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Optimization of Process Parameters for the Production of Biodiesel from Carbon dioxide Sequestering Bacterium

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ABSTRACT

In the study, the process of *in-situ* transesterification for biodiesel production from a carbon dioxide sequestering chemolithotrophic bacterial strain *Serratia*sp. ISTD04 was optimized. The optimization of process parameters was carried out in order to enhance the yield and reduce the cost of biodiesel production. *Serratia*sp. ISTD04 was grown in MSM in the presence of sodium bicarbonate as the sole carbon source. The bacterial biomass harvested after 48 hours was utilized for biodiesel production. Box–Behnken design and Response surface methodology were used to optimize the *in-situ* transesterification, by carrying out the optimization study on three process parameters- Temperature, Duration and Catalyst concentration. Under optimized conditions of- 40.52°C, 22.93hrs and 2.04% v/v of methanol, a biodiesel yield of 41.47% was obtained. The characterization of biodiesel was further done by GC–MS, FT-IR and NMR (H^1 and C^{13}).

1. Introduction

Green-house gases (GHGs) are one of the most serious environmental issues which raise a question mark on existence of the earth. Carbon dioxide (CO_2), one of the major GHGs, concentration was stable at about 270 ppm which has increased approximately 38% which is 380 ppm after industrial revolution. It is predicted that by the middle of this century the concentration of CO_2 will be reached to 600 ppm and by the end of the century it is likely to reach 700 ppm [Shrestha and Lal, 2006]. Increase in CO_2 concentration may be mitigated by autotrophic and heterotrophic carbon fixationby plants and microorganisms. CO_2 mitigation strategy includes- improving energy efficiency, capturing and sequestering CO_2 and use of alternative fuels (biohydrocarbon, biodiesel, etc.) [Bharti et al., 2014a]. One of the most effective and alternative methods to check the increasing levels of CO_2 is by using microorganisms as some of them are capable of fixing atmospheric carbon dioxide in to valuable products with the help of their enzymatic machinery [Kumar et al., 2016a]. Some microbes synthesize valuable products such as biodiesel and polyhydroxyalaka notes along with sequestration of CO_2 [Kumar et al., 2016a].Hydrocarbons (Alkanes/alkenes) have been widely distributed among organisms, that are including bacteria, fungi, algae, higher plants, and animals [Han et al., 1969] and they are the metabolic product of some chemolithotrophic bacteria which are produced from fatty acidsand triacylglycerol (TAG). Triacylglycerols (TAG) are fatty acid trimesters of glyceroland its properties vary depending on their fatty acid composition. The occurrence of TAG as energystock is widespread among eukaryotic organisms such as yeast, fungi, plants and animals, whereas, occurrence of TAG in bacteria has only rarely been described [Alvarez et al., 2000]. Biosynthesisand accumulation of TAG has been reported in bacteria belonging to the actinomycetes group, such as Streptomyces, Nocardia, Rhodococcus, Mycobacterium, etc. [Alvarez et al., 2002].These microorganisms utilize diverse types of carbon sources such as sugars, organic acids, alcohols, n-alkanes, branched alkanes, phenylalkanes, oils and coal for biosynthesis of TAG[Alvarez et al., 2000]. The excess availability ofcarbon and limiting nitrogen in the growth medium enhances the synthesis of TAG inside bacterium, because in such a situation, cellular growth is impaired and the cells utilize the carbon sourcemainly for the biosynthesis of neutral lipids [Alvarez et al., 2002].The extraction of lipids from bacterial biomass and further its transesterification is one of the challenging and cost intensive process. Lipids extracted by Sonication [Belarbi et al., 2000] helps to disrupt the cells and encourage better lipid extraction, but this method also adds significant mechanical and chemical costs. Bligh and Dyer method of lipids extraction [Bligh and Dyer, 1959]

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a second step is required to transesterify the lipids and is typically accomplished with the help of an alcohol and acid or base as catalyst. Thus this overall production step requires significant amounts of solvents as well as large processing time. Although these methods can potentially be scaled up to extract intracellular lipids, the cost and toxicity of the solvents are always a cause of concern.

The *in situ* transesterification method reduces the steps of production by combining the extraction and transesterification into a single step, which leads to lower solvent use and processing time[Ashford et al., 2000]. In this process, raw material is treated with a mixture of alcohol and acid or base as catalyst and the end product obtained in the form of fatty acid alkyl esters (typically fatty acid methyl ester, or FAME). Alkali catalyzed transesterification reactions have certain advantage like they are much faster than acid catalyzed reaction which ultimately reduces the processing time. However, they run the risk of water and soap formation from cellular free fatty acids (FFAs)[Vincente et al 2004]. The formation of soap in base catalyzed transesterification greatly increases the cost of production and makes product separation more tedious [Carrapiso and Garcia, 2000]. Soap formation can be avoided with the use of strong liquid acid-catalyzed transesterification processes where the FFAs are converted to the ester form through acid mediated esterification. Based on these considerations, the acid-catalyzed *in situ* transesterification process is likely to be more economically viable during large-scale processing. Therefore, the existing methodology needs to be modified and optimized for large scale production.

For improving the performance of a system, a process, or a product in order to obtain the maximum benefit from it, determination of the optimum conditions is important. Traditional approach of optimizing one-variable-at-a-time (OVAT) for a multivariable system is not only time and labor intensive but often results in missing out the interactive effects between the components [Bandaru et al., 2006]. Response surface methodology (RSM) is a suitable multivariate statistical technique which not only assists in understanding the interactions of different variables and predicts maximized response, but also is rapid and economical with fewer experiments and minimal resource utilization. Amongst the various RSM designs available, Box–Behnken design (BBD) has been found to be more efficient than the central composite and full factorial designs [Ferreira et al., 2007] and has been effectively applied for the optimization of various processes such as dye decolorization and degradation, biosorption, enzyme, drug and biodiesel production [Kumar et al., 2016b].

The present study aimed to optimize the *in situ* transesterification reaction for biodiesel production from a carbon dioxide sequestering bacterium which sequestered carbon dioxide at the rate of 0.756 x10^{-9} µmol CO_2 fixed $cell^{-1}$ h^{-1} [Srivastava et al., 2015]. The process parameters were optimized in order to enhance the biodiesel yield and make the process of biodiesel productionmore cost effective. Response surface methodology and Box–Behnken design were used to design the experiments, build models and determine the optimum conditions for increased yield of biodiesel. The statistical design was based on three factors (temperature, duration and catalyst concentration) at three levels.

2. Methodology

2.1 Microorganism and culture condition

Pre-LB culture of bacterial strain *Serratia* sp. ISTD04 (gene bank accession number- JF276275) isolated from marble rocks of the palaeoproterozoic metasediments of the Aravali Supergroup, Rajasthan [Srivastava et al., 2015]was centrifuged (7000 rpm, 10 min) and the cell pellet transferred in Minimal salt medium (MSM) containing (g L^{-1}): $Na_2HPO_4 \cdot 2H_2O$, 7.8; KH_2PO_4, 6.8; $MgSO_4$, 0.2; $NaNO_3$, 0.085; $ZnSO_4 \cdot 7H_2O$, 0.05; $ZnCl_2$, 0.02; Ca $(NO_3)_2 \cdot 4H_2O$, 0.05 and 20 mM $NaHCO_3$ as the sole carbon source at 30 °C, 150 rpm and pH 7.6. After 48 h, the bacterial biomass was collected for the *in situ* transesterification. For this, the cultures were centrifuged at 8000 rpm for 10 min, followed by washing twice with distilled water and drying at 60 °C until a constant weight was obtained.

2.2. In situ Transesterification

Fixed amounts of dried bacterial biomass were weighed into screw-capped vials. Then, methanol (25 mL) and catalyst sulfuric acid 3% v/v of methanol was added. The mixture was heated at 70 °C for 15 hrs in an incubator shaker. Thereafter, the mixture was allowed to cool down to room temperature followed by centrifugation at 7000 rpm for 5 min and the supernatant was collected. Further, for complete recovery of FAMEs from biomass residue, it was washed thrice with 5 mL of methanol, vortexed and centrifuged at 3000 rpm for 5 min. The supernatants were pooled and the methanol was removed at 55 °C. Then, the residue was re-dissolved in 15 mL of n-hexane and washed thrice with 5 mL distilled water. The hexane layer was then collected and passed through anhydrous sodium sulfate column to remove traces of water. The samples were then evaporated to dryness at room temperature using a vacuum rotator evaporator. After the hexane was completely removed, the flask was flushed with nitrogen to remove any remaining hexane in the gas phase. The residual weight was then determined and used to calculate the biodiesel yield [Bharti et al., 2014b].

The yield of biodiesel (Y) was calculated as:

$$Y = \frac{\text{residual weight (grams)}}{\text{biomass (grams)}} \times 100$$

2.3. FAME Analysis

2.3.1. Gas chromatography-mass spectroscopy analysis

The purity of biodiesel obtained from *in situ* transesterification was confirmed by GC-MS. For this, the biodiesel residue was dissolved in 1mL hexane and analysed on a Shimazdu GC-MS QP2010 Plus equipped with Rtx-5MS column (dimensions: 0.25-µm film thickness, 0.25 mm ID, 30 m in length). 1 µL of the sample in hexane was injected into GC200 MS injector port. The column temperature was held at 60 °C for 3 min; then temperature was increased from 60 to 320 °C at a rate of 12 °C / min for 30 min. Identification of the fatty acids was based on retention times and fragmentation patterns. Data were matched with the GC-MS inbuilt standard mass spectra library of NIST-08 and Wiley-8. The FAME standards (FAME –mix Sigma Aldrich) were prepared in a hexane solution at concentrations of 250µg, 500 µg, 750 µg, and 1mg ml^{-1}. This linear range of concentration standards was used to develop a standard curve equation by which all unknown fatty acid concentrations were determined.

2.3.2. Fourier Transform Infrared Spectroscopy (FT-IR)

An infrared spectrum of the biodiesel produced was obtained using Varian 7000 FTIR spectrometer (Perkin-Elmer Inc., Wellesley, MA, USA).For this, the sample was dried, powdered with the help of mortar and pestle and mixed with KBr in a ratio of 5:100 to make the KBr discs for spectrum analysis. The analysis was done with the help of Infrared spectra were recorded in the range of 4000 - 400 /cmwith a resolution at 4 /cm [Bharti et al., 2014a].

2.3.3. ^{1}H NMR and ^{13}C NMR analysis of FAMEs

NMR experiments were performed using Varian Mercury Plus NMR spectrometer equipped with 5 mm Varian probes (ATB and SW) using deuterated chloroform ($CDCl_3$) as solvent [Guillen and Cabo, 1997]. ^{1}H (300 MHz) spectrum was recorded with pulse duration of 45°, a recycle delay of 1.36 s and 16 scans. ^{13}C (75.46 MHz) spectra was recorded with a pulse duration of 45°, a recycle delay of 0.28 s and 300 scans.

2.4.Optimization of process parameters- Experimental design, analysis and validation

In the extraction of biodiesel from bacterial biomass, relatively high product yields are expected for economical feasibility. The yield of biodiesel can be increased by optimizing the *in situ* transesterification reaction conditions. For optimizing the reaction condition, Response Surface Methodology (RSM) and Box-Behnken design were applied using Design Expert software version 10 (Stat-Ease Inc., Minneapolis, USA). A total of 17 experiments were carried out to assess the effects of the three variables: temperature, duration and catalyst concentration, each at three levels (low, medium and high), with biodiesel yield taken as a response as shown in Table 1. The experimental design matrix obtained from the Box-Behnken model is also shown in Table 1. Prediction of optimal conditions can be done using the following second order polynomial equation:

$$Y = \beta_0 + \sum \beta_i x_i + \sum \beta_{ij} x_i x_j + \sum \beta_{ii} x^2_{ii} + \varepsilon \quad (1)$$

3. Results and Discussion

3.1 FAME Analysis

3.1.1. GC-MS Analysis

The composition analysis of the biodiesel produced after optimization of the process of *in-situ* transesterification showed (Table 2) that, the fatty acid methyl ester content was 95.56%, giving a very high purity value of the biodiesel obtained. GC-MS results indicated that the major constituents

Table1 The Box- Behnken design matrix for experimental design observed and predicted values of FAMEs Yield (%)

Experimental Run	Temperature (code)	Duration (code)	Catalyst conc. (code)	FAME Yield (%)	
				Actual	Predicted
1	100 (+1)	24 (+1)	3 (0)	30.3	32.04
2	70 (0)	15 (0)	3 (0)	35.32	35.31
3	100 (+1)	15 (0)	5 (+1)	25.24	24.23
4	100 (+1)	15 (0)	1 (-1)	23.91	24.27
5	40 (-1)	6 (-1)	3 (0)	40.61	38.86
6	70 (0)	24 (+1)	5 (-+1)	32.41	31.67
7	70 (0)	15 (0)	3 (0)	35.23	35.27
8	70 (0)	24 (+1)	1 (-1)	37.32	35.21
9	70 (0)	15 (0)	3 (0)	35.38	37.13
10	40 (-1)	24 (+1)	3 (0)	39.74	40.83
11	70 (0)	15 (0)	3 (0)	35.27	35.38
12	70 (0)	6 (-1)	1 (-1)	32.44	33.17
13	70 (0)	6 (-1)	5 (+1)	24.87	26.97
14	100 (+1)	6 (-1)	3 (0)	28.38	27.27
15	70 (0)	15 (0)	3 (0)	35.17	35.21
16	40 (-1)	15 (0)	5 (+1)	29.97	29.60
17	40 (-1)	15 (0)	1 (-1)	38.29	39.29

of the biodiesel obtained were the methyl esters of palmitic acid (C16: 0), heptadecanoic acid (C17:0) and oleic acid (C18: 1).Biodiesel compositions with high percentage of polyunsaturated methyl esters are not suitable for vehicle use as they have low cetane number and reduced oxidative stability, with European standard EN 14214 limiting it to 12 % [Knothe et al., 2011]. It has been reported that with the increase in the content of unsaturated fatty acids, there is an increase in hydroperoxide generation leading to the risk of polymerization, acidification and appearance of insoluble sediments and gums, which lead to filter plugging and deposits in the fuel systems [Gopinath et al., 2010]. Although, higher levels of saturated fatty acids may lead to poor low temperature behavior of the biodiesel, blending with mineral diesel could overcome this problem [Angerbauer et al., 2008].Higher levels of saturated fatty acids was observed which may present a problem in cold weather owing to gelling, but the higher saturated content will also lead to better burning properties [Bharathi et al. 2014]. Methyl palmitate (58.55 %) present in biodiesel is advantageous especially with regards to low-temperature properties [Knothe, 2008]. Exhaust emissions can be at least partially related to cetane number (CN). Also, nitrogen oxide (NOx) exhaust emission decreases with increasing CN, i.e., with increasing saturation of the fatty

Table 2 FAME profile of Biodiesel carried out by GC-MS analysis

R. Time	FAME	Carbon chain length	Area%	Relative FAME content (%)
13.202	Methyl nonanoate	C10:0	3.77	3.95
16.477	Methyl hexadecanoate	C17:0	55.95	58.55
17.497	Hexanoic acid, 3,5,5-trimethyl-, methyl ester	C9:0	1.06	1.11
18.112	Octanoic acid, methyl ester	C9:0	0.07	0.07
19.778	Heptadecanoic acid, 16-methyl-, methyl ester	C19:0	28.28	29.60
20.710	Methyl 9-octadecenoate	C18:1	4.76	4.98
21.741	Octanoic acid, 2-methoxy-, methyl ester,	C10:0	1.67	1.74
	Total		95.56	100

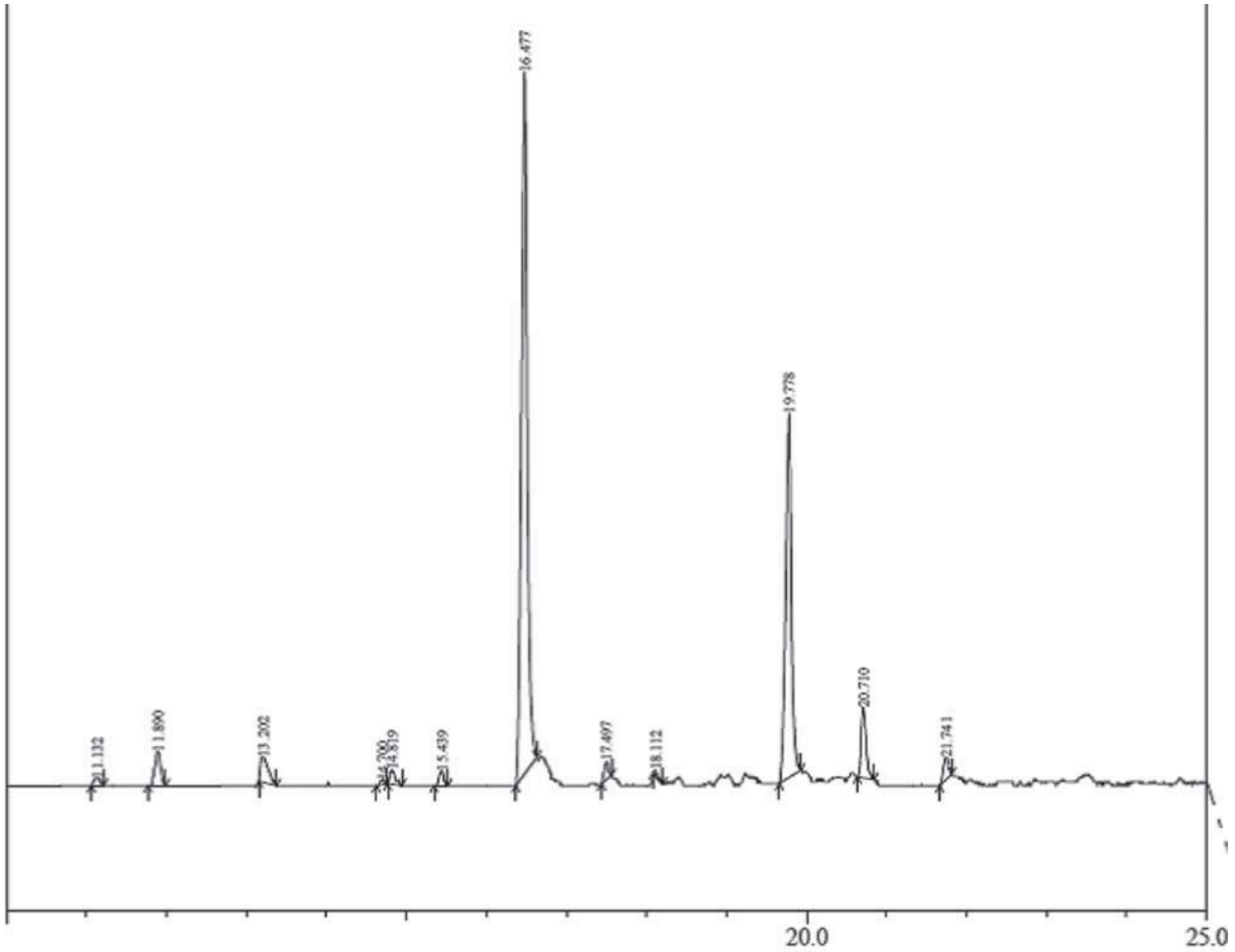

Fig. 1 GC-MS chromatogram of FAME in Biodiesel, produced from *in-situ* transesterificationof *Serratia* sp.ISTD04

ester chain [Ladommatos et al., 1996]. It has also been reported that reduction in particulate matter emissions occurs due to the presence of methyl palmitate. Another advantage of having a high content of saturated fatty esters is that they are oxidatively stable. High percentage of long chain methyl esters in the biodiesel produced increases the heat of combustion which reduces the fuel consumption [Knothe, 2008].

3.1.2. FTIR Analysis

The FTIR spectrum of biodiesel obtained is shown in Fig. 2. Since biodiesel is mainly monoalkyl ester, intense C=O stretching band of methyl ester appears at 1730 cm^{-1}. Strong bands at 2915 and 2846 cm^{-1} are due to the C–H stretching absorptions of the methylene and terminal methyl groups of fatty acid chain [Kumar et al., 2016b]. Absorption of some bending vibrations of the methylene group is produced at 1372 cm^{-1}. The C–C(=O) –O band of saturated esters appears at 1219 cm^{-1}. The O–C–C band of esters derived from primary alcohols appears in the zone between 1064 and 1031 cm^{-1}, while for those derived from secondary alcohols the band appears approximately at 1100 cm^{-1} [Kumar et al., 2016b]. The FTIR spectrum thus indicates the presence of both saturated as well as unsaturated esters in the biodiesel.

3.1.3. ^{1}H NMR and ^{13}C NMR Analysis

The ^{1}H NMR spectrum of FAME is shown in Figs. 3 a and b. A singlet signal at 3.67 ppm represents methoxy protons of the methyl esters and the triplets around 0.9 ppm are from the terminal alkyl methyl in each of the methyl esters [Horst et al., 2009]. The pentet at 2.33 ppm represents the α-methylene protons to ester, while the multiplet between 1.58-1.69 ppm represents the β-methylene protons [Basumatary et al., 2013; Horst et al., 2009]. The singlet signal at 1.30 ppm is due to the protons of backbone methylenes of long chain fatty acids [Basumatary et al., 2013].

Table3 FT-IR Bonds and Corresponding annotation of biodiesel produced after *in situ* transesterification

Peak (cm-1)	Bonds and Corresponding annotation
1031-1064 1100	O-C-C band of esters derived from primary alcoholsO-C-C band of esters derived from secondary alcohols
1219	C-C(=O) -O band of saturated esters
1372	Bending vibrations of the methylene group
1730	C=O stretching band of methyl ester
2915 and 2846	C-H stretching absorptions of the methylene and terminal methyl groups of fatty acid chain

The ^{13}C NMR spectrum of biodiesel is shown in Fig. 4 a and b. The signal at 178.62 ppm represents the carbonyl carbon of the ester molecules and the olefinic carbons appear at 129.94 ppm. The methylene and methyl carbons of fatty acid moiety appear in the range from 14.10 to 33.98 ppm [Basumatary et al., 2013].

3.2. Regression models and statistical testing

The relationship between independent variables and the response was drawn by second-order polynomial equation. The coded equations obtained from Box-Behnken design for biodiesel yield as suggested by the software is given below:

$$\text{Biodiesel Yield} = 35.27 - 5.09A + 1.68B - 2.46C - 0.7AB + 2.41AC + 0.66BC - 1.46A^2 + 0.94B^2 - 4.45C^2 \quad (2)$$

where A = Temperature (°C), B = Duration and C = Catalyst concentration (%)

The role of individual variables or their double interactions on the response is revealed by comparing the coefficients in the above coded equation. Negative coefficient values indicate a negative effect on the response, whereas positive coefficient values indicate that the variables increase the response in the tested range. Thus, the coefficient values in Eq. (2) reveal that the biodiesel yield decrease with variables –temperature and catalyst concentration whereas increase with increase in duration. Also, the duration had maximum influence on the biodiesel yield as indicated by the large value of its factor coefficient.

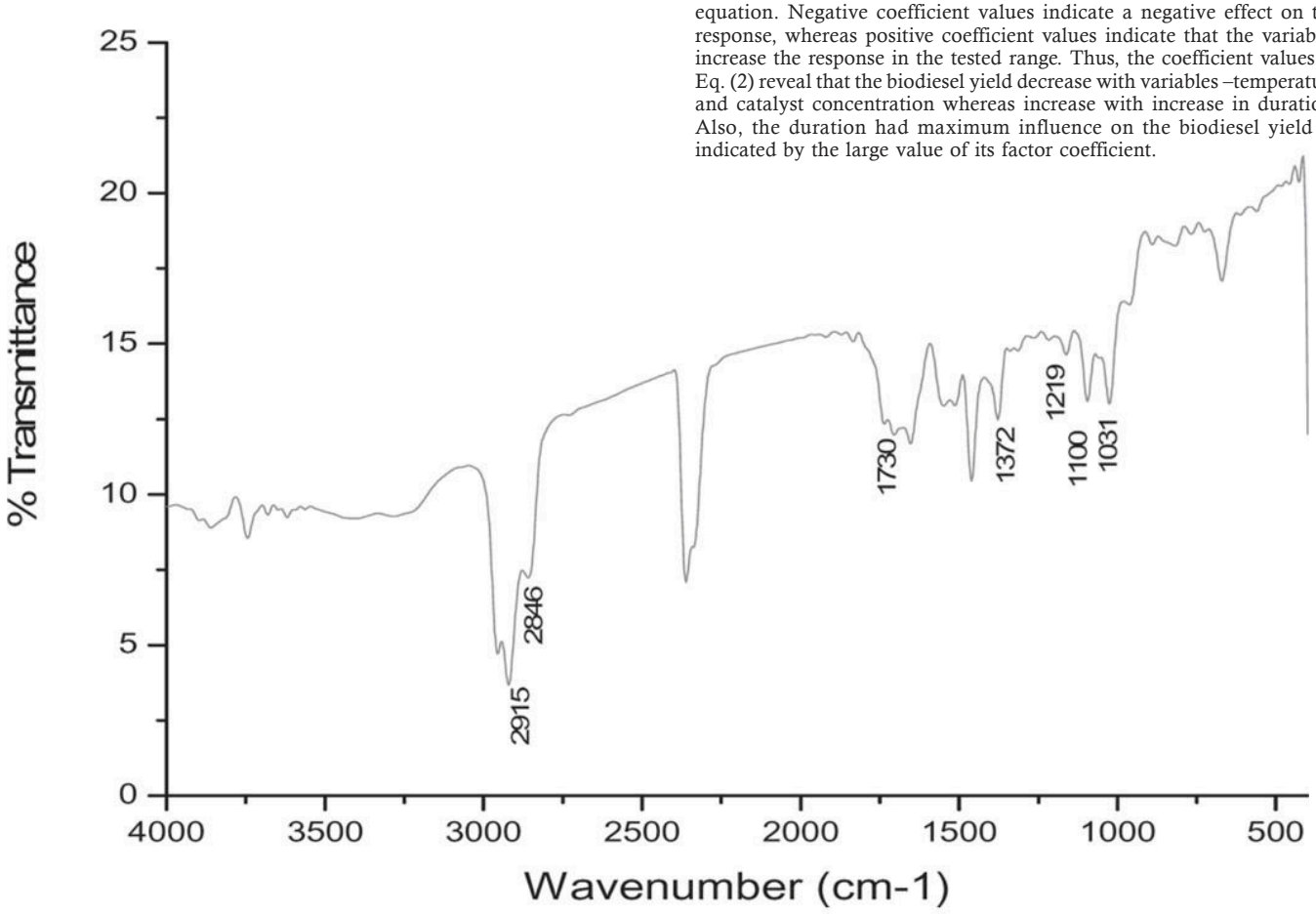

Fig. 2. Fourier transform infrared spectroscopy (FTIR) analysis of FAME in biodiesel produced by *Serratia* sp. ISTD04

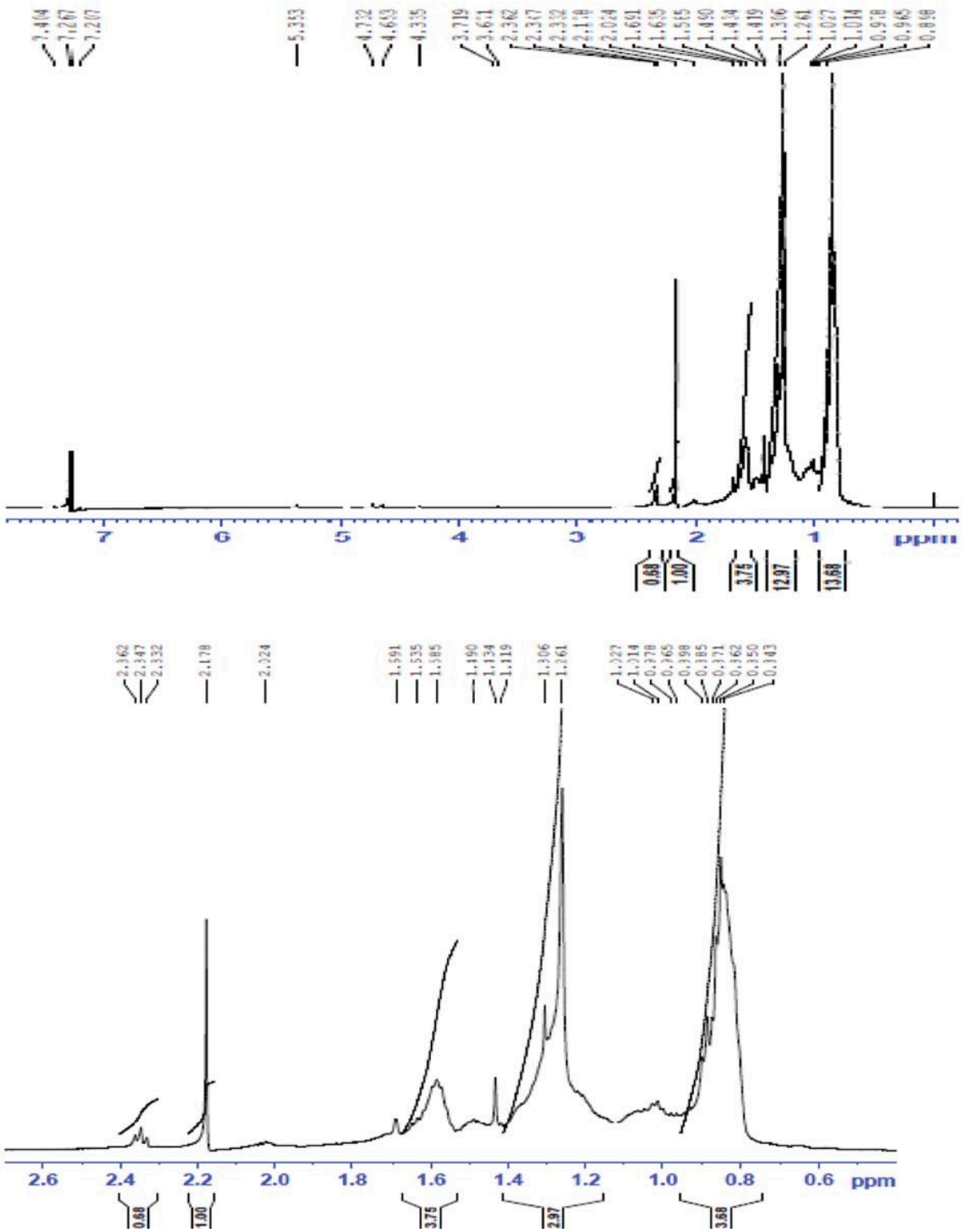

Fig. 3 ^{1}H NMR spectra of Biodiesel produced from *in-situ* transestrification of *Serratia* sp.ISTD04

The analysis of variance (ANOVA) results of the model are presented in Table 4**.** The Model F-value of 15.046implies that the model is significant. There is only a 0.09 % chance that an F-value this large could occur due to noise. The fit of the model was expressed by the coefficient of regression R^2, that was found to be 0.95, which was in agreement with the adjusted R^2 value which is 0.89. A high R^2 value close to 1 is desirable and a reasonable agreement with adjusted R^2 is necessary **[**Nordin et al., 2004**]**. The adequate precision (AP) value is a measure of the "signal to noise ratio" and values higher than four are desirable. The AP value for the model was found to be 12.55. The coefficient of variance (CV) which is the ratio of standard error of estimate to the mean value of response, defines the model's duplicability. A model normally can be considered reproducible if its CV is not greater than 10 % [Beg et al., 2003]. As shown in Table 4, the value of CV is less than 10 % (5.23) confirming the reproducibility of the model.

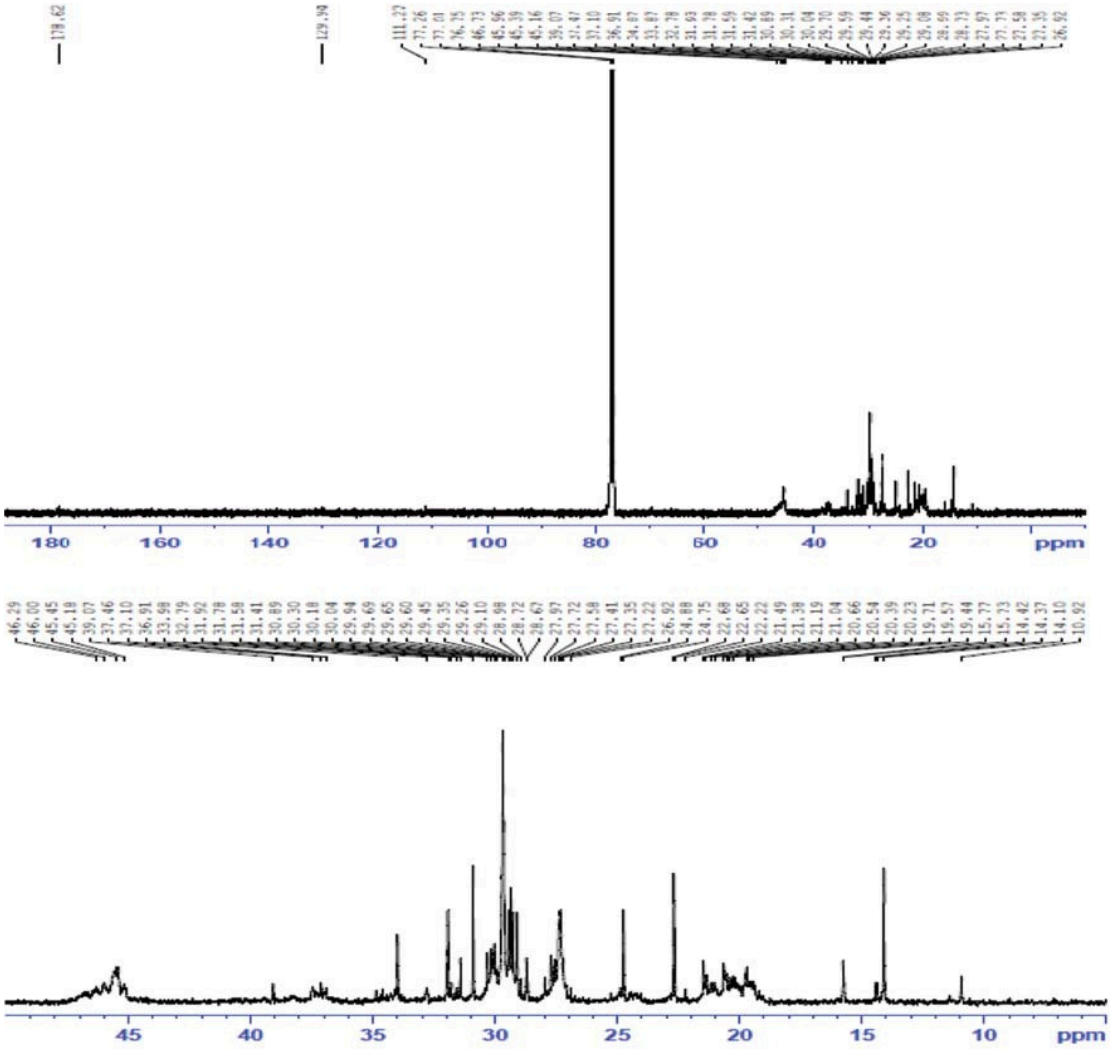

Fig. 4 ^{13}C NMR spectra of Biodiesel produced from *in-situ* transesterification of *Serratia* sp. ISTD04

Table4 Analysis of variance for RSM variables fitted to quadratic model

Source	Sum of squares	Degree of freedom (d.f.)	Mean square	*F*-value	*P*-value	*Prob*> F
Model	402.2789521	9	44.69766	15.04646	0.000856	Significant
Residual	20.794495	7	2.970642			
Pure error	0.02612	4	0.00653			
R^2	0.950					
$R^2_{adjCV\%}$	0.8905.23					

3.3. Interactive effects of factors on biodiesel yield

Three-dimensional graphical responses were generated on the basis of the model equations to visualize the interactive effects of the factors on biodiesel yield. The response surface plots are shown in Fig. 5. These plots illustrate the relative effects of any two factors by keeping the third factor constant. Increasing the duration along with a decrease in temperature had a positive influence on biodiesel yield (Fig. 5a). The biodiesel yield was positively affected with an increase in concentration of catalyst and decrease in temperature(Fig. 5b). Compared to catalyst concentration, an increase in duration had a more significant effect on biodiesel yield(Fig. 5c). Thus, these plots suggest that high temperature and high catalyst concentration had negative effect on biodiesel yield, whereas long duration of *in situ* transesterification increases the yield of biodiesel. The curve of response surfaces clearly indicate that duration of *in situ* transesterification was the most critical factor for maximum response generation.

Influence of temperature can be explained by the fact that unsaturated fatty acids and their esters can undergo polymerization at high temperature [Revellame et al 2010]. Sulfuric acid is a known catalyst for polymerization of unsaturated fatty acids. Significant polymerization of unsaturated fatty acids and their derivatives could have caused a decline in the biodiesel yield at temperatures above 40 °C.Sulfuric acid as an acid catalyst was chosen in the study, in order to maximize the biodiesel yield and avoid soap formation which occurs due to use of base catalysts. Furthermore, sulfuric acid has been shown to be an effective catalyst for the *in situ*transesterification of rice bran oil even in the presence of significant amounts of moisture (13.40 % weight) [Özgül-Yücel and Türkay, 2002].

3.4. Validation of the model

As shown in Fig. 6, numerically optimized conditions predicted by the model for highest desirability were, temperature = 40.52 °C, duration. = 22.93hrs and catalyst concentration = 2.04. Table 5 shows process parameters before and after optimization. Under these conditions, 41.47

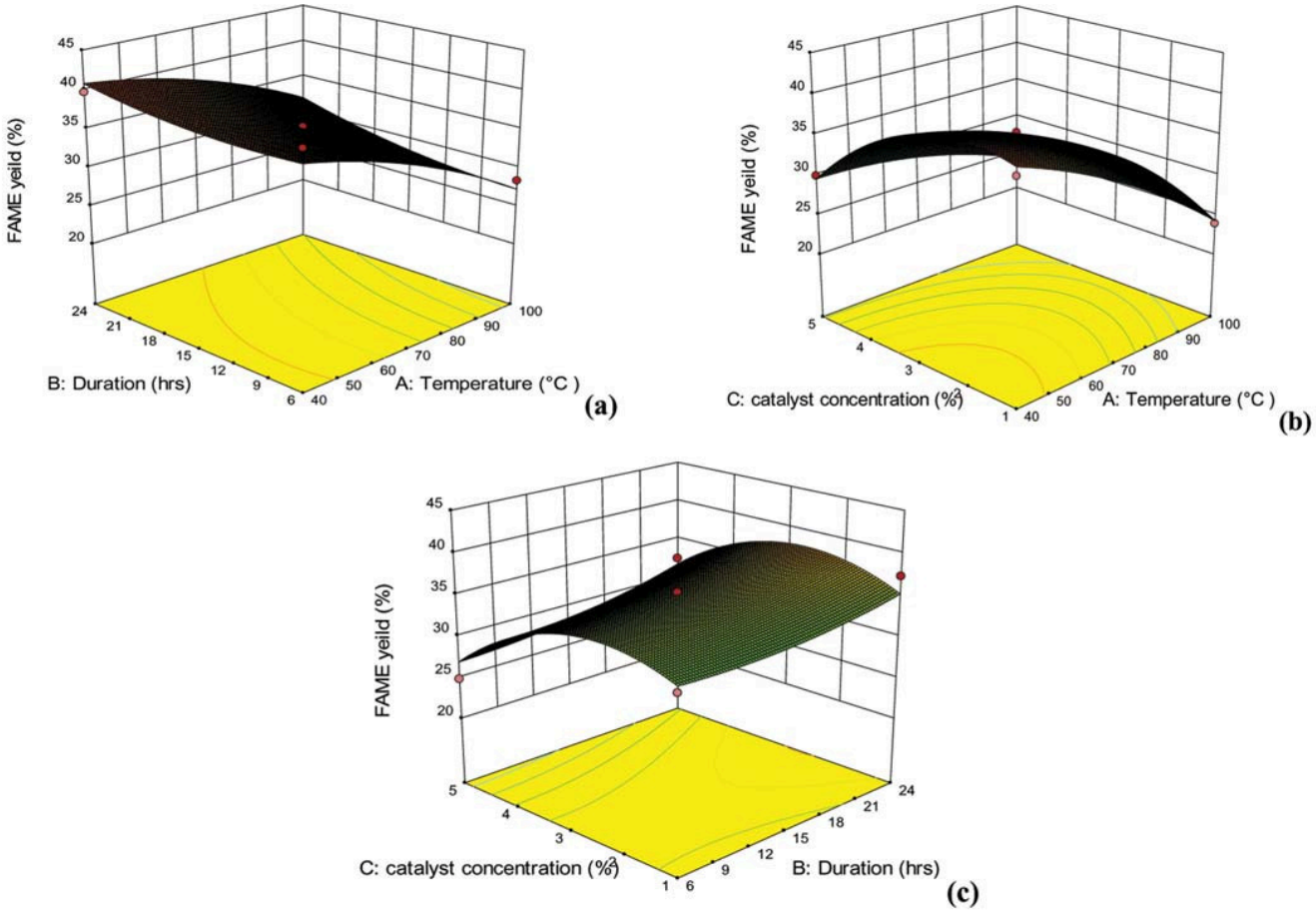

Fig. 5 3-D surface plot showing the interaction of (A) Duration (hrs) and Temperature (°C) (B) Catalyst conc. (%) and Temperature (°C) (C) Catalyst conc. (%) and) Duration (hrs)

% of biodiesel yield was predicted. Validatory experiment was performed in triplicates, which confirmed the accuracy of the predicted model with biodiesel yield equal to 41.31 ± 0.04 %. It was observed that the experimental value obtained was in good agreement with the value predicted from the model, with relatively small errors between the predicted and the actual value, which were only 0.16± 0.02 % for biodiesel yield. Without optimization of the process parameters, the biodiesel yield ranged from 24.23 % - 40.83 %. Thus, there was a significant increase in biodiesel production under optimized conditions and clearly indicates the significance of optimization of process pararameters via response surface methodology.

As transesterification efficiency increases after optimization, a reduction in the cost per litre occurs. The authors feel that production of biodiesel from *in situ* transesterification by carbon dioxide sequestering bacterium *Serratia* sp. ISTD04 under the conditions optimized in this study can surely enable commercialization of the biodiesel.

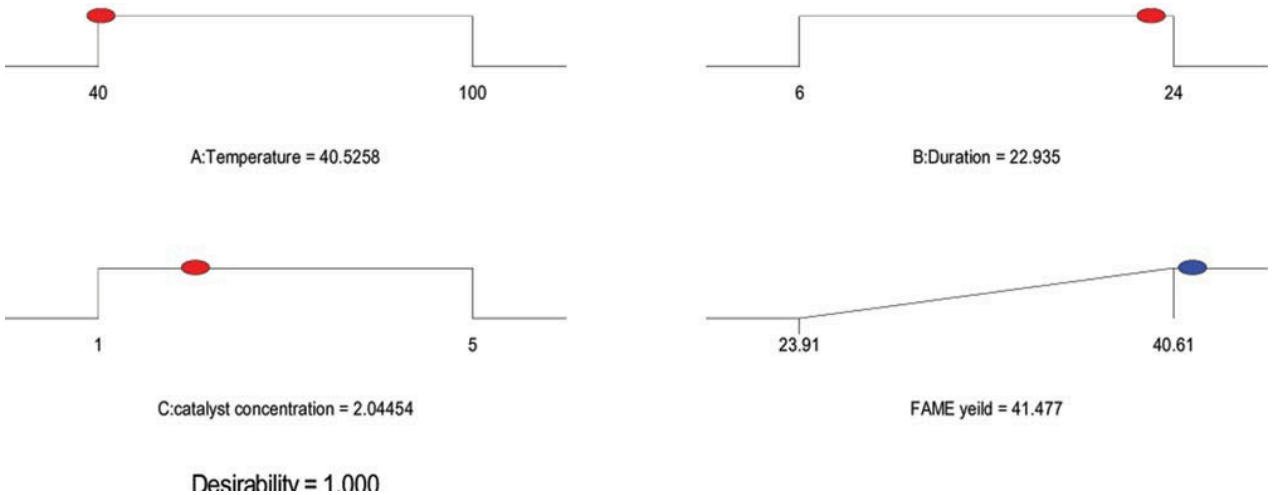

Fig. 6 Optimized condition and FAMEs yield predicted by model

Table 5 Before and after optimization of process parameters and yield of FAMEs

Variable	Optimization condition		Biodiesel yield (mean %)		
	Before	After	Optimization		
			Before (mean)	After	
				Predicted	Experimental (mean)
Temperature (°C)	70	40.52	35.27	41.47	41.31
Duration (hrs)	15	22.93			
Catalyst concentration (%)	3	2.04			

4. Conclusions

The biodiesel produced from carbon dioxide sequestering *Serratia sp.* ISTD04, under optimized conditions showed a very high purity (95.56%). Duration of the process of *in-situ* transesterification had the maximum influence on the biodiesel yield, followed by that of temperature and catalyst concentration. The biodiesel yield was enhanced by 1.15 folds post optimization. Enhanced yield along with the use of acid as catalyst reduced the production steps as well as the production cost of biodiesel.

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