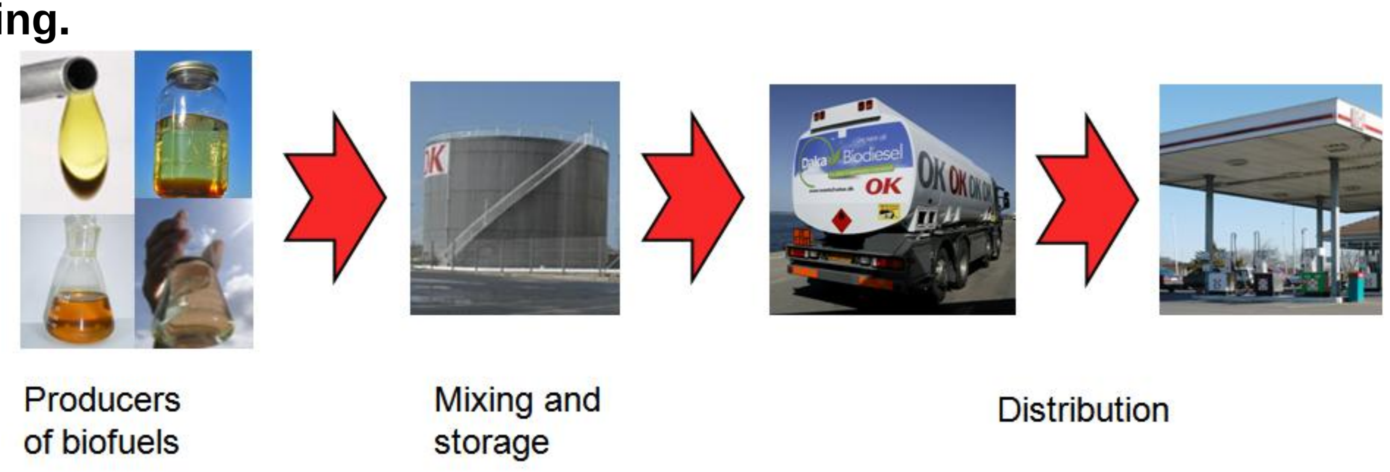
Assessing Microbial Spoilage of Biodiesel Blends Under Aerobic and Anaerobic Conditions

Sune Nygaard,† Ketil Sørensen,† Hans Ove Hansen,† Uffe Thomsen,† Thomas Lundgaard,† Torben L. Skovhus,† and Gitte Sørensen†

† Danish Technological Institute, Kongsvang Allé 29, DK-8000 Aarhus, Denmark

Introduction

Global warming and climate changes have become everyday words and concerns. To efficiently counter the negative changes associated with the increased CO_2 level in the atmosphere, significant measures are needed. In the European Union beginning in 2010, all transportation has to be substituted with 5.75% of renewable fuel on an energy basis. In order to meet this goal and future even more ambitious goals a number of chemically different renewable fuels have to be introduced into the existing fuel infrastructure. This will significantly complicate the fuel matrix and handling.



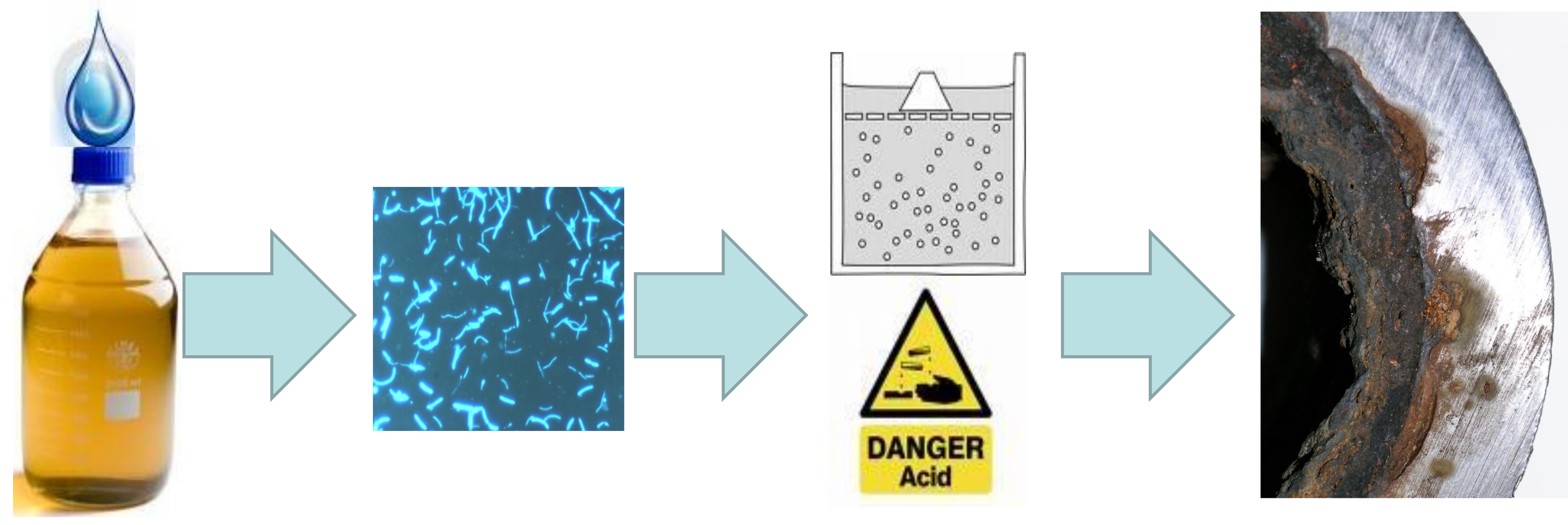
Fuel spoilage is a key concern! This can occur either through chemical degradation of the fuel or as a result of microbial growth in the fuel mixture. Most renewable fuels are biocompatible, which is beneficial from an environmental perspective, but in the case of long-term storage a challenge. This is due to the fact that some biofuels are degraded by microorganisms at a rate comparable to that of sugar [1]. Such degradation might lead to undesired fuel properties, i.e. acid formation, and particle formation in the biofuel.

Purpose of the setup

To gain knowledge about the amount of biomass, nature of the bacterial population and the associated effects on fuel quality we study microbial growth in petrochemical diesel and biodiesel blends by varying storage conditions.

Microbiologically Influenced Corrosion

Because the chemical properties of biofuels are different from petrochemical fuels, the shift in fuel mixtures may have undesired effects both regarding fuel blends and materials throughout the distribution system. Biofuels are hygroscopic in nature and therefore contain more water than what is usually found in petrochemical fuels. Increased water content in the fuel matrix will lead to increased growth of microorganisms in the fuel-water interphase.

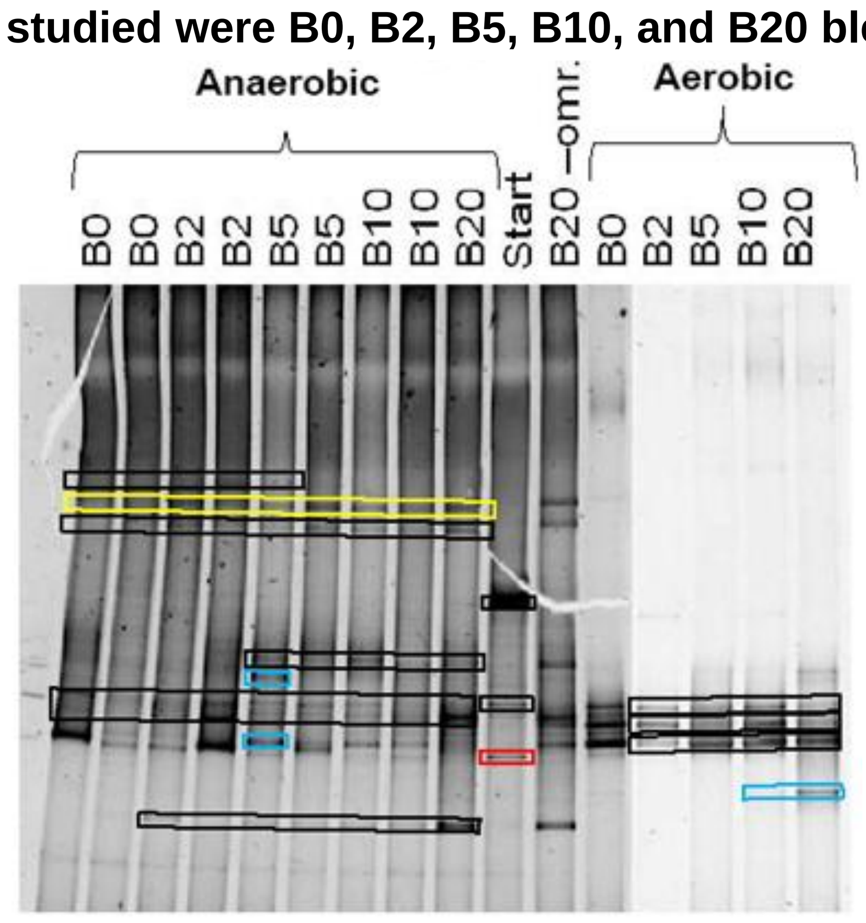


An increase in microbiological growth and activity is known to speed up corrosion rates, a phenomenon known as Microbiologically Influenced Corrosion (MIC) in the fuel handling infrastructure. With the switch towards increased proportions of sustainable fuels, MIC caused by biofilm formation will be a major challenge to storage and distribution facilities. Furthermore, biofuels promote corrosion more directly through increased presence of water or the adhesion of precipitates.
In order to counter these effects it is of paramount importance to obtain detailed knowledge on the types of microorganisms (bacteria and fungi) that grow in biofuel blends in order to evaluate how the growth of these organisms can effectively be countered.

Microbial Growth in Biofuel Blends when Subjected to Water

Initial studies have been performed in order to study the effect of biodiesel blends on microbial growth at various conditions, including biodiesel blend type, aeration and temperature.
The experiment was carried out by inoculating 400 ml BX with 100 ml water obtained from a heavy fuel storage tank. The temperature was held at -5, 5, 10, 20, 30, and 40 °C, respectively; and the BX blends studied were B0, B2, B5, B10, and B20 blends.

The photograph shows the aerobic experimental setup with the biodiesel blends (to the left) and the anaerobic setup (to the right).



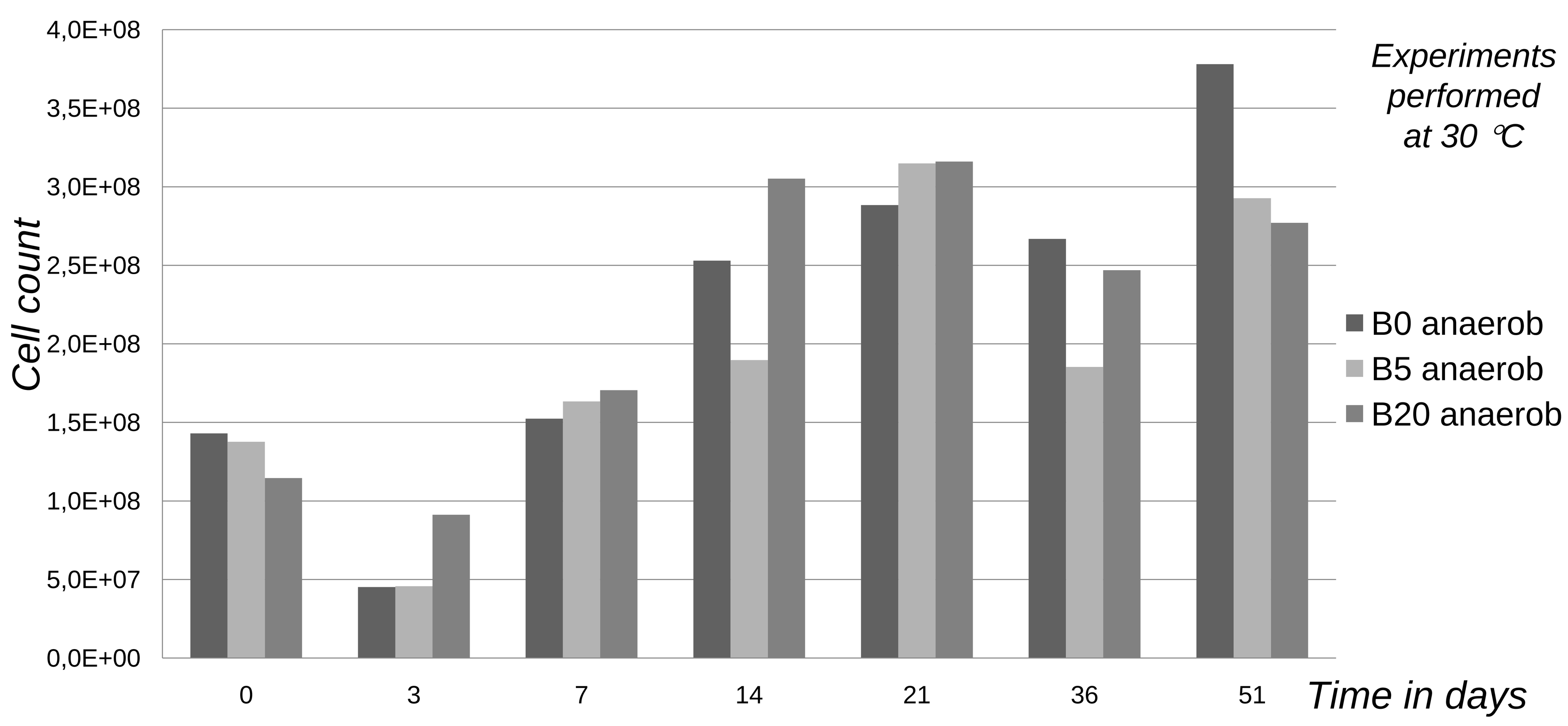
DGGE gel showing samples with bacteria present as bands in each vertical lane

Bacterial community analysis:
Bacterial populations in the incubations were compared by DGGE analysis (left). This revealed that:
1) the bacterial populations changed dramatically during all incubations
2) different strains of bacteria were present in aerobic compared to anaerobic incubations
3) several strains of microorganisms were present only in incubations with biodiesel

The identified bands were affiliated with the ***Alphaproteobacteria*** (black boxes), ***Bacteriodetes*** (yellow boxes), ***Clostridia*** (red boxes), and ***Gammapoteobacteria*** (blue boxes).

Anaerobic results (O_2 absent):
- Significant growth in all fuel types following addition of water
- No significant difference in the number of bacteria when comparing biofuel-containing blends to pure petrochemical diesel fuel
- Not possible to obtain chemical evidence by GC-MS for degradation of the fuel matrix
- Highly increased turbidity as a function of time and amount of added biodiesel
- Additionally, the observed turbidity peaked in samples stored at 20 °C

Aerobic results (O_2 present):
- Fewer bacteria in the aqueous phase as compared to the anaerobic samples
- Growth of filamentous fungi in all samples
- Highly increased turbidity due to microbial growth



Summary of microbiology

Aerobic incubations
- Growth of fungi was observed
- Low bacterial diversity was observed
- Bacterial growth appeared to be limited, probably due to competition or the with fungi
- The addition of high levels of biofuel (10% or more) resulted in growth of additional strains of *Bacteria*

Anaerobic incubations
- Relatively high overall bacterial diversity
- The addition of biofuel resulted in growth of additional strains of *Bacteria*
- All detected groups of *Bacteria* are widely distributed in both marine and freshwater environments
- Several of the detected bacterial groups are known to degrade complex organic molecules and polymers.
- Sporeforming *Clostridia* are widely distributed in oil reservoirs but were not detected in any of the incubations in this study

General Conclusions

- Addition of biodiesel to the fuel matrix results in growth of new types of bacteria than what is normally found in the fuel system.
- The turbidity of the fuel is correlated to the amount of biodiesel blended into the petrochemical base fuel.
- Further studies are necessary to identify the effect of the new types of identified bacteria in biodiesel blends and other renewable fuel types. This needs to be done in order to assure a trouble free implementation of existing and future biofuels in the existing fuel infrastructure.

References:
[1] **(a) Blin, J., Volle, G., Girard, P., Bridgwater, T., Meier, D.;*Biodegradability of biomass pyrolysis oil: Comparison to conventional fuels and alternative fuels in current use;* Fuel, 2007, *86*, 2670–2686; (b) DeMello, J. A., Carmichael, C. A., Peacock, E. E., Nelson, R. K., Arey, J. S., Reddy, C. M.; *Biodegradation and environmental behavior of biodiesel mixtures in the sea: An initial study*; Marine Pollution Bulletin, 2007, *54*, 894–904.3**