Ecotoxicity of biodiesel derived from waste cooking oil

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Introduction: Biodiesel is a fuel composed of fatty acid alkyl esters made by transesterification of fatty acids (triglycerides) from plant oils or animal fats. Biodiesel was proposed as a "green" alternative for fossil fuel and with the intention of causing lower impact on human health and the environment by reduction of combustion derived hydrocarbons, carbon monoxide, polycyclic aromatic hydrocarbons (PAHs), and particulate matter (PM) (Murugesan et al., 2009). However, concrete evidence which supports the reduced toxicity of biodiesel emissions is lacking. It is also apparent that biodiesel toxicity depends on the chemical composition and properties of the biodiesel made from diverse feedstocks which in turn can vary considerably. The impact of biodiesel on living systems is under intensive investigation using a variety of bioassays.

Residues of oils after cooking and from food waste, cleaning residues and animal fat wastes are a cheap source for biodiesel production and are widely used on an industrial scale. Used oils contain a high concentration of free fatty acids (FFA). According to the FFA content, used cooking oils are divided into two groups: yellow fat (FFA <15%) and brown fat (FFA> 15% and water). Yellow fats are a common source of biodiesel and can be used after filtration and cleaning (Canacsi and van Gerpen, 2001; Clark et al., 2013; Leung et al, 2006). The world population growth has led to an increase in the volume of food industry wastes and by-products. The use of these wastes for biodiesel production has been encouraged as an effective utilization of an otherwise wasted resource. Thus, biofuels from waste edible oils, which can differ in the initial composition of fatty acids and, consequently, in properties and levels of toxicity often enter the market.

To date only limited information is available on the aquatic toxicity of biodiesel or other biofuels. Petroleum-related pollutants are a worldwide threat to aquatic organisms and humans (Bellas et al., 2013), so the ecotoxicity of biodiesel biofuel needs to be examined in terms of its impact on such species. In our study, we used an algae culture growth-inhibition test for evaluation of the toxicity of a petroleum diesel (B100) and a B20 blend (20% biodiesel and 80% petroleum diesel) of biodiesel from waste cooking oil. The use of a phytoplankton for the assessment of marine pollution is well documented (Metting, 1996). These algae are one of the primary organisms in the food chain, playing a key role in toxin biomagnification to higher predators, such as zooplankton, fish and ultimately humans.

Materials and methods: Fuel samples were obtained from the Primorsky Krai (Russian Federation) market as B100 (pure biodiesel), produced by methanol transesterification of waste cooking oil (yellow grease), B0 (diesel fuel) and B20 (diesel-biodiesel blend of 20% biodiesel and 80% diesel fuel by volume). Hexane and methanol were used for comparison control. Potassium bichromate ($K_2Cr_2O_7$) (source) was used as the positive control.
Propidium iodide (PI), from Molecular Probes (Eugene, Oregon, USA), was used to measure cell death (or Growth-inhibition/vitality).

Four species of seawater microalgae isolated from the Peter the Great Gulf (Sea of Japan, the Primorsky Krai, Far-Eastern Russia) were used as the test organisms. The test species included two marine diatoms Attheya ussuriensis and Chaetoceros muelleri, a red algae Porphyridium purpureum and Raphidophyte Heterosigma akashiwo and were cultured with Guillard's f/2 medium. Microalgal culture medium was provided by National Scientific Center of Marine Biology, Far Eastern Branch of the Russian Academy of Sciences (NSCMB FEB RAS). Culturing and toxicity test conditions were according to OECD 201 standard. The suitability of each microalgae species as a test organism for the assay was carried out with potassium bichromate. Exposure of cells was carried out in 24- well plates. Each assay was run at concentrations of $K_2Cr_2O_7$ of 1, 2, 4, 8, 16, 32 mg/L. For each concentration and control group (without toxicant), the experiment was conducted in 4 replications. The volume of microalgae aliquots in each replication was 1.5 mL. The number of living cells was determined using a CytoFLEX flow cytometer (Beckman Coulter, USA) after 96 h (acute toxicity) and after 7 days (chronic toxicity).

Diesel fuel (B0), blended sample (B20), and straight biodiesel (B100) were analyzed by liquid chromatography–mass spectrometry (LC-MS). The algae-bioassay (24h, 96h and 7 days) was carried out to test the toxicity of the fuel samples. The accurate counting of algal cells was provided at 24h, 96h and 7 days by flow cytometer CytoFLEX (Beckman Coulter, USA) with the software package CytExpertv.2.0. Each sample was measured with a flow rate 40 µL/min during 75 sec. Cell viability was determined by staining with Propidium Iodide (PI).

Results and discussion: The diesel fuel B0 consisted of alkanes (mainly n-heptane and n-hexane) plus methanol and ethanol (with latter two alcohols representing more than 10%) as additives. An insignificant content of unsaturated hydrocarbons was observed, including 0.1% of several aromatic compounds. The blended sample B20 also consisted of alkanes (predominantly n-heptane), alcohols (about 23.5%) and a small amount of methyl esters of fatty acids. The main components of the biodiesel sample B100 were methyl esters of fatty acids (77.6%). The predominant components were methyl esters of hexadecenoic acid (C16: 0) and methyl esters of saturated and unsaturated octadecanoic acids (C18:0, C18: 1, C18: 2, C18: 3). In addition, BA100 contained ethanol (3.8%) and a significant amount of volatile organic compounds, such as 3-methylhexane (18.4%).

According to the results of the toxicity bioassays on microalgae A. ussuriensis, C. muelleri and H. akashiwo, the maximum level of toxicity was shown by a sample of pure biodiesel B100 and the diesel-biodiesel blend B20 sample for the red alga P. purpureum. P. purpureum was the most sensitive of the tested species for potassium bichromate. n-Hexane and methanol proved to be the most stable for B100 and B20. In addition, P. purpureum and H. akashiwo showed the ability to adapt to the presence of the tested pollutants. So, for P. purpureum, the EC50 increased for hexane and methanol following 7 days of exposure (chronic toxicity) compared to 96 h (acute toxicity), while for B20 and B100, an increase in the EC50 was observed from 24 h to 96 h. On the 7th day of the experiment the EC50 decreased. H. akashiwo during the experiment adapts only to B20, showing an increase in the EC50 from 24 h to 96 h and a sharp decrease in the EC50 by the 7th day. For the diatoms of C. muelleri and A. ussuriensis, the level of toxicity increased in direct proportion to the increase of cells exposure time. C. muelleri and H. akashiwo demonstrated the highest acute toxic effect for B100. The highest chronic toxicity was shown by A. ussuriensis for B20 and B100.

For all microalgae species tested, the toxicity level of samples containing biodiesel from waste cooking oil (B20 and B100) was higher than it was for hexane, methanol or diesel fuel. The results of our studies showed that the sample B20 containing 68.1% n-heptane, 15% methanol and 8.5% ethanol was significantly more toxic for all species than pure hexane, pure methanol and the sample B0 containing about 70% n-heptane, but with a fraction of methanol and ethanol (~5%). Quality biodiesel consists mainly of fatty acid methyl esters, the low toxicity and biodegradability of which determines their safety for the environment. However, based on our results, sample B100 consisting of 77.6% of FAMEs exhibited the greatest aquatic toxicity among all the samples tested. This was probably caused by the presence of the aliphatic iso-paraffinic constituent 3-methylhexane (18.4%) included in C7-C9 petroleum products. These paraffins have a high aquatic ecotoxicity potential, possibility due to the increasing concentration of methanol following FAME hydrolysis.

Conclusions: The properties of biodiesel as a non-toxic and environment-friendly alternative fuel may differ depending on the feedstock, the method of production and the chemical composition of the final product. The combination of individual components with relatively low toxicity can cause synergistic effects. Our results showed that the individual components of the biodiesel (n-hexane, n-pentane, methanol, FAMEs) had a lower toxicity threshold than in the complex mixture. B100 sample showed the highest level of toxicity for the microalgae A. ussuriensis, C. muelleri and H. akashiwo in comparison with hexane, methanol, B0 and B20. B20 proved to be the most toxic for the red algae P. purpureum. Overall, our findings provide the basis for further investigation into the effects of cooking waste oil-derived biodiesel on coastal aquatic ecosystems.

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