

Title	A collaborative research on bio-inspired approaches to lubricate engineering materials on nanometer scale
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Citation	Short term scientific mission, COST Action TD1003, scientific report, (2011)
Date	2011
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Scientific report

Short term scientific mission, COST Action TD1003

A collaborative research on bio-inspired approaches to lubricate engineering materials on nanometer scale

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Experiments were carried out with Dr Kirsi Pakkanen and Mr Troels Røn, Technical University of Denmark, Kgs. Lyngby (DK)

Introduction

Bio-inspired lubrication has gained great interest among researchers due to the fact that there are several examples in nature where biomolecules can enhance lubrication and provide low friction and low wear properties. VTT is currently running two projects on bio-based lubrication ("*New Bioinspired and Bio-based Solutions for Lubrication (NEWLUB)*") and "*Biomimetic Water Lubrication*" (BIOWAL)) that aim to use biomolecules as lubricant additives to be used in industrial applications. Some results are very promising, but there is still lack of understanding of lubrication mechanisms.

To understand how lubrication works on micro- and macroscale it is important to understand basic phenomena that occur in molecular level or in nanoscale. Technical University of Denmark (DTU) provided a great opportunity to study the lubrication mechanisms in more detail.

Materials and experimental

VTT manufactured two types of hydrophobin proteins that were studied in more detail. One hydrophobin is a wild type protein without carbohydrate part and the other is a glycosylated hydrophobin protein. Both proteins were added into 50 mM sodium acetate buffer pH 5 and concentration was 1.0 mg/ml.

At DTU, a few instrumental approaches are available that enable to investigate the lubrication effects in more detail.

Optical waveguide lightmode spectroscopy (OWLS) was used to study the adsorption of the proteins on chromium oxide and polydimethylsiloxane (PDMS) surfaces. Chromium oxide was chosen to be the primary material because it mimics the surface of stainless steel that was used in most of the tribological experiments carried out earlier at VTT. At the inlet of OWLS, hydrophobin sample solution was injected into the device and was allowed to mix with the flow of buffer solution (50 mM sodium acetate buffer pH 5), finally reaching target surfaces. Adsorption of the proteins was determined as the change in refraction index at the vicinity of the surfaces.

Atomic force microscopy (AFM) was used to measure frictional properties of the proteins on stainless steel surface at nanometer scale.

Pin-on-disc device (POD) and Mini-Traction Machine (MTM) were used to study the macroscopical lubrication properties of the proteins in sliding and rolling contacts. POD provides pure sliding contacts only, but MTM provides possibilities to control the sliding/rolling-ratio and observe how the proteins are able to reduce friction in this type of contact. For MTM experiments both disc and ball materials were stainless steel, AISI440C.

Circular dichroism (CD) spectroscopy was used to study the effects of temperature and tribological contacts on the conformation of the hydrophobin proteins.

Results and discussion

Tribological studies

It was observed that friction measurements with AFM were difficult to perform. Stainless steel is not very smooth material on nanoscale and that caused some problems. Friction coefficient could not be obtained as it is observed on micro- and macroscale due to the lack of AFM cantilever calibration, but friction forces can be compared with a standard sample when the same tip is used. However, there is an uncertainty due to possible wear of the tip and other factors that can affect the results. Preliminary tests (Data not shown) indicated a similar trend that had been earlier observed in experiments on microscale. In addition, in AFM experiments the buffer solution was able to reduce the friction on stainless steel surface compared to ambient. However, this needs to be confirmed by repeated measurements in near future. Reduction in friction was observed but due variation in measured points, a clear trend cannot be drawn yet. To employ molecularly smooth surfaces that are adequate for nanotribology measurements (other than stainless steel), further experiments will be conducted in near future.

Friction coefficient experiments with MTM clearly showed that both the hydrophobins were able to reduce friction also in rolling contact (Fig. 1, 2 and 3). Due to the high amount of lubricant required for one measurement, no more than one experiment with each lubricant was possible to perform.

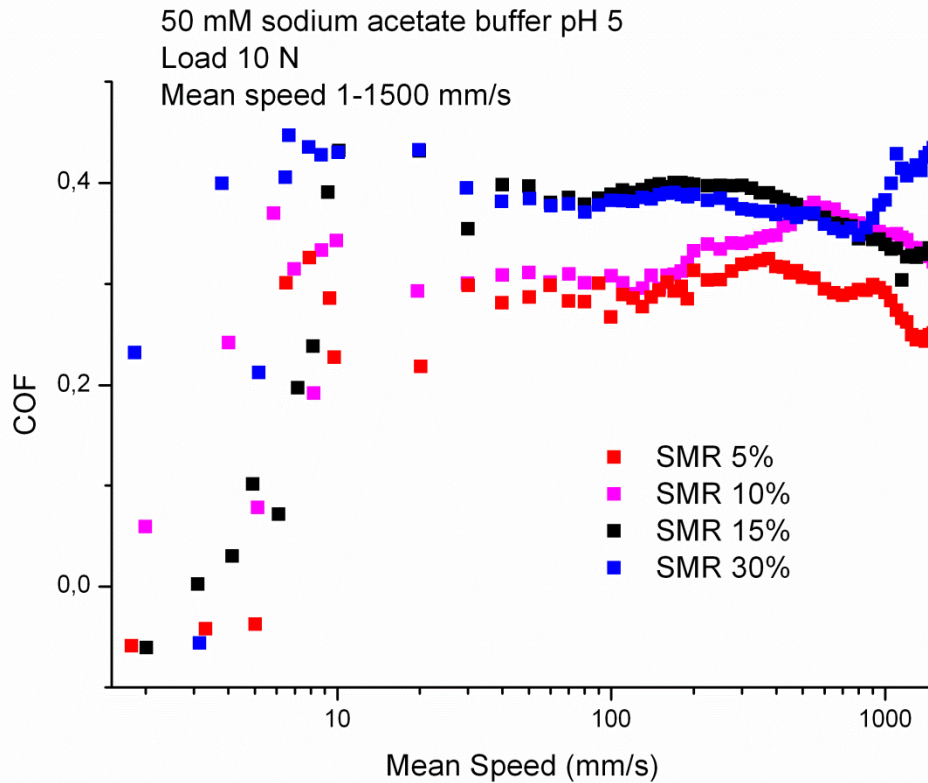


Figure 1. Friction coefficient (COF) as a function of mean speed and different slide/mean speed-ratios (SMR) of MTM experiments. Lubricant was 50 mM sodium acetate buffer pH 5.

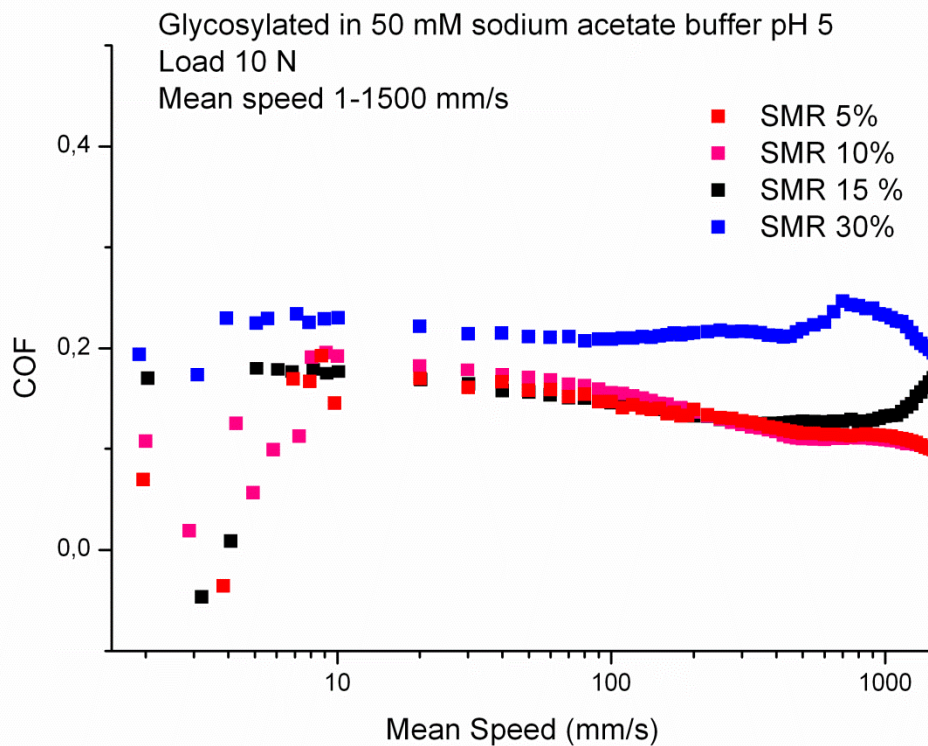


Figure 2. Friction coefficient (COF) as a function of mean speed and different slide/mean speed-ratios (SMR) of MTM experiments. Lubricant was glycosylated hydrophobin in 50 mM sodium acetate buffer pH 5.

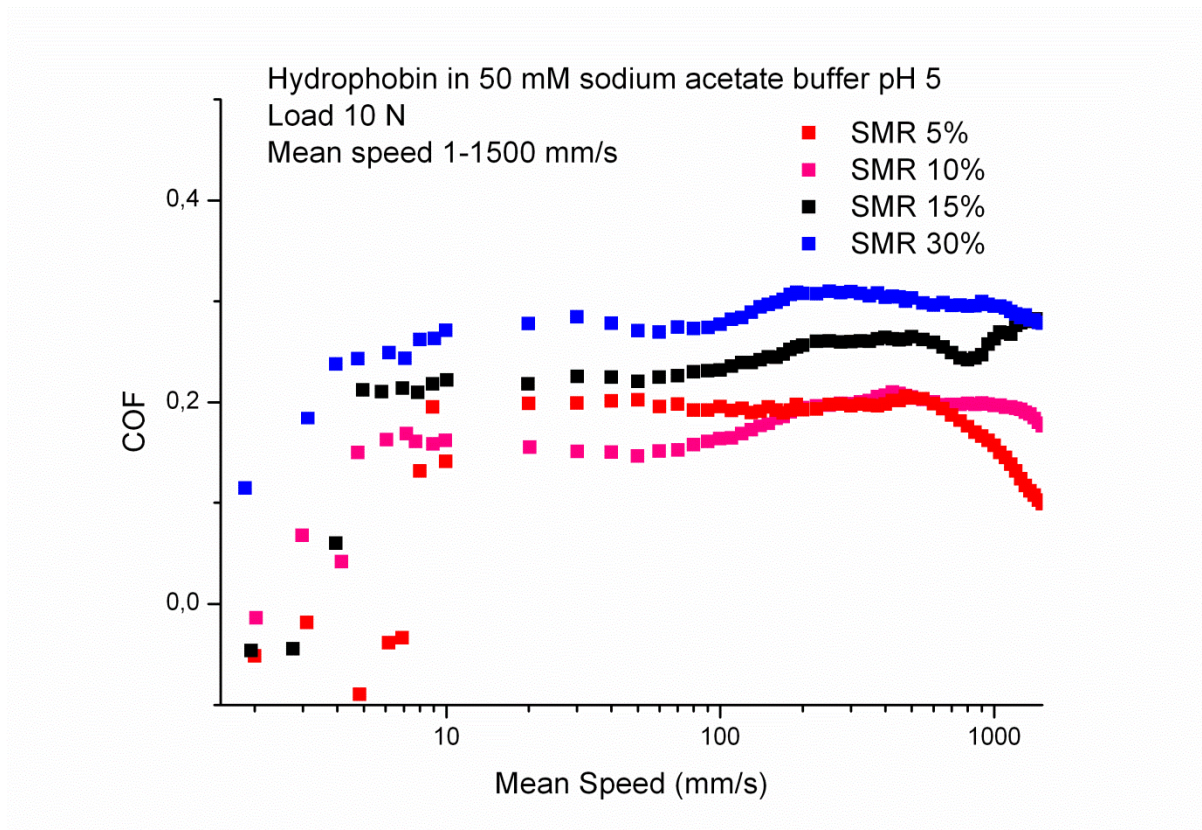


Figure 3. Friction coefficient (COF) as a function of mean speed and different slide/mean speed-ratios (SMR) of MTM experiments. Lubricant was wild type hydrophobin in 50 mM sodium acetate buffer pH 5.

As can be observed from the figures 1-3, the friction coefficient is reduced from 0.3-0.4 to close to 0.2 when hydrophobin proteins were added into the 50 mM sodium acetate buffer. In higher speeds the protein solutions started to form foam and it might have had an effect on the lubrication.

POD was used for two different types of experiments. First it was used to gain lubricants with hydrophobins that have been in tribological contact (data not shown) between stainless steel surfaces to be studied in more detail with CD to observe if there occurred some changes in the protein structure. POD was used also for preliminary test for lubrication of soft PMDS surfaces by hydrophobins. The results were very promising and the friction coefficient was reduced from 0.8 to close to 0 when the hydrophobins were added into the buffer solution (Fig. 4). Friction coefficient remained less than 0.1 even with velocities less than 1 mm/s (Fig. 5).

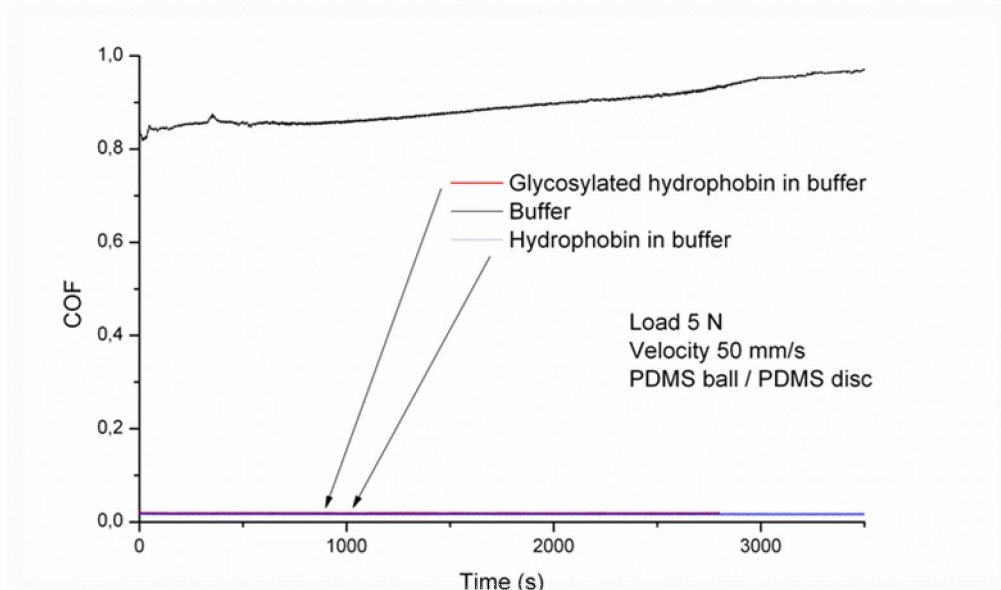


Figure 4. Lubrication of soft PDMS surfaces by hydrophobins reduced the friction coefficient from ~0.8 to almost 0 in POD tests.

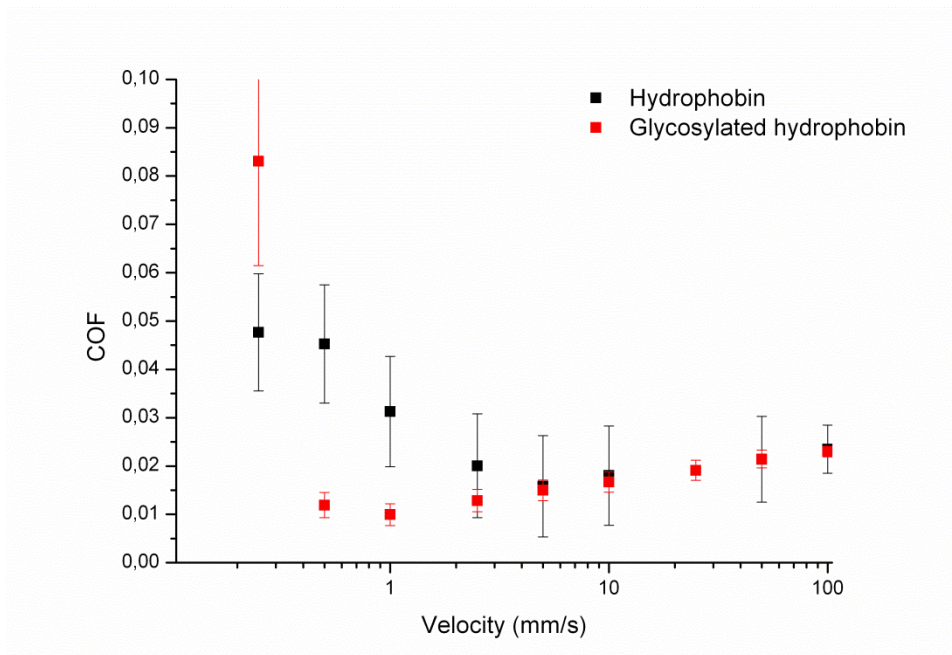


Figure 5. Friction coefficient of soft PDMS surfaces lubricated by hydrophobins as a function of velocity.

Adsorption studies

OWLS was used to study the protein adsorption on chromium oxide and PDMS surfaces. It was observed that adsorbed amount of the proteins was higher on PDMS surface than on chromium oxide surface (Fig. 6). Also the desorption from chromium oxide surface by buffer rinsing was higher than from PDMS surface after incubation for the protein solution.

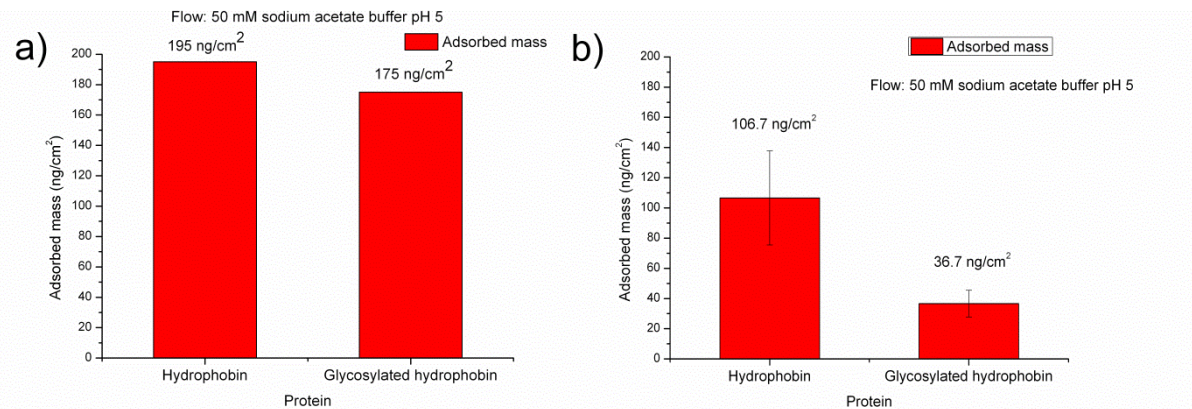


Figure 6. The adsorbed mass of the proteins onto a) PDMS and b) chromium oxide surfaces measured by OWLS.

Studies of protein structure

CD spectroscopy was used to study the changes in the protein structure both due to the tribological contact and increased temperature. There were small changes in the conformation, but interpretation of the results was not straightforward. However, it can be concluded that increase of the temperature to 60-80 °C causes some type of reversible conformational change (Fig. 7).

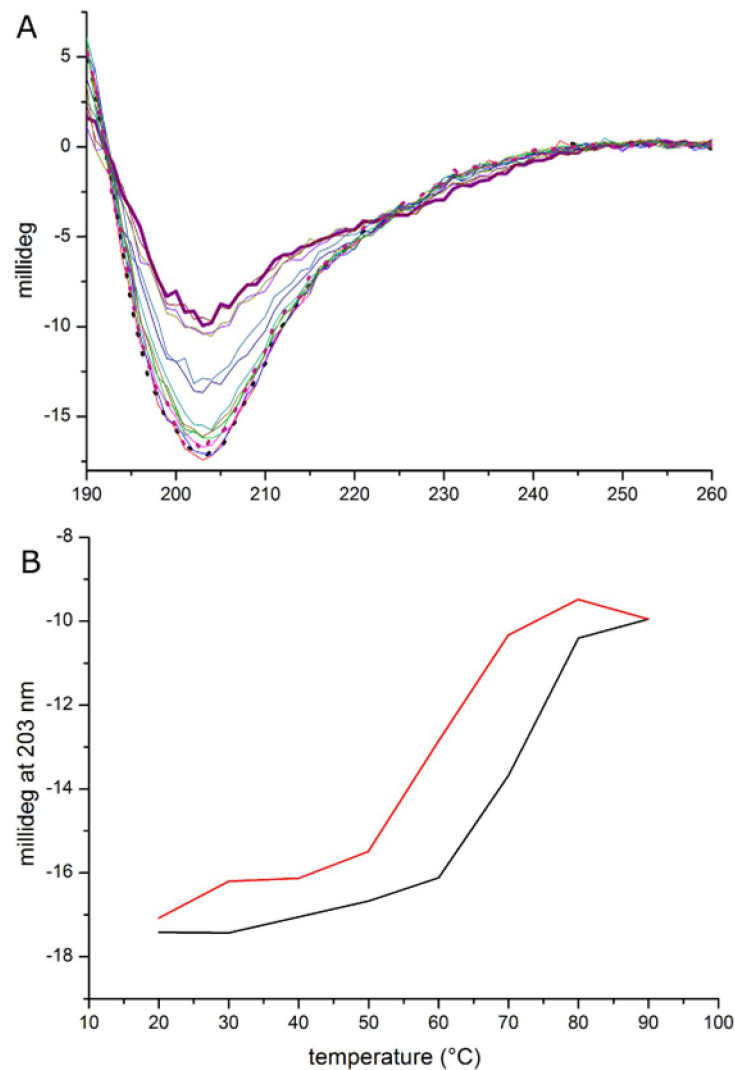


Figure 7. a) CD spectra of glycosylated hydrophobin at different temperatures (20-90°C) and b) change in spectra at 203 nm as a function of temperature. Black and red line: heating and cooling of the sample, respectively.

Conclusions

It was observed that protein adsorption and lubrication performance was surface dependent. In view of hydrophobicity alone, more hydrophobic surface appeared to increase the adsorption significantly and reduced the desorption by buffer solution rinsing. Increase in temperature had effect on the protein structure and it might have explained some variation observed in tribological experiments done earlier at VTT. AFM was somewhat difficult to be used in the friction measurements on stainless steel surface.

Some of the results will be published in the future.