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Oil removal from water by fungal biomass: A factorial design analysis

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ABSTRACT

A fractional factorial design analysis was conducted to screen the significant factors influencing removal of three emulsified oils from water, namely, standard mineral oil (SMO), canola oil (CO) and Bright-Edge 80 cutting oil using non-viable biomass of fungus *Mucor rouxii* rich with chitosan in its cell wall. Factors investigated were pH of the solution (3–9), temperature (5–30 °C), adsorbent dose (0.05–0.5 g), concentration of oil (50–350 mg/L) and rotational speed of the shaker (100–200 rpm). It was observed that pH of the solution was the most influencing parameter on the removal of all the three oils studied. Higher oil removal efficiencies (80–99%) were obtained at a pH of 3.0 by *M. rouxii* biomass for all the three oils studied. Temperature had an effect on SMO and Bright-Edge 80 removal while adsorbent dose was found to influence the removal of SMO. Average removals of SMO and Bright-Edge 80 were higher by 13% at a solution temperature of 30 °C compared to removals at 5 °C. Oil concentration had an effect on the removal of CO. The average removal of CO was found to be higher by approximately 15% at an initial oil concentration of 50 mg/L than at 350 mg/L.

1. Introduction

Major industrial sources of oily wastewater are petroleum refineries, metal manufacturing and machining, and food processors. Sources of oil in municipal wastewater are kitchen and human wastes [1]. Unlike free or floating oil spilled in sea, most of the industrial wastewaters contain oil-in-water emulsions as basic contaminants. The presence of emulsified oil in the wastewater is of real concern as it often results in fouling of process equipment and becomes part of the organic load that must be handled in biological treatment of such wastewaters. Conventionally, gravity separation, dissolved air flotation, chemical coagulation, filtration, membrane processes, biological processes, adsorption are used for the removal of oil and grease. Emulsified oils can be effectively removed from water by adsorption. Traditionally, activated carbon has been widely used as an adsorbent for removing oil. Various adsorbents such as crushed and/or processed plant materials [2–4], horticultural peat [5], bentonite organoclay [6], vermiculite [7], chitosan [8], reed canary grass, flax and hemp fiber [9] and walnut shell media [10] have been examined for their oil adsorption capacities.

Biomass of fungus *Mucor rouxii* is a type of biomaterial which has been used for many applications in separation technology. *M. rouxii* is a filamentous fungus in which chitosan is the most abundant component of the cell wall. Large quantities of positively charged chitosan and negatively charged phosphate and glucouronic acid on the cell wall of *M. rouxii* have been found to offer extensive possibilities for binding heavy metals [11]. No work has been conducted so far on the removal of oil from water by non-viable fungal adsorbents, although, a few studies have been conducted on uptake of oil by live fungi [12]. Preliminary studies conducted so far at the University of Regina showed that non-viable biomass of the fungus *M. rouxii* exhibited good potential for removal of oil from water. The present study was initiated to further explore the potential of *M. rouxii* to remove oil from water. Temperature, pH, initial concentration of oil and adsorbent particle dosage are important parameters in adsorption [8,13,14]. The objective of the present study was to conduct a factorial design analysis to screen the significant factors that influenced the removal of oil from water by *M. rouxii* biomass and understand their impact on the process.

2. Materials and methods

2.1. Experimental materials

The following oils were used in the study:

1. Standard (light) mineral oil (SMO) marketed by Fisher Scientific Company, USA, emulsified with oleic acid and triethanolamine using Regina tap water according to the procedure used by Biswas [15].
2. Vegetable oil, Canola oil (CO) marketed in Canada, emulsified in the same manner as SMO.
3. DoALL Bright-Edge 80, a cutting oil manufactured by DoALL Company, IL, USA, emulsified in the same manner as SMO.

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Table 1
Coded and uncoded values of the factors.

Factor	Coded low level	Corresponding uncoded low values	Coded high level	Corresponding uncoded high values
pH	−1	3	+1	9
Temperature	−1	5 °C	+1	30 °C
Dose	−1	0.05 g	+1	0.5 g
Concentration	−1	50 mg/L	+1	350 mg/L
Speed	−1	100 rpm	+1	200 rpm

The characteristics of the three oils used and the methods used for characterizing them are described elsewhere [10].

2.2. Preparation of non-viable fungal biomass

M. rouxii strains were routinely maintained on potato dextrose agar plates. The biomass was grown by shake flask method in an aerobic condition. *M. rouxii* was cultivated using the growth medium comprising of yeast extract (3 g/L), peptone (10 g/L) and glucose (replaced by dextrose) (20 g/L) [16,17]. The pH of the growth medium was maintained at 4.5 using 1.0N HCl. The culture was grown in an aerobic condition at room temperature (22 ± 2 °C) with 100 mL of the liquid medium in 250 mL conical flasks on a rotary shaker agitated at 125 rpm. *M. rouxii was* harvested after 3 days of growth by filtering the growth media through a 150 μm sieve. The harvested fungal biomass was washed with generous amounts of deionized water and autoclaved for 30 min at 121 °C and 103 kPa. The autoclaved biomass was allowed to cool down and dried in an oven at 60 °C for 24 h. The dried biomass was powdered into a fine size using a grinder. The biomass passing through a 400 mm sieve was used for the experiment. The surface area and pore size measurement of the powdered autoclaved *M. rouxii* biomass were carried out using Micromeritics® ASAP 2020 accelerated surface area and porosimetry analyzer. Surface charge of autoclaved *M. rouxii* biomass was measured using Zetasizer, model HSA 3000 (Malvern, Worcestershire, England).

2.3. Design of experiments

In order to evaluate factors that influence the percent removal of oil by *M. rouxii* biomass, a two level five factors fractional factorial experiment was designed. Five factors, pH of the solution, temperature, adsorbent dose, concentration of oil and rotational speed of the shaker, were chosen to study the response as percentage removal of oil by sorption on *M. rouxii* biomass. Each factor was studied at two levels – low level and high level. To analyze the factorial design, the original measurement units for the experimental factors (uncoded units) were transformed into coded units [18]. The factor levels were coded as −1 (low) and +1 (high). The response was expressed as the percent removal of oil by *M. rouxii* biomass.

The minimum number of experimental runs that has to be carried out for a two level five factor design is $2^5 = 32$ runs. This is called a 2^5 full factorial design. With two replicates, the number of test runs increases to 64, which is large. When the number of factors is more than four, fractional factorial designs can be used. The information on the main effects and two-order interactions can be obtained by running only a fraction of the full factorial design [20]. A fractional factorial design is represented by $2^{(k-p)}$, where k is the number of factors and $1/2^p$ represents the fraction of the full factorial 2^k. A $2^{(5-1)}$ fractional factorial design is ½th fraction of a 2^5 full factorial experiment. By this way, one may be able to study five factors at two levels in just 16 (i.e. $2^{(5-1)}$) experimental trials instead of 32 trials (2^5).

MINITAB™ release 15 [18] statistical software was used to create and analyze the experimental data, in order to measure the effect of various factors (shown in Table 1) on removal of oil from water. Five factors were analyzed at two levels using a ½ fraction

Table 2
Uncoded design table for the factors and response.

Run order	pH	Temperature (°C)	Dose (g)	Concentration (mg/L)	Speed (rpm)	% removal of SMO	% removal of CO	% removal of Bright-Edge 80
1	3	5	0.05	350	100	99.90	99.97	99.90
2	3	5	0.50	50	100	80.20	99.40	89.14
3	9	30	0.05	50	200	38.00	54.00	87.60
4	3	5	0.05	50	200	94.80	99.60	91.80
5	9	5	0.50	50	200	27.00	59.60	68.80
6	9	5	0.50	350	100	51.70	46.80	13.40
7	9	30	0.50	50	100	60.00	61.60	57.40
8	3	30	0.50	50	200	96.40	98.00	88.20
9	3	5	0.50	350	200	99.77	99.77	98.80
10	9	5	0.05	350	200	9.10	23.10	8.00
11	9	30	0.50	350	200	58.00	79.70	69.40
12	9	30	0.05	50	200	39.10	56.00	88.00
13	9	5	0.05	50	100	23.40	90.40	44.60
14	3	30	0.50	50	200	96.70	97.70	88.00
15	3	5	0.05	350	100	99.70	99.80	99.10
16	3	30	0.05	350	200	98.40	99.80	99.60
17	3	30	0.05	50	100	99.60	99.80	90.60
18	9	5	0.50	50	200	28.00	60.10	68.90
19	9	30	0.50	350	200	58.40	80.10	70.00
20	3	5	0.50	50	100	80.60	99.60	89.90
21	3	30	0.05	50	100	99.50	99.30	91.00
22	3	30	0.50	350	100	97.40	97.90	97.00
23	9	5	0.50	350	100	52.00	47.10	14.20
24	3	5	0.50	350	200	99.40	98.60	98.60
25	3	30	0.05	350	200	98.80	99.70	99.30
26	9	30	0.05	350	100	37.10	55.40	36.80
27	9	5	0.05	50	100	25.00	91.00	44.40
28	9	30	0.05	350	100	37.40	55.10	37.20
29	3	30	0.50	350	100	97.70	97.20	97.20
30	9	30	0.50	50	100	61.20	62.00	58.00
31	9	5	0.05	350	200	10.00	22.80	9.00
32	3	5	0.05	50	200	95.10	99.40	92.00

Table 3
Estimated effects and coefficients for removal of SMO (% coded units).

Term	Net effect	Regression coefficient	Standardized effect (T)	p-Value
Constant		67.17	753.50	0.000
pH	−57.41	−28.71	−322.02	0.000
Temperature	12.38	6.19	69.42	0.000
Dose	8.72	4.36	48.93	0.000
Concentration	3.76	1.88	21.09	0.000
Speed	−3.46	−1.73	−19.43	0.000
pH × temperature	8.00	4.00	44.86	0.000
pH × dose	13.43	6.71	75.31	0.000
pH × concentration	−2.26	−1.13	−12.68	0.000
pH × speed	−6.56	−3.28	−36.80	0.000
Temperature × dose	1.01	0.51	5.69	0.000
Temperature × concentration	−4.67	−2.34	−26.21	0.000
Temperature × speed	2.70	1.35	15.15	0.000
Dose × concentration	6.77	3.39	37.99	0.000
Dose × speed	1.32	0.66	7.42	0.000
Concentration × speed	−1.66	−0.83	−9.34	0.000

Standard error coefficient for all cases = 0.08914.

factorial 2^{5-1} Resolution V design resulting in 16 runs. The resolution is a description of the design that gives the extent to which interactions will be confounded with other factors and interactions [18]. In Resolution V design, no main effect or two-factor interaction is confounded with any other main effect or two-factor interaction [18]. All the 16 experimental trials were replicated and thus 32 experiments were conducted in random order that was generated by MINITAB.

2.4. Biosorption studies

A solution of volume 100 mL was taken in a conical flask of 250 mL capacity for each run and the temperature will be controlled using an air bath. All the three oil-in-water emulsions of 100 mL volume was contacted for 6 h with the *M. rouxii* biomass at a desired rotational speed in a platform shaker (Model: Classic C2), manufactured by New Brunswick Scientific, New Jersey, USA. pH was adjusted using 0.1 M HCl or 0.1 M NaOH solution. The experiments were conducted under controlled pH conditions using buffer solutions. 0.2 M of sodium phosphate and 0.1 M of citric acid were used in different ratios [19] to adjust the pH. The oil-in-water emulsions were vacuum-filtered through a 1.5 mm glass micro-filter after biosorption experiments. A control (oil-in-water with no biomass) was also set up for each run. All experiments were conducted in duplicate and the mean values were used in the analysis of data. The filtrate was analyzed for oil concentration using Horiba OCMA-350 oil content analyzer (Horiba Instruments Inc., CA). Horiba OCMA-350 has an inbuilt NDIR spectrophotometer and displays oil concentration directly in mg/L on a digital panel. Oil was extracted with tetrachloroethylene (ultra-resi analyzed) before being analyzed by OCMA-350. The measurement range of Horiba OCMA-350 is from 0 to 200 mg/L and 0 to 1000 mg/g.

3. Results and discussion

The design matrix of uncoded values for the factors and the response in terms of the percent removal of SMO, CO and Bright-Edge 80 for all experimental runs including replicates, are shown in Table 2. A linear regression model was fitted for the experimental data using the least square technique using MINITAB. The model coefficients for the removal, the effects and standardized effects of the factors and interactions, and p-values of the effects in the model are shown in Tables 3–5 for SMO, CO, and Bright-Edge 80, respectively. The net effect is a difference between the responses of two levels (high and low level) of factors; the regression model coefficients are obtained by dividing the net effects by two. The standardized effects are obtained by dividing the regression coefficients by standard error coefficient [20]. p-Value is the probability value that is used to determine the effects in the model that are statistically significant. The significance of the data is judged by its p-value being closer to zero (0.00). For a 95% confidence level the

Table 4
Estimated effects and coefficients for removal of CO (% coded units).

Term	Net effect	Regression coefficient	Standardized effect (T)	p-Value
Constant		79.07	922.86	0.000
pH	−40.05	−20.02	−233.69	0.000
Temperature	3.52	1.76	20.52	0.000
Dose	2.50	1.25	14.59	0.000
Concentration	−7.79	−3.90	−45.47	0.000
Speed	−4.65	−2.32	−27.13	0.000
pH × temperature	4.36	2.18	25.44	0.000
pH × dose	3.65	1.83	21.30	0.000
pH × concentration	−7.78	−3.89	−45.42	0.000
pH × speed	−4.60	−2.30	−26.84	0.000
Temperature × dose	4.39	2.19	25.60	0.000
Temperature × concentration	12.35	6.18	72.09	0.000
Temperature × speed	9.24	4.62	53.90	0.000
Dose × concentration	8.94	4.47	52.15	0.000
Dose × speed	12.40	6.20	72.34	0.000
Concentration × speed	5.19	2.59	30.27	0.000

Standard error coefficient for all cases = 0.08568.

Table 5
Estimated effects and coefficients for removal of Bright-Edge 80 (% coded units).

Term	Net effect	Regression coefficient	Standardized effect (T)	p-Value
Constant		71.43	1095.02	0.000
pH	−45.90	−22.95	−351.83	0.000
Temperature	14.05	7.02	107.67	0.000
Dose	3.00	1.50	23.01	0.000
Concentration	−11.93	−5.96	−91.42	0.000
Speed	10.39	5.19	79.60	0.000
pH × temperature	15.09	7.55	115.66	0.000
pH × dose	5.06	2.53	38.78	0.000
pH × concentration	−20.53	−10.27	−157.40	0.000
pH × speed	10.08	5.04	77.24	0.000
Temperature × dose	−3.62	−1.81	−27.71	0.000
Temperature × concentration	6.64	3.32	50.89	0.000
Temperature × speed	5.23	2.61	40.07	0.000
Dose × concentration	5.71	2.86	43.77	0.000
Dose × speed	6.42	3.21	49.23	0.000
Concentration × speed	−3.15	−1.57	−24.12	0.000

Standard error coefficient for all cases = 0.06523.

p-value should be less than or equal to 0.05 for the effect to be statistically significant.

A statistical analysis (normal probability plot) of the data in terms of the standardized residual was also conducted to verify the normality of the data. The absolute value of the estimated effect determines its relative strength over the response. Higher the value of the effect higher is the influence over the response. The significance level for this model was chosen to be 0.05 (95% confidence level). Solution pH had the highest effect on the removal of all the three oils. A negative sign of the effect indicates that a low factor setting (−1) would result in a higher removal [18]. A decrease in solution pH to 3.0 from 9.0 increased percent removal of SMO, CO and Bright-Edge 80 to as high as 99%. It was found that pH 3.0 is the point at which the zeta-potential of autoclaved *M. rouxii* was zero. Acidic pH has been found to increase the percentage of residue oil adsorption to 99% by Ahmad et al. [8]. Similar trends were observed with bentonite and activated carbon which when used for adsorption of residue oil from palm oil mill effluent (POME)

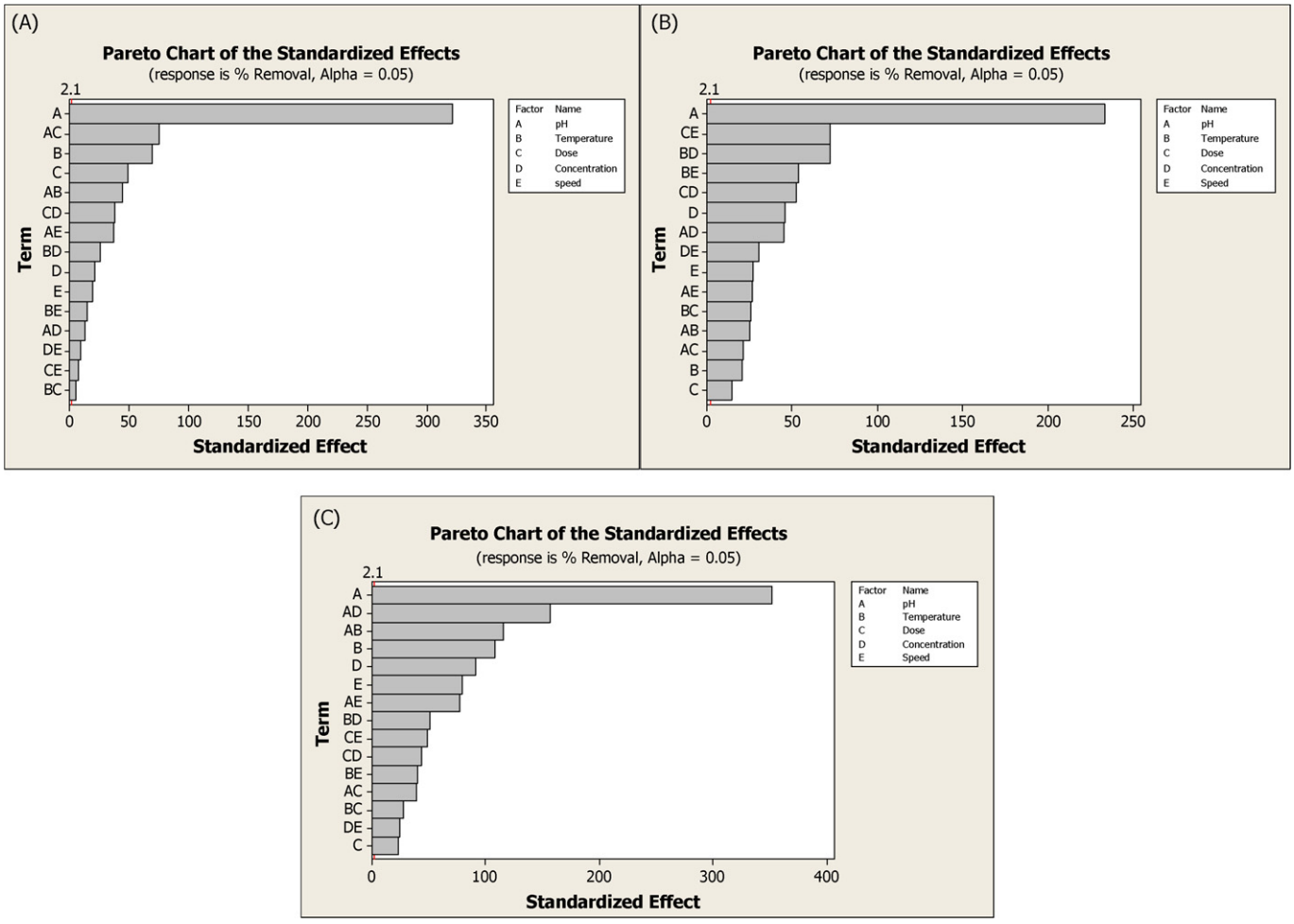

Fig. 1. Pareto chart for standardized effects for (A) removal of SMO, (B) removal of CO, and (C) removal of Bright-Edge 80.

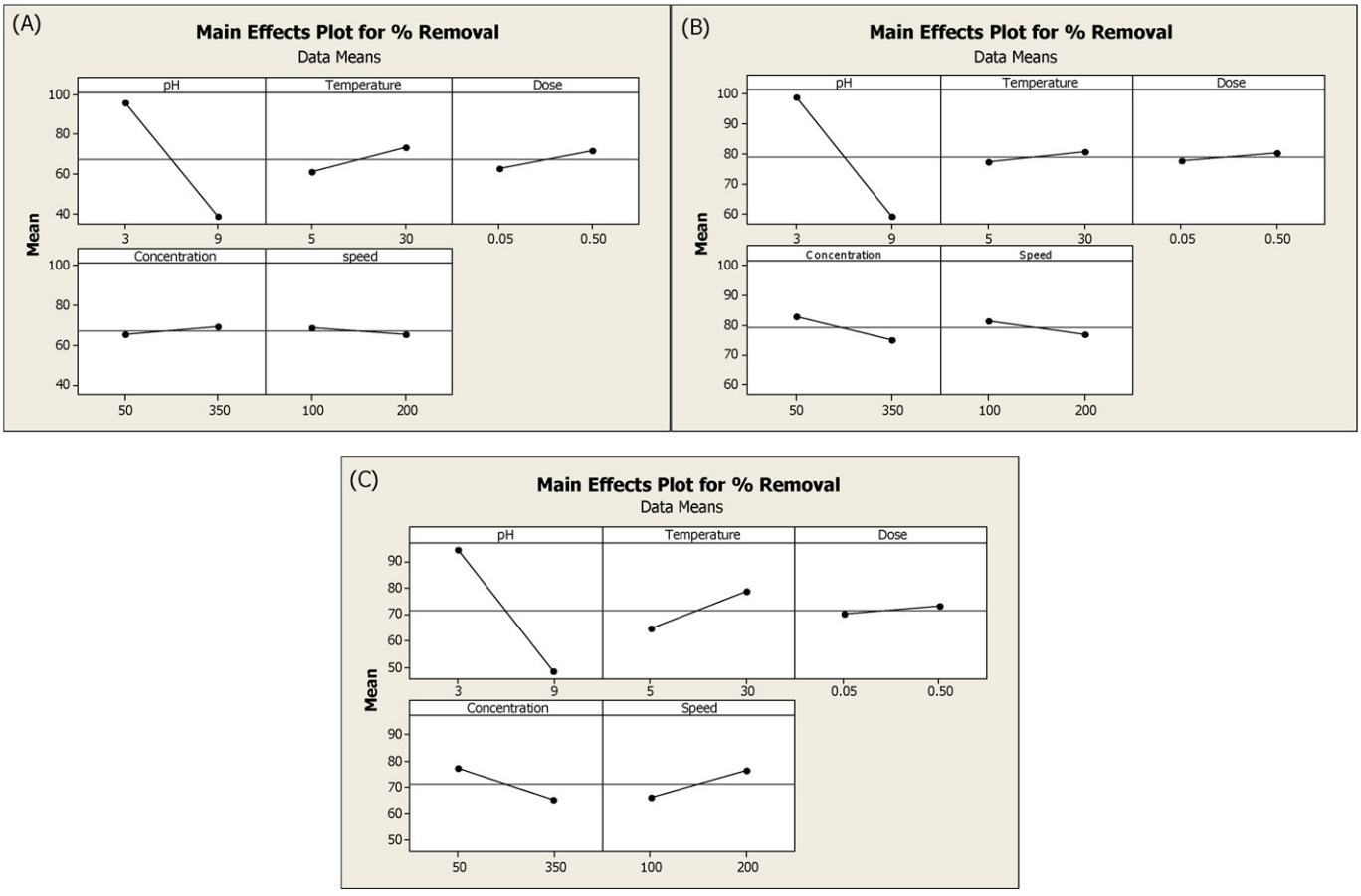

Fig. 2. Main effects plot for (A) removal of SMO, (B) removal of CO, and (C) removal of Bright-Edge 80.

showed higher oil removal at pH less than 5.0 [14]. In the case of fungal biomass, their surfaces have been generally observed to be negatively charged because of the ionization of functional groups present in them [21]. At acidic pH, some of the functional groups present in the fungal cell wall will be positively charged and negative charge intensity on the sites will be reduced, which might have an effect on the sorption characteristics of the biomass [21]. The higher adsorption capacity for oil by *M. rouxii* could be due to a high BET surface area value of 20.55 m^2/g. An adsorbent with higher surface area has been found to facilitate the adsorption of residue oil [8]. Mycelium of *M. rouxii* grows in the form of suspended growth leading to a larger surface area of the biomass for adsorption [21].

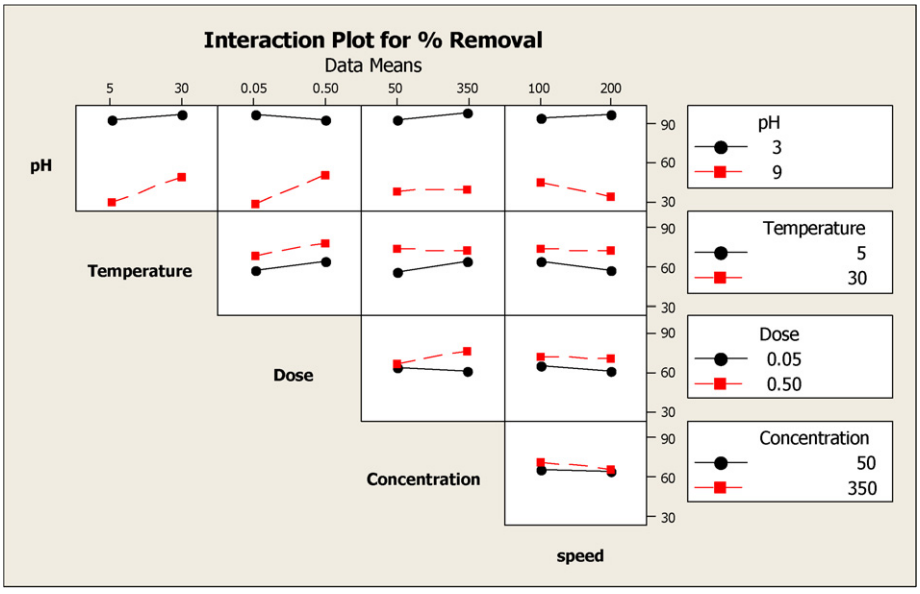

Fig. 3. Interaction effects plot for removal of SMO.

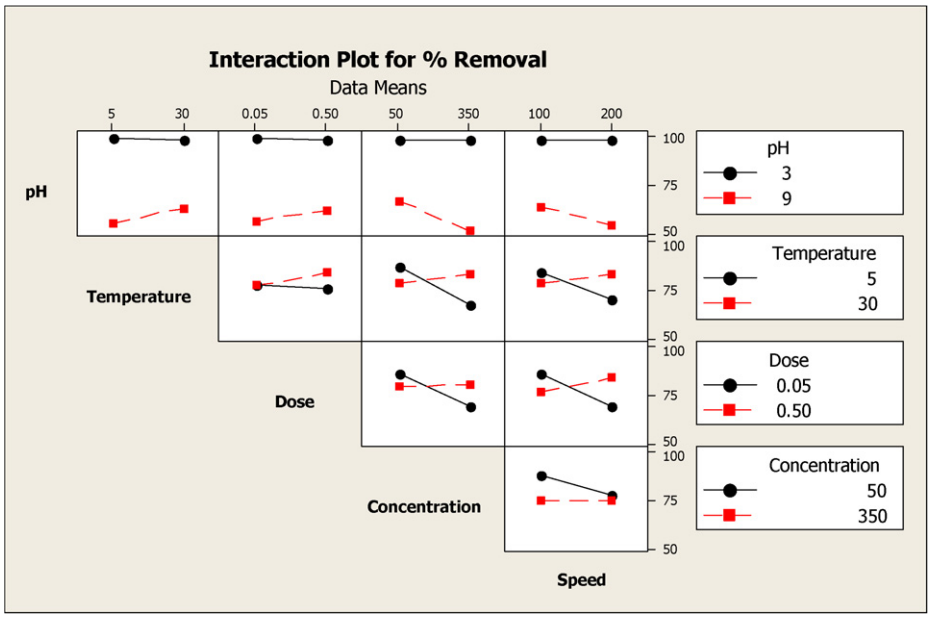

Fig. 4. Interaction effects plot for removal of CO.

For the removal of SMO, the magnitude of effects of each factor and their interactions were found to increase in the following order: pH > pH × dose > temperature. For the removal of CO, the increasing order of the effects is given by: pH > dose × speed > temperature × concentration. For Bright-Edge 80, it was given by: pH > pH × concentration > pH × temperature > temperature. The *p*-values in the estimated effects and coefficients were used to determine statistically significant individual and interaction effects. It can be observed from Tables 3–5 that all factors or combination of factors were found to be statistically significant (p-values ≤ 0.05) for removal of SMO, CO and Bright-Edge 80.

3.1. Pareto plot of effect

Pareto plot visually represents the absolute values of the effects of main factors and the effects of interaction of factors. A reference line is drawn to indicate that the factors which extend past this line are potentially important [20]. It can be seen from Fig. 1 that pH had the greatest effect on the removal for all the three oils. The effects that are above the reference line are statistically significant at 95% confidence level. It can again be seen from Fig. 1 that all effects are statistically significant. The relative importance of each of the factors and the combination of factors can be observed from pareto plots for all the three oils.

3.2. Main effects plot

The main effects plot is shown in Fig. 2 for removal of SMO, CO, and Bright-Edge 80, respectively. It indicates the relative strength of effects of various factors. A main effect is present when the mean response changes across the level of a factor. The sign of the main effect indicates the directions of the effect. It can be seen from Fig. 2 that for all the three oils, the effect of pH was characterized by a greater degree of departure from the overall mean. pH had a nega-

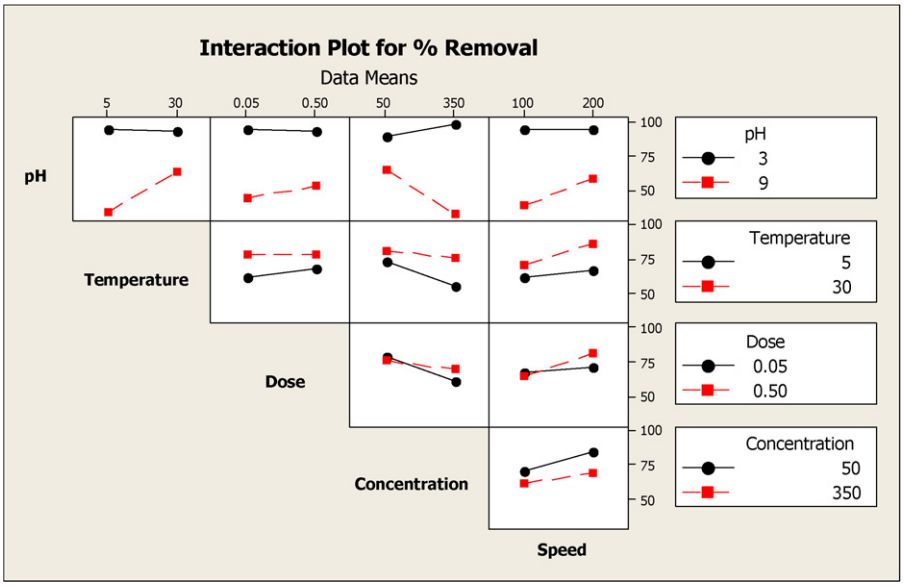

Fig. 5. Interaction effects plot for removal of Bright-Edge 80.

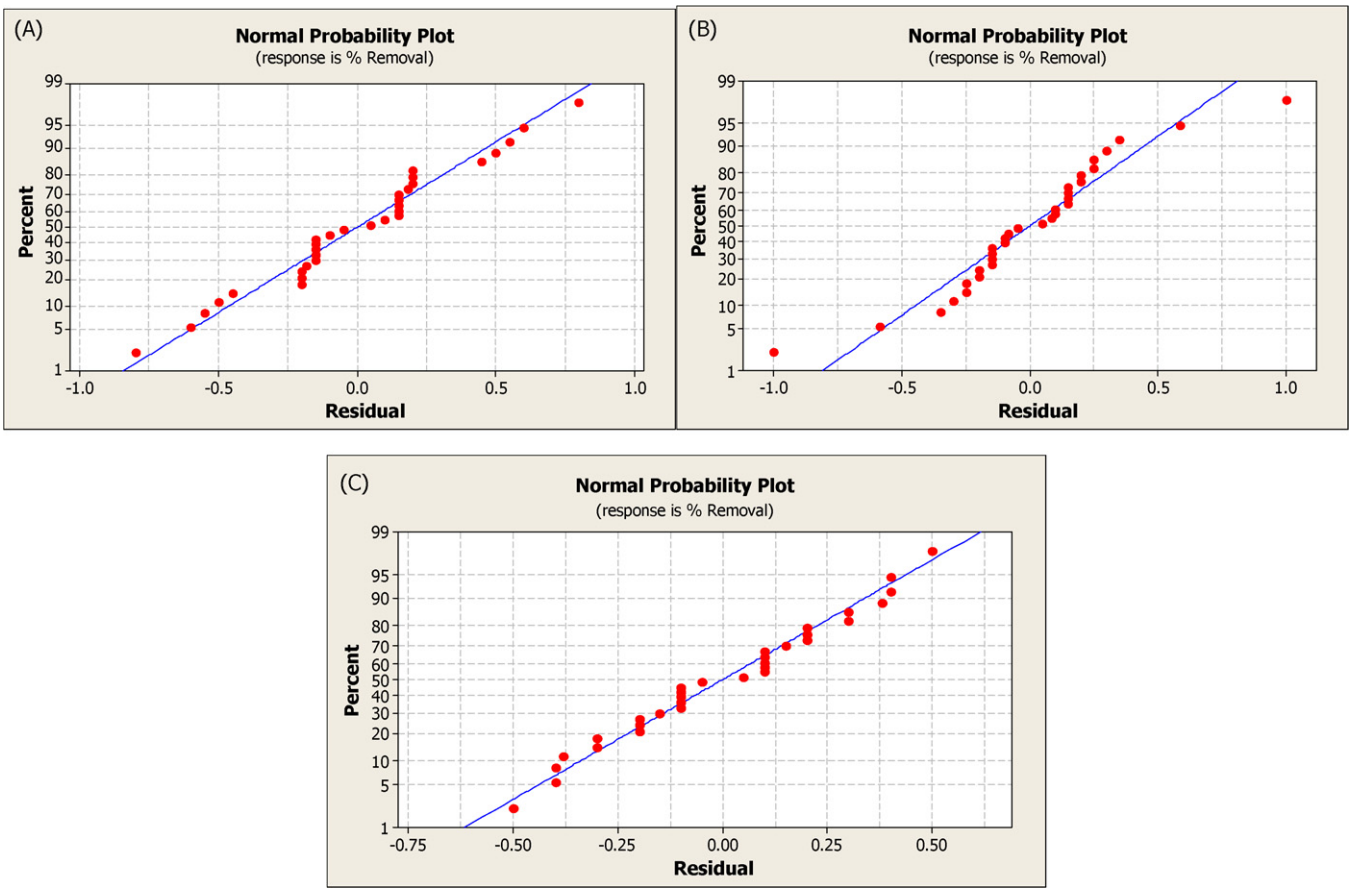

Fig. 6. Normal probability plot of the residuals for (A) removal of SMO; (B) removal of CO; and (C) removal of Bright-Edge 80.

tive effect on the removal of all the three oils. For SMO, temperature and dose showed a slight positive effect on removal. For CO, concentration had a slight negative effect on removal. For Bright-Edge 80, temperature and speed had a slight positive effect while concentration was found to have a slight negative effect. All other factors showed a smaller change. These patterns were previously identified by statistical significance. Statistical analysis of experimental data showed that the effects of the factors on removal percentage of three oils were not similar. This could be due to the fact that the composition of three oil-in-water emulsions used in the study was different. Mineral oils consist of mixtures of high molecular paraffins, naphthene and aromatic hydrocarbons with certain admixture of tar and asphaltene substances [22]. Vegetable oils are essentially triglycerides consisting of straight chain fatty acids attached, as esters, to glycerol [22]. A detailed study on oil removal by *M. rouxii* biomass involving the significant factors obtained by the fractional factorial analysis will help in understanding the process better.

3.3. Interaction effects plot

The interaction effects plots are shown in Figs. 3–5 for removal of SMO, CO, and Bright-Edge 80, respectively. The plots provide the mean response of two factors at all possible combinations of their settings. If the lines are not parallel, it is an indication of interaction between the two factors [20]. The interaction plots for all the three oils showed that pH interacted strongly with all other factors indicating pH to be a predominant influencing factor in removal. Decrease in the solution pH to 3.0 increased the percent removal of the three oils. Concentration of SMO and dose of the adsorbent showed minor interaction with each other (Fig. 3). When an adsorbent dose of 0.5 g was used, the percent removal of SMO decreased at SMO concentration of 50 mg/L and the percent removal of SMO increased at SMO concentration of 350 mg/L. It was possible that the system was not optimal with the factors involved. Further research is necessary to optimize the system. Percent removal of SMO seemed not to be affected at a low dose of 0.05 g irrespective of SMO concentration. Higher removal of SMO was observed at a dose of 0.5 g for both concentrations than at a dose of 0.05 g. Adsorbent dose was previously found to be statistically significant for removal of SMO. For CO, combinations of adsorbent dose and rotational speed, solution temperature and rotational speed, solution temperature and CO concentration and adsorbent dose and CO concentration showed antagonistic effects (Fig. 4). Concentration of CO and solution temperature showed a slight interaction with each other. Percent removal of CO was found to be not affected by rotational speed at a CO concentration of 350 mg/L while percent removal of CO increased at a CO concentration of 50 mg/L and a rotational speed of 100 rpm. Interaction effect between temperature and adsorbent dose showed that percent removal of CO was higher at a solution temperature of 30 °C and an adsorbent dose of 0.5 g. The percent removal of CO remained the same at a solution temperature of 5 °C irrespective of the adsorbent dose. In the case of Bright-Edge 80, solution temperature and Bright-Edge 80 concentration, solution temperature and adsorbent dose, and solution temperature and rotational speed had interaction with each other (Fig. 5). In all the three cases, percent removal of Bright-Edge 80 was found to be higher at 30 °C. Adsorbent dose and Bright-Edge 80 concentration and adsorbent dose and rotational speed were also found to have interaction with each other. In both cases, percent removal of Bright-Edge 80 was found to increase at a higher adsorbent dose. Other interactions showed no prominent features for a discussion.

3.4. Prediction of regression model

A model is proposed based on the regression coefficients (for coded units) for removal of SMO, CO and Bright-Edge 80. The regression models proposed are as follows:

$$\text{SMO removal}(\%) = 113 - 9.57\ \text{pH} + 0.495\ \text{temperature} + 19.4\ \text{dose} + 0.0125\ \text{concentration} - 0.0346\ \text{speed} \quad (1)$$

$$\text{CO removal}(\%) = 127 - 6.67\ \text{pH} + 0.141\ \text{temperature} + 5.6\ \text{dose} - 0.0260\ \text{concentration} - 0.0465\ \text{speed} \quad (2)$$

$$(3)\text{Bright-Edge 80 removal}(\%) = 98.0 - 7.65\ \text{pH} + 0.562\ \text{temperature} + 6.7\ \text{dose} - 0.0398\ \text{concentration} + 0.104\ \text{speed}$$

The applicable range of all the parameters of the regression model is provided in Table 1; the dose varied from 0.05 to 0.5 g, the temperature varied from 5 to 30 °C, the concentration varied from 50 to 350 mg/L and the speed varied from 100 to 200 rpm.

3.5. Normal probability plot of residuals

One of the key assumptions for the statistical analysis of data from experiments is that the data come from a normal distribution [20]. The normality of the data can be checked by plotting a normal probability plot of the residuals. If the points on the plot fall fairly close to a straight line, then the data are normally distributed [20]. The normal probability plot of the residual for SMO, CO and Bright-Edge 80 are shown in Fig. 6. It can be seen that for all the three oils, the points fall fairly close to the straight line. Therefore, the data from the experiments come from a normally distributed population.

4. Conclusions

The following conclusions are drawn based on data analysis:

1. pH was found to be the most influential parameter for removal of SMO, CO and Bright-Edge 80 by *M. rouxii* biomass. Lower the pH of the oil better the removal efficiency.
2. Adsorbent dose and temperature were found to have an effect on the removal of SMO, oil concentration had an effect on the removal of CO and temperature was found to have an effect on the removal of Bright-Edge 80.
3. *M. rouxii* biomass showed a good potential to remove oil from water.

It is important to note that these statements are valid within the lower and upper limits of the factors: adsorbent dose (0.05–0.5 g), temperature (5–30 °C), concentration (50–350 mg/L) and speed (100–200 rpm).

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