EI Research Report

Guidelines for the investigation of relationship between water content in biodiesels and microbial growth and contamination

energy institute

RESEARCH REPORT: GUIDELINES FOR THE INVESTIGATION OF RELATIONSHIP BETWEEN WATER CONTENT IN BIODIESELS AND MICROBIAL GROWTH AND CONTAMINATION

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FOREWORD

EN 590. *Automotive fuels. Diesel. Requirements and test methods* specification currently allows for blending of up to 7 % (v/v) of fatty acid methyl esters (FAME) into standard automotive diesel fuel. Some vehicle fleet operators also use biodiesel blends containing significantly higher FAME concentrations. Fuel users have reported operational problems such as filter plugging and corrosion of fuel tanks as a result of microbiological growth and contamination of diesel fuel systems.

The Energy Institute (EI) *Research report: Investigation of microbiological susceptibility of biodiesel and biodiesel blends* concluded that FAME at concentrations of 2 % and above increased the susceptibility of fuel to microbial growth, most notably for fungal growth. As FAME has an increased propensity to hold water (both as dissolved water and as free water dispersed as microdroplets), there is an increased potential for microbial contamination of bulk fuel in tanks.

Following on the findings of EI *Research report: Investigation of microbiological susceptibility of biodiesel and biodiesel blends*, the EI Microbiology Committee commissioned further research on the topic. This publication reports on the second phase of the laboratory research undertaken to investigate the influence of water content on the extent of microbiological contamination in hydrocarbon diesel (B0) and two biodiesel blends (B10 and B20). The investigation consisted of two parts: the principal part of the study investigated the relationship between microbiological contamination and water content (100, 400, 1 000 and 10 000 ppm total water) in laboratory microcosms for each fuel, which simulated fuel stored in tanks. The microcosms were held under defined conditions of temperature and humidity over a 14-week test period. For the second part, the settling time for water and microorganisms in microcosms containing B10 and B20 at 400 ppm total water content was investigated. For both parts of the investigation, the vertical profile of microbiological contamination and water content was determined by analysing sub-samples drawn from four distinct depths of fuel in each microcosm.

For all fuels, increase in the total water content of microcosms resulted in an increase in the amount of microbial biomass in aqueous phase; this increase in biomass was most noticeable on increasing the total water content from 100–400 ppm. Contrary to expectations, at the end of the 14-week study microbiological contamination was not detected in bulk fuel layers for the biodiesel blends, whereas for B0 some contamination was detected in bulk fuel. The water content of bulk fuel was observed to increase with increasing FAME concentration; this increase was found to be predominantly due to dissolved water, not free water. The settling rate of microorganisms in fuel was found to decrease considerably from B10 to B20.

This publication presents the results of the laboratory study. While every reasonable route has been taken to ensure the accuracy of its contents, the EI cannot accept any responsibility for any action taken, or not taken, on the basis of this information. The EI shall not be liable to any person for any loss or damage which may arise from the use of any of the information contained in any of its publications.

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This project was commissioned by the EI's Microbiology Committee, chaired by Joan Kelly (Conidia Bioscience Ltd.) and formerly chaired by Elaine McFarlane (Shell).

The work was carried out by Graham Hill, Gareth Williams and Leon O'Malley from ECHA Microbiology and steered by members of the Microbiology Committee, who during the project included:

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Carol Devine	NECE
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Bob Eden	Rawwater
Alex Few	ExxonMobil
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Geert Van der Kraan	Dupont
Leanne Walker	Rawwater
Neil Whitehead	Minton, Treharne & Davies
Gareth Williams	ECHA Microbiology

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The EI would like to thank Ramya Venkataraman of ExxonMobil for supplying the base fuel used to prepare blends and FAME used during the investigation. The EI would also like to express its gratitude to Minton, Treharne & Davies for carrying out the IP 387, IP 139 and Infrared analysis of the settled precipitate at their facilities in Cardiff allowing the completion of this research report.

The project coordination and technical editing were carried out by Kerry Sinclair (EI) and Marcela Betancur-Diaz (EI).

1 INTRODUCTION

1.1 TECHNICAL BACKGROUND

European Union (EU) regulations mandating the use of fuels from renewable sources for road transport have seen increasingly wide use of FAME in automotive diesels. EN 590 now enables up to 7 % (v/v) FAME to be blended into standard automotive diesel fuel and some vehicle fleet operators use blends containing significantly higher FAME concentrations. Sporadic serious operational and fuel quality problems due to microbiological contamination have been experienced by some retailers and vehicle operators (e.g. filter clogging, poor fuel stability, tank gauging malfunction and rapid corrosion of fuel tanks). In many cases, these problems have led to a requirement for more rigorous maintenance practices when storing, handling and using fuels containing FAME, in particular improved controls over the ingress and accumulation of water.

It is well documented that the presence of free water in fuels is the main factor influencing the extent of microbial growth in fuel tanks and systems. However, less is known about the correlation between levels of water contamination, as measured in representative fuel samples, and levels of microbial growth and contamination. Additionally, there has been little understanding of how the propensity of biodiesel blends to hold water influences the ability of microbial contamination to grow and disperse in the bulk fuel phase.

In conventional fuels, microbial growth is usually restricted to areas of free water accumulation in tank bottoms or surface condensate films where it forms slimes, known as biofilm, when adhered to surfaces. Any microbial contamination detected in the bulk fuel phase is usually a consequence of physical disturbance of this growth, and consequent dispersion of microbial contamination (e.g. by turbulence when a tank is filled). The microbial biomass breaks up to form a freely suspended particulate contamination, but with time, this contamination will usually settle out.

Conversely, biodiesel blends may hold water, both as dissolved water and as free water dispersed as microdroplets. This may increase the ability of microbial growth to foul and contaminate bulk fuel in storage tanks and ultimately impact on fuel quality. If free water is held in suspension in fuel, microbial growth may be possible even when water is no longer detected by routine dipping. In addition, it may be more difficult to mitigate contamination by conventional routine housekeeping practices, as it will be more difficult to remove water by tank draining as the water and microbial contamination may not settle out as readily.

Industry experience suggests that diesel fuels containing FAME can have an increased susceptibility to microbial growth, as stated in the IASH conference paper *Strategies for resolving problems caused by microbial growth in terminals and retail sites handling biodiesels*. The EI's review paper *Implications of biofuels on microbial spoilage and corrosion within the fuel distribution chain and end use,* highlighted the need for further research. The EI also issued a technical bulletin *Microbial growth in diesels and other fuels containing fatty acid methyl esters (FAME)*, which discusses the implications of FAME on microbial growth and provides provisional recommendations for the maintenance of fuel handling facilities.

The technical bulletin expands on the EI *Guidelines for the investigation of the microbial content of petroleum fuels and for the implementation of avoidance and remedial strategies* and *Research report: Investigation of microbiological susceptibility of biodiesel and biodiesel blends,* which demonstrate that a number of factors unique to biofuels are pertinent to

the increase in operational problems; therefore, this research report describes a second phase of laboratory research to establish a better understanding of the relationship between microbiological contamination in biodiesel blends and water content.

1.2 SCOPE

In order to investigate different aspects of the relationship between water content and microbiological contamination in biodiesel blends, the research was split into two parts:

– Part 1 (the main part of the study): investigated the relationship between microbiological contamination and water content (in both inoculated and uninoculated microcosms) for three fuels (B0, B10 and B20) held under defined conditions of temperature and humidity over a 14-week test period. The microcosms simulated diesel stored in tanks on a small laboratory scale. The vertical distribution of microbiological contamination and water content was determined by analysis of sub-samples drawn from four distinct depths (representing upper, middle, lower and bottom layers) in each microcosm over this time.
– Part 2 (conducted at the end of the 14-week period): two microcosms were chosen for a shorter study to investigate the influence of settling time on the vertical distribution of microbial contamination and water within the fuel layers. In this second part, microbial contamination present in the bottom layer was disturbed into the bulk fuel; the settling of microorganisms and water was monitored by analysis of sub-samples drawn from each depth over a 48-hour period.

Key findings from this investigation may assist in developing good practice guidelines for the handling and storage of biodiesel. Fuel suppliers have an obligation to implement effective control measures at fuel terminals, which ensure the quality and fitness-for-purpose of fuel. This publication should assist industry in gaining a better understanding on how measured values of water content in fuel could influence microbial growth and contamination in the downstream infrastructure. In addition, the settling study provides information on settling parameters for microbiological contamination in biodiesel, which can be used to form a basis for estimating the effectiveness of routine product settling practices.

This publication provides details on the influence of:

– FAME concentration on the vertical distribution and concentration of water in fuel;
– FAME concentration on the vertical distribution and extent of microbial contamination in fuel;
– total water content on the vertical distribution and extent of microbial contamination in fuel, and
– settling time on the vertical distribution of microbiological contamination and water content in fuel.