Emissions from urban bus fleets running on biodiesel blends under real-world operating conditions: Implications for designing future case studies

Mohammad Ali Rajaeifar\textsuperscript{a,\textasteriskcentered}, Meisam Tabatabaei\textsuperscript{b,c,d,e}, Mortaza Aghbashlo\textsuperscript{e}, Abdul-Sattar Nizami\textsuperscript{f}, Oliver Heidrich\textsuperscript{g}

\textsuperscript{a} School of Engineering, Newcastle University, Newcastle upon Tyne, NE1 7RU, United Kingdom
\textsuperscript{b} Faculty of Plantation and Agrotechnology, University Teknologi MARA, 40450, Shah Alam, Selangor, Malaysia
\textsuperscript{c} Biofuel Research Team (BRTeam), Karaj, Iran
\textsuperscript{d} Microbial Biotechnology Department, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education, and Extension Organization (AREEO), Karaj, Iran
\textsuperscript{e} Department of Mechanical Engineering of Agricultural Machinery, Faculty of Agricultural Engineering and Technology, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran
\textsuperscript{f} Center of Excellence in Environmental Studies (CEES), King Abdulaziz University, Jeddah, Saudi Arabia
\textsuperscript{g} School of Engineering, Tyndall Centre for Climate Change Research, Newcastle University, Newcastle upon Tyne, NE1 7RU, United Kingdom

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ABSTRACT

The present study provides firstly a comprehensive review of studies on measuring the impacts of different biodiesel blends on exhaust emissions characteristics of urban buses under real-world operating conditions. Secondly, this paper discusses the errors that can be made in conducting case studies. Thirdly and finally, it shows lessons learned and provides guidelines to setup case studies, conduct the measurements, perform the statistical analysis and report the results to policy makers and the wider audience. To achieve climate change mitigation targets, using alternative fuels, e.g., biodiesel, hydrogen or electric (EVs) for the urban fleets requires an in-depth analysis of the impacts under real-world operating conditions. Such experiments are generally very complex as numerous factors could directly or indirectly interfere with the results produced and potentially jeopardize the integrity of the research and the conclusions drawn. Results of the present research show that some vital parameters were ignored by many of the studies performed including the statistical uncertainties, driving cycle uncertainties and fuel uncertainties. Lack of appropriate experimental designs or clear assertions about the level of significance for differences in emissions/fuel consumption between alternative fuels (i.e., biodiesel) and the reference fuel used (i.e., diesel) could be regarded as the main weaknesses. Moreover, many other overarching and very influential factors (e.g., covariates/confounders) can interfere with the research outcomes as these were mostly overlooked by the reviewed studies. A careful and complete experimental design for assessments of alternative fueled vehicles (are critical when conducting real-world operating condition tests. The study findings help to formulate the guidelines for assessing real-world operating condition experiments to achieve the most feasible and meaningful research outcomes that will have significant implication for local and global policy makers. The guidelines are of use for all types of research studies that want to evaluate the effects of alternative fuels for any transportation fleet.

1. Introduction

The rate of urbanization is continuously increasing worldwide \cite{1,2}, and more than half of the world’s population is living in cities \cite{3}. Urban life is inevitably integrated with public transport systems that are predominantly (approximately 95%) relying on fossil fuel resources \cite{4}. Generally, fossil fuel combustion is regarded as one of the main culprits of anthropogenic air pollution and greenhouse gas (GHG) emissions, that have intensified the global environmental concerns in cities and towns \cite{5-8}. In the cities of developing nations, estimations show an increase in the share of urban energy use to 73% (from 66% of the world’s energy use in 2006) and of CO\textsubscript{2} emissions to 76% (from 71% of the global energy-related CO\textsubscript{2} emissions in 2006) by the year 2030 \cite{4}. The transportation system including urban transportation is considered...
as one of the urban energy contributors and the primary source of GHG emissions, contributed to 14% of global GHG emissions in the year 2010 [9]. According to International Energy Agency (IEA) estimates, over 50% of global nitrogen oxide (NOx) emissions as well as 24% of global CO2 emissions are generated only by the transport sector [10, 11]. Beside CO2 and NOx, this sector is also responsible for air pollutants that can have local impacts first by creating air pollution issues e.g., SO2, particulate matter (PM), CO, volatile organic compounds (VOCs), and indirectly, ozone. This situation could be more challenging in cities where people are directly exposed to street-level air pollution of road transport origin [9], leading to numerous human health problems and deaths. Air pollution could also pose significant threats to the environment, the economy and food security [10].

The strategies implemented in the transportation sector especially in the cities in preventing local air pollution and GHG emissions are critical as they could consequently reduce the progression of health hazards (local) and climatic change (global). Implementation of renewable energy in urban transportation system is a promising strategy that would simultaneously ensure the reduction of exhaust emissions as well as the conservation of energy resources [5, 12]. Compared to other renewable energy sources, biofuels have emerged as a viable option to the indigenous availability of raw material (biomass) all year around. It is argued that they are the only renewables that could partially or wholly replace the existing conventional fuels used in the transportation sector in an internal combustion engine [13–17]. Other low carbon strategies for the transport sector such as the electrification, using e.g., lithium-ion batteries, fuel cells, etc. are also pursued and operationalized by some governments [18, 19]. Electrification and Electric Vehicles (EVs) could also bring significant benefits in terms of reducing non-renewable energy demand for urban transportation fleets [20]. However, such strategies have unintended consequences such as supply chain issues and end-of-life treatment (reuse-recycling-disposal) of the spent batteries which need further research and strategic planning. Such research projects are still ongoing paving the way toward optimization of the full life cycle of lithium-ion batteries utilized in the automotive sector, from material management issues, through use phase, to their reuse/recycling stage [21].

Biodiesel is the most promising alternative fuel for diesel engines [22, 23] and can be produced from a variety of vegetable oils and animal fats [24–26]. Among the different methods for biodiesel production, transesterification is regarded as the most popular process in the industry since it provides the highest conversion efficiency and at the lowest cost [27, 28]. Transesterification is a reaction in which triglycerides reacts with an alcohol in the presence of a catalyst (to improve the reaction rate and yield) to produce esters and glycerol [29–32]. Different types of enzymes as well as homogeneous/heterogeneous acids or base catalysts are conventionally used for transesterification reaction. Nevertheless, some advanced methods for biodiesel production has been proposed in order to improve the efficiency, simplicity or cost of the process, such as nanocatalytic [33], ultrasound-assisted [34, 35], microwave-assisted [36], in-situ transesterification [37], supercritical fluid (SCF) [38], superheated vapor (SHV) [39], subcritical [40], and membrane-assisted technologies [41]. Since catalytic reactions come with a lot of disadvantages [42], researchers developed some non-catalytic alternative methods such as SCF and SHV. These processes could facilitate the easy separation of products [42], increasing the rate of reaction (and thus efficiency) [42], and eliminating the necessity of pre-treatment steps for various feedstock (and thus reducing costs) [43–45]. Further explanation of SCF and SHV technologies could be found in Karmakar and Halder [42]. Beside the mentioned development in biodiesel production processes, there is also a great focus on plant genetic engineering for sustainable biodiesel production. In such context, production of high performance-oil plants varieties for commercial biodiesel production could be achieved through next generation sequencing (NGS), omics technologies, and genetic engineering pathways [46].

Biodiesel combustion results in less air pollution compared to petroleum diesel due to its higher oxygen content and lacking of sulfur and aromatic compounds [47, 48]. More specifically, biodiesel combustion results in less PM, CO, and unburned hydrocarbons (UHC) emissions [49–51]. In this regard, biodiesel could also be a promising alternative for urban buses that are the primary source for high levels of PM and CO emissions in urban areas. Apart from the mentioned advantages of biodiesel, a number of studies have also reported increases in NOx emissions in response to biodiesel inclusion in fossil diesel [52–54]. However, there is no general agreement in this regard in the published literature [55–58], and the results differ with fuel properties, engine characteristics, as well as the operating conditions. For example, a comprehensive review on NOx formation mechanism showed that several factors could contribute to an increase in NOx emissions from biodiesel combustion [59]. These factors encompassed: rising adiabatic flame temperature, advanced injection timing, increasing heat release rate, more stoichiometric burning, decreased radiative heat transfer from soots, growth in premixed burn fraction, widespread high-temperature distribution areas and reduced spray cone angle [59]. Therefore, judgment about NOx emissions is much more complex.
Moreover, it has been reported that biodiesel can provide lower carbon deposits and wears of the vital engine parts [49,60,61]. However, some long-term endurance engine tests showed an increase in the carbon deposition on some engine components as well as wear while using biodiesel [62,63]. For example, a recent review conducted by Hoang and Le [64] showed that biodiesel-based fuel consumption could lead to an increase of deposits in the injector nozzles, injector tips, and injector holes of diesel engines compared to neat diesel. The review also highlighted the effects of fuel components, temperature (in the injector tip) and injector configuration as the three main factors affecting the level of deposit formation. In another study, Dhar and Agarwal [65] performed 250 h long endurance test on the effect of 20% Karanja oil methyl ester blend (KOME20) on engine wear and found that biodiesel increases carbon deposits on piston top, cylinder head, and injector tip. While visual inspection determined that biodiesel could reduce wear of pistons, piston rings, valves, liners, and small end bearings of the connecting rods, an increase in wear of big end bearing of the connecting rods, main bearings and crank pins was recorded [65]. Overall, literature review conducted herein highlighted that the performance and emission characteristics of biodiesel could vary depending on variations in fuel properties (i.e., feedstock, blending ratio, density, cetane number, viscosity, and transesterification route), engine characteristics, as well as the operating conditions (i.e., load percentage, and driving cycles) [13,66-72]. These are the main factors behind different results observed for some emissions/engine performance indices mainly reported in the literature.

Studies on the effects of biodiesel on performance and emission characteristics of diesel engines fall into two categories such as laboratory chassis dynamometer tests (steady-state operating condition, also known as bench-scale examinations) and real-world operating condition tests. However, there are only a few studies performed under real-world operating conditions. Comparatively, steady-state operating condition studies are commonly carried out based on standard driving cycles at considerably less costs and experimental burdens. In these tests, driving cycles are generally classified into two main groups of transient driving cycles and modal driving cycles. Transient driving cycles involve numerous speed variations, representing the typical conditions experienced in on-road driving (e.g., EPA Federal Test Procedure, American FTP-75, unofficial European Hyzem driving cycles). Whereas modal driving cycles involve straight acceleration and constant speed periods (e.g., Japanese 10–15 Mode and JC08 cycles). The driving cycles are measured and modeled directly from the driving patterns for a specific country/region or using approved international test standard cycles for the whole world that are mostly derived theoretically.

These standard driving cycles practiced in steady-state operating condition studies allow repeatable conditions and could further facilitate the comparison of the results among the fuels and vehicles investigated [73]. When scrutinizing the effects of a dedicated blend of biodiesel on a diesel engine’s performance and emission characteristics, standard driving cycles could effectively help to explore all possible impacts of the alternative fuels. Nevertheless, the results especially the emission values achieved by these types of measurements could not be used as the exact representation of the emission values under real-world operating conditions [74]. This is mainly due to the fact that there are many influencing factors in real-world operating condition that significantly affect the exhaust emissions and the fuel consumption of a vehicle, making the results different from those estimated using standard drive cycle methods [74,75]. In addition, the employed driving cycles are mostly theoretical cycles, or they are based on the average data collected on drivers’ behavior and vehicle vs. road conditions for a specific region. It is also evident to some extent that tunnel tests and transient test studies are not accurate representatives of real driving conditions either; they are comparatively far closer to real-world operating conditions though. Therefore, measuring the emission impacts of various biodiesel blends under real-world operating conditions is critical in order to decide on the best scenario for an intended country/region/city. Such findings could realistically show the engine performance and emission properties, especially in urban transportation fleets and this was the focus of this study.

Considering the rapid development in the biofuel production technologies which offers different biofuel combinations, investigating the potential benefits/impacts from use of these fuel in real-world operating condition in an urban fleet could have a significant impacts on achieving a sustainable public transportation system [6,76]. This study, for the first time, has comprehensively examined and discussed the studies conducted on measuring the impacts of biodiesel blends on emission characteristics of diesel engines under real-world operating conditions. Furthermore, the key issues in conducting real-world operating condition tests are presented and guidelines to setup case studies, conduct the measurements, perform the statistical analysis and report the results are recommended. The proposed guidelines could be of use for all types of engines and research studies aimed at evaluating the effects of different alternative fuels for any transportation fleet.

2. Methodology

2.1. Collection and filtration criteria

The focus of the present research was on the procedures of performing real-world operating condition experiments rather than discussing the trends of different exhaust emissions and their significance. These procedures are of utmost importance in order to highlight key effective issues during real-world operating condition experiments and to consequently, develop and present a set of scientific guidelines for future studies. These guidelines would be expected to assist with generating more complete, consistent, and reliable real-world emission results.

In order to identify the most common errors in real-world operating condition studies as well as effective factors, a literature review was conducted using a systematic search method. Accordingly, the review was pursued through collecting, filtering, evaluating and discussing existing literature related to exhaust emissions of biodiesel in real-world operating condition studies. As the first stage, the paper collection was performed by selecting papers/reports published from 1990 to 2018. This step was conducted through searching combined keywords on major databases and publisher websites, such as Google Scholar, Scopus, Science Direct, Springer link, and Wiley Online Library. ‘Biodiesel’, ‘Real-world operating’, ‘On-road emissions’, ‘Urban buses’, ‘Bus idling’, ‘Urban mass transit buses’, ‘Tailpipe emissions’, ‘Mobile diesel emissions testing’, ‘Public transport bus emissions’, ‘Vehicle operation parameters’ were the main keywords employed in order to collect the related information.

In the second step, a filtration of literature studies was performed based on the following criteria:

- At least one bus was recruited during the experiments.
- At least one engine operation mode under real-world operating conditions (idle and/or motion modes) was included in the experiments. Moreover, studies based on fleet data were also considered.
- At least one combination of biodiesel with diesel (BXYD with X representing biodiesel percentage and Y denoting diesel percentage; ranging from B1 to B100) or with diesel and other fuels (e.g., ethanol) was examined under real-world operating condition.
- Research studies examining tailpipe emissions or fuel consumption (or both) for buses operating under real-world condition.

2.2. Evaluation criteria and development of guidelines

The next step was to evaluate the selected papers. The evaluation was performed to identify the critical points in performing real-world operating condition tests using urban buses fueled with biodiesel
blends. The evaluation criteria were as follows:

- Number of buses used in the experiments and their grouping quality,
- Existence of any pre-test inspection/recording of experimental materials,
- Test conditions (idle or motion engine mode, test route(s), state of exhaust emission/fuel consumption recording, year -and time considerations-of performing the experiments),
- Fuel/s used,
- The existence of any covariate/cofounder recording during the experiments,
- Type of statistical analysis used.

Accordingly, the critical points including the most common errors in real-world operating condition studies as well as the effective factors on the outcomes were determined.

Finally, a set of guidelines were developed based on the identified critical points under three main key issue categories. The guidelines are expected to assist with performing more accurate experimental studies on the effects of different alternative fuels for any transportation fleet.

3. Global experiences on experimenting biodiesel application in urban buses

3.1. United States of America (USA)

Based on the latest available data on biofuels production, United States of America (USA) was the largest producer of biofuels in the year 2017 producing 36936 thousand tonnes oil equivalent (Ktoe) that is 43.9% of the world total biofuel production [77]. The USA biofuels production amount has increased 3-folds between the years 2007 and 2017-reaching from 39% of the world total biofuel production to 43.9% in the same period-, showing the country's great interest in biofuels production (Fig. 1). On the other hand, the USA consumed more than 2285 trillion Btu of biofuels in the year 2017 that is more than 132% growth compared with the year 2007; out of this, about 249.4 trillion Btu was related to biodiesel consumption (Fig. 2).

Since the use of biodiesel has increased considerably in the USA, there are many studies conducted on different aspects of biodiesel production and consumption. Nevertheless, the number of real-world operating condition studies is much lower than the laboratory (steady-state operating condition) ones. Table 1 has shown the results of studies focused on emission characteristics of bus engines fueled by different types of biodiesel through real-world operating conditions in the USA.

In a study conducted by Schumacher et al. [79], the real-world impacts of a biodiesel blend on urban bus engine exhaust emissions, maintenance, reliability, cost, fuel economy, and safety were examined at St. Louis (Missouri). A total of 10 buses (6V92 TA Detroit Diesel) were selected for the experiments in which 5 buses were operated on 20% biodiesel in combination with 80% diesel (B20) fuel blend while 5 other buses were run on petroleum-based low sulfur diesel fuel (LSD) as the control of treatment. While most of the steady-state engine operating condition tests argue that the application of biodiesel-diesel blends would reduce PM emissions and increase NOx emissions [41], their examination led to unexpected results. More specifically, PM emissions did not change while the NOx level was decreased by nearly 10% when B20 blend was used. Moreover, fuel consumption rate was lower for the buses powered by B20 blend, again contrary to most of the steady-state engine operating condition tests claiming fuel consumption increases when using biodiesel-diesel blends [41]. Whereas the maintenance costs were slightly higher for the measured buses. Although some of the differences observed among the reported values may have originated from the driving cycles used in the steady-state operating condition, this study also failed to perform a careful and complete experimental design that increased the statistical uncertainty of the results. For example, based on their report, examining the data by excluding those concerning the bus No. 8441 led to opposite results indicating a 2% increase in NOx and a 29% reduction in total PM value. Therefore, it could be concluded that the implementation of appropriate experimental design (as completely discussed in Section 3 of this manuscript) could be critically vital to the correctness and accuracy of the conclusions drawn. In addition to the above-mentioned shortcomings, it should also be noted that in the study conducted by Schumacher et al. [79], the covariate/confounder factors (e.g., mileage that each bus traveled before the experiments, ambient temperature, humidity, etc.) were also overlooked which could have caused a deflection in the average values. Covariate/confounder factors are continuous variables that are not part of the main experimental error and cannot be randomized but can be measured/recorded before or during the test. These factors are also introduced and discussed in Section 4.

In a different study, Hearne [80] carried out a comprehensive study on bus idling and mobile emissions with a focus on using alternative fuels in mobile emissions testing for different school buses in New Jersey. Exhaust gas emissions measurements for both types of experiments included CO, CO2, NO2, NO, SO2, O2, UHC, and PM. In order to measure the emissions from the school buses during idling conditions, they selected three buses from the most commonly-used types of buses in New Jersey school transportation system (i.e., International T-444E, International DT-466E, and Ford Cummins 5.9L ISB Series) and fueled them with neat diesel. Same three school buses were also examined in the mobile emission testing, running on B20 (in combination diesel No.2), B20 (in combination with ultra-low sulfur diesel (ULSD)), neat diesel. Fig. 1. USA biofuels production between 2007 and 2017 [78].
Table 1
Emission characteristics of bus engines fueled by different types of biodiesel reported in real-world operating condition studies in the USA.

<table>
<thead>
<tr>
<th>Region</th>
<th>Number of buses</th>
<th>Reference fuel</th>
<th>Tested fuel blends</th>
<th>Emission results</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Missouri (St. Louis)</td>
<td>10</td>
<td>LSD</td>
<td>LSD80B20</td>
<td>↓</td>
<td>[79]</td>
</tr>
<tr>
<td>New Jersey</td>
<td>3</td>
<td>LSD</td>
<td>LSD80B20 (engine: International T-444E)</td>
<td>↑ / ↓</td>
<td>+ / −</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ULSD80B20 (engine: International T-444E)</td>
<td>↑ / ↓</td>
<td>+ / −</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ULSD (engine: International T-444E)</td>
<td>+</td>
<td>− / −</td>
</tr>
<tr>
<td></td>
<td></td>
<td>LSD</td>
<td>LSD80B20 (engine: International DT-466E)</td>
<td>↓ / ↑</td>
<td>− / −</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ULSD80B20 (engine: International DT-466E)</td>
<td>−</td>
<td>− / −</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ULSD (engine: International DT-466E)</td>
<td>−</td>
<td>− / −</td>
</tr>
<tr>
<td>Texas</td>
<td>5</td>
<td>ULSD</td>
<td>ULSD80B20 (biodiesel type: market blend)</td>
<td>nc</td>
<td>− / −</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ULSD80B20 (biodiesel type: Soybean oil)</td>
<td>nc</td>
<td>− / −</td>
</tr>
<tr>
<td>Idaho (Meridian city)</td>
<td>200</td>
<td>ULSD</td>
<td>ULSD80B20</td>
<td>+</td>
<td>− / −</td>
</tr>
<tr>
<td>Ohio (Toledo)</td>
<td>14</td>
<td>ULSD</td>
<td>ULSD80B20</td>
<td>↓ / +</td>
<td>− / −</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ULSD95B5 (Idle engine operation)</td>
<td>↑</td>
<td>− / −</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ULSD90B10 (Idle engine operation)</td>
<td>+ / −</td>
<td>− / −</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ULSD80B20 (Idle engine operation)</td>
<td>+ / −</td>
<td>− / −</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ULSD95B5 (On-road operation)</td>
<td>+ / b</td>
<td>− / −</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>ULSD90B10 (On-road operation)</td>
<td>+ / b</td>
<td>− / −</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>ULSD80B20 (On-road operation)</td>
<td>+ / b</td>
<td>− / −</td>
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</table>

<table>
<thead>
<tr>
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</thead>
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<td>38</td>
<td>LSD &amp; ULSD</td>
<td>ULSD80B20</td>
<td>−</td>
<td>[85]</td>
</tr>
<tr>
<td>Ohio (Toledo)</td>
<td>10</td>
<td>ULSD</td>
<td>ULSD80B20</td>
<td>−</td>
<td>[86]</td>
</tr>
<tr>
<td>Ohio (Toledo)</td>
<td>10</td>
<td>ULSD</td>
<td>ULSD80B20</td>
<td>−</td>
<td>[87]</td>
</tr>
</tbody>
</table>

DXBY = X% diesel in combination with Y% biodiesel, LSD = Low sulfur diesel, ULSD = Ultra low sulfur diesel.

The symbols ↑/↓ and +/- represent the trend and the intensity of variations observed in each study, respectively, and cannot be used as a means of comparison among different studies.

nc = no change in comparison with the reference fuel.

a Only NO emissions was reported.

b The level of emissions under this operation condition was lower than that of the idle engine operation.
ULSD, and diesel No. 2 while their actual on-road emissions data were acquired. The results of the idle-engine testing showed that the measured CO emissions decreased from 10 to 40% by increasing the ambient temperature while NOx emissions decreased with an increase in the humidity. This simply confirmed the effect of confounder factors on some of the dependent variables. Moreover, HC and PM emissions did not show any relationships with temperature, humidity, or fuel consumption. The results of the mobile testing revealed that the emission impacts of NOx originated from biodiesel combustion varied depending on the employed engine and the type of base fuel to which biodiesel was added. Moreover, using the B20 blends led to decreases in UHC emissions in all the investigated engines. On the contrary, the B20 blends reduced CO and PM emissions for the Cummins and T444E engines but showed no effect on the CO emissions from the DT466E engine (a 22% reduction in PM for the DT466E was observed though). In view of CO2 emissions, no exact correlation was reportedly found between this emission and the application of biodiesel. Overall, although interesting results were produced in the study, the main weakness of the study lies in the lack of appropriate experimental design as well as using a low replication number (sample size) for each type of engine (only 1 replication), collectively leading to the low statistical certainty of the results obtained. Another deficiency would be ignoring to correct the average of each emission type based on the recorded covariate/confounder factors (e.g., the mileage each bus traveled before the experiments, ambient temperature, and humidity). This deficiency could be the driving factor behind the partial (increasing or decreasing) trends observed for emissions making it difficult to draw an overall trend pattern for a given emission. Similar defects could also be found in the CO and PM emissions in mobile emissions testing [80].

In a comprehensive study, Proc et al. [81] examined nine identical 40-ft transit buses (Cummins ISM 2000) fueled by B20 and diesel for two years in Colorado. The mileage accumulation, fuel economy, maintenance costs, road calls (a call-in to dispatch unit mainly reporting a mechanical problem happened), delivered fuel properties, and engine oil performance were the factors monitored during real-world operating conditions in the study. The engine emission test was only performed using a chassis dynamometer (steady-state operating condition) while the fuel economy was recorded under both steady-state and real-world operating conditions and the results obtained were compared. Although the study did not examine the tailpipe emissions under real-world operating conditions, its concluding remarks on fuel economy based on the real-world data would still be of interest. More specifically, their results showed that the fuel economy was the same for the buses fueled by diesel and B20 under the real-world operating condition while the steady-state emission testing showed a 2% reduction in the fuel economy when using B20. The differences in engine and fuel systems maintenance costs for the buses running on B20 and diesel as well as differences in the mileage between road calls were not significant. Engine oil analysis results revealed that there were no additional wear metals because of B20 application compared with neat diesel. Moreover, the steady-state emission testing showed that B20 reduced emissions of all regulated pollutants (i.e., NOx, Total hydrocarbon –THC, CO, and PM). Comprehensiveness of the study and the application of statistical analysis for testing the significance level of differences for each item discussed were among the main points of the investigation conducted by Proc et al. [81]. However, the authors failed to monitor and exclude the effects of covariate/confounder factors taking place under the real-world operating condition from the average results, and this could have led to some degrees of uncertainty in the results produced. In spite of that, it is worth highlighting that the long test duration (2 years) and the high mileage practiced in their study must have assisted in reducing the uncertainty of the results mentioned above. Overall, it could be concluded that the reported results would have been more convincing if they had added the impacts of covariates/confounders as well.

In the year 2006, Farzeneh et al. [82] investigated the impacts of biodiesel (B20 produced from market oil blend and soybean oil) on NOx emissions emitted from 5 diesel school buses in Texas. The data collection part of the study took place at TTTi test track located at Riverside Campus of Texas A&M University, Texas. However, to simulate the real driving condition, two driving cycles were developed based on the real rural and urban drive cycle data collected using a global positioning system (GPS). The buses were equipped with in-line, six-cylinder international engines (Series D466 and D466E), and each bus was loaded with 56 sandbags (total load of 1270 kg) to simulate an average loading situation (approximately equal to 30 children on-board). The findings of the study indicated that B20 did not significantly affect NOx emissions for Texas school bus fleet. However, the buses older than 1994 emitted approximately 1.6 times the amount of NOx compared with the buses manufactured after the year 1994. Likewise, B20 had a significantly higher HC reduction rate for older buses investigated in the study (i.e., older than 1994). This result showed the significant effect of the vehicle’s age (engine model year) on the tailpipe emissions level. Their results also showed that the differences in the driving cycles led to differences in NOx, CO, CO2, and HC emissions. They argued that more transient driving profiles (generally taking place in urban driving conditions), i.e., more stops, more acceleration/deceleration periods, and shorter cruising intervals could increase such emissions. Overall, although each tested scenario was repeated for three times which increased the robustness of the results, a better study would have also examined the significance level of the average results while monitoring and excluding the effects of covariate/confounder factors as well. Moreover, in order to be able to generalize the results to the whole bus transportation fleet in a given region, it is essential to consider buses that are the most available on the market of interest. This would ensure the possibility of future purchases as well as the ability to generalize the results to the whole bus transportation fleet.

In a field study conducted by Mazzoleni et al. [83], a fleet of school buses (i.e., 200 school buses) in Meridian, Idaho, was considered to examine the effects of biodiesel usage (B20) on gaseous and PM fuel-based emission factors under real-world operating conditions. The pilot plan involved a variety of engines, e.g., Detroit Diesel 8.2 L, Ford Brazilian, Cummins B-Series 5.9 L, Ford DT 466/International, and Caterpillar. The results implied that the use of B20 fuel instead of 100% neat diesel substantially increased PM and CO emissions while decreased NO and HC emissions. However, the biodiesel used in the study was analyzed at the end of the on-road experiment and was found to be off-specification, i.e., did not meet the ASTM D6751 biodiesel standards. This fact shows the necessity of rigorous quality control in the biodiesel production stage before actual utilization. The results also revealed that cold-start CO emissions and hot-stabilized HC emissions were also higher when using B20 while the other tailpipe emissions were not significantly different [83]. Although the study employed a correct and robust statistical analysis (with a huge number of buses), the off-specification fuel as well as the lack of monitoring and excluding the covariate/confounder factors could be regarded as the main weakness of the study.

Nerella [74] examined the exhaust emission variations from the public transit buses running on biodiesel blends (B5, B10, B20, and ULSD as the control of the treatment) in the city of Toledo, Ohio. The tests involved 14 buses (including 2 engines from ISB 275 Cummins and 12 engines from MBE900 Mercedes Benz), but the repeats were unequal for the different treatments. Two test cycles were designed for the real-world idle-engine and real-world on-road operating conditions. The idle-engine emission test results (only available for MBE engines) revealed that all the monitored pollutants (except CO2) increased in proportion to the biodiesel concentration in the base fuel (Table 1). This contradictory result showed that idle engine operation of a vehicle could have significant negative impacts on the environment and that the idling time of vehicles should be reduced. It is also worth mentioning that the idling operating condition must be exactly reported to
avoid wrong conclusions by the other researchers, i.e., cold-start or hot-start, fast or normal idle (revolutions per minute). The differences in idling conditions seem to have led to different results reported in Vijayan et al. [84] study, in which similar buses (37 Thomas buses from MBE900 Mercedes Benz) were examined in the fast-idle mode under B20 (19 buses) and ULSD (8 buses) fuels in the city of Toledo, Ohio. The average results from the idling study showed that B20 emitted lower concentrations of all the monitored pollutants (except for NOx), as compared with ULSD-fueled buses [84]. The results of the on-road testing (when the vehicle was in motion) revealed that as biodiesel concentration in the base fuel increased, the level of tailpipe emissions decreased (except for NOx emissions). More specifically, the tailpipe emission levels were less when the bus was in motion (compared with the idle-engine mode). This could be ascribed to the fact that vehicles in motion delivered appropriate amounts of fuel into the cylinder leading to complete combustion [74]. Moreover, when the engine worked in the idle mode, it did not run at its optimum temperature and conditions leading to incomplete combustion. It could be highlighted that the primary outcome of the Nerella's study was the identification of the important variables affecting the exhaust emission levels of buses running on biodiesel (in both on-road and idle-engine operation modes) using statistical regression analysis [74]. However, lack of monitoring and excluding the effect of covariate/confounder factors remained as the main weakness of the study.

In a study by Vijayan and Kumar [85], the main focus was placed on characterizing in-vehicle fine PM (≤PM2.5) behavior inside public transit buses operating on B20 in Toledo, Ohio. The bus fleet selected for the experiment involved 38 identical Thomas-built buses (Mercedes Benz MBE900 engines), and the experiment was conducted during two time periods. In the first time period, in the year 2003, all the 38 buses were operated on conventional diesel (petroleum diesel No.2) while in the second period at the end of the year 2006, the entire fleet was switched to alternative fuels, i.e., 19 buses used ULSD and the other 19 buses ran on B20/ULSD. The results demonstrated that -when the bus windows were kept open-the in-vehicle PM1.0 mass concentrations were lower inside the biodiesel-powered buses compared with diesel-fueled buses. The main conclusion of the study was that vehicle’s fuel type, operating periods (time of day when a vehicle operates), operation status, passenger counts, traffic conditions, and meteorological variations were the main factor affecting in-vehicle PM emissions [85]. Accordingly, it could be suggested that these factors could be recorded and monitored as confounder factors when considering PM emissions in total.

In a similar case study, Kumar et al. [86] used 10 different transit buses running on B20 (soybean biodiesel/ULSD) in order to evaluate the PM concentration and analyze its elements in the tailpipe emissions. The findings of this study indicated that using B20 could significantly help to decrease PM emissions by 17% on average. Moreover, newer transit buses showed a greater PM reduction (more than 98% on average) than old buses when using ULSD. Based on the elemental analysis results, the major elements found in the PM emissions were calcium (Ca), sodium (Na), and iron (Fe) both in the field and laboratory experiments. The main finding of the study was introducing positive matrix factorization (PMF) showing that four parameters, i.e., sources of oil (including fuel and engine oil), lubricant, engine parts, and ambient conditions significantly contributed to the generation of PM in the exhaust. The study assumed that the effects of ambient temperature and humidity on NOx emissions were negligible due to the high exhaust temperature and their collection method [46]. However, ambient temperature and humidity could also affect the intake air quality and consequently change the NOx emissions level. Therefore, a better study should have excluded the effect of these parameters as covariate factors on final results to enhance their statistical accuracy. Moreover, considering only the idle mode in PM measurement (hot and cold idle modes) and lack of appropriate experimental design are the other drawbacks associated with the study conducted by Kumar et al. [86]. A similar test procedure was also performed by Omidvarborna et al. [87]. A similar test procedure was also performed by Omidvarborna et al. [87] in which the total particulate matter (TPM) emissions from public transit buses using B20 (soybean biodiesel/ULSD) in idle modes was evaluated and compared with that of neat diesel. Similar to Kumar et al. [86], the findings of the study indicated that using B20 could help to decrease TPM emissions significantly. Similar suggestions made regarding the outcomes of Kumar et al.’s study [86] could also be considered in the case of the study performed by Omidvarborna et al. [87].

3.2. The European Union (EU)

The European countries experienced a significant shift in favor of diesel engines in the passenger car market in recent years. Besides the higher fuel efficiency of diesel engines and their technological improvements, restrict regulations and directives in the Member States are another important driving force behind this transformation [88]. Likewise, using biofuels in the transportation sector as well as biomass in the heat and power market are outlined in the EU through energy

![Fig. 3. EU biofuels production between 1990 and 2015](image-url)
and climate change package (CCP) as well as different directives. Based on the data presented in (Fig. 3), the EU biofuels production (liquid biofuels and biogas) increased by approximately 4260% between the years 1990 and 2015 (and more than 405% between the years 2004 and 2015) implying the synergic effect of different policies and programs implemented in favor of biofuels production in the union [89]. For instance, biodiesel production in the EU in the year 2015 stood at more than 14 billion liters accounting for about 40% of the global total [90]. Meanwhile, biofuel consumption (liquid biofuels and biogas) in the EU was more than 1234 trillion Btu in the year 2015 (showing more than 4531% growth compared with the year 1990) while biodiesel consumption was estimated at 458 trillion Btu (showing a great increase compared with the year 1990 in which the biodiesel consumption was almost very low, i.e., 0.25 trillion Btu; Fig. 4).

As biodiesel consumption has increased considerably in the EU, there have also been many studies conducted on many aspects of biodiesel production and consumption. Nevertheless, like as was presented and discussed for the USA earlier, the number of published reports on real-world operating condition tests is much lower than that of the laboratory ones. Table 2 shows the studies on the emission characteristics of bus engines fueled by different types of biodiesel reported under real-world operating condition in the EU. In a study conducted by Carraretto et al. [91], potentialities of biodiesel as an alternative fuel in boilers and diesel engines were investigated trough bench-test and on-road experimentation. The diesel engine used for the investigations was a UNIC 8220.12; a widely installed engine on local urban buses in Padova, Italy. For the means of on-road experiments, 4 urban buses were selected and examined under B30 and neat diesel (two urban buses for each fuel type) during December 2001–May 2002. Distances traveled, fuel consumed and emissions (CO2, CO, HC, and NOx) were monitored during the on-road experiment. The results of the study revealed that using B30 blend could reduce HC and CO emissions by 13.5% and 3% (on average), respectively, thanks to the higher oxygen content of biodiesel (compared with neat diesel). Whereas, this fuel blend led to an increase in NOx emissions by 9%. It should also be noted that although the average tailpipe emissions of CO2 showed no changes, the authors claimed that CO2 emissions throughout the biodiesel lifecycle would be reduced [91]. Lack of appropriate experimental design, as well as lack of information about routes, traveled and type of statistical analysis used, could be regarded as the main deficiencies jeopardizing the validity of the study results/judgments. Moreover, none of the covariate/confounder factors were recorded and subsequently excluded from the results of the real-world operating condition analysis.

Branco et al. [92] performed a comparative study on the quantification of energy consumption and emissions using petroleum diesel, B5, B20, and B100 in the urban Bus fleet of Évora Municipality, Portugal. For this purpose, 13 Euro 3 urban buses (types of engines were not mentioned) and four Euro 2 minibuses from the Évora transport fleet were selected. The major finding of the study was that biodiesel led to an increase in the NOx and CO2 emissions while decreased the other pollutants such as CO, PM, and VOCs. Unfortunately, the type of statistical analysis was not mentioned by the authors, making judgments on the validity of the results impossible. Moreover, the covariate/confounder factors were not monitored, and their effects were not excluded from the results either.

In a different investigation conducted by López et al. [93], on-road emissions of urban buses fueled by diesel and biodiesel (B20 and B100) in Madrid, Spain, were explored. Two Euro 4 urban buses (both with direct injection diesel engines, but with different exhaust gas after-treatment technologies) were selected for the experiments, and each fuel was examined for five test runs. The examination involved recording the data on CO, CO2, UHC, and NOx emissions, as well as fuel consumption and the speed data for the vehicle. In order to simulate the real driving condition, a driving cycle was developed for fuel economy and emission testing with onboard measurement equipment. The findings of the study demonstrated that by increasing the amount of methyl ester (biodiesel) in the blend, the CO, PM and UHC levels decreased while the NOx and CO2 levels increased. However, due to the lack of appropriate experimental design and a low number of replications (sample size), the results were deemed uncertain. Moreover, there was no information reported on the covariate/confounder factors. Some fluctuations were observed when shifting from B20 to B100 which was uncommon and could be originated from the experimental errors or the overlooking of the effects of the covariate factors. Examples of such uncommon observations were increases in PM emissions from 0.025 to 0.026 when shifting from B20 to B100 using exhaust gas recirculation (EGR) alongside diesel particulate filter (DPF) technology.

Biodiesel usage in the public transport system in Belgrade was investigated by Tica et al. [94] through the framework of a project called BIO-PEX. Four urban buses (MAND2866LUH22/Euro2) were involved in the experiments for a period of 38 days using 13000 l of B100. In order to compare diesel and B100, the tailpipe emissions including UHC, CO, SOx, polycyclic aromatic hydrocarbons (PAHs), nitrated polycyclic aromatic hydrocarbons (nitro-PAHs), NOx, and PM were examined. The results depicted that in comparison with the diesel-powered vehicles, biodiesel-powered vehicles showed significant

![Fig. 4. EU biofuels and biodiesel consumption between 1990 and 2015 [89].](image-url)
However, the CO₂ results were contradictory to the literature mainly indicating increases in CO₂ tailpipe emissions (and decreases in CO) as well as a moderate/slight decrease in NOₓ and CO₂ emissions [94,95]. Nevertheless, the authors presented no clear logic justifying the CO₂ increase observed and instead they only confined to mentioning that neat biodiesel could help to reduce CO₂ emissions by more than 75% by referring to the literature. Such justification is based on the result of some lifecycle assessment (LCA) studies such as Sheehan et al. [96] who claimed that B100 reduced net emissions of CO₂ by 78.45% compared with petroleum diesel during its lifecycle (from well to wheel). Likewise, Balat [97] reported that biodiesel could help to reduce CO₂ with petroleum diesel during its lifecycle (from well to wheel). Like-wise, Balat [97] reported that biodiesel could help to reduce CO₂ with petroleum diesel during its lifecycle (from well to wheel). The response to biodiesel inclusion mentioned in the literature has been calculated based on the LCA of biodiesel and not the combustion stage solely. Overall, the conclusions drawn by Tica et al. [94] could be calculated based on the LCA of biodiesel and not the combustion stage solely. Overall, the conclusions drawn by Tica et al. [94] could be

### Table 2

Emission characteristics of bus engines fueled by different types of biodiesel reported in real-world operating condition studies in Europe.

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of buses</th>
<th>Reference fuel</th>
<th>Tested fuel blends</th>
<th>Emission results</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>NOₓ</td>
<td>CO</td>
</tr>
<tr>
<td>Italy</td>
<td>4</td>
<td>Petroleum diesel</td>
<td>D70B30</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Portugal</td>
<td>17</td>
<td>Petroleum diesel</td>
<td>D95B5</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>D80B20</td>
<td>↑</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B100</td>
<td>↑</td>
<td>–</td>
</tr>
<tr>
<td>Spain</td>
<td>2</td>
<td>Petroleum diesel</td>
<td>D80B20 (EGR + DPF)</td>
<td>↑</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B100 (EGR + DPF)</td>
<td>↑</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>D80B20 (SCR + Urea)</td>
<td>↑</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B100 (SCR + Urea)</td>
<td>↑</td>
<td>–</td>
</tr>
<tr>
<td>Serbia</td>
<td>4</td>
<td>Petroleum diesel</td>
<td>B100 (Idle motion)²</td>
<td>↓</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B100 (50% load)²</td>
<td>↓</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B100 (100% load)²</td>
<td>↓</td>
<td>–</td>
</tr>
<tr>
<td>Belgium</td>
<td>1</td>
<td>Petroleum diesel</td>
<td>D95B5</td>
<td>↓</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>D90B10</td>
<td>↓</td>
<td>↑</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>D70B30</td>
<td>↓</td>
<td>++</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>B100</td>
<td>↓</td>
<td>–</td>
</tr>
<tr>
<td>Spain</td>
<td>1</td>
<td>Petroleum diesel</td>
<td>D92.3E7.7³</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>D60E10B30 ³</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

**DXBY = X% diesel in combination with Y% biodiesel.**

The symbols ↑/↓ and +/− represent the trend and the intensity of variations observed in each study, respectively, and cannot be used as a means of comparison among different studies.

(nc = no change in comparison with the reference fuel, na = not available.

|     |     |     |     |     |
|-----|-----|-----|-----|
| a   | Exhaust gas recirculation. |     |     |
| b   | Diesel particulate filter. |     |     |
| c   | Selective catalytic reduction. |     |     |
| d   | Idle motion of the engines fueled by B100 compared to idle motion of the same engines fueled by Euro diesel. |     |     |
| e   | 50% load of the engine fueled by B100 compared to 50% load of the engine fueled by Euro diesel. |     |     |
| f   | 100% load of the engine fueled by B100 compared to 100% load of the engine fueled by Euro diesel. |     |     |
| g   | Ethanol (7.7% v/v)-diesel. |     |     |
| h   | Ethanol (10% v/v)–biodiesel (30% v/v)-diesel blend. |     |     |
| i   | Smoke opacity. |     |     |
| j   | K-value. |     |     |

reductions in CO, SO₂, soot, and organic substances emissions in all the three operation regimes (idle motion, 50% load, and 100% load), as well as a moderate/slight decrease in NOₓ and CO₂ emissions [94,95]. However, the CO₂ results were contradictory to the literature mainly indicating increases in CO₂ tailpipe emissions (and decreases in CO emissions reflecting complete combustion) when running on biodiesel. Nevertheless, the authors presented no clear logic justifying the CO₂ increase observed and instead they only confined to mentioning that neat biodiesel could help to reduce CO₂ emissions by more than 75% by referring to the literature. Such justification is based on the result of some lifecycle assessment (LCA) studies such as Sheehan et al. [96] who claimed that B100 reduced net emissions of CO₂ by 78.45% compared with petroleum diesel during its lifecycle (from well to wheel). Likewise, Balat [97] reported that biodiesel could help to reduce CO₂ emissions by up to 75% (from well to wheel). The CO₂ reduction in response to biodiesel inclusion mentioned in the literature has been calculated based on the LCA of biodiesel and not the combustion stage solely. Overall, the conclusions drawn by Tica et al. [94] could be referred to the literature. Such justification is based on the result of some lifecycle assessment (LCA) studies such as Sheehan et al. [96] who claimed that B100 reduced net emissions of CO₂ by 78.45% compared with petroleum diesel during its lifecycle (from well to wheel). Likewise, Balat [97] reported that biodiesel could help to reduce CO₂ emissions by up to 75% (from well to wheel). The CO₂ reduction in response to biodiesel inclusion mentioned in the literature has been calculated based on the LCA of biodiesel and not the combustion stage solely. Overall, the conclusions drawn by Tica et al. [94] could be
considered as uncertain because of two reasons. First, in spite of recording the weather condition during the test time, there was no evidence presented on how the effect of such covariate/confounder factors was excluded from the average values or if the average values reported were corrected by excluding the covariate/confounder factors effect at all. Second, two urban buses were fueled by biodiesel only while two other buses were fueled by diesel. As for the latter drawback, it would have been more statistically-appealing if the authors had used the same vehicles and drivers for each fuel type tested. This could help to eliminate the respective experimental errors originating from the differences between the experimental materials, i.e., buses.

In a comprehensive study by Pelkmans et al. [98], the impacts of biodiesel and bioethanol blends on emissions and fuel consumption of modern engines were investigated through a Belgian research project called ‘BIOSES’. The on-road tests on diesel engines running on different biodiesel-diesel blends were performed on a delivery van, a passenger car, and a city bus. The city bus (VanHool A360-engine: MAN D2866) was tested on three bus cycles – De Lijn cycle; the DUBDC; and SORT (the description of the driving cycles could be found in Ref. [99])-in Lommel, Belgium using different fuel blends, i.e., neat diesel, B5, B10, B30, and B100. The test results for the impact of biodiesel on the urban bus showed that increasing biodiesel proportion in the blends, the volumetric fuel consumption was increased up to 4–5% for B100 compared with neat diesel. The emission results revealed that the use of biodiesel instead of diesel decreased NOx emissions as well as THC and PM emissions (except for B5 and B10 for which only PM emissions were unchanged and fluctuated, respectively). Moreover, CO emissions were slightly increased when using B10 and B30 while using B5 and B100 led to decreased CO emissions (fluctuations). The fluctuations were also observable for CO2 emissions while their changes were not in line with those of CO for some fuel blends. Despite the diversity of the project in term of the use of different fuel blends and engine types as well as driving cycles, a better study would have also tried to include appropriate experimental design, a higher number of replications (sample size-more than one bus) and well-explained experimental conditions. Moreover, recording and excluding the covariate/confounder factors were not reported by the authors either which could have also negatively affected the outcomes reported. In better words, it should be noted that although the test was performed based on the standard driving cycles, covariate/confounder factors could have deflected the average results. This could be the driving factor behind some fluctuations observed throughout the study.

Serrano et al. [100] employed a methodology similar to that of Proc et al. [81] in which a laboratory chassis dynamometer test was performed alongside a fleet test, and the results were compared. Similarly, the emission test was also only performed under steady-state operating condition while fuel economy was explored under both steady-state and real-world operating conditions. The examined bus fleet consisted of 201 buses working mainly in the north of Portugal operated as two different groups, i.e., urban areas (working through traffic, with constant stop/go operation) and extra-urban type of circulation (with a more diversified operation conditions, varying between sloped to smoother roads, including motorways). Neat diesel, B10, B15, B20, B30, B50, and B100 were tested under steady-state operating condition while neat diesel, B6, B10, B20, and B30 were tested in the fleet. The authors also mentioned that the fuel used for tests were identical and provided by the same supplier. Moreover, three common types of engine were used under the real-world operating condition while only one of them was considered in the steady-state operating condition experiments [100]. The results of the study highlighted that using biodiesel blends reduced the fuel consumption in the urban circulation while increased the fuel consumption in the extra-urban fleet. This finding revealed the importance of the driving cycle on fuel consumption and consequently emissions when using biodiesel. Moreover, the real-world operating condition test results agreed with those of the steady-state operating condition experiments. Despite the interesting findings reported by Serrano et al. [100], one could criticize the reported outcomes by highlighting the fact that the authors did not prepare an appropriate experimental design. Moreover, none of the covariate/confounder factors were recorded and excluded from the results of real-world operating condition analysis.

In a recent investigation, Nanaki et al. [103] compared the environmental emissions of urban buses running on diesel, CNG, and biodiesel in Athens, Greece. The study used the fleet data for the year 2009 for Athens’s bus fleet using five alternative biodiesel blends, i.e., B10, B30, B50, B80, and B100. The results demonstrated that increasing the biodiesel proportion in the fuel blend led to a reduction in CO, PM, and HC emissions while increasing the level of NOx emissions. The authors also claimed that as the percentage of biodiesel in the fuel mix was increased, the CO emissions decreased as well, e.g., they argued that the CO2 emissions associated with B100blendstood 78.45% lower compared with that of neat diesel [103]. As mentioned earlier, this conclusion could be misleading as it rather concerns the well to wheel lifecycle of soybean biodiesel than the combustion process itself. This was more comprehensively elaborated when discussing the findings of Tica et al. [94] earlier. Overall, Nanaki et al.’s study [103] would have been more appealing if the number of buses involved, experimental conditions, uncertainties, and the type of statistical analysis had also been mentioned. All these deficiencies have consequently reduced the robustness of the results presented.

In a study conducted by Macian et al. [104], the effect of low viscosity engine oil (LVO) on fuel consumption and CO2 emissions of Spanish urban buses fueled by biodiesel and compressed natural gas (CNG) were assessed. The most important results driven from their study was that the viscosity of engine oil could affect fuel consumption and CO2 emissions of the investigated urban buses under real-world operating condition. This finding could be important for future tests to consider this factor in their statistical analysis as a covariate factor as well.

As the first reference in Hungary, impacts of biodiesel usage on engine performance and emission characteristics of public urban buses were investigated by Bai et al. [105]. For the means of experiments, four buses were selected including two solo buses and two articulated ones fueled by diesel, B10, B20, and B50. One important criterion to be observed before any real-world tests are to fully examine each bus in terms of the whole vehicle status, as well as to inspect oil and air filter and to replace them if necessary. This criterion was well observed by Bai et al. [105], and moreover, before the real-world on road tests, the buses underwent a specific diesel test bench (steady-state operating condition test) for measuring engine performance and emission characteristics. These measurements could be considered as the basis for future comparisons and could also simply show the differences between the steady-state and real-world operating condition tests. The results obtained by Bai et al. showed that the real-world fuel consumption of
the buses using B10 and B20 increased compared with neat diesel fuel, while, the higher biodiesel blend (B50) led to less fuel consumption in several cases [105]. More interestingly, the steady-state operating condition test results did not show any statistical differences in terms of fuel consumption among various blends further highlighting the importance of testing fuels under real-world operating conditions as well.

The engine performance results showed that as the level of biodiesel inclusion increased, engine performance decreased proportionally. Pollution-related observations of the biodiesel blends used were also investigated in ideal and full speed modes of the engine. The results obtained showed reductions in HC, smoke opacity, and K-value (both at idle and full speed), but these values did not change proportionally with the biodiesel inclusion rate in the blends. Overall, the study by Bai et al. [105] offers some important points over the other studies reviewed earlier, including employing an analysis of variance (ANOVA) test and pre-test inspection of buses. However, there are some deficiencies to be mentioned as well, i.e., the number of replications (sample size) in each group (solo and articulated) was low, the routes traveled were not reported, the driving cycles were not proposed, and finally there was no mention of any corrections for the covariate/confounder factors.

### 3.3. Other countries

Apart from the substantial interest and advancements in biofuel production and application in the USA and EU, biofuels have also attracted a great deal of attention in many other countries/regions. In better words, out of the 84121 Kioe biofuel production in 2017, 33141 Kioe (about 39.4%) was produced outside of the USA and EU [77]. Brazil and Argentina in South America, Indonesia, Thailand, and China in Asia and Canada in North America are among the countries with considerable biofuel production capacities. The production capacity is very low in Africa (probably due to the low technological development in biofuel production industry in this continent) as well as the Middle East and Commonwealth of Independent States (CIS) due to the existence of oil and gas reserves.

Despite the considerable amounts of biofuels produced outside the USA and EU, there is a very limited number of investigations carried out on urban buses running on biodiesel blends during real-world operating conditions in the other regions of the world (Table 3). Among these few examples was the work of Correà and Arbilla [106] who strived to determine the mercaptans (methyl, ethyl, n-propyl and n-buty1 mercaptans) emissions from a bus engine (MMW 185 HP) fueled by diesel and biodiesel blends (B2, B5, B10, and B20). Mercaptans emissions detection could be of great interest as these types of emissions exhibit high levels of toxicity, could affect the nervous system and could cause convulsion and narcosis [106]. Their results showed that using biodiesel led to decreased mercaptans emissions. Moreover, as the biodiesel content in the blend was increased, the mercaptans reduction rates decreased proportionally. Although the authors mentioned that the test was performed under real-world operating conditions across the Rio de Janeiro city (Brazil), the routes specifications (distance, geographical location, and bus stops) and operational conditions of the real-world conditions (average number of passengers, test time, temperature, humidity, and season) were not mentioned in their report. A sample size of only 1 and not including the impacts of the covariate/confounder factors could be considered as the other deficiencies of the study.

In a different study also conducted in Brazil by Martins et al. [107], PM concentration at a bus station located in Londrina was determined. All the buses traveled to the station were fueled by B3 blend. In this type of real-world operating condition study, a different approach was employed. More specifically, the authors aimed to investigate emissions at a certain place, i.e., a bus station (which is very important since generally, the passenger's traffic is very high at the stations) and not by focusing on vehicles. Nevertheless, since PM emissions were measured when the buses were in the idle mode but under operational conditions, the nature of the study could be regarded the same as real-world operating condition test studies. Their results showed that biodiesel decreased PAHs while also increased fine and ultrafine particles when compared with diesel. Although measuring emissions at bus stations could show the impact of a dedicated fuel blend in an idling condition. However, it was not clear to what extent the other factors such as bus engine type/technology/model year, engine repair and maintenance, ambient temperature and PM concentration, and humidity could have affected the emissions measured by Martins et al. [107]. Moreover, they did not compare the results of biodiesel blend and diesel statistically. Therefore, it could be concluded that their study could have been more conclusive if they had considered a number of certain buses and determined the impacts of the other factors on emissions level by conducting an appropriate experimental design. A similar study was performed by Mkoma et al. [108] in which major ions in PM2.5 and PM10 released from buses fueled by B5 were detected in Lapa bus terminal, Salvador, Brazil. However, the same deficiencies existed in this study as well. Moreover, no comparisons were made between diesel and biodiesel in terms of their impacts on PM emissions and concentration at the intended terminal.

In a field experiment carried out in the state of Morelos, Mexico, fuel efficiency and tailpipe engine emissions of an urban bus fueled by diesel and B20 were evaluated by Hernández et al. [109]. The real-world operating condition experiments performed under two different driving conditions, i.e., a highway and an urban area located in the state of Morelos. The results revealed that using B20 in the urban driving cycle reduced tailpipe emissions of CO, NO, and CO2 while increased O2 and NOx emissions. Similar observations were made in the highway driving cycle except for the NOx emissions, which were reduced. From the fuel economy point of view, diesel fuel showed higher fuel efficiency than B20 regardless of the test driving conditions. Overall, one could deduce that although the study benefited from two different driving cycles and an appropriate emission monitoring method, there were some deficiencies existed associated with this study as well which could have increased the uncertainty of the results. Those include using only one sample, lack of appropriate experimental design, unclear significance level of the results, not proposing the exact specifications of the routes.

### Table 3

<table>
<thead>
<tr>
<th>Country</th>
<th>Number of buses</th>
<th>Reference fuel</th>
<th>Tested fuel blends</th>
<th>Emission results</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Brazil 1</td>
<td>Petroleum diesel</td>
<td>D98B2</td>
<td>– – – –</td>
<td>↑a</td>
<td>[106]</td>
</tr>
<tr>
<td>Brazil 1</td>
<td>Petroleum diesel</td>
<td>D95B5</td>
<td>– – – –</td>
<td>✗</td>
<td>a</td>
</tr>
<tr>
<td>Brazil 1</td>
<td>Petroleum diesel</td>
<td>D90B10</td>
<td>– – – –</td>
<td>✗</td>
<td>a</td>
</tr>
<tr>
<td>Brazil 1</td>
<td>Petroleum diesel</td>
<td>D80B20</td>
<td>– – – –</td>
<td>✗</td>
<td>a</td>
</tr>
<tr>
<td>Mexico 1</td>
<td>Petroleum diesel</td>
<td>D97B3</td>
<td>– – – –</td>
<td>↑b</td>
<td>[107]</td>
</tr>
<tr>
<td>Mexico 1</td>
<td>Petroleum diesel</td>
<td>D80B20</td>
<td>↑+d</td>
<td>✗</td>
<td>✗</td>
</tr>
</tbody>
</table>

DXBY = X% diesel in combination with Y% biodiesel, LSD = Low sulfur diesel, USLD = Ultra low sulfur diesel.

The symbols ↑/↓ and +/– represent the trend and the intensity of variations observed in each study, respectively, and cannot be used as a means of comparison among different studies.

- a Mercaptans emissions are the result of sulfur compounds combustion.
- b Fine and ultrafine particles.
- c PAH emissions.
- d NOx emissions decreased in the highway driving condition.
- e In both highway and urban areas driving conditions.

traveled and cycles, and finally not recording and excluding the effect of covariate/confounder factors.

4. Lessons learned and guidelines for future case studies

4.1. Key issues in conducting real-world operating condition tests

Having highlighted the critical parameters that are overlooked by most of the studies performed on urban buses powered by biodiesel blends under real-world operating conditions, key issues to be observed when conducting real-world tailpipe emission tests (also applicable for real-world engine performance tests) in urban buses are presented herein. Furthermore, a set of guidelines were provided in the following sections regarding the implementation of statistically-accurate and reliable tests. Fig. 5 summarizes key issues and guidelines for reducing experimental uncertainties in real-world operating condition tests. As mentioned above, the main weaknesses of the previous studies could be classified into three distinct groups, 1) statistical uncertainties, 2) driving cycle uncertainties, and 3) fuel uncertainties which are comprehensively analyzed in this work.

4.2. Statistical considerations and guidelines

One of the primary deficiencies with most of the conducted real-world operating condition studies is the lack of careful and complete experimental designs. Such experimental designs generally should include five basic stages [110,111], i.e.:

1) testing a null hypothesis,
2) designing an experiment,
3) performing the experiment,
4) conducting statistical analysis,
5) interpreting and reporting the results.

Similar experimental designs are commonly used in many research studies across many fields of science as well [110,112]. Any deficiencies in these five mentioned stages would lead to statistically-uncertain results. For example, in most studies that were reported here, it is not clear that the differences between biodiesel blends and the reference fuel used (i.e., diesel) were statistically significant or not. Based on the statistic principles, reporting the average results without any accompanying information on uncertainty could be highly misleading [110,113].

Overall, the statistical defects of most real-world operating condition studies on urban buses concern the stages 2, 3 and 4. These statistical defects include lack of testing the homogeneity of experimental materials, i.e., buses before conducting the experiment (mileage traveled prior to/throughout the experiments and engine type/model year), frequent lacking of appropriate replication number (sample size), long time gaps when conducting experiments, lack of reporting the significance level of the differences among treatments, and lack of recording and excluding the effects of covariate/confounder factors.

4.2.1. Experimental setup

In comparison with the observational studies, in experimentally-designed experiments, experimental units (i.e., buses) are designated by
different treatments (e.g., different fuels used) and then the effect of the treatments on the experimental units through an appropriate statistical analysis will be investigated [114]. Therefore, all the principles and criteria necessary to perform a careful and complete experimental design should be observed. In line with that, a set of guidelines and recommendations to achieve this are presented herein:

4.2.1. Prior test inspections. Generally, the experimental materials should be homogenous [115] but in many cases, procuring experimental materials of similar specifications is not practically feasible. For example, providing buses with the same mileage traveled, same model year, and engine status is rarely possible. Therefore, it is recommended to test the homogeneity of the experimental materials first, and if the materials are heterogeneous, one of the following actions could be taken in order to overcome this challenge. First, Randomized Blocks Design or Latin Square Design [115,116] could be used and second the extreme materials which would otherwise increase the magnitude of heterogeneity prior to the experiments, could be excluded. If it would not be possible to do the latter, the heterogeneity factors (as potential covariate factors after data collection) such as different mileage traveled, different engine model year, engine type, engine oil type, and viscosity could be taken into account and analyzed [117]. Overall, it is essential to take an exact note of the factors which could potentially affect the engine performance and emission levels of the buses under investigation before conducting real-world operating condition experiments [74,82,118].

4.2.1.2. Replication (sample size). Replication is the key to the accuracy and certainty level of the results obtained in any experiments and real-world case studies using urban buses are no exception [115,116]. In fact, replication or having a sample size of more than one (unlike what reported by some of the above-mentioned studies, e.g. Refs. [98,101,106,109]) could assist in obtaining an accurate estimate of experimental error, increasing the precision of the experimental (residual) variance estimation while also significantly influencing the sensitivity of the experiments [116]. Moreover, replication could enable researchers in estimating the mean effect of any experimental factors [115]. Principally, sample size should be small enough so that negligible treatment differences would not be declared statistically significant and should also be large enough so that statistically significant treatment differences would be identified [111]. An appropriate number of replications should be selected based on the desired precision of a given experiment and the type of intended experimental design. This is ascribed to the fact that the number of replications could directly affect the degree of freedom and consequently the mean square error –MSE– of the experiments [119].

4.2.1.3. Randomization. Through replications, one could estimate the statistical significance level of the results. However, even a statistically-favorable replication number could not ensure the validity of the results [115] and randomization (i.e., random assignment of treatments to experimental units) is also essential to achieve that. Randomization prevents the introduction of systematic bias into the experiments while establishing a link between the actual experiment and the statistical model used. As mentioned earlier, in the studies reviewed herein, there was no evidence of observing random assignment of the treatment to the experimental units. Having highlighted this, it is recommended to randomly assign the fuels under investigation to buses without any prior selection. In cases that a Randomized Blocks Design or Latin Square Design to be used, the randomization should be performed after blocking or after arranging the Standard Latin Square map [119].

4.2.1.4. Local control. Is the degree of control over the placement of subjects in experimental units and the organization of those units [115]. Although replication and randomization could to some extent ensure the validity of the test results, the magnitude of the experimental error could still deflect the results. Therefore, local control is a way to reduce the magnitude of the experimental error through blocking or balancing the experimental units [115].

Blocking is, in fact, the same action taking place when performing the Randomized Blocks Design in which experimental units are allocated to blocks or groups so that the units within a block are relatively homogeneous [115]. It should be mentioned that when only one factor is investigated, and the experimental units are homogeneous, completely randomized design should be used according to which blocking would not be needed [115] as the magnitude of the experimental error would be sufficiently low.

Balancing is an optional activity in which an equal number of subjects are assigned to each treatment. Balancing is desirable because it could simplify the statistical analysis. Nevertheless, design scan also is performed correctly using little or no balancing as well [115].

4.2.2. Covariate/confounder factors

A covariate is a variable related to the experimental units which could interfere with the outcome of the study [120]. If the covariate is related to both dependent and independent variables studied, then the covariate becomes a confounder. For example, if the impacts of fuel type (independent variable) on engine performance/emission characteristics (dependent variable) of a bus engine are investigated, factor such as mileage traveled, and model year are considered as covariates while factors such as ambient temperature, humidity, and passenger counts (load) are considered as confounders. The presence of covariates/confounders could affect the studied variables to the extent that the results may not reflect the actual relationships [117]. For example, a 2% increase/decrease in a given emission by using biodiesel (compared with diesel fuel or other reference fuels) may not be exactly 2%, because of the mentioned factors.

As for covariates, it is recommended to exclude or control these factors through prior test inspections, i.e., randomization, restriction, and matching. Using randomization, any links between the main variable and covariate could be eliminated. Generating groups that are comparable concerning known and unknown covariates could help to reduce the potential for interfering with these factors with the test outcomes [117]. Through restriction, variations in the covariates could be eliminated. For instance, this could be done by selecting buses of the same age or engine type. Matching is also commonly used in case-control studies and involves the selection of a comparison group concerning the distribution of one or more potential covariates. It should be noted that as mentioned earlier, all these methods are applicable at the time of the study, i.e., prior data gathering. Therefore, in cases where data gathering has already been concluded, statistical methods are recommended to adjust for potential covariate effects.

As for confounders, one should record the values related to these factors during the experiments, and then, it would be possible to employ statistical methods to exclude their effects on the research outcomes [121]. Among the statistical methods suggested to eliminate the effects of confounders, Logistic Regression, Linear Regression, and Analysis of Covariance are the most promising ones [117].

4.2.3. Ambient and vehicle factors

Since real-world operating condition experiments on buses are performed in the open air, therefore, all the relevant ambient factors could potentially emerge as confounders affecting the main results through influencing the combustion quality. Based on the findings of engine performance and emissions studies, variations in fuel properties, engine characteristics, and the operating conditions could significantly affect the engine performance and emissions characteristics [41,74,118]. Therefore, any factors capable of affecting fuel properties, engine characteristics, and the operating conditions must be recorded, and their effects must be excluded from the results. Based on the reviewed studies herein, ambient temperature, humidity, vehicle speed, and the number of passengers are the main factors potentially affecting

engine performance and emission profiles [74,85,86,104]. Therefore, it is recommended to record these factors and exclude their effects using different statistical methods as mentioned above.

Since buses are the experimental materials, any prior test differences in the buses under investigation must be avoided or if not possible, recorded, and then excluded using different statistical methods mentioned earlier. Based on the reviewed studies herein, the engine model year, engine type, mileage traveled before the experiments, mileage traveled during the experiments, engine oil type and viscosity are the main factors affecting the engine performance and emission profiles [74,80,82,83,104]. Among these variables, the effect of mileage could be simply excluded as the covariate factor. While, for the rest of the variables, it is possible to avoid the covariate effects by means of blocking or grouping the subjects.

4.3. Real-world driving considerations and guidelines

As an important and influential factor, engine operation conditions could affect engine performance and tailpipe emissions significantly [41,74,118]. Therefore, to reach correct, reliable, and extensible results, the engine operating conditions should be 1) constant for the different fuels compared during a test and 2) a good representative of the real-world driving conditions. Although such ideal engine operation conditions seem hard to achieve, the following guidelines should assist in reaching the possible conditions:

Accordingly, it is recommended to:

- The selected routes must be the regular bus routes with their ordinary passengers on-board [122].
- Precisely record and thoroughly report the routes tested along with their details including a general map, length, number of stations, number of stops, average number of passengers, average road grade, and type of route, i.e., urban, suburban, rural, and highway [74,75].
- If possible, when studying an urban fleet, various routes should be used to increase the validity and extensibility of the results [123].
- The selected routes must cover all the geographical and road circumstances (e.g., low passenger routes and crowded ones, urban cycles and highway or rural ones). This could help to achieve more reliable and extensible data under real-world traffic conditions.
- Select the bus types from the intended fleet which are still available on the market (or in the other targeted markets) to ensure the possibility of future purchases [80].
- When it is not possible to use real-routes, and instead, it is intended to design a driving cycle and perform the test on a special and exclusive test track, the driving cycles must be carefully designed based on the road specifications of the city fleet under investigation. For instance, loading the buses with sandbags could simulate an average loading situation of passengers [82] or designing different driving cycles (e.g., urban, suburban, highway, and rural driving cycles) based on GPS data from the fleet could increase the precision of the designed cycles [80,82].
- As peak traffic and off-peak traffic conditions, e.g., on various weekdays, could result in different tailpipe emission levels [75], collect data in multiple traffic situations and on different weekdays. For this purpose, it is important to include time variation in the desired experimental design by considering time variation as an independent variable or by using specific types of experimental designs which include different time horizons.

4.4. Fuel considerations and guidelines

Fuel type in the real-world engine performance and tailpipe emission experiments is generally considered as a primary reason for uncertainty. The main aim of these types of experiments is to examine the impacts of fuel type, e.g., diesel, biodiesel, and their blends as the main treatment on tailpipe emissions (and engine performance) of urban buses. Moreover, the type of biodiesel (i.e., type of feedstock), the type of base fuel to which biodiesel is added, and biodiesel properties (blend percentage, density, cetane number, viscosity, and transesterification route) could affect the level and type of engine tailpipe emissions [41,74,124]. For example, Polanga biodiesel, Neem biodiesel, and Jatropha biodiesel have been shown to exert different impacts on NOx emissions [125]. Similarly, the tailpipe emission levels of B20 + LSD was reported to be different from those of B20 + ULSD [80]. In better words, fuel specifications could reduce or even eliminate the emission benefits expected from using biodiesel and its blends [83]. Therefore, the fuel used for tests should be identical (i.e., the biodiesel used in preparing different fuel blends should be of the same feedstock with the same standard properties) and should also be provided by the same supplier. This should also be observed for the diesel used in the experiment. Moreover, the specification of the tested biodiesel must meet the criteria defined by biodiesel quality standards such as ASTM D6751 and EN 14214. This is also applicable for fossil diesel based on its commercial grade. Any types of the variations mentioned above among the fuel blends used could reduce the precision and consequently result in misleading judgments/conclusions.

4.5. Future perspectives

Since the use of alternative fuels (e.g., biofuels, electricity, and hydrogen) for the urban transport fleet is gaining more interest worldwide, establishing appropriate guidelines to properly test alternative fuels on a given fleet under the real-world operating condition is essential. In line with that, the present review was aimed to provide such standards for performing more precise real-world operating condition tests on alternative fuels. Considering the thriving interest toward electrification of urban fleets, the guidelines, and recommendations provided through the course of this study could be of practical use for testing different types of EVs through transportation fleets as well. In fact, reliable and precise real-world operating condition tests could exhibit the degree of real-world on-road benefits of EVs in comparison with previously calculated or examined ones using standard driving cycles. It should be noted that there is a necessity of further adjusting the proposed guidelines herein based on the type of EVs used. For example, no direct emissions would be assessed when examining battery electric buses on a fleet and therefore the fuel considerations (fuel type was realized as the main source of uncertainty in exhaust emission testing) would change to considerations regarding the electricity mix (to recharge the vehicle’s battery during the use phase). In line with that, future research studies required to focus on adjusting such guidelines with testing different type of EVs. Also, future studies must focus on practical experiments aimed at discovering the other related covariate/cofounder factors effective on tailpipe emissions/fuel consumption of different alternative fuels when examining urban transport fleets. Moreover, since testing alternative fuels on urban transportation fleets is a costly and time-consuming procedure, substantive research must be carried on experimental methods capable of lowering the required cost and time. For example, this might be realized through designing driving cycles whose attributes are most like those of a given real-world operating condition.

5. Conclusions

Alternative renewable energy carriers have gained considerable interest recently in response to motorization and increasing primary energy demands especially in urban areas. Transportation is among the major contributors to global warming by emitting 14% of the global GHG emissions. Therefore, employing renewable energies in the transportation sector are needed. In such a context, biofuels have gained increasing interest owing to the indigenous availability of raw materials (biomass) throughout the year and capability to completely or partially replace the existing fossil fuels used in the transportation
Biodiesel is one of the most promising alternatives to petroleum diesel as it could potentially result in less air pollutants compared with its petroleum counterpart. In this regard, biodiesel and its blends have been increasingly investigated to obtain more in-depth understandings of their environmental benefits. Among various experimental approaches is the real-world operating condition whose results would be of more practical interest vs. laboratory scale tests. Such experiments are generally very complex, and numerous factors could directly or indirectly interfere with the results produced potentially jeopardizing the integrity of the research conclusions drawn. The results of the review showed that some important parameters are overlooked by many of the studies performed on urban buses powered by biodiesel blends under real-world operating conditions. These parameters could be classified into three distinct groups, 1) statistical uncertainties, 2) driving cycle uncertainties, and 3) fuel uncertainties and are comprehensively reviewed and discussed in this work. Accordingly, it could be realized that employing a careful and complete experimental design (through obtaining all the required principles and criteria necessary to perform a careful and complete experimental design) alongside excluding, controlling, or recording covariates/confounders could be among the key issues for conducting real-world tests. Moreover, guidelines regarding real-world driving considerations as well as fuel considerations to achieve the most feasible ideal research outcomes should be considered in future studies of vehicle fleets. The study findings help to formulate the guidelines for assessing real-world operating condition experiments to achieve the most feasible and meaningful research outcomes that will have significant implication for local and global policy makers. The guidelines are of use for all types of research studies that want to evaluate the effects of alternative fuels (e.g., biodiesel, EVs or hydrogen) for any transportation fleet.

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