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In This Issue

On Our Cover

Inauguration of 15th Education course on "Automotive Greases"
September 21-22 , at Indian Oil R& Centre, Faridabad

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Title

A Study of Speciality Grease lubrication in High Speed Power tools

Prepared by:- Francis ,Mr. Viren ,Mr. S.Mahanti

Abstract

Lubrication of high speed power tools requires development of a high performance long life lubricant to increase the reliability of high productivity tools. The lubricant applied on the power tools is required to protect the surface and reduce friction during the severe hammering of power tools at high speeds and at high surface temperatures generated during the process. Conventional greases or semi-fluid lubricants fail to meet these severe requirements.

Various candidate lubricants derived from greasy, semi-fluid lubricants with solid lubricants were considered. Effect of candidate lubricants on the friction and vibration of power tools was studied, in addition to run off characteristics at the point of impact and adhesion to the surface, in order to arrive at effective re-lubrication intervals with specific types of products.

A semi fluid synthetic grease with complex soap thickener provided the most effective lubricant and significantly enhanced the life and productivity of the power tools. Based on laboratory evaluation, field studies were carried out with a leading power tool manufacturer at multiple locations to ascertain frictional as well as vibration related performance. Number of impacts of the power tool was taken as measure of the effective life of the developed grease. Field studies confirmed nearly two fold increase in the performance life and significant reduction in friction and surface temperature of the power tools during operation. Developed product showed favourable performance compared to alternative imported product. Work in in hand to extend the application of the developed product to chop saws, single grinders and a number of other diverse power tools.

Introduction

The problem of lubricating high speed power tools is to satisfy the following conditions

1. To reduce heat generation, wear and tear between sliding surfaces and rotary components.
2. To reduce vibration levels as they are directly handled by operators
3. To improve reliability and productivity of the high cost machines

The lubrication requirements of the power tools were fully studied in detail and friction coefficient tests were conducted with various greases to recommend the greases for sliding & rotary lubrication in power tools. It was important to exceed the performance of the grease currently being used as it would meet the stringent requirements.

The challenge was to conduct the field trials at 5 different locations under different conditions.The current grease used met their requirements but it was necessary that an import substitute was required to enhance brand equity with cost effective solutions.

The key components of our approach is to

1. Evaluate the current lubricant recommendations and conduct compatibility with machine components along with friction coefficient tests.

2. To select a suitable lubricants and conduct similar tests to match or exceed the performance of the target grease.

3. To conduct field trials at various locations and evaluate the results

4. To freeze on the lubricant recommendations.

Scope of experiments

- The continuous duty machines were identified and were earmarked for study at 5 different locations. namely Chennai, Hyderabad, Jaipur, Delhi & Bangalore.

- During service the existing lubricants were completely cleaned and selected lubricants were applied under close supervision of their service engineers.

- The power tools were lubricated and assembled and trial run was conducted to asses the performance.

- The power tools were sent to the field and their performance was monitored.

- The trials were run for 200 working hours to assess the performance of the new lubricants.

- After 200 hours of operation the power tools were opened to check for wear and tear.

- Conclusion of trials.

Performance experiments

In order to understand the lubrication requirements of the high speed power tools the section view of the gear drive is shown below Figure 1

Next the sliding portion of the power tool before application of the grease

Before Application of Gear Grease

After application the candidate grease

After Application of Silding Grease

Assembly of Sliding Rams

Power Tool Components Grease Compatibility

Assembled Power tool View

Trials Conclusions

1. **The vibration levels of the power tools had been considerably reduced .**
2. **The service interval period have been doubled**
3. **The performance report in all 5 locations has been in the affirmative and better than the existing grease used.**
4. **Commercialization in progress.**

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Biodegradability and toxicity studies of synthesized polyol ester lube base stocks

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ABSTRACT

Synthetic esters are used as environmentally acceptable base fluids in high performance lubricants.Compromise between viscosity, volatility and cost influences the choice of base stocks for their effective ecofriendly formulation.Manufacture and use of ester from natural fatty oils through chemical transformations have emerged in the recent past as an interesting area.

Biodegradability characteristics of the synthesized lube base stocks were determined using ASTM-D-5864-95 method.Bacterial toxicity of the samples was determined by the modified method of Algal inhibition test. It was taken from official journal of the European Communities No.L383A/179-185(1993).The samples were found to be completely biodegradable and also non toxic to the sewage bacteria NS/8.This paper will provide the biodegradability and toxicity studies of synthesized ester lube base stocks.

Key words: Synthetics, polyol esters, characterization, biodegradability, toxicity,

environment friendly.

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Introduction

New demand placed on lubricants is changing rapidly.From the view point of better performance these should be friendly to the environment and be eventually biodegradable. There is a potential for developing novel biodegradable and ecologically harmless base stocks for new generation of lubricants.Before ecological aspects became part of lubricant development, ester oils were used in special lubricants for technical reasons.The inherent biodegradability of these ester molecules offers added benefits to those of performance (1).

To initiate and boost the use of biodegradable product, government incentives and mandatory regulations are needed to put pressure on the industries that release lubricants into the environment. Several countries are awarding environmental seals, which are nothing but the environmental acceptability eco-labelling schemes. The first seal was awarded by Germany by the name as Blue Angel. Similarly "White Swan", "Green Cross" and "Ecomark" are the environmental seals of Scandinavia, USA, Japan and India respectively.These ecolabelling schemes include ecological test requirements, prohibitions and manufacturers declarations which often differ and are being continuously updated (2).

Currently available biodegradable base stocks are: Highly unsaturated or light oleic vegetable oils (HOVO), Low viscosity polyalpha olefins (PAO), Poly alkylene glycols (PAG), Di basic acid ester (Diesters) and Polyol esters (PE).

Biodegradation: Degree and Assessment

A lubricant is considered to be biodegradable only when it is having proven capability to decompose into the most common environment with in a specific time period through natural biological processes.Biodegradability extent may either be primary, i.e., loss of a particular functionality or ultimate conversion to simple molecules such as CO_2, H_2O and NH_3 leaving inorganic salts and new microbial mass. The lubricants / base fluids may be readily biodegradable, i.e., exceeding a certain level of degradation or inherently biodegradable having unequivocal evidence of degradation in any biodegradability test (3).There are a number of tests available for the assessment of biodegradability (Table 1).

Table 1: Tests for Assessment of Biodegradability

Sl.No.	Tests	Pass Level %	Duration (Days)	Biodegradability Parameters
1.	**OECD Test for Ultimate Biodegradability**			
	301 A : AFNOR Test	70	28	DOC Removal
	301 B : Modified Sturn Test	60	28	CO_2 Production

	301 C : MITI Test	-	28	O_2 Uptake
	301 D : Closed Bottle Test	-	28	O_2 Uptake
	301 E :	70	28	DOC Removal
	301 F : Manometric Respirometery EPA Gled Hill Aquatic Shake Flask Test	-	28	CO Production
2.	**OECD Tests for Inherent Biodegradability**	**For classification as inherently**		
	302 A : SCAS; Semicontinuous activated sludge test	20	-	DOC Removal
	302 B; ZAHN Wallens - EMPA test	20	28	DOC Removal
	302 C; Modified UK/MITI 11 test	20	-	BOD/COD
3.	**CEC Tests for Relative Biodegradability**			
	CEC-L-33-T-82	70-80	21	IR absorption CH_3CH_2 loss
	CEC-L-33-A-93	70-80	21	IR absorption CH_3CH_2 loss
	CEC-L-33-F-94	70-80	21	IR absorption CH_3CH_2 loss

Methods of Biodegradation

Although there are as yet no universally accepted standards to determine biodegradability, several test methods exist to determine the extent to which materials are biodegradable. To define ecofriendly lubricant more accurately, their toxicity and bioaccumulation are also required to be determined along with their biodegradability. In addition, nature of emissions source is also considered. As a general rule, biodegradable compounds have linear non aromatic, short chain molecules with no branching.

The three most common methods include the CEC-L-33-T-82, EPA 560/6-82-003 shake flask test and the OECD 301 series of methods. The CEC Method was designed for oily substances, while the OECD methods were designed for water miscible substances.

The CEC method is the only method designed to assess the biodegradation of lubricants. The test method involves adding the sample oil to an open container of water containing natural salts and an un-acclimated sewage innoculum. The rate of biodegradation is assessed by comparing an infrared spectrum of the mixture at 21 days and comparing the hydrocarbon signature with a scan taken before the test. The oil is considered to be readily biodegradable if at least 70% of the hydrocarbons are removed after 21 days. The biodegradability as per CEC-L-33-A-93 test of typical mineral lubricants, synthetic base fluids are relatively poor biodegradable, while tri-glyceride vegetable oils, Polyol esters and other are easily biodegraded.

The EPA method involves a flask containing the sample, water and bath sewage and soil innoculum. The flask is shaken for 28 days and the amount of carbon dioxide produced is the basis for the measure of biodegradability of the sample. In order to pass the test, a minimum of 60% of the sample must be converted to CO_2 in the test period.

The OECD methods are designed for fluids which are miscible in water. They are not relevant for the mostly immiscible industrial oils. The assessment is done through the amount of CO_2 produced, the dissolved hydrocarbon content, or the biological oxygen demand during the process. Greater than 60% biodegradability in 28 days is considered acceptable.Laboratory tests only allow a comparison of relative optimal biodegradability.True biodegradability can only be determined in realistic spill conditions (4).

Bacterial Toxicity

Fluids which are considered environmentally friendly must not only be biodegradable, but also relatively non toxic, in both their initial form and their degradation products. Their effects on flora and fauna must be minimal. There are two common tests to evaluate toxicity on fauna - the microtox^{TM} (Microbics Corporation, Curlsbad, CA) test analyze the effects of the sample on photosensitive bacteria and provides information on the microorganisms found in soil and water which form the foundation of plant and aquaitic life. This test is only semiquantitative and very dependent upon the bacteria species used.

The 96 hours rainbow trout bioassay (EPS-1-RM-13, AGAT Laboratories) is the most common toxicity test.The results are quoted as the LC_{50} or lethal concentration, the concentration of sample which causes mortality in 50% of a trout population after 96 hrs. Specifically raised rainbow trout approximately one month old, are used because they are more suceptible to toxic substances. The higher the LC_{50}, the lower the toxicity of the product. An LC_{50} of greater than 1000 ppm (1%) is considered acceptable for an environmental friendly designation (5).

Other tests evaluate the subliming response of daphnia, a species of water flea used as food by fish (6). Several methods are popular for lubricant testing, the most common aquatic tests using various species of algae. Other tests evaluate the germination and length of seedlings. The measurement of seedling length permits identification of

certain compounds as being toxic which would be over looked if considering germination percentage alone (7).

Experimental

All the polyols and acids used were commercial products from Atlas and Godrej. A number of polyol ester lube base stocks have been synthesized by using various aliphatic carboxylic acids and alcohols ranging from C_6-C_{10} & C_5-C_7 respectively.The physico chemical properties of the synthesized esters were determined according to the American society for Testing and Materials (ASTM) standard procedures.Finally the biodegradability of lube base stocks has been assessed by ASTM-D-5864 method.The bacterial toxicity was determined by the modified method of Algal inhibition test, official Journal of the European Communities No.L 383 A/179-185 (1993).

Results and Discussion

The ASTM-D-5864 method is used to determine ultimate biodegradability. In this, biodegradability is measured by collecting the CO_2 produced when the lube oil is exposed to micro organisms under controlled aerobic aquatic conditions. The value is then compared with the theoretical amount of CO_2. The results are given in the following tables 2&3.

Table 2: Biodegradation Profile of Synthesized Ester Lube Base Stocks

Sample code	Total No. of Days	% of Theoretical CO_2 evolved			No. of Days for Plateau	Inference
		I	II	Average		
PN-1	18	88.1	59.4	73.7	18	Readily
PN-2	18	59.2	39.3	49.2	18	Partially
PN-3	18	55.7	49.9	52.8	18	Fairly
PN-4	23	82.5	82.5	82.5	14	Readily
PN-5	10	60.2	60.2	60.2	14	Readily
PN-6	16	54.8	54.8	54.8	12	Readily
PN-7	16	56.7	95.6	76.1	12	Readily
PN-1	17	91.6	91.6	91.6	11	Readily
PN-9	18	72.6	76.5	74.0	10	Readily
PN-10	10	58.8	58.8	58.8	9	Readily
PN-11	18	72.45	73.55	73.0	8	Readily

Table 3: Time Requirement for Complete Biodegradability of Ester Lube Base Stocks

Sample	Time (days)													
	0	2	4	6	8	10	12	14	16	18	20	22	24	26
PN-1														
PN-2														
PN-3														
PN-4														
PN-5														
PN-6														
PN-7														
PN-1														
PN-9														
PN-10														
PN-11														

From the Table 2&3 it is evident that all the samples were degraded before recommended (28 days) period of incubation. For PN-11 the degradation rate is very high, while PN-4, PN-5, PN-6, PN-7, PN-8, PN-9 and PN-10 took moderate time. PN-1, PN-2, and PN-3 required maximum time, i.e., 18 days for complete biodegradation.Out of 11 samples one sample is partially, two samples fairly and eight samples were readily biodegradable.

Toxicity Measurements

Micro organisms are important members of many eco systems. Micro organisms are easy to culture and provide rapid results. Toxicity tests conducted using Micro organisms provide better understanding of the toxic effect of substance on eco system. The purpose of toxicity test was to determine the effects of the test lubricant on the growth of bacteria isolated from sewage, with an aim to report the LC_{50} value.

Algal inhibition test reported by official journal of the European communities was modified in which algae was replaced with a bacteria. The culture of bacteria isolated from sewage, coded as NS8 was used. It is gram, negative rod shaped bacteria fast growing aerobically on glucose.It forms white irregular circular colonies.Toluene was used as reference material for standardization of protocol.

Conclusion

Polyol esters as base stock were found to be completely biodegradable. However, the time requirement for complete biodegradation of all the 11 samples is different.All the polyol esters were found to be non toxic to the sewage bacteria NS/8.

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Studies in Grease and Lubricant Wastes as Substrates for Growth of Metabolites

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Introduction

The use of nutrient rich wastes as substrates for the growth of metabolite producing microbes has been attempted earlier. The biodegradability, eco-friendliness and easy availability of microbial metabolites from these sources are making them increasingly relevant today [1].

Grease can, along with other lubricant wastes be a major pollutant. The biodegradation of grease using a bacterial consortium has been studied [2]. Other lubricant wastes like putrid metalworking fluids have also been recognised as a medium that permits microbial growth [3].

Lubricants and greases provide a favourable environment for a variety of microorganisms. Microbial growth results in biodegradation of its components. In order to do so, microbes produce a variety of biomolecules in course of their metabolic processes, which have potential benefits for several industries.

Studies of water based MWF mixes have shown that *Pseudomonas* is the most common inhabitant which is also a known biosurfactant producer [4].

Production of Biosurfactants in MWF wastes

Work was undertaken by us to produce a biosurfactant using MWF waste as a substrate. Biosurfactants are biological compounds that exhibit high surface active properties and constitute a diverse group of surface active molecules and are known to occur in a variety of chemical structures. The biosurfactant produced by *Pseudomonas sp*. has been characterized as a rhamnolipid, consisting of an ester of rhamnose sugar and a lipid tail providing hydrophilic and lipophilic moieties that are responsible for its surface active properties [5].

The current work used putrid metalworking fluid, a potential disposal hazard, as a substrate for the production of bacterial surfactant simultaneously leading to the degradation of the metalworking fluid waste. Optimization of biosurfactant production was undertaken, first one factor at a time, and later using response surface methodology.

Preliminary Study of Grease as Substrate

Further work was undertaken to evaluate the potential of using waste grease as a growth substrate. This work was of a preliminary nature. Two types of greases were tested as potential substrates on the basis of their support of bacterial growth and presence of catalase activity, catalase being an enzyme produce by virtually every aerobic organism [6].

Materials and Methods

Biosurfactant from MWF waste

The microorganism *Pseudomonas aeruginosa* ATCC 9027 was procured from the collection of NCIM, Pune. The cultures were stored on nutrient agar slants and were transferred to fresh slants every two months. Nutrient broth, agar agar, urea, potassium dihydorgen phosphate and rhamnose were procured from HiMedia Laboratories, Mumbai. A *Psuedomonas* lipase was obtained from Zytex, Mumbai. Silica gel plates for TLC were obtained from Merck. Soluble cutting oil was procured from M/s Sarbi Petroleum and Chemicals Pvt. Ltd.. The formulation was given to M/s Hydrotechnoque where a 5% emulsion of the cutting oil in water was used for lathing operations. Putrid emulsion was filtered using Whatmann filter paper no. 1. The broken emulsion was then separated using a separating funnel.

Reagents were procured from Fisher Scientific Ltd. and alcohol was procured from Changshu Yangyuan Chemicals.

Biosurfactant Production and Optimization

Pseudomonas aeruginosa ATCC 9027 was grown in nutrient broth for 24 hrs. 150 micro litres of 24 hr. old cultures were inoculated in 20 ml of autoclaved (120°C at 15psi) growth medium. The growth medium was composed of 1g waste metalworking fluid (MWF) oil, which was made up to 20 ml using the waste water recovered. These flasks were incubated for 120 hrs.

Biosurfactant was detected qualitatively using the drop collapsing test for surfactant activity [7][8]. One factor at a time optimization was carried out crudely using surface tension measurements using a stalagmometer. Surface tension was calculated as follows:

$$\frac{ST_w}{ST_s} = \frac{N_s}{N_w}$$

Where, ST_w is surface tension of water, ST_s is surface tension of the solution, N_s is the no. of drops of soln and N_w is the no. of drops of water.

The factors optimized for one factor at a time production were temperature (25°C, 35°C, 40°C, 45°C), pH (3, 4, 5, 6, 7, 8) nitrogen source (urea, 0.5g/l, 1.0g/l, 1.5g/l and 2.0g/l), carbon source (amount of waste oil, 2%, 4%...10%), quantity of phosphate (KH_2PO_4, 2.5g/l, 3.0 g/l, 3.5 g/l, 4.0 g/l). On the basis of these results, statistical optimization using response surface methodology (RSM) was undertaken.

Estimation of biosurfactant using RSM was done using phenol-sulphuric acid method, which estimated rhamnolipid indirectly by estimating the sugar (rhamnose) moiety of rhamnolipid.

1 ml of cell free supernatant of the centrifuged medium (at 6000 rpm for 30 min.) was taken in a test tube. 1 ml of 5% phenol was added, to which 1 ml of conc. (98%) sulphuric acid was added. This was mixed and read after 15 min in a spectrophotometer at 490 nm. This gave the rhamnose content in the mixture. Multiplying by a factor of 3 yielded the rhamnolipid content [9].

Purfication and Characterization

The cell free broth (prepared by centrifuging at 8000 rpm for 30 min) was acidified with 1N HCl to a pH of 2 and extracted using ethyl acetate to yield crude biosurfactant. Emulsification Index [9] and Surface Tension reduction was measured.

Preliminary Study of Grease as Substrate

Wheel Bearing Grease, and Chassis Grease were procured from the market. Hydrogen Peroxide was procured from Ashwin Fine Chemicals Pvt. Ltd. *Bacillus Subtilis* was procured from NCIM Pune, and stored on slants.

5 g of each grease was taken in flasks to which 5 g of water were added. This medium was autoclaved at 120°C at 15psi. 500 micro litres of 24 hr. old culture was used to inoculate the greases. The grease was tested for presence of bacterial growth after 24, 72 and 120 hrs. turbidimetrically using nutrient broth as a culture medium. This was done qualitatively. Un-inoculated (control) samples were compared with inoculated samples and tested for catalase activity.

Results and Discussion

Biosurfactant Production from MWF Waste

One factor at a time optimization yielded optimum conditions as follows: 4% waste oil at 35°C at pH 5 with a presence of 1g/l urea and 3g/l KH_2PO_4.

RSM optimization was undertaken using Design Expert software and was done in two blocks.

The pair-wise combination of the four selected factors: carbon, nitrogen, phosphate and pH have been shown by the three-dimensional graphs generated by the software wherein two factors were varied while keeping the other two at their optimum levels for biosurfactant production. The graphs (Figures) are given here to highlight the roles played by the selected factors in the final yield of biosurfactant production. Significant factors were carbon, nitrogen, pH. The interaction of the carbon source with pH was also found to be significant.

Figure 1 shows the interaction of MWF waste oil and potassium dihydrogen phosphate when varied with fixed values of Urea (0.02 g) and pH (6). Increase in phosphate showed no significant increase in biosurfactant production, while there was an increase in

biosurfactant production with increase in MWF waste oil between 2-5% after which it started to decline.

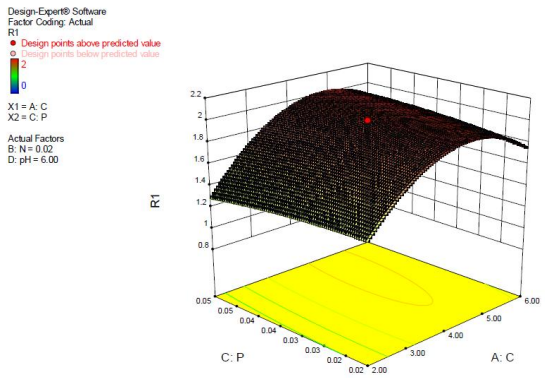

Fig 1: Interaction of Urea and pH with KH_2PO_4 and MWF oil waste

Figure 2 shows the interaction of MWF waste oil and pH when varied, with urea (0.02 g) and potassium dihydrogen phosphate (0.04 g). Production of biosurfactant declined when the pH became alkaline. There was an increase in biosurfactant production with increase in MWF waste oil between about 2-5.5% after which it started to decline.

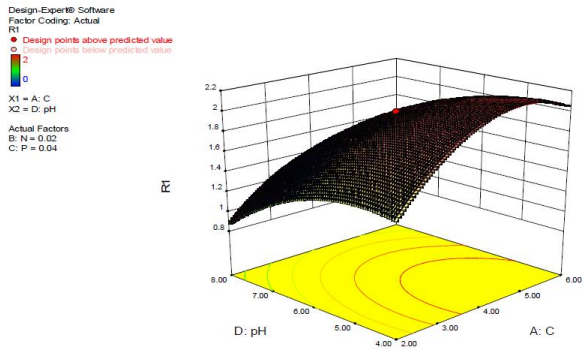

Fig 2: Interaction of Urea and KH_2PO_4 with MWF oil waste and pH

Figure 3 shows the interaction of MWF waste oil and urea when varied, with fixed values of pH (6) and potassium dihydrogen phosphate (0.04 g). Production of biosurfactant increased with increase in urea, though it seemed to decrease slightly at 0.03g/20ml. An increase in biosurfactant production also seen with increase in MWF waste oil between about 2-5.5% after which it started to decline. The maximum biosurfactant production occurred when the MWF waste oil was approximately between 4-5%

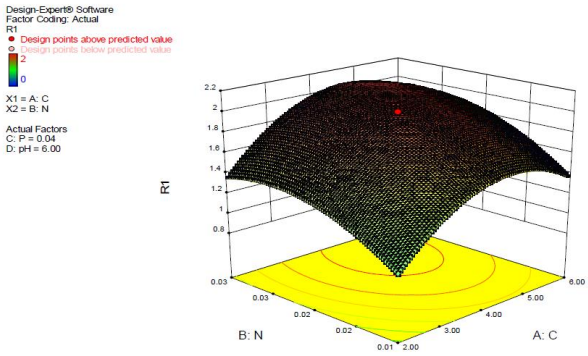

Fig 3: Interaction of Urea and MWF oil waste with KH_2PO_4 and pH

Figure 4 shows the interaction of potassium dihydrogen phosphate and urea when varied, with constant values of MWF waste oil (4%) and pH (6). Production of biosurfactant increased with increase in urea and remained nearly constant when potassium dihydrogen phosphate was varied in the specified range.

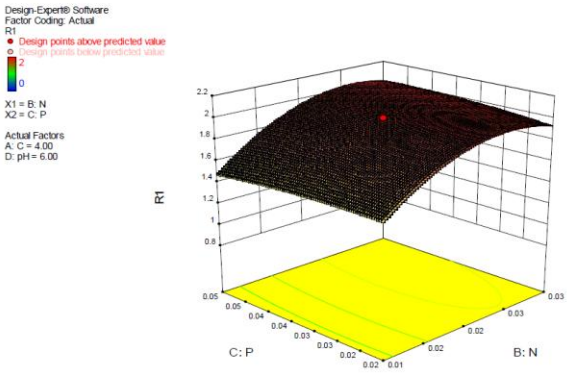

Fig. 4: Interaction of MWF oil waste and pH with KH_2PO_4 and Urea

Figure 5 shows the interaction of potassium dihydrogen phosphate and pH when varied with MWF waste oil (4%) and urea (0.02 g). Biosurfactant production declined as the medium became alkaline and again remained nearly constant when potassium dihydrogen phosphate was varied.

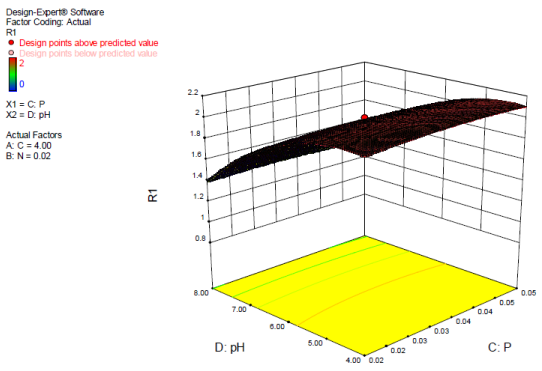

Fig. 5: Interaction of KH_2PO_4 and pH with MWF oil waste and Urea

Figure 6 shows the interaction of urea and pH when varied with contant amounts of MWF waste oil (4%) and potassium dihydrogen phosphate (0.04 g). Biosurfactant production declined with increase in pH, drastically at alkaline pH. Increase in urea increased biosurfactant production.

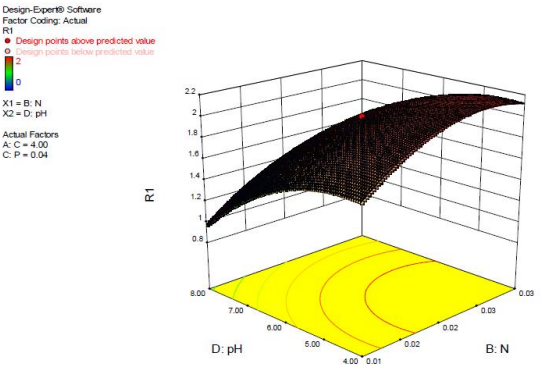

Fig. 6: Interaction of Urea and pH with MWF oil waste and KH_2PO_4

RSM optimization yielded the following optimized media composition:

1. MWF waste oil: 5.06%
2. Urea: 1.5g/l
3. Potassium di-hydrogen phosphate: 2g/l
4. pH: 4.74

The RSM validation batch was prepared using the above media composition and rhamnolipid estimation was done using phenol-sulphuric acid method. The OD corresponded to 1.464 g of rhamnose which was calculated as an equivalent of 4.4g/l rhamnolipid, a yield higher than that using molasses (0.25g/l), comparable to sunflower oil medium (4.31 g/l), but lower than waste soybean stock (11.72g/l) and oil refinery waste (9.5g/l) [10].

Emulsification Index

The stabilization of an oil and water emulsion is commonly used as a surface activity indicator. Emulsification index measurements of olive oil and diesel were carried out using the E24 method. The rhamnolipid emulsified diesel (46%) better than olive oil (24%). Biosurfactant isolated from *Bacilli* BiBi B 5 which showed an emulsification index (E24) of corn oil as 64% but showed low emulsification towards crude oil and short chain hydrocarbons [11]. Oil degrading bacteria of the genera *Marinococcus* was isolated. In that study, the highest emulsifying index of 68% was observed with M6 after 10 min which remained stable after 24 hours. Similarly isolates M4 and M7 registered stable emulsification activity of 68.23% and 63.85% respectively. Emulsification activity of isolates M1, M5 and M10 was 56.75%, 47.75% and 62.44% respectively after 10 min which drastically declined after 24 hrs. M9 showed emulsification activity of 49.35% only after 10 minutes while M2 did not show any such activity [12]. A rhamnolipid isolated from *Pseudomonas aeruginosa* L2-1 showed excellent emulsification activity of 100% with soybean oil [13].

Reduction of surface tension

The activity of a biosurfactant is largely defined by its property of surface tension reduction. Surfactin isolated from two *Bacillus* sp. reduced the surface tension of water to 28 mN/m [14]. Two rhamnolipids isolated from *Pseudomonas aeruginosa* UW-1, decreased the surface tension of water to 27.7 and 30.4 dynes/cm respectively [15]. In this study, the crude rhamnolipid extract was added to distilled water. The biosurfactant reduced the surface tension of water from 72.8 dynes/cm to 33.37 dynes/cm.

Grease as a Substrate

Wheel Bearing Grease failed to show significant growth of *Bacillus subtilis*, whereas Chassis grease showed bacterial growth within 24 hours, and could sustain growth for 120 hours. The inoculated chassis grease also gave a positive reaction for catalase at 24 hours (Fig. 7). As these studies were very preliminary, both greases needs to be studied thoroughly before before their suitability can be evaluated. From this study however, chassis grease shows better potential as a

substrate for bacterial growth and metabolite production.

Fig. 7 Effervescence (circled) showing Catalase Activity

Conclusion

This study highlights the possibility of using micro-organisms to utilize wastes for producing industrially important metabolites. Biosurfactant was successfully produced in a waste metalworking fluid, and characterised. It showed good emulsification and surface tension reducing properties.

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