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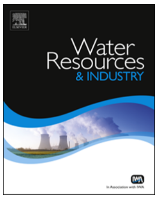

Application of Taguchi method for optimizing the process parameters for the removal of copper and nickel by growing *Aspergillus* sp.

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ABSTRACT

In the present study, the process parameters affecting biosorption were optimized by adopting Analysis of Mean (ANOM) approach for maximizing the percentage removal of copper and nickel by growing *Aspergillus* sp. in batch reactor using Taguchi method. The process parameters include inoculum concentration, initial metal concentration, pH and temperature. The optimized conditions were found to be 15% v/v inoculum concentration, 50 mg l^{-1} concentration of copper/nickel, pH 4 and temperature 30 °C. The percentage contribution of each process parameter on the removal of copper/nickel determined using Analysis of Variance (ANOVA) method followed the order: concentration of copper/nickel > inoculum concentration > pH > temperature. The percentage removal of copper and nickel realized in the confirmatory experiments carried out at optimized conditions was found to be higher than that obtained in all the test runs of Taguchi design, thereby supporting the accuracy of optimization of process parameters under the given set of experimental conditions.

1. Introduction

Wastewater generated from various industries such as metal plating, storage batteries, alloys, dying, textile, fertilizers, smelting, mining, pigment, metallurgical, etc. is a major source of heavy metals. The uncontrolled discharge of this wastewater enriched with heavy metals beyond the prescribed standards, leads to serious environmental pollution and results in severe health hazards for plants, humans and animals [1–4]. Thus, the increased awareness of toxicity of heavy metal and the stringent environmental safety regulations demand the removal of them from the various discharges to avoid contamination of the biological ecosystem.

Several methods such as chemical precipitation, ion-exchange, adsorption and reverse osmosis are the commonly used processes for heavy metal removal from wastewater. However, these methods suffer from several setbacks in the form of technological and/or economic constraints [5,6]. Among these methods, biosorption is considered to be an alternative to the conventional methods for heavy metal removal from industrial wastewater and sewage sludge [7,8]. The process offers several advantages such as low operating cost, minimal chemical and/or biological sludge generation, and a higher efficiency in detoxifying dilute effluents [9]. Of all the micro-organisms, fungi are well suited for the biosorption process as they

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exhibit remarkable tolerance towards heavy metals. Therefore, the ability of fungi as biosorbents for heavy metals has been extensively investigated [10–15]. The biosorption process has been found to be affected by various factors like pH, temperature, initial metal concentration of solution, type of microorganisms and their functional groups, structure, etc.[15–17].

Among the various heavy metals, copper is an essential micronutrient for all living organisms including humans, whereas nickel is an essential trace element present in many species. Copper occurs naturally in rocks, soils, sediments, water as well as in plants and animals and is involved in biochemical processes such as detoxification and oxidation [18]. Beyond the permissible levels, both the heavy metals are known to be toxic and carcinogenic having serious impact on human health as well as on the ambient environment [19–21]. Prolonged exposures to copper can cause irritation of the nose, mouth and eyes, headache, stomach ache, dizziness, vomiting and diarrhea. Atrophic changes in nasal mucous membranes are diagnosed resulting in metal fume fever because of severe exposure to copper fumes, dusts, or mists in industries. Chronic copper poisoning may also result in Wilson's disease. Intentional high uptakes of copper may cause liver and kidney failure resulting in death. Excessive levels of copper in copper rich soils adversely affect the survival rate of plant life as well as deteriorate the health of animals [22]. However, the other heavy metal nickel occurs naturally in the molten core of earth, sea water and tea leaves, etc. Nickel is an essential element to all the biotic life at lower concentrations. However, excessive levels of exposure make it quite carcinogenic in addition to various ailments related to heart and thyroid [23]. Direct contact of nickel may sometimes cause nickel dermatitis with symptoms of skin rashes. Nickel emissions from various industrial activities become air-borne affecting the public health [24]. Large scale usage of both the heavy metals (Cu & Ni) in large number of industries manufacturing products ranging electroplated goods, electrical appliances, metal alloys, etc. shows the pollution potential of these metals, thereby affecting the ambient environment and public health. Therefore, the need of the hour is to alleviate the pollution effects by removal of these heavy metals from heavy metal contaminated wastewaters.

Conventional optimization studies involving variation of one parameter while keeping the other parameters constant is often considered an exhaustive and expensive ordeal. However, statistical design of experimental methods provide an easier and equally efficient approach to optimize several operational variables. The frequently applied experimental design methods include the evolutionary operation, Response surface methodology [25–27] and Taguchi method [28,29]. Taguchi's optimization technique is a unique and powerful optimization discipline that allows optimization with minimum number of experiments. The Taguchi experimental design reduces cost, Improves quality, and provides robust design solutions. The advantages of Taguchi method over the other methods are that numerous factors can be simultaneously optimized and more quantitative information can be extracted from fewer experimental trials. Taguchi methods have been used for optimization in various fields of wastewater treatment. Barrado et al., [30] have reported application of Taguchi method for optimizing the conditions for treatment of metal contaminated wastewater. Studies were also reported on optimization of process parameters for color removal from textile dye effluents [31]. The optimization of experimental conditions for recovery of coal fines from the wastewater generated in coal cleaning operation has been reported by Chary and Dastidar [32] using Taguchi method. This method can also be applied to designing factorial experiments and analyzing their outcomes [33]. Therefore, we investigate for the remove of copper and nickel ions by growing *Aspergillus* sp. was optimized using Taguchi method.

In the present study, the biosorption of copper and nickel by the growing *Aspergillus* sp. isolated from industrial wastewater was investigated in a batch bioreactor using Taguchi experimental design with L_9 orthogonal array. The study is primarily aimed at statistical optimization of various controllable factors like inoculum concentration, initial concentration of heavy metals, pH, and temperature for maximizing the removal of copper and nickel using Analysis of Mean (ANOM) approach. The percentage contribution of each parameter on percentage removal of copper and nickel by biosorption is analyzed by adopting Analysis of Variance (ANOVA) approach. A confirmation experiment was also carried out at the optimized conditions.

2. Material and methods

2.1. Media and Growth condition

Aspergillus sp. used in the present study was cultured over potato dextrose agar (PDA) plates. The PDA plates of the stock culture were then stored at 4 °C. The fungal biomass was cultivated in 250 ml Erlenmeyer flask containing 100 ml of growth media of the following composition (g/l^{-1}): K_2HPO_4, 0.5; NaCl, 0.5; $MgSO_4$, 0.5; NH_4NO_3, 0.5, yeast extract, 5.0. The *Aspergillus* sp. was cultivated in 250 ml Erlenmeyer flask with 100 ml working volume at 30 °C with shaking at 180 rpm. A one day old culture (10% v/v) was used for all the batch biosorption experiments. The pH of the growth media was adjusted by using 1 N H_2SO_4 and 1 N NaOH.

2.2. Batch biosorption experiments

2.2.1. Preparation of solutions of copper and nickel

Stock solutions of Cu (1000 mg l^{-1}) and Ni (1000 mg l^{-1}) were prepared by dissolving appropriate quantities of pure metal salt powders ($CuSO_4 \cdot 7H_2O$) and ($NiCl_2 \cdot 6H_2O$) respectively, in double distilled water. The solutions of different

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Table 1
Controllable factors and their levels.

Factor	Description	Level 1 (L1)	Level 2 (L2)	Level 3 (L3)
A	Inoculum v/v (%)	5	10	15
B	Copper/Nickel concentration (mg/L)	50	75	100
C	pH	3	4	5
D	Temperature (°C)	20	30	40

Table 2
Test runs.

Run	Inoculum in v/v (%) (A)	Initial copper/Nickel concentration in mg/L (B)	pH (C)	Temperature in °C (D)
1	5	50	3	20
2	5	75	4	30
3	5	100	5	40
4	10	50	4	40
5	10	75	5	20
6	10	100	3	30
7	15	50	5	30
8	15	75	3	40
9	15	100	4	20

concentrations were obtained by adequate dilution of the respective stock solutions. All the chemicals used were of analytical grade.

2.2.2. Batch biosorption

Batch biosorption studies were carried out in 250 ml Erlenmeyer flasks with working volume of 100 ml containing sterile growth media supplemented with heavy metals (50, 75 and 100 mg l^{-1}). The media was inoculated with one day old culture (5%, 10% and 15% v/v). The pH (3, 4 and 5) was adjusted by using 1 N H_2SO_4 and 1 N NaOH. Each flask was incubated at temperature (20 °C, 30 °C and 40 °C) with shaking at 180 rpm. The samples were drawn at regular time intervals (12 h) and subsequently centrifuged at 4000 rpm for 3 min. The supernatant fraction from the centrifuging process was then analyzed for residual concentration of metals (Cu & Ni) using Atomic Absorption Spectrophotometer (Perkin Elmer A Analyst 200). The settled biomass was separated out and gravimetric analysis was carried out to determine the biomass concentration. The process was monitored for 7 days beyond which equilibrium was found to be attained in the metal removal. All the experiments were carried out in triplicates.

2.3. Optimization studies by ANOM approach

In the present experimental investigation, four controllable factors were considered with each factor at three levels as shown in Table 1. An L-9 orthogonal array designed by Taguchi has been used to determine the optimum experimental conditions for maximum removal of heavy metal and the designed experimental runs are given in Table 2. To achieve a statistical measure of the process performance, Signal-to-Noise (S/N) ratio was evaluated. In every statistical approach, depending on the desired output quality characteristics, three types of Signal-to-Noise (S/N) ratios are available: (1) smaller is better, (2) nominal is best, and (3) bigger is better [34]. Since the present study involves maximizing the removal of heavy metal, the S/N ratio for the case of bigger is better was evaluated as per the formula given below:

$$\frac{S}{N} = -10\log\left(\frac{1}{n}\sum_{i=1}^{n}\frac{1}{R_i^2}\right) \tag{1}$$

where 'n' represents total number of replications of each test run and Y_i represents the percentage removal of heavy metal realized in replication experiment 'i' carried out under the same experimental conditions of each test run.

Analysis of mean (ANOM) statistical approach was adopted to optimize the process parameters. In this approach, the average value of S/N ratio of each parameter at a certain level is evaluated and is mathematically represented as given below:

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Table 3
Percentage removal of copper and nickel & corresponding S/N ratios.

Run	Cu & Ni Removal (%)								S/N	
	Removal (1) (R_1)		Removal (2) (R_2)		Removal (3) (R_3)		Average removal ($\bar{A}$)			
	Cu	Ni	Cu	Ni	Cu	Ni	Cu	Ni	Cu	Ni
1	96	86	92.1	87	92.02	88	93.37	87	39.39	38.79
2	90	86	96	88	91	86	92.33	86.67	39.29	38.76
3	82	78	86	80	85	77	84.33	78.33	38.51	37.88
4	98	96	98.3	98	99.2	98	98.5	97.33	39.87	39.76
5	91	91	93	90	91.4	88	91.8	89.67	39.26	39.05
6	93	88	91.5	86	90	85	91.5	86.33	39.23	38.72
7	99	98	97.5	96	98.2	97	98.23	97	39.84	39.73
8	98	92	96	90	98	93	97.33	91.67	39.76	39.24
9	94	91	91	90	92	92	92.33	91	39.30	39.18

Table 4
S/N ratio response table.

Factor/ Level	$\left[\left(\frac{S}{N}\right)^{Factor}_{Level}\right]_j$						$\left[(M)^{Factor}_{Level}\right]$	
	j=1		**j=2**		**j=3**			
	Cu	Ni	Cu	Ni	Cu	Ni	Cu	Ni
A/1	39.39	38.79	39.29	38.76	38.51	37.88	39.07	38.47
A/2	39.87	39.76	39.26	39.05	39.23	38.72	39.45	39.18
A/3	39.85	39.73	39.76	39.24	39.30	39.18	39.64	39.39
B/1	39.39	38.79	39.87	39.76	39.85	39.73	39.70	39.43
B/2	39.29	38.76	39.26	39.05	39.76	39.24	39.44	39.02
B/3	38.51	37.88	39.23	38.72	39.30	39.18	39.02	38.59
C/1	39.39	38.79	39.23	38.72	39.76	39.24	39.46	38.92
C/2	39.29	38.76	39.87	39.76	39.30	39.18	39.49	39.23
C/3	38.51	37.88	39.26	39.05	39.85	39.73	39.21	38.89
D/1	39.39	38.79	39.26	39.05	39.30	39.18	39.32	39.01
D/2	39.29	38.76	39.23	38.72	39.85	39.73	39.46	39.07
D/3	38.51	37.88	39.87	39.76	39.76	39.24	39.38	38.96

$$(M)^{Factor=F}_{Level=i} = \frac{1}{n_{Fi}} \sum_{j=1}^{n_{Fi}} \left[\left(\frac{S}{N}\right)^{Factor=F}_{Level=i}\right]_j \tag{2}$$

where, $\left[(M)^{Factor=F}_{Level=i}\right]$ represents the mean of the S/N ratio with factor F at level *i*,

$\left[\left(\frac{S}{N}\right)^{Factor=F}_{Level=i}\right]_j$ represents the value of S/N ratio with factor F at level 'i' in its jth appearance in Table 3 and is the jth value in Table 4 (where j=1, 2. 3… n_{Fi}) and

n_{Fi} represents the number of appearances of factor F in level i.

The mean of the S/N ratios of various factors at various levels were determined to construct the S/N response table (Table 4). The optimum process conditions for maximum removal of Cu & Ni were determined from Table 4, by identifying the level at which a process parameter has maximum value of S/N ratio.

2.4. Percentage contribution of various factors by ANOVA approach

Analysis of Variance (ANOVA) statistical method has been used in the present study to determine the percentage contribution of each controllable factor on percentage removal of heavy metal [1]. The percentage contribution of each factor, ρ_F is given by:

$$\rho_F = \frac{SS_F - (DOF_F\ V_{Er})}{SS_T} x100 \tag{3}$$

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Table 5
Percentage removal of copper & nickel response table.

Factor/ Level	$\left[\left(\bar{A}\right)_{Level}^{Factor}\right]_j$						$\left[\left(\bar{R}\right)_{Level}^{Factor}\right]$	
	j=1		**j=2**		**j=3**			
	Cu	Ni	Cu	Ni	Cu	Ni	Cu	Ni
A/1	97.37	87	92.33	86.67	84.33	78.33	90.01	84
A/2	98.5	97.33	91.8	89.67	91.5	86.33	93.93	91.11
A/3	98.23	97.00	97.33	91.67	92.33	91.00	95.97	93.22
B/1	93.37	87.00	98.5	97.33	98.23	97.00	96.70	93.78
B/2	92.33	86.67	91.8	89.67	97.33	91.67	93.82	89.33
B/3	84.33	78.33	91.5	86.33	92.33	91.00	89.39	85.22
C/1	93.37	87.00	91.5	86.33	97.33	91.67	94.07	88.33
C/2	92.33	86.67	98.5	97.33	92.33	91.00	94.38	91.67
C/3	84.33	78.33	91.8	89.67	98.23	97.00	91.45	88.33
D/1	93.37	87.00	91.8	89.67	92.33	91.00	92.50	89.22
D/2	92.33	86.67	91.5	86.33	98.23	97.00	94.02	90
D/3	84.33	78.33	98.5	97.33	97.33	91.67	93.38	89.11

where SS_F is the factorial sum of squares and is given by Eq. (4):

$$SS_F = \frac{mn}{L}\sum_{k=1}^{L}\left(\bar{R}_k^{F} - \bar{R}_T\right) \qquad (4)$$

In the above equation, $\bar{R}_T$ represents the cumulative average of heavy metal removal achieved in the present study and is mathematically represented as:

$$\bar{R}_T = \frac{\sum_{j=1}^{m}\left(\sum_{i=1}^{n} R_i\right)_j}{mn} \qquad (5)$$

'm' in Eq. (5) represents the number of experiments carried out in the present study and 'n' represents the number of replications of each experiment. R_i represents the percentage heavy metal removal realized in replication experiment 'i' carried out under the same experimental conditions of each test run.

In Eq. (4), $\bar{R}_k^{F}$ is the cumulative average of percentage removal of heavy metal with a certain factor F at kth level and is mathematically represented as:

$$\left(\bar{R}\right)_k^{F} = \frac{1}{n_{Fk}}\sum_{j=1}^{n_{Fk}}\left[\left(\bar{A}\right)_{Level=k}^{Factor=F}\right]_j \qquad (6)$$

In Eq. (6), $\left[\left(\bar{A}\right)_{Level=k}^{Factor=F}\right]_j$ represents the average percentage removal of heavy metal $(\bar{A})$ with a factor F at level k in its jth appearance sequence in Table 3 and is the jth value in the percentage heavy metal removal response table shown in Table 5 (where j=1, 2. 3… n_{Fk}). n_{Fk} represents number of appearances of factor F at level k.

In Eq. (3), DOF_F represents the degrees of freedom for each factor, which is obtained by subtracting one from the number of levels of each factor (L). V_{Er} used in Eq. (3) is the variance of error and is evaluated by Eq. (7).

$$V_{Er} = \frac{SS_T - \sum_{F=A}^{D} SS_F}{m(n-1)} \qquad (7)$$

SS_T appearing in Eq. (3) & Eq. (7) represents total sum of squares which is given by:

$$SS_T = \sum_{j=1}^{m}\left(\sum_{i=1}^{n} R_i^2\right)_j - mn\left(\bar{R}_T\right)^2 \qquad (8)$$

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3. Results and discussion

The average removal of heavy metal ($\bar{R}_i$) was determined by taking the average of the heavy metal removal (R_i) realized in experimental runs replicated thrice under the same experimental conditions in the order shown in Table 2. The S/N ratio in Table 3 was evaluated by using Eq. (1). The average value of S/N ratio with a certain parameter at a certain level i.e.,$(M)_{Factor}^{Level}$ was calculated using Eq. (2). The evaluated mean S/N ratios with various factors at various levels are summarized in the S/N ratio response table given in Table 4. The heavy metal removal response table (Table 5) was also constructed in a similar fashion after calculating the cumulative average heavy metal removal with a particular factor at a particular level ($\bar{R}_k$) using Eq. (6). The above procedure was followed for Cu & Ni separately. Figs. 1–4 show the percentage removal of heavy metal and S/N ratio response graphs for copper and nickel at various levels of different parameters under study.

3.1. Analysis of experimental data

Fig. 1 shows the effect of Inoculum concentration (% v/v) on percentage removal of Cu and Ni & S/N ratio. Lower percentage removal observed at L1 (5% v/v Inoculum concentration) could be due to lower microbial population. Higher percentage metal removals observed at higher inoculum concentrations of 10% v/v (L2) and 15%v/v (L3) might be an outcome of increased microbial colonies. Similar results were observed for the metal removal by fungal biomass [17,35].

The effect of initial metal concentration (mg/L) on percentage removal of Cu and Ni is shown in Fig. 2. With an increase in initial metal concentration from L1 (50 mg/L) to L3 (100 mg/L), the percentage removal of metal decreased due to the inhibitory effect of metal on the growth of the fungal biomass at higher initial metal concentrations. The availability of the binding sites present on the surface of the biomass is also reduced because of reduced growth of the fungal biomass. The other reason for lower metal removal is the reduced accessibility of available binding sites by metal ions due to the presence

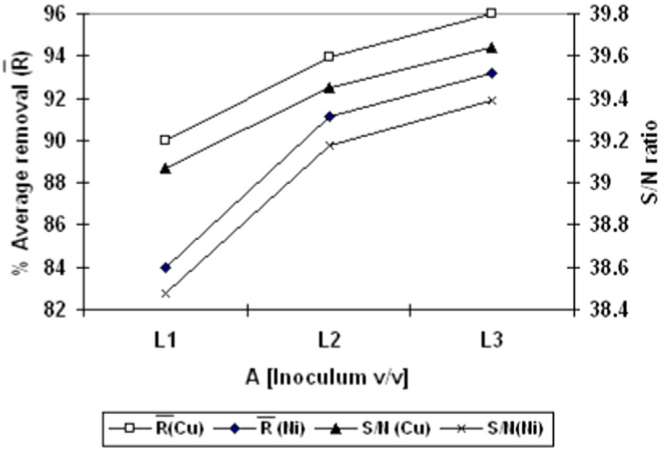

Fig. 1. Inoculum (% v/v) Vs Percentage removal of Cu and Ni & S/N ratio.

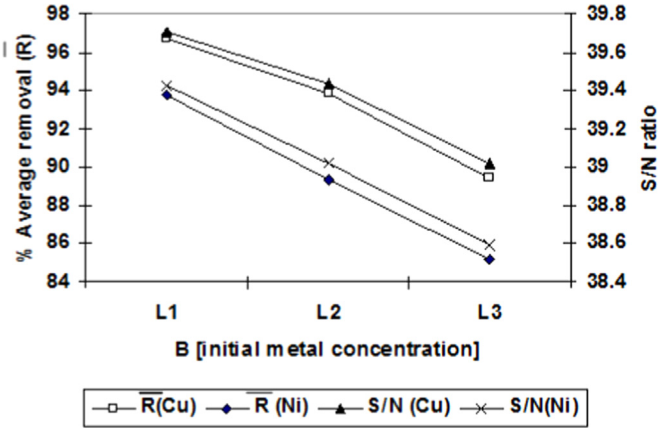

Fig. 2. Initial metal (Cu) concentration (mg/L) Vs Percentage removal of Cu and Ni & S/N ratio.

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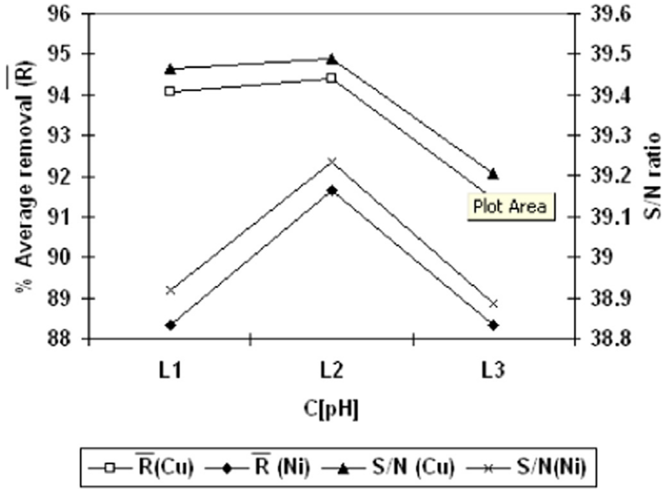

Fig. 3. pH Vs Percentage removal of Cu and Ni & S/N ratio.

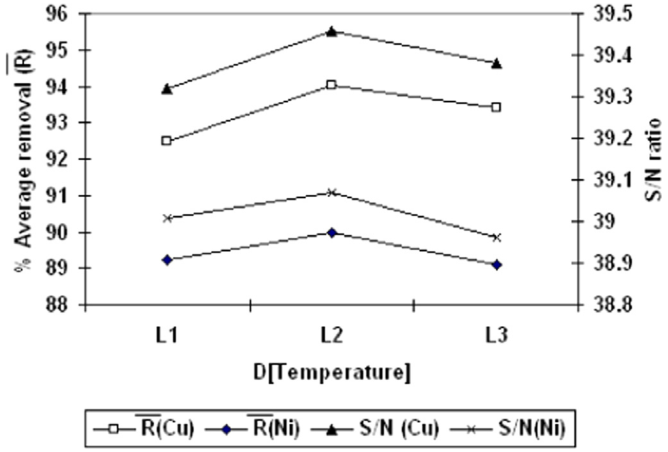

Fig. 4. Temperature Vs Percentage removal of Cu and Ni & S/N ratio.

of excessive metal ions in the liquid at higher metal concentrations. AÇIKEL and Alp [15] also reported that bioaccumulation of Cu and Ni depends upon initial concentration of metal as it affects the growth of the *Rhizopus delemar.* Similar findings were observed for the Cu and Ni removal by fungal biomass [16,19].

Fig. 3 shows the effect of pH on the percentage removal of Cu and Ni. At lower pH 3 (L1), some of the functional groups present on the outer most layer of the fungal biomass may be positively charged and may not interact with metal ions resulting in lower metal removal [35]. In a study reported on biosorption of zinc, cadmium and lead using living fungal biomass, it was also observed that metal biosorption was inhibited below pH 3.0 due to the repulsion between metal cations with the positively charged metal binding ligands on the surface of fungal biomass [36]. Similarly, Tian-Wei et al., [37] also reported lower metal removal at lower pH range by *Penicillium chrysogenum*. With an increase in pH from L1 (pH 3) to L2 (pH 4), the percentage metal removal increased due to an increase in the number of surface negative charges at higher pH. Yakup [38] also attributed the higher metal removal at higher pH to the strong relations of biosorption on the number of surface negative charges which depends on the dissociation of functional groups. The decrease in removal of metal above pH 5 is due to the formation of metal hydroxide. Dönmez and Aksu [17] also reported that pH value 4.0 was the optimum condition for maximum growth of *Kluyveromyces marxianus* and copper removal by the organism, which is in close agreement with the findings of the present study.

Fig. 4 shows the effect of temperature on percentage removal of Cu and Ni. With an increase in temperature from L1 (20 °C) to L3 (40 °C), no significant difference was observed in the percentage metal removal. The temperature is an important factor affecting the growth of the organism. At lower temperatures, enzymatic reactions get affected. Above certain temperature, the proteins are irreversibly damaged and thereby decrease the microbial growth and metabolic activities. The minimum and maximum temperatures for the microbial growth reflecting the optimum temperature range vary widely

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among microorganisms. The optimum temperature range for the growth of edible fungi was reported to be 25–35 °C [39]. Sharma and Sharma [40] reported 28–30 °C as the most favorable temperature range for the growth of *Trichophyton mentagrophytes* and *Chrysosporium tropicum*. Srivastava and Thakur [41] reported that *Aspergillus* sp. can grow between 10 and 40 °C temperatures. Similar observations have been reported by other researchers [42–44]. In the present study, no significant change in the metal removal was observed in the temperature range (20–40 °C), although 30 °C appears to be the optimum temperature for maximum removal of heavy metal.

3.2. *Optimization of process parameters by ANOM approach*

According to ANOM approach, the factor-level combination at which the S/N ratio has the highest value is the optimum condition. The factor-level combination at which removal of copper is optimum is A3, B1, C2, and D2 i.e. inoculum concentration (15%), initial metal concentration (50 mg l^{-1}), pH (4) and temperature (30 °C). A similar trend is observed for optimum removal of nickel as well.

3.3. *Determination of percentage contribution by Analysis of Variance*

For evaluation of the percentage contribution of each factor towards the removal of heavy metal, $\bar{R}_T$ was calculated using Eq. (5), which is found to be 93.30 for copper and 89.44 for nickel. The values of $\bar{R}_k$ and $\bar{R}_T$ were substituted in Eq. (4) to obtain the factorial sum of squares, SS_F, for each factor and these values were listed in Table 6 for copper and nickel. The total sum of squares, i.e., SS_T was calculated from Eq. (5) and is found to be 521.89 for copper and 850.67 for nickel. The calculated values of SS_F and SS_T were used in the calculation of variance of error (V_{Er}) using Eq. (7). The calculated variance of error for copper and nickel is 3.093 and 1.667 respectively. The percentage contribution of each factor 'ρ_F' was obtained by substituting $DOF_F=2$, V_{Er}, SS_F and SS_T in Eq. (3), the values of which are tabulated in Table 6 for copper and nickel separately.

Table 6
SS_F and ρ_F.

Factor	SS_F		ρ_F	
	Cu	Ni	Cu	Ni
A	164.82	420.22	30.39	49.01
B	244.30	329.56	45.62	38.35
C	46.61	66.67	7.75	7.45
D	10.49	4.22	0.825	0.10

Table 7
Summary of % heavy metal (Cu & Ni) removal in test runs and confirmatory experiment.

Run	**Inoculum, %v/v (A)**	**Initial Cu/Ni concentration (mg/L) (B)**	**pH (C)**	**Temperature (D)**	**Actual heavy metal removal (%)**	
					Cu	**Ni**
1	5	50	3	20	93.37	87
2	5	75	4	30	92.33	86.67
3	5	100	5	40	84.33	78.33
4	10	50	4	40	98.5	97.33
5	10	75	5	20	91.8	89.67
6	10	100	3	30	91.5	86.33
7	15	50	5	30	98.23	97
8	15	75	3	40	97.33	91.67
9	15	100	4	20	92.17	91
Confirmation under optimized conditions	15	50	4	30	98.8	97.9

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From the Table 6, the percentage influence of the parameters on the removal of copper was found to be in the following order: initial metal concentration (45.62%) > concentration of inoculum (30.40%) > pH (7.75%) > Temperature (0.83%) and on the removal on nickel was found to be in following order: initial metal concentration (49%) > concentration of inoculum (38.4%) > pH (7.45%) > Temperature (0.10%) respectively. The initial metal concentration is an important parameter in biosorption as it affects the growth of the organisms [15,16,19]. The extent of heavy metal removal is mainly determined by the growth of the organism and therefore, the prime importance of initial metal concentration is evident from its higher percentage contribution value. Inoculum concentration occupies the second place in the order of percentage contribution as the microbial population also determines the extent of metal removal which is evident from a number of studies [17,33]. The importance of pH on heavy metal removal by biosorption process has been highlighted by a number of studies [16,17]. However, the percentage contribution of pH range under examination would come only next to initial metal concentration and inoculum concentration evident from its third place in the order. The copper uptake was independent of temperature in the range of 20–45 °C [45]. In the present study, significant change in the heavy metal removal was not observed with changes in temperature. The above observation is supported by a lower percentage contribution of temperature towards heavy metal removal.

A confirmatory experiment carried out under the optimized conditions showed a higher removal of heavy metals (Cu, 98.8; Ni, 97.9) than that was achieved in all the test runs, thereby supporting the accuracy of optimization. The Table 7 shows the summary of percentage removal of heavy metals realized in all test conditions and that during the confirmation experiment.

4. Conclusions

Taguchi experimental design with L_9 orthogonal array was used to optimize the process parameters for maximum percentage removal of heavy metals (Cu & Ni). The optimized experimental parameters were inoculum concentration (15%), initial metal (Cu/Ni) concentration (50 mg l^{-1}), pH 4.0 and temperature (30 °C). The contribution of each parameter towards the percentage removal of copper is of the following order: initial metal concentration (45.62%) > inoculum concentration (30.40%) > pH (7.75%) > Temperature (0.83%) and the contribution of each parameter towards the percentage removal of nickel is of the following order: initial metal concentration (49%) > concentration of inoculum (38.4%) > pH (7.45%) > Temperature (0.10%). The percentage removal of heavy metal realized in the confirmation experiment is higher than all the test runs. Thus, the accuracy of the optimization of process parameters carried out to maximize the percentage removal of heavy metal (Cu & Ni) is ascertained. However, a more comprehensive 'Design of Experiment' technique with more number of experimental runs and more parameters would provide a better understanding of the process of biosorption. In addition to the above, optimization using a different set of process parameters may further help in the enhancement of process efficacy.

Competing interest

The authors declare that they have no competing interests.

Author's contribution

RP carried out the experimental work as well as analysis work of the results of biosorption studies, participated in the sequence alignment and drafted the manuscript. GHVCC executed the designing of the experiments in line with the Taguchi experimental methodology and performed the statistical analysis by application of Analysis of Mean and Analysis of Variance approaches to the experimental results. MGD provided guidance in the execution of biosorption studies as well as participated in the sequence alignment and in the finalization of the submitted manuscript. All authors read and approved the final manuscript.

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