microbes for Cr(VI) reduction

Kinetics of Cr(VI) reduction was carried out under aerobic condition with an initial Cr(VI) concentration of 50 mg/L for all the seven isolated bacterial strains and the results are presented in Fig. 1. Among the seven strains screened, the strain isolated from a clay mat near the old effluent treatment plant, which had a high Cr(VI) concentration (Sample E), showed highest Cr(VI) reduction potential. This strain was used for further studies.

5.2. Cr(VI) reduction in aerobic and anaerobic conditions

Earlier studies on Cr(VI) reduction potential under aerobic and anaerobic conditions showed that 100% reduction of Cr(VI) was possible when the concentration of Cr(VI) was lower than 20 mg/L [5]. It was also observed that Cr(VI) reduction was high under aerobic conditions for higher Cr(VI) concentrations. However, provision of aeration may not be economically feasible when large volumes of contaminated soil have to be bio-remediated in the field. Therefore, the facultative anaerobic reduction/bioremediation option was adopted in all further studies though it is slightly less efficient than the aerobic system.

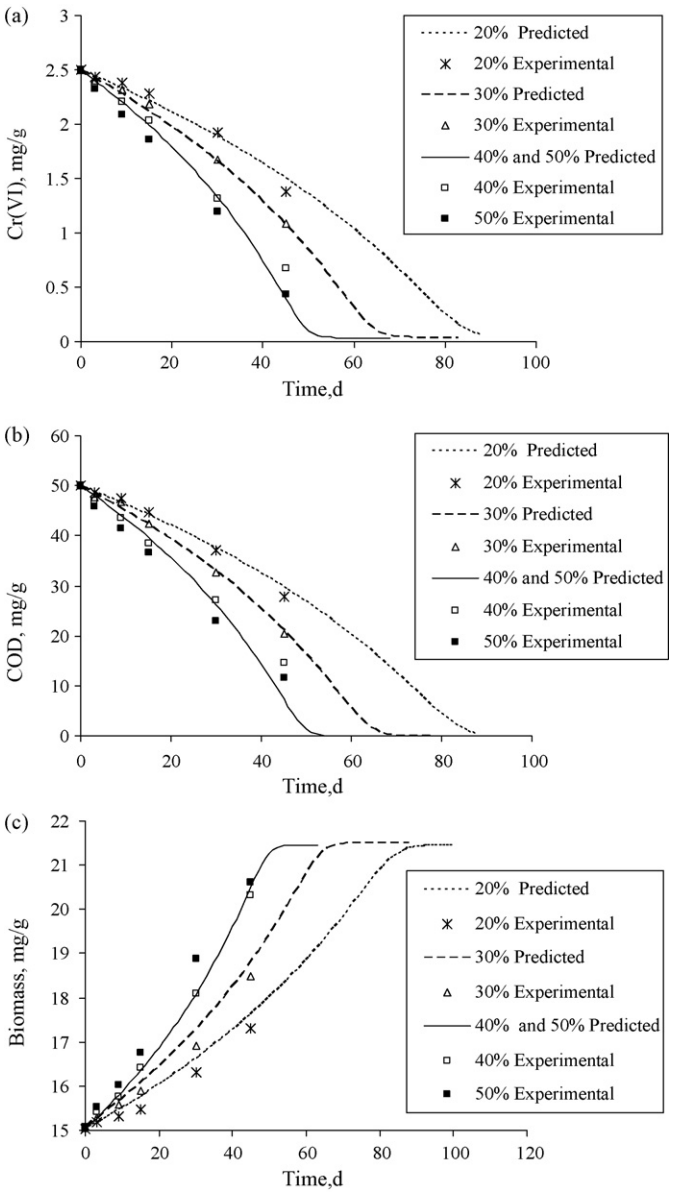

Fig. 2. (a) Experimental and predicted results of Cr(VI) reduction for different moisture contents. (b) Experimental and predicted results of COD reduction for different moisture contents. (c) Experimental and predicted results of growth curve with different moisture content.

5.3. Optimum moisture content for bioremediation

Studies were carried out to find out optimum moisture content under facultative anaerobic condition as described earlier. Experimental and model fitted results are presented in Fig. 2(a–c). It can be observed from these figures that chromium reduction rate increased with increase in moisture content up to a value of 40%. There was no significant improvement in the chromium reduction rate when the moisture content was increased beyond this value. Similar trend was observed in the case of substrate utilization (Fig. 2(b)) as well as biomass growth (Fig. 2(c)). A minimum moisture content is essential for availability of pollutant/substrate in the liquid phase for the microbial utilization. Beyond this minimum moisture content, the adsorption/desorption process might be the controlling process for the availability of pollutant/substrate. From the results of the present study, it may be inferred that this minimum moisture content is 40%. Hence, 40% moisture content was used as the reference moisture content for developing the model using Eq. (4). Biokinetic parameters were estimated from experiments for 40% moisture content ($\mu_{max,o} = 0.030$ (1/d); $K_s = 8.127$ mg/g; $K_i = 0.646$ mg/g; $\eta = 0.049$ and $Y_t = 0.129$) and μ_{max} is calculated for all other moisture contents keeping other parameters constant. The values of a and b obtained were 0.983 and 0.759, respectively. It has been suggested by Kohne et al. [25] that the performance of any mathematical model can be statistically evaluated using the dimensionless modified coefficient of efficiency, E. Details of computation of E are given elsewhere [17].

Table 2
E values for the model fitting of batch experiments with different moisture contents.

Parameters			
Moisture content	Biomass	Substrate	Cr(VI)
20%	0.733	0.898	0.863
30%	0.833	0.945	0.936
40%	0.952	0.751	0.980
50%	0.857	0.778	0.867

Values of E for the simulations in the above batch experiments are presented in Table 2. The E value for chromium reduction ranges from 0.86 to 0.98. It ranges from 0.75 to 0.94 for substrate utilization and from 0.73 to 0.95 for microbial growth. E values for all the validation studies are greater than 0.5, indicating a good performance of the proposed mathematical model.

5.4. Effect of initial molasses and biomass concentrations

Effects of initial biomass and molasses concentrations on Cr(VI) reduction are presented in Figs. 3 and 4. It was found from the experimental results that a biomass concentration of 15 mg/g was sufficient to achieve Cr(VI) reduction and there was no significant increase in Cr(VI) reduction rate beyond this microbial concentration (Fig. 3a). It can be observed from Fig. 4 that an initial molasses concentration of 50 mg/g resulted in significantly higher Cr(VI) reduction rate as compared to that when the molasses concentration was only 25 mg/g. It may be noted here that optimization of carbon source for bioremediation of Cr(VI) contaminated soils was earlier reported by the same group [5,6]. The experimental data obtained for different biomass and molasses concentrations was also used to validate the proposed mathematical model. There is a good agreement between experimental and predicted results as observed form Figs. 3 and 4. E values in these validation runs were greater than 0.5 indicating a good model performance. The disagreement in Cr(VI) reduction curve, when 40 mg/g biomass was used may be due to the fact that the rate of desorption of Cr(VI) from the soil was much less than the possible Cr(VI) reduction rate by the biomass present in the system. As a result, the model predicted a much higher rate of Cr(VI) reduction compared to that observed in the experiments. It may be noted that the model presented in this study does not consider the adsorption–desorption mechanism and the availability of Cr(VI) for microbial reduction. As discussed earlier, the soil used in the experiments was already contaminated with Cr(VI) where as molasses was added externally to the contaminated soil. Hence, availability of molasses could be much higher than that of Cr(VI). This is evident from Fig. 3(b and c) for substrate utilization and microbial growth, where model predictions were closer to the observed values. It is also observed (Fig. 3(b)) that as the time progresses, the deviation between the predicted and observed values of residual COD increased. This may be due to the fact that some part of the molasses was not easily biodegradable and this was not considered in the present model [17].

Table 3
Concentrations of total chromium and Cr(VI) in eluents after the leaching.

Hexavalent chromium			Total chromium		
	Treated (mg/L)	Untreated (mg/L)		Treated (mg/L)	Untreated (mg/L)
Distilled water	5.69	89.48	Distilled water	8.15	94.13
	5.12	91.24		7.23	91.69
Tap water	6.11	92.87	Tap water	8.56	94.62
	6.25	91.41		9.25	92.21
TCLP	9.21	134.96	TCLP	10.26	171.92
	9.56	142.94		11.35	174.35
Acid digestion	–	–	Acid digestion	615.24	627.3
	–	–		619.35	634.2
Alkali digestion	10.93	228.73	Alkali digestion	12.42	232.4
	10.80	226.25		11.79	234.2

5.5. Cr(VI) reduction in soil reactors

Chromium reduction studies were conducted in composting mode as explained earlier. The experimental and model fitted results are presented in Fig. 5(a–c). It was observed in these experiments that the rate of chromium reduction increased with each cycle of operation, indicating a better adoptability of the chromium reducing bacteria to Cr(VI). It can be observed from Fig. 5 that the model predicted results for Cr(VI) reduction (Fig. 5(a)) and biomass growth (Fig. 5(c)) matched with the experimental results very well. *E* values for the prediction of Cr(VI) reduction and biomass growth were 0.86 and 0.93, respectively. However, in the case of residual COD, the matching between the predicted and observed values is not as good (*E* value = 0.70). As discussed earlier, this could be due to the fact that the proposed model did not consider the effect of non-degradable part of the substrate. As the time progresses, this component might have accumulated in the system.

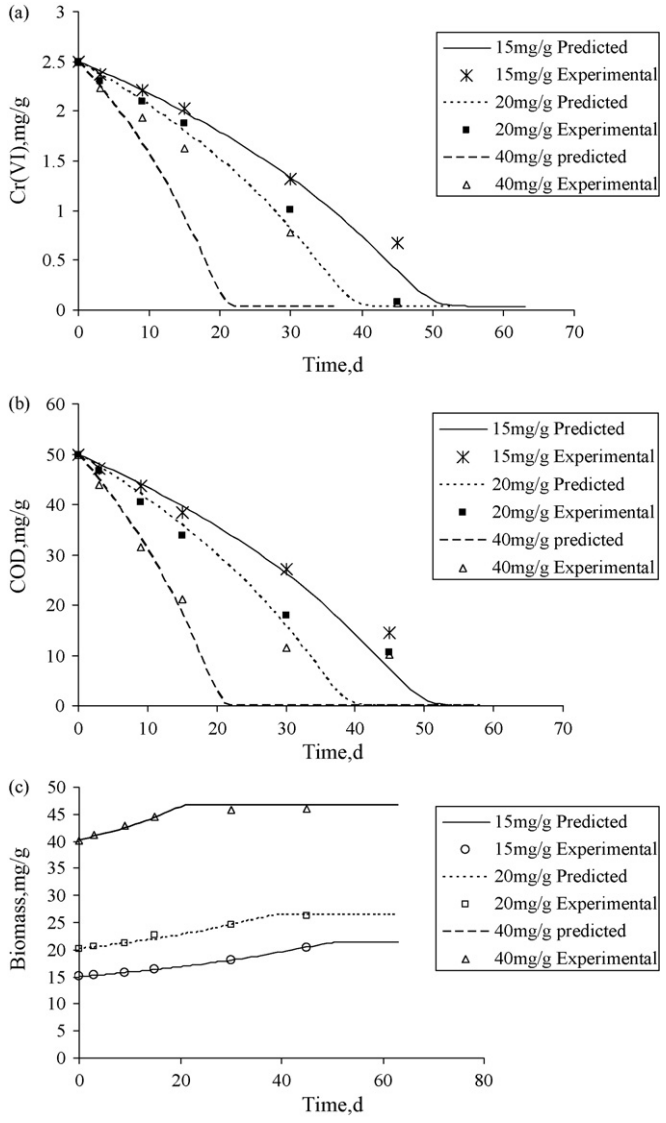

Fig. 3. (a) Experimental and predicted results of Cr(VI) reduction for different initial biomass concentrations. (b) Experimental and predicted results of COD reduction for different initial biomass concentrations. (c) Experimental and predicted results of bacterial growth for different initial biomass concentrations.

5.6. Leaching study with remediated and unremediated soil

Leaching studies were conducted using soil taken from the reactor after approximately 92% of Cr(VI) reduction. Distilled water, tap water, TCLP, acid and alkali solutions were used as eluents in these studies. Results from these studies are presented in Table 3. Cr(VI) concentrations in distilled water, tap water, TCLP and alkali solutions after the leaching from the remediated soil was in the range of 5–9% (considering an average concentration of 2.5 mg of Cr(VI) per gram of untreated soil). In untreated soil, tap water could leach approximately 71% of Cr(VI) where as TCLP could leach almost all the Cr(VI) present in the soil. Cr(VI) concentration after alkali digestion was significantly high. Though an average concentration of 2.5 mg of Cr(VI) per gram of soil was taken, the presence of small lumps of chromate ore in the soil might have increased the Cr(VI) concentration significantly. TCLP solution consists of dilute acetic acid, which might have caused some Cr(VI) reduction, which in turn reduced Cr(VI) concentration in the leachate. Total chromium concentrations in eluents of treated soil were similar to that of Cr(VI) because Cr(VI) is significantly more mobile than Cr(III). Acid digestion resulted in a total chromium concentration above 600 mg/L

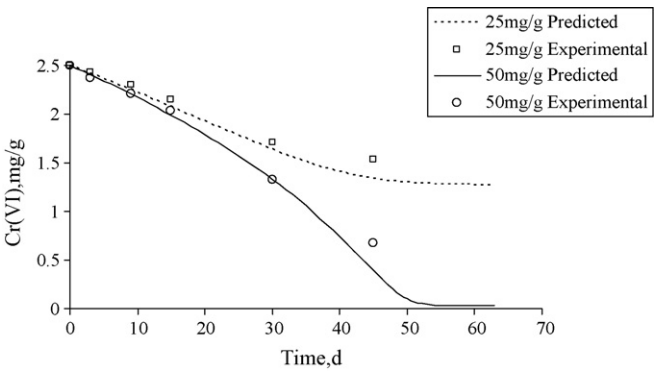

Fig. 4. Experimental and predicted results of growth curve for different initial substrate concentrations.

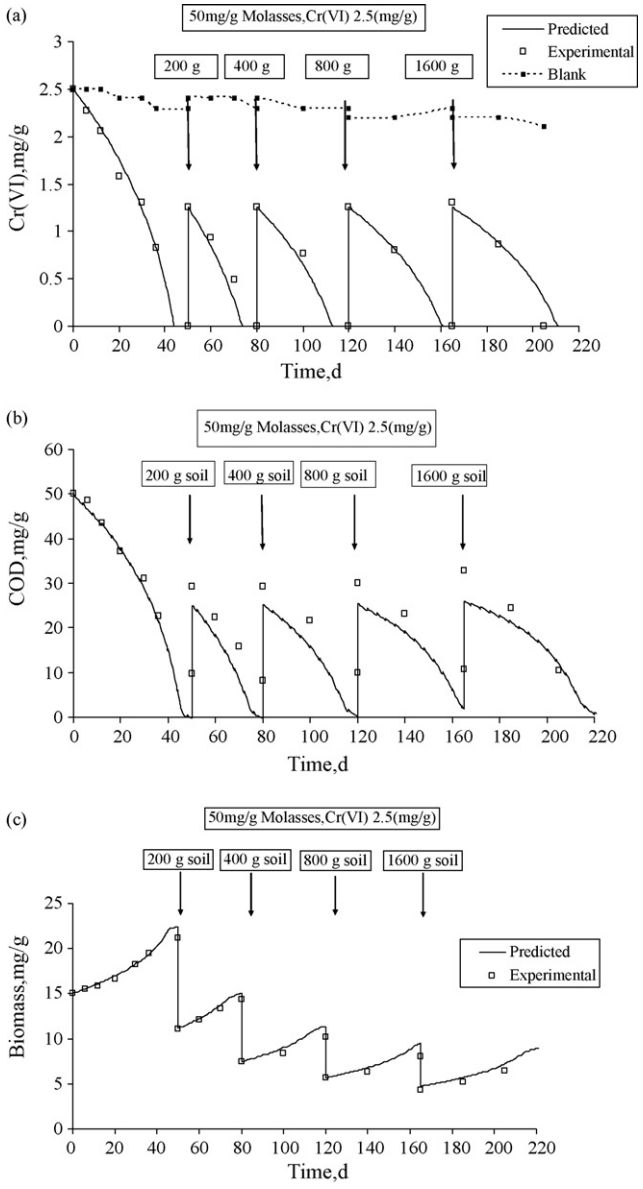

Fig. 5. (a) Experimental and predicted results of chromium reduction during five cycles of operation. (b) Experimental and predicted results of COD reduction during five cycles of operation. (c) Experimental and predicted results of biomass growth during five cycles of operation.

for both treated and untreated soils. This corresponds to an average total chromium concentration of 12 mg/g of soil. It is evident from these results that chromium is highly immobile in remediated soils even under highly adverse environmental conditions.

5.7. Optimal operation of soil reactors

The cost of operation of soil reactors and time taken for remediation depend upon the five design variables: $W_{initial}$, $M_{initial}$, $S_{initial}$, f_{inter} and COD_{last}. In the present study, management models were used for obtaining optimal solution for the treatment of 200,000 tons of chromium processing residue. The optimization problem was solved using the GA available in the MATLAB tool box. The GA input parameters and the initial condition for all the three management models are presented in Table 4.

Application of Management Model-I to the soil remediation problem minimized the time of treatment. Optimal results for all the decision variables, along with their upper and lower bound values, are presented in Table 5. Total time taken for treatment was 803 days (2.2 years), at a total treatment cost of Rupees 379.5 million ($7.6 million) and with a residual COD in the treated soil less than 0.1 mg/g of soil. The simulated results for chromium reduction corresponding to the optimal solution are shown in Fig. 6. It can be observed from these figures that the time taken for treatment during a particular cycle increased as the cycle number increased because the unit biomass concentration reduced as the number of cycles increased. This was because the rate of increase in biomass was much lower than the amount of soil added at the beginning of each cycle. In the present study, the permissible residual COD at the end of treatment was limited to 0.3 mg/g. This value was chosen based on adsorption/desorption characteristics of the contaminated soil. At any time after the completion of remediation, the COD desorbed from the soil should not exceed the permissible level of 20 mg/L (BOD standard for effluent discharge to any water body). The value of 0.3 mg/g was calculated based on the Freundlich isotherm. It should be noted here that (results not shown) addition of lesser amount of COD in the last cycle was very much essential to meet the requirement on residual COD in the treated soil (Fig. 7).

Application of Management Model-II for this problem considered minimization of the cost, and the time taken for treatment was not an issue. Optimal results for all the decision variables, along

Table 4
GA input parameters and initial condition for all the management models.

S. No.	GA parameters	Values
1	Initial population	1000
2	Scaling function	Rank
3	Selection function	Tournament of size five
4	Cross-over fraction	0.8 (two point cross-over)
5	Mutation	0.1 (uniform mutation)
6	Migration (forward)	Fraction (0.2) and interval (20)
7	Elite count	2
8	Generations	500
9	Function tolerance	10^{-6}
10	Distance measure function	Distance crowding
11	Fraction of Pareto-optimal front population	0.75
12	Total amount of soil to be treated	2×10^8 kg
13	μ_{max}	0.030 (1/d)
14	K_s	8.13 mg/g
15	K_i	0.65 mg/g
16	η	0.049
17	Y_T	0.129
18	Cost of 1 kg of COD (molasses)	Rupees 20.0
19	Cost of 1 kg of biomass	Rupees 120.0

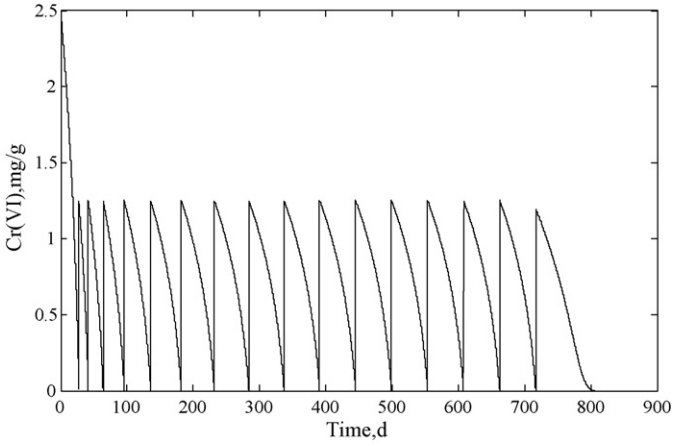

Fig. 6. Simulated results for Cr(VI) reduction corresponding to the optimal solution obtained using Management Model-I.

Table 5
Optimal results obtained using Management Model-I.

	Initial amount of soil to be treated (kg)	Initial biomass (mg/g)	Initial COD (mg/g)	Fraction of initial COD to be added when COD becomes less than 0.001 (f_1)	COD to be added in the last cycle (COD last) (mg/g)
Upper bound	5000	25	100	1	50
Lower bound	500	15	10	0.1	0.1
Optimum value	3189	25	69.5	0.87	2.7

Table 6
Optimal results obtained using Management Model-II.

	Initial amount of soil to be treated (kg)	Initial biomass (mg/g)	Initial COD (mg/g)	Fraction of initial COD to be added when COD becomes less than 0.001 (f_1)	COD to be added in the last cycle (COD_{last}) (mg/g)
Upper bound	5000	25	100	1	50
Lower bound	500	15	10	0.1	0.1
Optimum value	685	15.4	10.4	0.25	19.0

with their upper and lower bound values, are presented in Table 6. With these decision variables, optimal cost for the total treatment was Rupees 203.8 million ($4.1 million), which was almost half the cost of treatment for the solution obtained using the Management Model-I. However, the time for total treatment in the present case was 28 years, which was significantly longer than the time for treatment obtained using the Management Model-I. Time variation of residual concentrations of chromium corresponding to the optimal solution is presented in Fig. 7. It was observed from results for time variation of molasses concentration (not shown here) that minimization of cost was mainly achieved by adding just sufficient amount of substrate when required. This kept the residual concentration of substrate in the system very low most of the time, and as a consequence, the total time for treatment was much longer when compared to the time of treatment obtained using Management Model-I. Thus it is obvious that there is a trade off between the cost of treatment and the time taken for treatment.

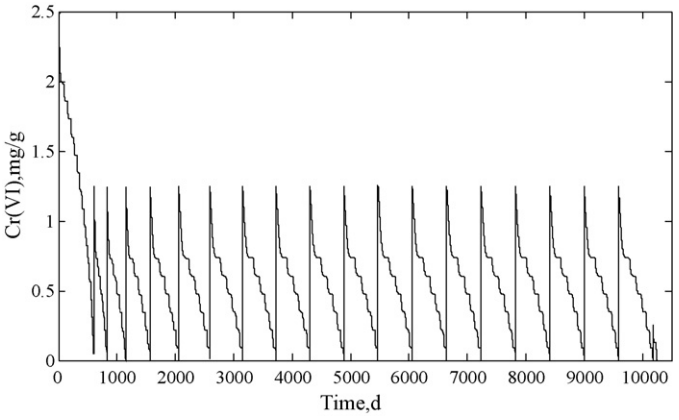

Fig. 7. Simulated results for Cr(VI) reduction corresponding to the optimal solution obtained using Management Model-II.

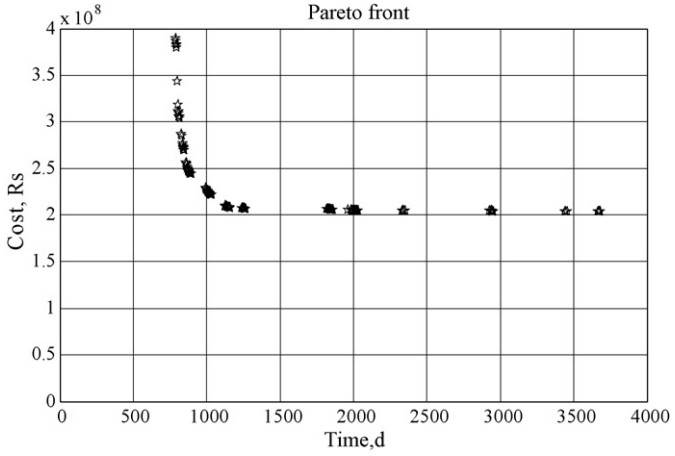

Fig. 8. Pareto-optimal front obtained using the Management Model-III.

Management Model-III considers both objectives of minimization of cost and time taken for treatment. A trade off curve between the two objectives i.e., the Pareto-optimal front was obtained by applying this model to the present case of soil treatment. The Pareto-optimal front is presented in Fig. 8. It can be seen from this figure that the cost of treatment can be reduced if one is willing to wait for a longer time for treatment of all the contaminated soil. One can also conclude from Fig. 8 that probably the best trade off between the cost and time of treatment is obtained when one is willing to wait for 900–1000 days for complete treatment. It may be also noted here that the solutions presented are specific to the upper and lower bounds taken for decision variables. The bounds for decision variables are site specific, and a different optimal solution will be obtained by changing them. The purpose of this study is only to demonstrate the applicability of management models for obtaining optimal solutions.

6. Conclusion

The present study focused on bioremediation of chromium contaminated soil using the indigenous microorganisms isolated from a chromium contaminated site. The bacterial strain isolated from an old ETP of the potassium dichromate manufacturing unit showed high Cr(VI) reduction potential. Batch and continuous experiments were carried out using this strain to determine the effects of moisture content, initial substrate concentration and initial biomass concentration. Leachability study was conducted to assess the stability of biotransformed Cr(VI) in the treated soil. Experimental data was used to determine biokinetic parameters, and to develop and validate a mathematical model. The mathematical model describing the process was embedded into a management model using a simulation–optimization framework. The management models included (i) cost minimization, (ii) time minimization, and (iii) multiple objectives. Optimization problem was solved using a Genetic Algorithm, available in MATLAB tool box. Applicability of proposed management models was demonstrated. It was also demonstrated how the trade off between the cost of treatment and the time taken for treatment can be obtained using the multi-objective management model.

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