

Article

Analysis of Biological Degradation and Life Cycle Indicators of Mineral Diesel Fuel Mixtures, Containing 10% Biodiesel, Obtained by Simultaneous Oil Extraction and Transesterification

Violeta Makareviciene * , Migle Santaraite and Egle Sendzikiene 

Department of Environment and Ecology, Agriculture Academy, Vytautas Magnus University, K. Donelaičio Str. 58, LT-44248 Kaunas, Lithuania; migle.santaraite@vdu.lt (M.S.); egle.sendzikiene@vdu.lt (E.S.)
* Correspondence: violeta.makareviciene@vdu.lt; Tel.: +370-37-752292

Abstract: This article provides data on the environmental properties of biofuels obtained by the simultaneous extraction of oil from spoiled rapeseed and transesterification, with the addition of mineral diesel to the reaction mixture. The resulting reaction product contained 10% biodiesel: fatty acid methyl, ethyl, or butyl esters in mixtures with mineral diesel. The addition of biodiesel has been found to increase the rate of biodegradation of fuels. Such fuels are classified as partially biodegradable, according to the OECD classification. Life cycle analysis showed that the mixtures of biodiesel and mineral diesel have lower negative environmental impacts, compared to pure mineral diesel. The values of indicators such as abiotic depletion, acidification, global warming, ozone depletion, and human toxicity for these mixtures were 40–58% lower compared to the corresponding values for mineral diesel.

Keywords: biological degradation; life cycle; mineral diesel biodiesel mixture



Citation: Makareviciene, V.; Santaraite, M.; Sendzikiene, E. Analysis of Biological Degradation and Life Cycle Indicators of Mineral Diesel Fuel Mixtures, Containing 10% Biodiesel, Obtained by Simultaneous Oil Extraction and Transesterification. *Energies* **2021**, *14*, 8367. <https://doi.org/10.3390/en14248367>

Academic Editor: Dmitri A. Bulushev

Received: 12 November 2021
Accepted: 8 December 2021
Published: 12 December 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.



Copyright: © 2021 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (<https://creativecommons.org/licenses/by/4.0/>).

1. Introduction

Extraction of energy from fossil fuels has a negative impact on the environment (formation of ozone layer depletion, acid rain, negative impact on human health, etc.); therefore, ways are being sought to reduce this negative impact

In the production and use of pure biodiesel and its mixtures with mineral diesel, it is important to assess, not only the physicochemical properties of the fuels and their compliance with standards, but also environmental properties, such as the rate of biological degradation and values of life cycle indicators.

The rate of biodegradation of fuels to CO₂ and H₂O is different and depends on fuel origin and composition. In the natural environment, fuel is exposed to physical and chemical processes, during which microorganisms use the fuel for nutrition. Following the OECD methodology, biodegradation (BD) within 28 days is assessed and substances are divided into: fully degradable (BD > 60%), partially degradable (BD 20–60%), and non-degradable (BD less than 20%). The rate of biodegradation of biodiesel has been found to be five times higher than that of mineral diesel [1]. Silva et al. found that biodiesel produced from rapeseed, sunflower, and soybean oil was completely biodegradable over a 28-day period [2].

The results of some researchers show that biodiesel causes high cytotoxicity and inhibits seed development in artificially contaminated soil [3,4]. Meanwhile, biodiesel derived from animal fats is less phytotoxic, as determined after 120 days of soil incubation [5]. Biodiesel produced from a variety of feedstocks has been shown to exceed phytotoxicity limits for many test organisms, such as *Daphnia magna* [6], *Eisenia fetida* [7], marine microalgae [8], *Sinapis alba*, and *Unio pictorum* [9]. Various biodegradation studies of biodiesel are underway: microbial activity, phytotoxicity, and metabolite formation are

assessed by studying biodiesel-contaminated soil, to better understand the environmental impact of biodiesel [10].

The biodegradability of fuel mixtures containing mineral diesel and biodiesel was also analyzed [11]. Under anaerobic conditions, the rate of biodegradation of the B20 fuel mixture (containing 20% of soybean fatty acid methyl esters and 80% of mineral diesel) was high, 50% biodegradation was achieved in 6.8 days, and the mixture was almost completely degraded in 28 days [12]. Vauhkonen et al. analyzed the biodegradation of rapeseed oil and rapeseed oil methyl esters according to the OECD 301F methodology and found that more than 60% of these substances are biodegradable in groundwater within a 28-day period [13]. Other researchers have found that the biodegradation of pure rapeseed oil butyl esters was 70% over 28 days (meeting the requirements for biofuel degradation), while only up to 26% of mineral diesel is degraded during the same period. A fuel mixture of mineral diesel with up to 30% biodiesel is less biodegradable than pure biodiesel and is reported as a partially biodegradable material [14]. Most authors report that the addition of biodiesel to mineral diesel accelerates the biodegradation of fuel. When mixed with mineral diesel at more than 35% biodiesel, biodegradation was found to exceed 90% in 21 days [15].

Other researchers evaluated the biodegradation of mineral diesel and biodiesel mixtures, with different biodiesel levels in the mixture (2%, 5%, and 20%), using a respirometric method and a redox index and performed a 2,6-dichlorophenol indophenol (DCPIP) test. Measurements of CO₂ emissions showed that, although biodiesel is more readily and faster biodegradable than mineral diesel, among the mixtures evaluated, only the mixture containing the highest biodiesel concentrations was characterized by a significantly higher rate of biodegradation, compared to mineral diesel [16]. The same trend was observed by Horel and Schiewer [17].

Life cycle analysis (LCA) was performed to assess and compare the environmental impact of biodiesel throughout the product's life cycle, from the fuel's 'birth to death'. LCA of biodiesel fuel often involves the feedstock production, catalyst preparation and transesterification processes, product purification, and end use (Figure 1) [18–20]. The end use of the fuel is its combustion in the engine of the vehicle.

An analysis of the literature has shown that the majority of LCA studies are related to the assessment of the environmental impact of pure fuels compared to fuel mixtures [21,22].

Life cycle analysis of mineral diesel and rapeseed oil butyl ester (RBE) mixtures was performed. Sendzikiene et al. found that the use of RBE in a mixture with MD, compared to pure MD, allowed reducing negative impacts on the environment, such as abiotic depletion, acidification, global warming, and human toxicity [23].

Most biological decomposition studies and life cycle analyses were performed for fuel mixtures produced by the mixing of pure mineral diesel and biodiesel. No data have been found on life cycle analysis performed in the production of fuel mixtures using simultaneous oil extraction and a transesterification process (in situ). The aim of this study was to analyze the biodegradability of fuel mixtures produced by a simultaneous extraction and transesterification process of low quality rapeseed oil using methanol, ethanol or, butanol, and to evaluate their life cycle indicators in comparison with the indicators of pure biodiesel and mineral diesel.

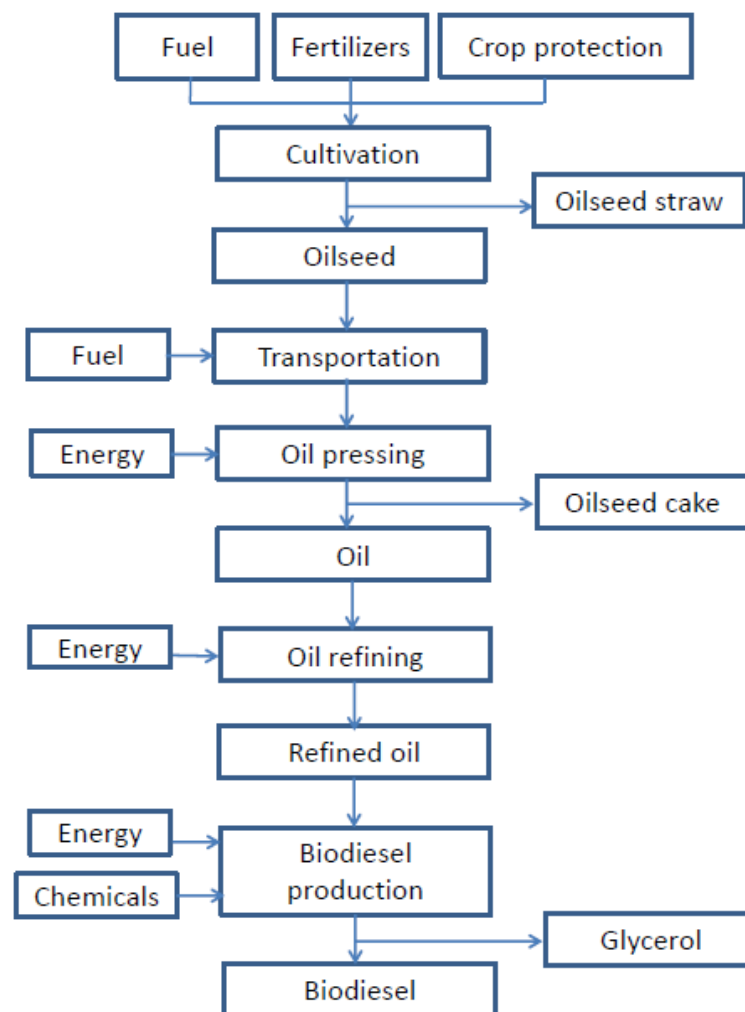


Figure 1. Life cycle stages of biodiesel.

2. Materials and Methods

2.1. Analysis of Biological Degradation

The fuel mixtures were obtained by simultaneous rapeseed oil extraction with methanol, ethanol, and butanol, by adding mineral diesel to the reaction mixture as an additional component, to accelerate oil extraction and allow a mixture of mineral diesel and biodiesel to be obtained immediately during the process. Spoiled rapeseed was used for the synthesis, the acidity of which exceeded the requirements for edible oil. The mineral diesel content was calculated by estimating the oil content of rapeseed, so that the biodiesel content of the final product in a mixture with mineral diesel would reach 10%. The crushed rapeseed was placed in conical flask and mixed with mineral diesel. The flask placed in a glycerol bath placed on magnetic stirrer. The samples were mixed at a constant (250 min^{-1}) rotation speed. When the required temperature was reached, a weighed amount of alcohol and biocatalyst was added to the reaction flask. The resulting fuel blends were filtered. The glycerol was separated in a separatory funnel (by decantation) and the resulting fuel blends were washed twice with distilled water. A rotary evaporator was used to remove residual water and unreacted alcohol. Silica gel was used for complete removal of moisture residues.

Biological degradation was assessed for mixtures of mineral diesel containing 10% rapeseed oil methyl esters (RME), rapeseed oil ethyl esters (REE), and rapeseed oil butyl esters (RBE). Biodegradation of pure RME and mineral diesel was also determined. The manometric respirometry method was applied for analysis of biological degradation of samples, which is specified in the OECD 301F methodology [24]. The bacterial culture used for the studies was wastewater after the initial mechanical treatment from the Kaunas

wastewater treatment plant (Lithuania). Samples with fuel mixtures and bacterial culture were incubated at 22 ± 1 °C for 28 days. The biodegradation results were measured with a BOD-System AL606 instrument, which determined the biochemical oxygen demand (BOD) of the test samples according to the change of the pressure difference in the closed system.

Biodegradation was calculated as the ratio of the oxygen demand during the experiment to the theoretical oxygen demand required to decompose the sample. The theoretical oxygen demand was calculated from the elemental composition of the samples, as determined using a CHNS/O Analyzer 2400 Series II.

Analysis of each sample was performed in triplicate. The arithmetical mean value of three independent determination results were taken as the test result.

2.2. Life Cycle Analysis of Fuel

Life cycle analysis of fuel mixtures and pure mineral diesel was performed using the SimaPro 9.1.1 software package (PRé sustainability, The Netherlands). Energy consumption was estimated for the production of 1 t of fuel in situ, with the catalyst immobilized lipase: lipozyme TL IM. Table 1 shows the values of the materials and processes used for the analysis.

Table 1. Values of materials and processes in the life cycle analysis of pure mineral diesel and fuel mixtures.

Material	Unit	Fuel Type			
		MD90-RBE10	MD90-REE10	MD90-RME10	MD100
Rapeseed	kg	279	279	279	-
Mineral diesel	kg	900	900	900	1000
Lipase (Lipozyme LT IM)	kg	5.022	4.185	4.185	-
Butanol	kg	21.606	-	-	-
Ethanol	kg	-	13.430	-	-
Methanol	kg	-	-	9.343	-
Process					
Electricity	MJ	1313	1313	1313	5000
Heating	MJ	1173	1173	1173	27,300

In the calculations of material costs, the data from the technical documentation of Lithuanian biodiesel producer JSC Rapsoila and the data presented in the dissertations of Sendžikienė [25] and Kazanceva [26] were taken into account. Data on mineral diesel production (electricity and heat consumption) are taken from research data reported by the U.S. Department of Energy [27]. It was assumed that poor quality rapeseed would be obtained free of charge. Quantities of electricity and heating energy (MJ) required for the production of fuel mixtures are taken from the data presented in the dissertation of Kazanceva [26].

In order to compare all cases of the production of fuels and their mixtures, the CML-2 baseline 2000 method was selected, which describes the problem in more detail and allows comparing the results with those of other researchers. The method was designed to assess impacts on human health, the ecosystem, and resources. The following impact categories were selected for the LCA assessment: resources (abiotic depletion), ecosystem (acidification), and human health (global warming, ozone layer depletion, human toxicity).

3. Results and Discussions

3.1. Properties of Fuel Mixtures

Fuel mixtures were produced under the optimal conditions previously established. Their quality indicators are presented in Table 2.

Table 2. Quality indicators of fuel mixtures.

Quality Indicator	Unit	MD90-RME10	MD90-REE10	MD90-RBE10	Method of Determination	EN 590 and EN 14214
Ester content in biological part of fuel	% (w)	98.75	99.89	99.08	EN 14103	-
Density at 15 °C	kg m ⁻³	821	819	831	EN ISO 3675 LST EN ISO 12185	820–845
Viscosity at 40 °C	mm ² s ⁻¹	2.17	2.11	2.33	EN ISO 3104	2–4.5
Flash point	°C	78	78	73	EN ISO 3679	Min 55
Sulfur	mg kg ⁻¹		0		EN ISO 20846 EN ISO 20884	Max 10
Cetane number	-	43.29	43.35	46.72	EN ISO 5165	Min 51
Copper strip corrosion (3 h at 50 °C)	grade		1 grade		EN ISO 2160	1 grade
Oxidation stability at 110 °C	h	17.87	7.49	12.84	EN 14112	Min 20
Cold filter plugging point	°C	−36	−37	−33	EN 116	−32
Monoglyceride	% (w)	0.46	0.00	0.03	EN 14105	-
Diglyceride	% (w)	0.07	0.08	0.09	EN 14105	
Triglyceride	% (w)	0.00	0.00	0.09	EN 14105	
Free glycerol	% (w)	0.00	0.00	0.00	EN 14105	

The data provided show that all the fuel mixtures produced comply with the requirements of the standards for mineral diesel and biodiesel fuel EN 590 and EN 14214. All mixtures have a low viscosity and density, so they have the ability to lubricate parts of the fuel system well. All fuel mixtures also meet the flash point requirements, which according to EN 590 should be at least 55 °C. The fuel mixtures are sulfur-free, which is a major environmental advantage.

Oxidation stability studies have shown that the most stable fuel mixtures are those produced from rapeseed using methanol (oxidation stability: 17.87 h). All fuel mixtures are of first corrosion grade and meet the requirements of both mineral diesel and biodiesel standards. All fuel mixtures are characterized by excellent cold temperature properties. Their cold filter plugging point is lower than minus 32 °C and meets the requirements of mineral diesel standard EN 590 for arctic climatic zones. Such fuel is suitable for use as winter diesel in Scandinavian countries.

3.2. Biological Degradation of Fuel

The results of the biodegradation studies for fuel mixtures and pure mineral diesel tested according to the OECD 301F methodology are presented in Figure 2.

Based on the results obtained, it can be stated that pure RME is a readily biodegradable substance, as 60% of the biodegradation threshold was exceeded after 8 days ($65.21 \pm 1.12\%$) and a biological degradation of 96.40 ± 1.07 was observed after 28 days. Mineral diesel, in contrast, is a slowly biodegradable substance, as a biological degradation of only $20.64 \pm 0.34\%$ was obtained within 28 days.

The addition of 10% biodiesel in a fuel mixture promotes the biodegradation process of organic matter. Compared to the biodegradation of pure mineral diesel, the presence of 10% rapeseed methyl esters in fuel mixtures results in a faster biodegradation of fuel: within 28 days, the biodegradation of such mixtures is $37.27 \pm 0.09\%$. Meanwhile, the biodegradation of mineral diesel during this period is only $20.64 \pm 0.34\%$. This biodegradation value for the RME and mineral diesel mixture was reached within 12 days. Over a period of 28 days, about 80% more of the fuel mixture decomposed compared to pure mineral diesel.

In the presence of 10% rapeseed ethyl esters in the fuel mixtures, a biodegradation of $23.58 \pm 1.04\%$ was achieved within 28 days. In this case, about 14% more fuel mixture decomposed compared to mineral diesel. The biodegradation value of this fuel mixture in 22 days ($20.75 \pm 0.06\%$) was equal to the biodegradation value of mineral diesel obtained in 28 days.

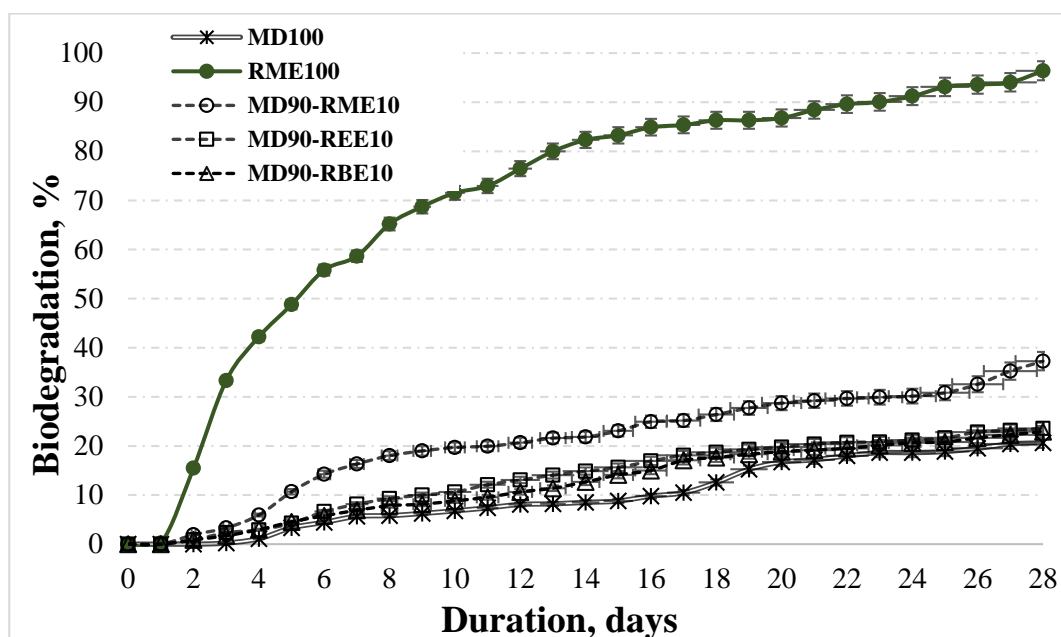


Figure 2. Biodegradation of mineral diesel, biodiesel, and fuel blends made from 90% mineral diesel and 10% rapeseed oil fatty acid methyl, ethyl, or butyl esters.

The biodegradation of fuel mixtures containing 10% rapeseed butyl esters was slightly slower than that of mixtures containing rapeseed methyl or ethyl esters; 22.91% of such mixtures were biodegradable within 28 days. However, the biodegradation of such mixtures was ~ 10% higher than the biodegradation of mineral diesel. In 28 days, 2.27% more fuel mixture decomposed than mineral diesel.

Based on the results of experimental studies, polynomial equations for the dependence of biodegradation on storage time were developed and coefficients of determination were calculated (Table 3).

Table 3. Dependence of the biodegradation of fuels and their mixtures in aqueous media on storage time.

Fuel Type	Polynomial Equations of Biodegradation	Coefficient of Determination, R^2
MD100	$y = 0.0039x^2 + 0.7001x - 0.7928$	0.9763
RME100	$y = -0.1804x^2 + 7.9273x + 6.6964$	0.9590
MD90-RME10	$y = -0.0332x^2 + 21322x - 0.1804$	0.9702
MD90-REE10	$y = -0.020x^2 + 1.466x - 1.5292$	0.9954
MD90-RBE10	$y = -0.0115x^2 + 1.1925x - 1.2278$	0.9928

Where y is the degree of biodegradation of mineral diesel (MD) and fuel mixtures containing 90% of mineral diesel and 10% rapeseed oil fatty acid methyl esters (RME), ethyl esters (REE), or butyl esters (RBE), %; x , time in days.

The polynomial equations show the actual biodegradation trends of pure MD and its mixtures with biodiesel. All coefficients of determination of the obtained results are $R^2 > 0.97$, which shows that the model accurately describes the data. Polynomial equations can be used to predict the biodegradation of fuel mixtures over time. The results obtained contradict some of the results obtained by other researchers. It was reported that mixtures of mineral diesel and biodiesel fuels containing more than 35% of rapeseed methyl, ethyl, or linseed and pork lard methyl esters can decompose up to 90% after as little as 21 days [15]. Prince et al. examined fuel mixtures containing 20% biodiesel and found that the rate of biodegradation was high and the fuel mixtures decomposed almost completely after 28 days [12]. Kazanceva et al. found that mixtures with mineral diesel containing up to 30% biodiesel were less biodegradable and found that such a fuel mixture could be classified

as a partially biodegradable substance, according to the OECD classification [24]. Such different results could be explained by the different methodologies used to determine the biodegradation of the fuels and their mixtures.

Summarizing the obtained results, it can be stated that, compared to pure mineral diesel, the biodegradation of its mixtures with biodiesel is faster. Mixtures with 10% rapeseed methyl esters had the highest biodegradation rate compared to mixtures containing ethyl esters or butyl esters. This could be due to the fact that the chain of the methanol molecule is shorter, making it easier to decompose and biodegrade. Although mineral diesel mixtures with 10% of biodiesel biodegraded faster than pure mineral diesel, a 60% biodegradation rate for biodegradable products was not achieved within 28 days; therefore, these fuel mixtures can be classified as partially degradable materials, according to the OECD classification.

3.3. Life Cycle Analysis of Fuel

The results of life cycle analysis of mineral diesel and fuel mixtures consisting of 90% mineral diesel and 10% fatty acid methyl-, ethyl-, or butyl esters obtained by using the program SimaPro (method CML-2 baseline 2000) are presented in Table 4 and in Figure 3. The analyzed fuel mixtures were produced using low-quality rapeseed, as this raw material is currently the most readily available and its quantities are sufficient to realize this process.

Table 4. Environmental exposure categories determined using the CML-2 baseline 2000 method.

Exposure Category	Units	Fuel Type			
		MD100	MD90-RME10	MD90-REE10	MD90-RBE10
Abiotic depletion	kg Sb eq/kg	53	24	24	24
Acidification	kg SO ₂ eq/kg	15.02	8.04	7.97	8.04
Global warming	kg CO ₂ eq/kg	0.0018	0.00107	0.00106	0.00107
Ozone layer depletion	kg trichlorfluormethane eq/kg	0.00138	0.000609	0.000605	0.000609
Human toxicity	kg 1,4-dichlorbenzene eq/kg.	1.85×10^3	691	666	691

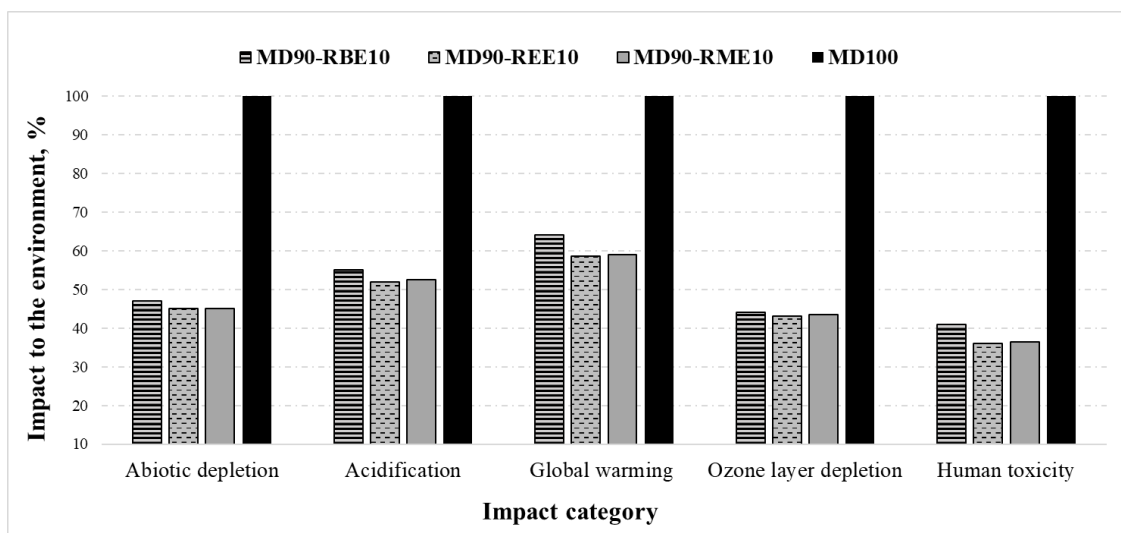


Figure 3. Comparison of the environmental impact of fuel mixtures with regard to the alcohol used in production, assessed using the CML-2 baseline 2000 method (values of mineral diesel are equated to 100%).

The data provided show that the addition of biofuels to mineral diesel reduced all values of the environmental impact indicators. Among the life cycle indicators examined, pure mineral diesel showed the highest values of the indicators having the greatest negative impact on the environment.

Abiotic depletion. This exposure category relates to the protection of human well-being, human health, and the health of ecosystems. This impact category indicator is related to the extraction of minerals and fossil fuels, resulting in the highest impact of mineral diesel. The data obtained show that in the case of mixtures with mineral diesel at 10% REE and RME, this indicator reaches 45% in comparison with pure mineral diesel, and in the case of mixtures with the same amount of RBE, the value of this indicator reaches up to 47% of the value of mineral diesel. Other researchers studied only the life cycle performance of pure biodiesel and mineral diesel and found that the value of this indicator for pure RBE is five-times lower than that of mineral diesel [23]. Harding and co-authors found that for pure RME and REE this indicator was equal to 15.4 kg Sb eq and 13.4 kg Sb eq/kg [28]. As can be seen from the data in the Table 4, the value of this indicator is almost twice as low than that of the mineral diesel. For the mineral diesel mixtures with REE and RME, the value of this indicator was 24.0 kg Sb eq, and for mineral diesel mixtures with RBE, the value of this indicator was 24.8 kg Sb eq. This can be explained by the high concentration of mineral diesel in the fuel mixtures.

Acidification. This is an important indicator, as increasing concentrations of acidic substances in the atmosphere do a lot of damage to ecosystems. Acidification is caused by emissions of gases such as sulfur and nitrogen oxides caused by human activity [29]. The data presented in Table 3 and Figure 2 show that the production and usage of mineral diesel mixtures with biodiesel reduces acidification, and range from 7.97 to 8.04 kg SO₂ eq/kg. The effect of pure mineral diesel on acidification is 15.02 kg SO₂ eq/kg. In order to further reduce the impact of fuel mixtures on acidification, the amount of biodiesel in mineral diesel should be increased.

Global warming. This is caused by carbon dioxide emissions from industry, transport, and so on. The assessment of this indicator identifies the global warming potential as an indicator that describes the value of the global warming potential of a greenhouse gas in relation to its carbon dioxide equivalent. The obtained data show that the production and application of fuel mixtures containing 10% of biodiesel reduced the global warming rate compared to the case of mineral diesel, by about 40% in the case the mixture containing REE or RME, and by about 35% for the mixture containing RBE. For pure RBE, the difference is up to 80% [23]. Spirinckx and Ceuterick found that the global warming effect can be reduced by up to 50% by using pure biodiesel instead of mineral diesel [30].

Ozone layer depletion. Ozone is an allotropic form of oxygen. It consists of three oxygen atoms. Ozone is formed from atmospheric oxygen, due to strong electric shocks or solar radiation. There is a tendency that, with the increase of ultraviolet radiation, more ozone is formed, and the higher the ozone concentration, the better the absorption of radiation. Some substances, such as nitrogen oxides, chlorine, and bromine compounds, promote ozone dissociation. The thickness of the ozone layer is affected by the sun's ultraviolet radiation and the amount of incoming pollutants, which deplete the ozone molecule. The presented data show that the presence of all types of esters (RME, RBE, REE) in mixtures with mineral diesel reduces the effect of ozone depletion by up to 55%, compared to the influence on ozone layer depletion of the production and usage of pure mineral diesel. The ozone layer depletion values for mixtures with different types of biodiesel are very similar, ranging from 43 to 44% when the value of mineral diesel is equated to 100%.

Human toxicity. The purpose of this indicator is to assess the negative effects on human health. Human health is affected by pollutants that can enter the body from the atmosphere, water, and soil. The value of this indicator is reduced by the production and usage of mineral diesel mixtures with RME or REE. Sendzikiene and others proved that the use of low-quality rapeseed oil for biodiesel production has a positive impact on all environmental impact categories, when using either biotechnological or chemical production methods [23]. It was found that the production and use of RBE produced from low quality oil (applying the biotechnological method) reduced the negative impact on the environment: abiotic depletion up to 79.1%, acidification up to 38.8%, eutrophication up to

18.33%, global warming up to 77%, ozone layer depletion up to 90.12%, human toxicity up to 71.8%, freshwater ecotoxicity up to 9.01%, seawater ecotoxicity up to 56.9%, terrestrial ecotoxicity up to 0.19%, and photochemical oxidation up to 36.8% [23].

The results of our research showed that the value of the human toxicity indicator for the mineral diesel mixture with biodiesel was about 60% percent lower compared to this indicator for pure mineral diesel. The data obtained showed that in the case of mixtures with mineral diesel at 10% REE and RME, the values of this indicator were lowest, and reached 36 and 36.5% in comparison with pure mineral diesel, while, in the case of mixtures with the same amount of RBE, the value of this indicator reached up to 41% of the value of mineral diesel. This reduction was relatively significant, even at low concentrations of biodiesel in the fuel mixtures.

According to the results of our life cycle analysis, one way to reduce the negative impact on the environment is to produce mixtures of mineral diesel and biodiesel fuels. Replacing as much as 10% of the esters in fuel mixtures could reduce the negative environmental impact of diesel fuel. The values were not significantly affected by whether the fuel mixtures contained 10% RME, REE, or RBE.

4. Conclusions

The addition of 10 percent biodiesel to mineral diesel increased the rate of biodegradation. More than 20% of fuel mixtures containing 10% rapeseed methyl-, ethyl-, or butyl esters was biodegradable within 28 days, and according to the OECD classification, they belonged to the group of partially biodegradable substances. Mixtures containing rapeseed methyl esters were characterized by higher biodegradation rates. The biodegradation of such mixtures was $37.27 \pm 0.09\%$. A biodegradation of $23.58 \pm 1.04\%$ was achieved within 28 days for mixtures containing rapeseed ethyl esters. When the mixtures contained butyl esters, a biodegradability of 22.91% was determined within 28 days. The biodegradation of mineral diesel during this period was only $20.64 \pm 0.34\%$.

A life cycle analysis of blends obtained by simultaneous oil extraction and transesterification showed that the presence of 10% biodiesel in blends significantly reduced the negative environmental impact, compared to the life cycle performance of mineral diesel. Values for indicators such as abiotic depletion, acidification, global warming, and ozone layer depletion, and human toxicity for mineral diesel and biodiesel blends were 40–58% lower compared to the corresponding values for pure mineral diesel.

For the first time, the environmental properties of fuel mixtures obtained by applying a simultaneous oil extraction and transesterification process (in situ) were analyzed. The obtained results showed that biodiesel blends with mineral diesel have a higher biodegradation rate than pure mineral diesel. The resulting fuel blends significantly reduced the negative environmental impact, in terms of life cycle performance. The results of the research also show that, not only conventional methanol, but also ethanol and butanol can be used for the in situ process. By using them in an in situ process, it is also possible to reduce the negative impact of fuels on the environment. Even the use of small amounts of biodiesel accelerates the biodegradation of fuels and reduces the values of the environmental impact indicators. An even higher addition of biodiesel could significantly improve the life cycle performance, but higher levels of biodiesel in blends with mineral diesel have been found to increase nitrogen oxide emissions.

Author Contributions: Conceptualization, V.M. and E.S.; methodology, V.M. and E.S.; software, M.S.; validation, formal analysis and investigation, V.M., M.S. and E.S.; resources, E.S.; data curation, M.S.; writing—original draft preparation, M.S. and E.S.; writing—review and editing, V.M.; visualization, V.M., M.S. and E.S.; supervision, V.M. All authors have read and agreed to the published version of the manuscript.

Funding: This research received no external funding.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Not applicable.

Acknowledgments: The authors wish to thank Pre Sustainability for providing the software SimaPro 9.1.1 for the study presented in this paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Yassine, M.H.; Wu, S.; Suidan, M.T.; Venosa, A.D. Aerobic biodegradation kinetics and mineralization of six petrodiesel/soybean-biodiesel blends. *Environ. Sci. Technol.* **2013**, *47*, 4619–4627. [[CrossRef](#)] [[PubMed](#)]
2. Silva, G.S.; Rezende, R.P.; Romano, C.C.; Dias, J.C.T.; Marques, E.L.S.; Lobo, I.P.; da Cruz, R.S. An outlook on microbial behavior: Mimicking a biodiesel (B100) spill in sandy loam soil. *Fuel* **2019**, *235*, 589–594. [[CrossRef](#)]
3. Hawrot-Paw, M.; Koniuszy, A.; Zaja, G.; Szyszlak-Darglowicz, J. Ecotoxicity of soil contaminated with diesel fuel and biodiesel. *Sci. Rep.* **2020**, *10*, 16436. [[CrossRef](#)] [[PubMed](#)]
4. Leme, D.M.; Grummt, T.; Heize, R.; Sehr, A.; Renz, S.; Reinel, S.; de Olivera, D.P.; Ferraz, E.R.A.; Rodrigues, M.R.; Machado, M.C.; et al. An overview of biodiesel soil pollution: Databased on cytotoxicity and genotoxicity assessments. *J. Hazard. Mater.* **2012**, *199*, 343–349. [[CrossRef](#)] [[PubMed](#)]
5. Cruz, J.M.; Montagnoli, R.N.; Bidoia, E.D. Biodegradation of Soybean Biodiesel Generates Toxic Metabolites in Soil. *Water Air Soil Pollut.* **2020**, *231*, 429. [[CrossRef](#)]
6. Bamgbose, I.A.; Anderson, T.A. Assessment of three plant-based biodiesels using a daphnia magna bioassay. *Environ. Sci. Pollut. Res.* **2017**, *25*, 4506–4515. [[CrossRef](#)] [[PubMed](#)]
7. Bamgbose, I.A.; Anderson, T.A. Ecotoxicity of three plant-based biodiesels and diesel using *Eisenia fetida*. *Environ. Pollut.* **2020**, *260*, 113965. [[CrossRef](#)]
8. Pikula, K.S.; Zakharenko, A.M.; Chaika, V.V.; Stratidakis, A.K.; Kokkinakis, M.; Waissi, G.; Rakitskii, V.N.; Sarigiannis, D.A.; Hayes, A.W.; Coleman, M.D.; et al. Toxicity bioassay of waste cooking oil-based biodiesel on marine microalgae. *Toxicol. Rep.* **2019**, *6*, 111–117. [[CrossRef](#)]
9. Eck-Varanka, B.; Kováts, N.; Horváth, E.; Ferincz, Á.; Kakasi, B.; Nagy, S.T.; Imre, K.; Paulovits, G. Eco and genotoxicity profiling of a rapeseed biodiesel using a battery of bioassays. *Ecotoxicol. Environ. Saf.* **2018**, *151*, 170–177. [[CrossRef](#)]
10. Cruz, J.M.; Tamada, I.S.; Lopes, P.R.M.; Montagnoli, R.N.; Bidoia, E.D. Biodegradation and phytotoxicity of biodiesel, diesel, and petroleum in soil. *Water Air Soil Pollut.* **2014**, *225*, 1962. [[CrossRef](#)]
11. Woźniak-Karczewska, M.; Lisiecki, P.; Białas, W.; Owsianiak, M.; Piotrowska-Cyplik, A.; Wolko, L.; Ławniczak, L.; Heipieper, H.J.; Gutierrez, T.; Chrzanowski, L. Effect of bioaugmentation on long-term biodegradation of diesel/biodiesel blends in soil microcosms. *Sci. Total Environ.* **2019**, *671*, 948–958. [[CrossRef](#)]
12. Prince, R.C.; Haitmanek, C.; Lee, C.C. The primary aerobic biodegradation of biodiesel B20. *Chemosphere* **2008**, *71*, 1446–1451. [[CrossRef](#)]
13. Vauhkonen, V.; Lauhanen, R.; Ventelä, S.; Suojaranta, J.; Pasila, A.; Kuokkanen, T.; Prokkola, H.; Syväjärvi, S. The phytotoxic effects and biodegradability of stored rapeseed oil and rapeseed oil methyl ester. *Agric. Food Sci.* **2011**, *20*, 131–142. [[CrossRef](#)]
14. Kazanceva, I.; Sendžikienė, E.; Sendžikaitė, I. Evaluation of biodegradability and stability of biodiesel fuel and its mixtures. *Agric. Sci.* **2017**, *24*, 101–107. [[CrossRef](#)]
15. Sendžikienė, E.; Makareviciene, V.; Janulis, P.; Makareviciute, D. Biodegradability of biodiesel fuel of animal and vegetable origin. *Eur. J. Lipid Sci. Technol.* **2007**, *109*, 493–497. [[CrossRef](#)]
16. Mariano, A.P.; Tomasella, R.C.; Oliveira, L.M.D.; Contiero, J.; Angelis, D.F.D. Biodegradability of diesel and biodiesel blends. *Afr. J. Biotechnol.* **2008**, *7*, 1323–1328.
17. Horel, A.; Schiever, S. Influence of constant and fluctuating temperature on biodegradation rates of fish biodiesel blends contaminating Alaskan sand. *Chemosphere* **2011**, *83*, 652–660. [[CrossRef](#)]
18. Hafzan, C.; Noor, Z.Z.; Hussein, N.; Sabli, N.S.M. Life cycle assessment of diesel blending production. *Environ. Eng. Res.* **2021**, *26*, 200297. [[CrossRef](#)]
19. Chung, Z.L.; Tan, Y.H.; Chan, Y.S.; Kansedo, J.; Mubarak, N.M.; Ghasemi, M.; Abdullah, M.O. Life cycle assessment of waste cooking oil for biodiesel production using waste chicken eggshell derived CaO as catalyst via transesterification. *Biocatal. Agric. Biotechnol.* **2019**, *21*, 101317. [[CrossRef](#)]
20. Farrell, S.; Cavanagh, E. An introduction to life cycle assessment with hands-on experiments for biodiesel production and use. *Educ. Chem. Eng.* **2014**, *9*, e67–e76. [[CrossRef](#)]
21. Collotta, M.; Champagne, P.; Tomasoni, G.; Alberti, M.; Busi, L.; Mabee, W. Critical indicators of sustainability for biofuels: An analysis through a life cycle sustainability assessment perspective. *Renew. Sustain. Energy Rev.* **2019**, *115*, 109358. [[CrossRef](#)]
22. Vargas-Ibañez, L.T.; Cano-Gómez, J.J.; Zwolinski, P.; Evrard, D. Environmental assessment of an animal fat based biodiesel: Defining goal, scope and life cycle inventory. *Procedia CIRP* **2020**, *90*, 215–219. [[CrossRef](#)]
23. Sendžikienė, E.; Makareviciene, V.; Kazanceva, I. Life Cycle Analysis of Rapeseed Oil Butyl Esters Produced from Waste and Pure Rapeseed Oil. *Pol. J. Environ. Stud.* **2018**, *27*, 829–830. [[CrossRef](#)]

24. Organisation for Economic Cooperation and Development OECD. *OECD Guideline for Testing Chemicals: 301 Ready Biodegradability*; Adopted by the Council on 17 July 1992-Ready; OECD: Paris, France, 2021; pp. 1–62. Available online: <https://www.oecd.org/chemicalsafety/risk-assessment/1948209.pdf> (accessed on 25 October 2021).
25. Sendžikienė, E. Usage of Fatty Wastes of Agricultural Origin for the Production of Biodiesel. Ph.D. Thesis, Lithuanian University of Agriculture, Akademija, Lithuania, 2005.
26. Kazanceva, I. Reduction of Environmental Pollution by Using Biobutanol for the Production of Biodiesel Fuel. Ph.D. Thesis, Lithuanian University of Agriculture, Akademija, Lithuania, 2012.
27. U.S. Department of Energy. 2020. Available online: <https://www.energy.gov/> (accessed on 11 September 2021).
28. Harding, K.G.; Dennis, J.S.; von Blottnitz, H.; Harrison, S.T.L. A life-cycle comparison between inorganic and biological catalysis for the production of biodiesel. *J. Clean. Prod.* **2008**, *16*, 1368–1378. [[CrossRef](#)]
29. Gade, A.L.; Hauschild, M.Z.; Laurent, A. Globally differentiated effect factors for characterising terrestrial acidification in life cycle impact assesment. *Sci. Total Environ.* **2021**, *761*, 143280. [[CrossRef](#)] [[PubMed](#)]
30. Spirinckx, C.; Ceuterick, D. Biodiesel and fossil diesel fuel: Comparative life cycle assessment. *Int. J. Life Cycle Assess.* **1996**, *1*, 127–132. [[CrossRef](#)]