Steady State Performance of a Bioreactor for Production of near Zero Sulfur Diesel (NZSD) and Bio-surfactant

Sujaya Bandyopadhyay, Ranjana Chowdhury, and Chiranjib Bhattacharjee

Abstract—**Kinetics of biodesulfurization of hydrotreated diesel using *Rhodococcus sp* has been studied with special reference to removal of organo-sulfur compounds in diesel and production of 2-hydroxy biphenyl. The identification of 2-HBP in treated diesel has been made using HPLC,FTIR and GC-MS .The sulfur concentration of feed diesel was in the range of 200-540 mg/L. Aqueous phase to diesel ratios were varied in the range of 9:1 to1:9. The optimum ratio was found to be 1:4 and the maximum conversion of sulfur was determined to be 95%. The values of Monod kinetic parameters namely, μ_{max}, maximum specific growth rate and K_s, saturation constant of the microbial growth and Yield coefficient of surfactant have been measured to be 0.096h^{-1} , 71mg/L , and 17 µmol/g dry cell weights respectively by conducting batch type experiments. A continuous chemostat was studied using different hydrodynamic and physico-chemical parameters like dilution rate, initial concentration of organo-sulfur compounds in diesel, stirring rate and aeration rate. Surfactant part was characterized by determination of surface tension (Ring method), E24, TLC, HPLC and GC-MS. The interfacial tension of the supernatant fermented by *Rhodococcus sp* decreased from 28 dynes/cm to 9 dynes/cm.The surface tension of aqueous nutrient phase and diesel was observed to decrease from 71 dynes/cm to 30 dynes/cm and to 20 dynes/cm from 30dynes/cm respectively. Values of emulsification index (E24) were determined to vary from 18 to 58 over the growth period of 2 to 48 hours in the chemostat. The critical miceller concentration was found to be 200mg/L. The simulated data have been compared with the experimental ones to validate the model.**

Index Terms—**Biodesulfuirzation of diesel, biosurfactant, emulsification index, mathematical model, Monod kinetics steady state, surface Tension.**

I. Introduction

All fossil fuels like coal and petroleum contain either elemental sulfur or sulfur in complex form. Ultra-deep desulfurization of fuels, particularly of diesel, has become a very important subject in petroleum refining industry due to the heightened concerns for cleaner air and more stringent environmental quality requirements. Sulfur is a detrimental source of air pollution. So desulphurization is a necessity for using coal or petroleum from the environmental point of view. In the present study our major concern is for diesel .There are some sulphur compounds like methylbenzothiophenes(MBTs) and dibenzothiophenes (DBTs) remaining unconverted in diesel oil after conventional hydro desulfurization. The sulfur level usually lies in the range of 100 ppm. These are even after applying stringent operating conditions i.e., high operating temperature and pressure. On combustion, these substituted organo-sulphur compounds cause acid rain and thus are extremely hazardous for the environment. As a consequence, US, Japan, European Union and other concerned countries have enforced stringent regulations on sulfur level which should be "near zero". It is very difficult and expensive for the hydro treatment process to reach such level [1]-[6]. So it is better to use bio desulfurization as a choice with milder and cheaper operating conditions. One added advantage of this process is production of biosurfactants as a byproduct. Biosurfactants like hydroxybiphenyl --- a hydrotrope, etc. are produced as byproducts [6]-[12]. The biosurfactants [13], [14] may be successfully utilized in the detergent,food processing and pharmaceutical industries as well as in advanced oil recovery from wells. These microbially produced surfactants have similar properties of synthetic surfactants but are less toxic, biodegradable and can be produced in situ at any contaminated petroleum sites. However, the microorganisms used for the biodegradation should be chosen judiciously [6]. Some of the anaerobic microorganisms desulfurize orgsanosulfur compounds through the breakage of C-C linkages resulting in lowering of calorific value of the ultimate product diesel. On the other hand, another category of microorganisms act through oxidative route without hampering the C-C linkage responsible for the heating value of diesel. *Pseudomonas acidovorans, Rhodococcus erythropolis, Rhodococcus rhodochrous, Nocardia sp. and Gordona sp* are some of the species which belong to the oxidative category [6]- [7]. Under the present research scheme, attempts would be made to desulfurize diesel using biodesulfurization with an aim to maximize the production of biosurfactants.

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The authors are with Chemical Engineering Department Jadavpur University, Kolkata, India (e-mail: chatterjeesujaya@yahoo.co.in; ranjana.juchem@gmail.com; c.bhatta@gmail.com).

II. Experimental

A. Materials

Beef extract (E. Merck), peptone (E. Merck), NaCl (Ranbaxy), methanol (E. Merck), acetone (E. Merck), dibenzothiophene (Aldrich Chemical), N_2 (Prakash traders), Di-ehyl ether (E. Merck), NaOH (E. Merck), Choloform(E. Merck), n-pentane (E. Merck), HCl (E. Merck), ethyl alcohol (Process chemical industries) and Bauxite(20-60 mesh) (E. Merck), Sillica Gel(100-200 mesh) (E. Merck),n-Hexadecane(Hi-Media) 2- hydroxybiphenyl (Fluka), acetonitrile(E. Merck) have been used during the present investigation.

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B. Diesel Used

Hydrodesulfurized diesel sample were purchased from Indian oil Corporation (IOC), Kolkata, India having the characteristics given inTable I.

TABLE I: CHARACTERISTICS OF HYDROTREATED DIESEL

Properties	Values
I.B.P. (º C)	140
F.B.P (º C)	370
Specific Gravity (Basis: Density of water = 1000 kg/m^3)	821.6
Sulfur (ppm)	50

C. Microorganism

The pure bacterial strain of Rhodococcus sp. (NCIM 2891) was purchased from National Collection of Industrial Microorganisms (NCIM), India. Cells were cultivated and enriched in sulfur free medium supplemented with diesel oil in 50 ml Erlenmeyer flasks.

D. Composition of the Growth Medium for Microorganisms

Basis: 1 dm^3, beef extract: 10 g, NaCl (AR): 5 g, peptone (for bacteriology): 10 g.

III. ANALYTICAL METHODS

A. Dry Weight Method for the Determination of Bacterial Mass

The biomass concentration in the reaction broth was determined by dry weight method. In this method, the broth was centrifuged at the rate of 10,000 rpm for 15 min at – 15 º C. The bacterial mass was then transferred to a pre-weighted aluminium cup and dried at 50 º C overnight. The exact weight of the bacterial mass was determined by subtracting the weight of dry cup from that of the cup containing dry bacterial mass. The cell concentrations were also determined using spectrophotometer (SPECTRASCAN UV 3600-Chemito)at 600 nm.

B. Sulfur Analysis

UOP 357-80 (trace sulfur in petroleum distillates by nickel reduction), X-ray fluorescence XRF and CHNSO method have been followed to determine the concentration of sulfur in the diesel samples.

C. Separation of Aromatics and Non aromatics by Elusion Chromatography

ASTM D2549 has been used for the separation of aromatics and non-aromatics faction in diesel sample.

2 grams of sample diesel is charged to the top of a glass chromatographic column packed with activated bauxite and Silica gel. n-Pentane is added to the column to elute the nonaromatics. When all the non aromatics are eluted , the aromatic fraction is eluted by addition of diethyl ether ,choloroform and ethyl alcohol.

D. Analysis of 2-Hydroxybiphenyl(HBP)and Other Extracellular Lipids

The concentration of 2-HBP in the feed and product diesel has determined using HPLC (Perkin Elmer Series 200) equipped with a reverse phase column at 215 nm using acetonitrile (50% v/v) as mobile phase. It was rechecked using GC-MS (Agilent Technologies model 6890 model) equipped with inert mass selective detector. Presence of hydroxy biphenyl group in treated diesel was validated using FTIR (VERTEX-70). Other extracellular lipids were extracted by chloroform ethanol solvent system and analysed by TLC.

E. Batch Experiments for the Determination of Kinetic Parameters

Batch type experiments were conducted in Erelenmeyer flasks. Ratio of aqueous medium (composition given in material section) to diesel oil was maintained in the range of 90:10 to 0:100 .The optimum ratio for the experiment was 80:20.The overall sulfur concentration was varied from 200 to 540 ppm. The kinetic parameters μ_{max}, K_s, $Y_{x/s}$ and $Y_{p/x}$ were determined.

IV. OPERATION IN A CHEMOSTAT

The experiment was carried out in a 2dm^3 B.Braun chemostat as continuous stirred tank reactor (CSTR) with working volume of 1.5 dm^3 .Hydrotreated diesels was used as sulfur source. The sterile sulfur free aqueous medium was used as nutrient broth of microorganisms. Hydrotreated diesel of desired concentration was exposed to UV radiation for 30 minutes to sterilize. After sterilization, chemostat was cooled to room temperature and adequate volume of diesel and the sterile medium was added in presence of alcohol flame into the chemostat. The chemostat was then inoculated by adding 10% v/v inoculaum i.e., the enriched bacterial strain under aseptic conditions inside a laminar flow chamber. After inoculation the chemostat was equipped with an air compressor and a stirrer .The stirring rate and aeration rate were maintained at 100 rpm and 25 L per hour respectively. The diesel to aqueous phase ratio was maintained at 80:20 during all runs in the chemostat. The dilutuion rate was varied in the range of 0.03 to 0.1 and the sulfur concentration in feed diesel was varied from 200 to 540 ppm. Under each operating condition the reactor was operated for 4 days.

V. CHARACTERIZATION OF BIOSURFACTANTS.

A. Measurement of Surface Tension and Interfacial Tension

The change in surface tension of culture was evaluated by using a Leconde Du Nouy Tensiometer (Ring method).

B. Measurement of Emulsification Index (E24)

Emulsification index, E24 of culture samples was determined by adding 2 ml of hydrocarbon to the same volume of culture broth and by subsequently mixing in a vortex for 2 minutes and leaving to stand for 24 hours. The E24 index was calculated as percentage of height of emulsified layer (mm) on the basis of total height of the liquid column (mm).

C. Measurement of Critical Micelle Concentration (CMC)

Critical micelle concentration was determined by plotting the discontinuity of surface tension as a function of concentration of the surfactant

VI. Theoretical Analysis

A. Determination of Kinetic Parameters Based on Growth Model

In the present investigation in order to understand the reaction engineering behaviour of the biodegradation process classical Monod type of substrate uninhibited kinetic model (as shown by equation 1) has been attempted during simulation work. The experimental data generated from the studies in a chemostat have been used to determine the kinetic parameters based on Monod model.

$$\mu = \frac{\mu_{\max} C_S}{K_S + C_S} \tag{1}$$

Now the rate equation in terms of microbial growth kinetics may be written as;

$$r_x = \mu C_X \tag{2}$$

$$r_s = -\frac{r_x}{Y_{x/s}} \tag{3}$$

Therefore,

$$\frac{dC_S}{dt} = -\frac{\mu C_X}{Y_{X/S}} \tag{4}$$

The mathematical model of the system has been developed on the basis of the following assumptions.

1) Influent stream of the bioreactor is sterile.
2) There is no external mass transfer resistance present in the system
3) Organo-sulphur compounds of diesel are the only growth limiting substrates.
4) Microbial growth follows the Monod Kinetics.

The system equations for batch mode are as follows,

Substrate

$$\frac{dS}{dt} = D(S_{in} - S) - \frac{1}{Y_{X/S}} \frac{\mu_m SX}{K_S + S} \tag{5}$$

Biomass

$$\frac{dX}{dt} = -DX + \frac{\mu_m SX}{K_S + S} \tag{6}$$

Product

$$\frac{dP}{dt} = -DP + Y_{P/X} \frac{\mu_m SX}{K_S + S} \tag{7}$$

under steady state the mass balance equations for substrate, biomass and product become**,**

$$S_s = \frac{K_S D}{\mu_m - D} \tag{8}$$

$$X_S = Y_{\frac{X}{S}} (S_{in} - \frac{K_S D}{\mu_m - D}) \tag{9}$$

$$P_S = \left[\frac{D}{Y_{P/X}} \cdot \left(\frac{K_S}{\mu_m} \frac{1}{S_s} + \frac{1}{\mu_m} \right) \right]^{-1} X_S \tag{10}$$

VII. Results and Discussions

The values of kinetic parameters, namely, μ_{max}, K_s, $Y_{x/s}$, $Y_{p/x}$ were determined by using batch type experimental data obtained with different initial sulfur concentrations (200 to 540 ppm) the values are $0.096h^{-1}$, 71mg/L, 0.2 and 17 µmol/g dry cell weights respectively. The optimum ratio of diesel to aqueous medium was found to be 80:20.

A chemostat was operated to control/optimise the microbial growth rate, surfactant production rate and the substrate utilization rate. These were achieved by adjusting the volumetric feed rate or dilution rate. After a certain operating period of 30 hours (>3τ) the concentrations of biomass, substrate and product become invariant with reaction time. This may be considered to be the onset of steady state. In Fig. 1- Fig. 3 concentrations of product, biomass and substrate have been plotted respectively against dilution rate. In Fig. 1 and Fig. 2 substrate concentration has been used as a parameter. The concentrations of product and biomass show a decreasing trend with dilution rate. This may be justified by the fact that as the dilution rate increases the residence time in the reactor decreases and as a consequence the rates of outlet of both product and biomass outweigh the generation rates of the respective components resulting in an ultimate decreasing trend of concentration with dilution rate. For both biomass and product the concentrations achieved at any dilution rate show increasing trends with an increase of inlet substrate concentration. This signifies that the generation rates increase with the increase of substrate concentration under the present range of inlet substrate concentration being studied. This is also clear from the growth kinetic equation (Equation 1) being used under the present study. The concentration of substrate shows an increasing trend with increase of dilution rate. This is also at per with the observation obtained from Fig. 1 and Fig. 2, wherefrom it is clear that at higher dilution rates, the rate of consumption is less than the rate of input of substrate.

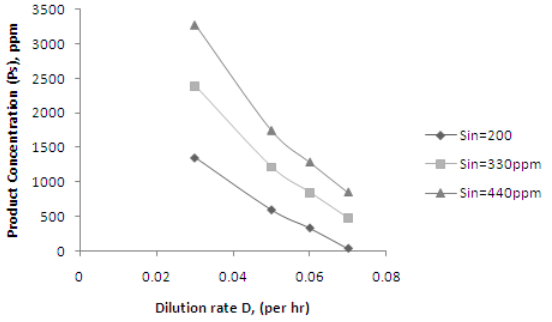

Fig. 1. variation of steady state product concentration with respect to dilution rate at different inlet sulfur concentration

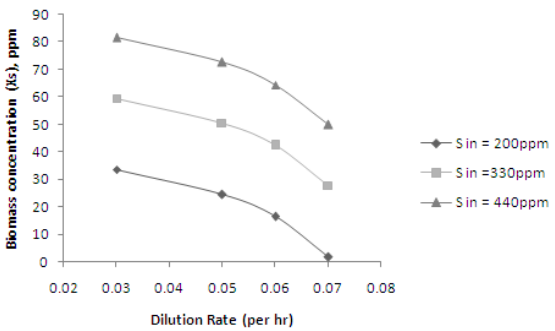

Fig. 2. Variation of steady state biomass concentration with respect to dilution rate at different inlet sulfur concentration

In Fig. 4 the experimental values of surface tension of aqueous phase and oil phases of the reaction broth, interfacial tension as well as the emulsification index of the broth obtained with initial substrate concentration of 330 ppm and diesel to aqueous ratio of 80:20 have been plotted with the operating time of the chemostat. It has been observed that the surface tension decreases and the value of E24 increases with the increase in reaction time. The surface tension of aqueous nutrient phase and diesel was observed to decrease from 71 dynes/cm to 30 dynes/cm and to 20 dynes/cm from 30dynes/cm respectively. Values of emulsification index(E24) were determined to vary from 18 to 58 over the growth period of 2 to 48 hours in the chemostat. The critical miceller concentration was found to be 200mg/L.This is an indication of the formation of more biosurfactant, namely, 2- HBP and some triglycerides, polar and nonpolar lipids with the propagation of reaction. The increase of values of E24 with reaction time also establishes the effectiveness of the biosurfactants produced as by-products of biodesulfurization of diesel.

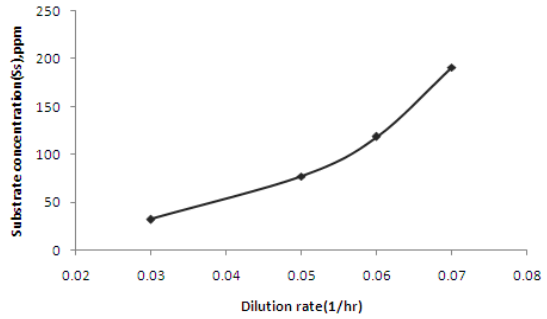

Fig. 3. Variation of steady state substrate concentration with respect to dilution rate.

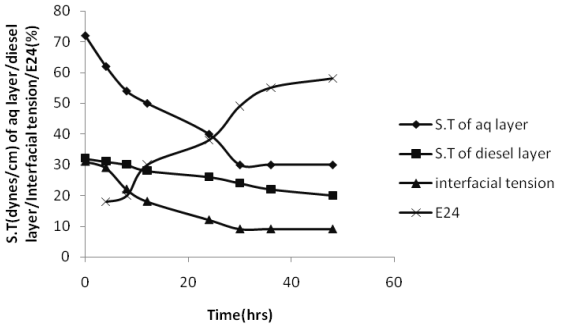

Fig. 4. variation of surface tension (S.T) of aqueous, diesel layer, interfacial tension, E24 with respect to operating time

VIII. Conclusions

Rhococcus sp was successfully utilized in desulphurizing HDS diesel to near zero level with simultaneous production of biosurfactant, 2-HBPand other extracellular lipids. Sulfur concentration of the hydrotreated diesel can be lowered upto 20 ppm with a maximum conversion of 95%. The concentration of both 2-HBP and biomass show a decreasing trend with an increase of dilution rate in the range of 0.03-0.07 hr $^{-1}$. The surface tension of aqueous nutrient phase and diesel was observed to decrease from 71 dynes/cm to 30 dynes/cm and to 20 dynes/cm from 30dynes/cm respectively. Thus the biosurfactant produced through biodesulfurization of diesel can be effectively utilized in detergents. The steady state model developed under present study may be further used in future studies dealing with similar systems.

Acknowledgment

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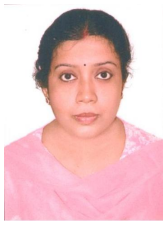

Sujaya Bandyopadhyay was born in Kolkata, West Bengal, India on 11th June, 1975. Sujaya Bandyopadhyay completed B.Sc (Chemistry Honours), Bachelor of Technology in chemical Technology (Specialization in Petrochemical and Petroleum Refinary Engineering) from Calcutta University, West Bengal, India in1996 and 1999 respectively. Ms. Bandyopadhyay completed Master of Business Administration (Specialization in Operation, Quality and transport Management) from Annamalai University, Tamil Nadu, India in 2005. Presently Ms. Bandyopadhyay is pursuing doctorate in philosophy in chemical engineering in Jadavpur University, West Bengal, India and is working as a senior research fellow.

She is working in a project entitled as "*Production of Biosurfactant through biodesulfurization of hydrotreated diesel and spent engine oil using Rhodococcus sp*". She received the senior research fellowship, sponsored by Council of Scientific and Industrial Research (CSIR). She had published her research article in one on-line journal; Journal of American Institute of Physics in 2010. She had communicated another research article in one

prestigious Elsevier journal. She already published many parts of her research in nine international and national conferences. Her thrust research area is sustainable energy development, environmental science and technology, mathematical modeling of biochemical engineering, biotechnology, waste treatment.

Ms. Bandyopadhyay had work experiences in different industries for eight years.

Ranjana Chowdhury was born in Kolkata, West Bengal, India on 17th June, 1963. Ranjana Chowdhury completed bachelor of engineering in chemical engineering from Jadavpur University, West Bengal, India in 1985. Ranjana Chowdhury completed doctorate in philosophy in chemical engineering from Jadavpur University, West Bengal, India in 1993.

She worked as a Lecturer, Reader and Professor for last 19 years in Jadavpur University, West Bengal, India and was head of the department from 2008-2010. Presently she is working as a Professor in same university. She is involved in several industrial consultancy works. She has delivered many invited lectures at different organizations, international and national conferences and symposium. Apart from these, she was involved in several project activities as principal investigator/ coordinator, funded by AICTE, DST and UGC. She has supervised more than 25 Master Degree projects. Already, 11 Doctoral Degrees have been awarded and works have been in progress for 10 other candidates. She is having one Indian patents to his credit. She has published more than 50 research articles in international journals of repute (published by Elsevier, Springer, AIChE, etc.). She has around 65 national research publications and numerous research papers in national/international conference to her credit. She received university gold medal for standing first in M. Ch.E examination. She obtained DAAD (German Academic Exchange Service) scholarship and carried out research work for about two years in reputed laboratories of Karlsruhe University, Germany. She has also carried out research work in Technical University of Naples FED-II, Italy for a month. Presently her research interest is on novel separation techniques, bioremediation process (biodesulfurization and bioleaching), pyrolysis, enzyme technology, production of alternative fuel and functional foods from waste materials, bioprocess technology and mathematical modeling of bioreactor and pyrolyzer.

Chiranjib Bhattacharjee was born in Kolkata, West Bengal, India on 30th September, 1966. Chiranjib Bhattacharjee completed bachelor of engineering in chemical engineering from Jadavpur University, West Bengal, India in 1989. Chiranjib Bhattacharjee completed master of technology in chemical engineering from Chemical Engineering Department from Indian Institute of Technology (IIT), Kanpur, India in 1991. Chiranjib Bhattacharjee completed doctorate in philosophy in chemical engineering from Jadavpur University, West Bengal, India in 1998.

He was worked as a professor for last 21 years and was head of the department from 2010-2012. Presently he is working as a professor in same university. He is involved in several industrial consultancy works. He has delivered many invited lectures at different organizations, international and national conferences and symposium. He was involved in collaborative research with University of Montpellier, France and University of Melbourne, Australia. Apart from these, he was involved in several project activities as principal investigator/ coordinator, funded by AICTE, DST, DBT, and UGC. He has supervised more than 20 Master Degree projects. Already, 7 Doctoral Degrees have been awarded and works have been in progress for 12 other candidates. He is having two Indian patents to his credit. He has published more than 65 research articles in international journals of repute (published by Elsevier, American Chemical Society, AIChE, etc.). He has around 30 national research publications and numerous research papers in national/international conference to his credit. Presently his research interest is on Environmental Remediation, novel separation techniques, bioremediation process, advance mass transfer, advace numerical analysis and production of functional foods from waste materials. He is also working on implementation of Zero Effluent Concept in Dairy and Pulp and paper industries.

Prof. Dr. C. Bhattacharjee has several awards to his credit, like Best Project National Award (first prize) from AICTE, Best Paper Award Institution of Engineers (India) (IIE) and Indian Institution of Chemical Engineers (IIChE).