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Micromixers for Wastewater Treatment and Their Life Cycle Assessment (LCA)

Olga P. Fuentes, Mabel J. Noguera, Paula A. Peñaranda, Sergio L. Flores, Juan C. Cruz and Johann F. Osma

Abstract

The use of micromixers and catalytically active nanocomposites can be an attractive alternative for the treatment of wastewaters from the textile industry, due to their high activity, low consumption of such nanocomposites, short reaction times and the possibility to work under continuous operation. In this study, 6 different designs of micromixers were modeled and evaluated for the treatment of wastewaters. Velocity profiles, pressure drops, and flows were analyzed and compared for the different devices under the same mixing conditions. In addition, Life cycle assessment (LCA) methodology was applied to determine their performance in terms of environmental impact. Considering the high environmental impact of water sources contaminated by dyes from the textile industry, it becomes critically important to determine when the proposed micromixers are a suitable alternative for their remediation. The LCA and operational efficiency studies results shown here provide a route for the design of novel wastewater treatment systems by coupling low-cost and high-performance micromixers.

Keywords: micromixers, wastewater, life cycle assessment, dyes, magnetite

1. Introduction

Microfluidics is the science that study fluid behavior on micro/nano scales that are circulating in artificial microsystems [1, 2]. Also, this science considers the fabrication of fluidic devices for the transport, delivery, and handling of fluids on the order of microliters or even smaller volumes [3]. Microfluidic techniques have shown advantages such as high performance, design flexibility, low reagent consumption, miniaturization, and automation [4]. The application of these techniques has led to microfluidic devices that have found application in several fields, such as medical and biochemical analysis, environmental monitoring, biochemical, and microchemistry [5–8].

Currently, there is a growing need to monitor water quality across a broad range of applications, including industrial wastewaters as well as drinking water and different surface waters (rivers, lakes, groundwater and marine) [9]. Water sources contaminated by dyes or phenolic compounds, which are present in textile industrial wastewater, represent a threat to human health and the environment [10]. For that reason, it is imperative to find efficient routes to monitor these pollutants in wastewater in order to avoid their discharge above permissible limits. A wide range of sensors and

analyzers are commercially available for wastewater monitoring, and they are based on different detection techniques, such as colorimetric, chemical, electrochemical or optical [11]. Here, a growing trend is emerging where microfluidic technologies are considered for environmental detection mainly due to their lower investment and operation costs, as well as reduced infrastructure requirements. Moreover, it has been shown that microreactors, help to maximize biodegradation processes due to the absence of dead volume, allow to perform continuous reactions, and enable to control the contact between the reagents by changes in the microchannel geometries [9, 12].

By handling fluids in microchannels, it is possible to achieve high production yields, and minimize waste generation. Moreover, with this approach it is feasible to operate under short reaction and analysis times, is relatively cheap and enable high-throughput schemes [13]. However, the manufacture of microfluidic devices generally relies on sophisticated cleanroom techniques [14], which is disadvantageous due to their high costs. This issue has been overcome with low-cost manufacturing methods such as polymer laminates, 3D printing, and laser cutting [15, 16]. In this approach, devices are often manufactured by cutting a piece of polymethylmethacrylate (PMMA) followed by engraving a pre-designed microchannel pattern on a separate PMMA. The device is then assembled by gluing the two pieces together. PMMA is one of the preferred thermoplastics for the manufacture of microfluidic devices, due to its optical transparency, superior mechanical properties, low cost and good workability in conjunction with its ease for prototyping and mass manufacturing [17]. In this study, we will show how PMMA can be used to manufacture micromixers and we will analyze and compare the potential environmental impact of implementing them for wastewater treatment.

Life cycle assessment (LCA) has been widely applied in the wastewater treatment industry due to its important role as a tool for the sustainability assessment of new technologies, processes and the improvement of waste management practices. On this, inputs, such as raw materials and energy, and outputs, such as waste and emissions, are collected in the form of elementary flows for the whole life cycle (Life Cycle Inventory – LCI step) and then converted into environmental impact indexes by means of characterization factors (Life Cycle Impact Assessment – LCIA step) [18]. According to Corominas et al. [19], LCA can be a useful decision-support tool for examining alternative future operational scenarios during strategic planning within the water sector. Also, LCA evaluates beyond the limit imposed by the trade-off between process efficiency and final effluent quality because it considers resource and energy consumption, air emissions and waste generation [20].

In this study, we explore the design and manufacture of micromixers for wastewater treatment to enable the enzyme-based degradation of dyes. In this regard, we propose a LCA assessment to establish the potential environmental impact of implementing these devices. Also, this analysis integrates the required chemical supplies, energy, and water needed for wastewater treatment. Through life cycle assessment (LCA), we compared six different designs of micromixers to identify the one providing the least environmental impact during operation. LCA analysis might therefore contribute significantly to improving wastewater treatment process by coupling micromixers capable of remediating wastewaters with high efficiencies.

2. Materials and methods

2.1 Materials

Iron (II) chloride tetrahydrate (98%) ($\text{FeCl}_2 \cdot 4\text{H}_2\text{O}$), Iron (III) chloride hexahydrate (97%) ($\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$), and dye Eriochrome Black T (EBt) (C.I. 14645) were obtained from PanReac AppliChem (Spain). 2,2-azino-bis(3-ethylbenzothiazoline-6)

sulphonic acid (ABTS), glutaraldehyde (25%), sodium hydroxide (NaOH) (98%), tetramethylammonium hydroxide (TMAH) (25%), 3-Aminopropyl-triethoxysilane (APTES) (98%) were purchased from Sigma-Aldrich (USA). Polymethyl methacrylate (PMMA), Methyl methacrylate, Ethanol (96%) and 345 mT Neodymium cylindrical magnets (ϕ : 6 mm x h: 7 mm) were purchased at a local shop.

2.2 Laccase

Laccases (*P. sanguineus* CS43) (EC 1.10.3.2) were obtained from tomato medium as described elsewhere [21]. Briefly, mycelia were removed from the culture supernatant by filtration using two tangential flow filters in series, one of them with pore size of 0.5 mm while the other of 0.2 mm. The obtained laccase cocktail was ultra-filtered using a membrane with a molecular weight cut-off of 10 kDa.

2.3 Synthesis of magnetite and laccase immobilization

Magnetite nanoparticles were synthesized by coprecipitation of 20 mL of 1 M FeCl₂ and 20 mL of 2 M FeCl₃ under agitation at 1,500 rpm and 90 °C. 40 mL of 8 M NaOH and 40 mL of 2% (v/v) TMAH were then added to the mixture during 3.5 h at a flow rate of 12 mL/h. Nanoparticles (Magnetite) were magnetically separated aided by a strong permanent magnet, then washed thoroughly with 2% (v/v) TMAH, and finally sonicated for 100 min in a VibraCell ultrasonic bath (Sonics, USA).

Magnetite nanoparticles were buffered by adding a NaOH solution until pH approached 11, then sonicated for 10 min. 50 μ L of 2% (v/v) TMAH was added and dispersed and then the mixture was sonicated for 10 min. Silanization of the nanoparticles was carried out by adding 50 μ L of 2% (v/v) APTES followed by sonication for 20 more min. 50 μ L of 2% (v/v) glutaraldehyde was added to the mixture as the crosslinker, and left to react for 30 min. Finally, 50 μ L of 960 U/L laccase was added and left overnight to immobilize the enzyme on the nanoparticles. The resulting bionanocompounds (i.e., Lac-Magnetite) were separated by magnetism and washed thoroughly with MilliQ water.

2.4 Geometry design and fabrication

Six different prototypes of micromixers were designed with different micro-channel geometries for the reaction chambers. This was achieved by varying the number of layers of PMMA sheets required to create the channel. In the case of one layer, the channel geometry was circular and triangular. In contrast the assembly of multiple layers enabled rectangular-3D, one loop, two horizontal loops, and two vertical loops (See **Figure 1**).

For the micromixers manufacture, each design was engraved and cut on sheets of polymethylmethacrylate (PMMA), with a thickness of 3 mm and an area of 75x25 mm, using a Speedy 100, 60 W laser cutting system (TROTEC, Germany). Sheets were glued together to assemble the devices by applying a few drops of 96% ethanol on the contacting surfaces and maintaining a constant pressure for 8 minutes at 105 °C.

2.5 Experimental test for wastewater treatment

To estimate the dye biodegradation, we selected the EBt dye as a model. The EBt solutions were prepared at pH 5.48 and three different concentrations, namely, 5 mg/L, 10 mg/L and 20 mg/L. Biodegradation tests were conducted by introducing 5 mg of the bionanocompound and 5 mL of dye solution into each micromixer for 25 minutes at a constant rate of 12 mL/h. A neodymium permanent magnet, of

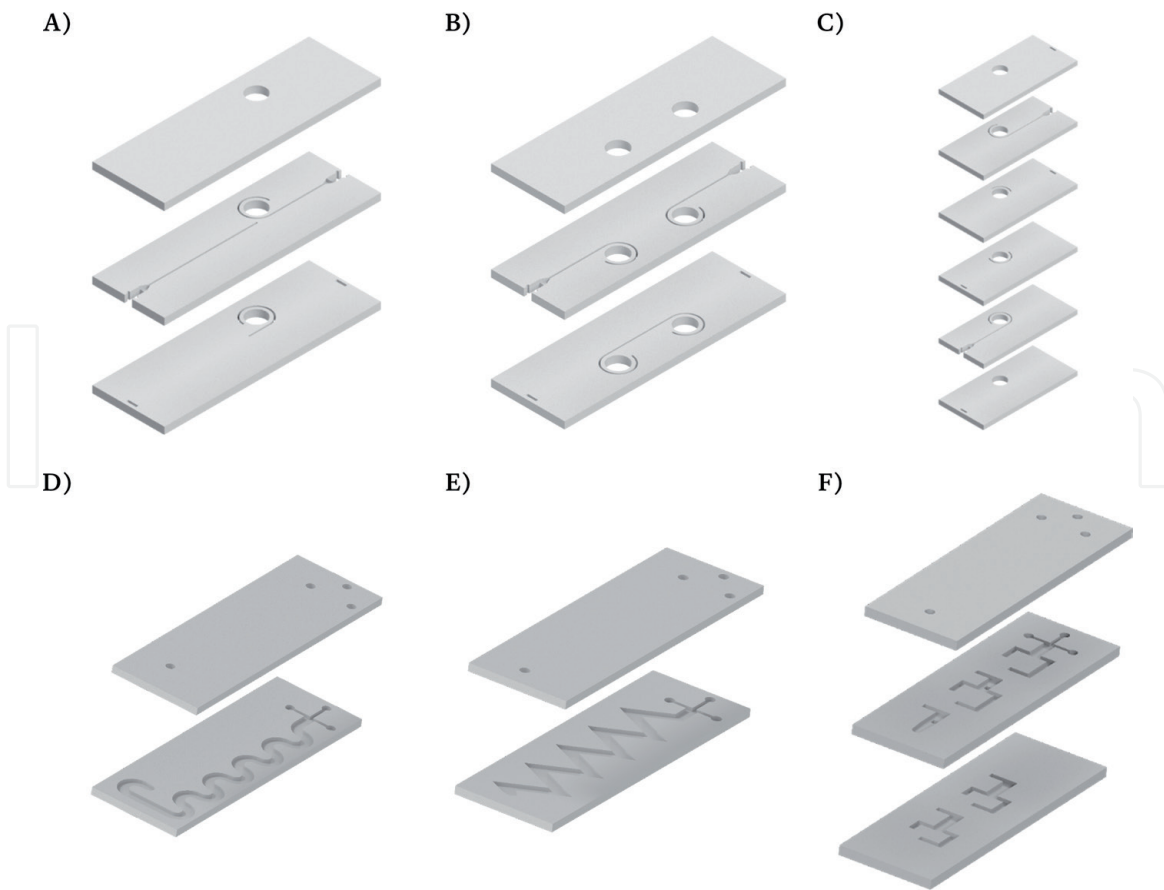


Figure 1. Micromixers geometries. A) One loop, B) two horizontal loops, C) two vertical loops, D) circular, E) triangular and F) rectangular-3D.

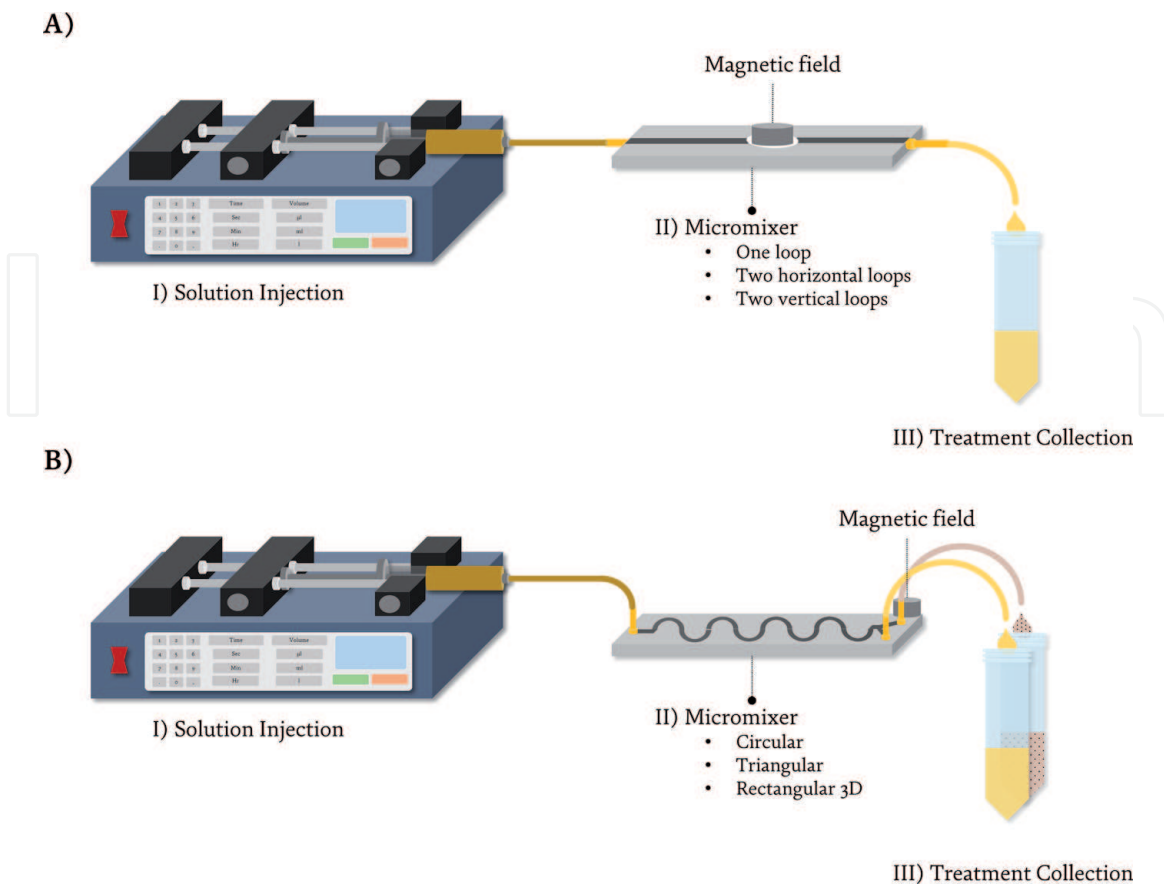


Figure 2. Experimental scheme performed for on the micromixers by topologies with loops (a) and without (B).

349.23 mT, was externally inserted into the loops of some of the devices to retain the bionanocompounds while the reaction occurred. For devices that lack loops for permanent magnets, separation of the bionanocompounds by placing the magnets at the outlets of the system (See **Figure 2**). All experiments were carried out in triplicate. After the treatment, each sample was collected and analyzed spectrophotometrically in a GENESYS 10S UV-Vis v4.004 2L5R078128 (Thermo SCIENTIFIC, USA) An absorbance peak was monitored at 545 nm and also the absorbance area of the entire visible spectrum in the range between 400 and 700 nm was calculated. All measurements were carried out in triplicate.

2.6 LCA requirements

2.6.1 Goal and scope

This life cycle analysis aimed to evaluate possible impacts associated with manufacture and operation of six different micromixers for wastewater treatment. This LCA was based on an attributional approach or descriptive “cradle to gate”

Stage	Inventory	Amount	Unit
	<i>Single layer micromixer</i>		
	Polymethyl methacrylate (PMMA)	0.011	m ³
	Ethanol	1	ml
	Energy	0.339	kWh
	Water consumption	53	ml
	<i>Two layers micromixer</i>		
	Polymethyl methacrylate (PMMA)	0.017	m ³
<i>Manufacturing</i>	Ethanol	2	ml
	Energy	0.577	kWh
	Water consumption	51	ml
	<i>Multiple layers micromixer</i>		
	Polymethyl methacrylate (PMMA)	0.034	m ³
	Ethanol	5	ml
	Energy	1.293	kWh
	Water consumption	51	ml
	<i>Enzyme activity assay</i>		
	Citric acid	0.060	g
	Disodium hydrogen phosphate	0.050	g
	ABTS	0.110	g
	Energy	0.057	kWh
<i>Operation</i>	Water consumption	15	ml
	<i>Dye preparation</i>		
	Eriochrome black	0.0001	g
	Water consumption	5	ml
	<i>Operation</i>		
	Energy	0.020	kWh

Table 1.
 Inventory report of micromixers manufacturing and operation.

of the laboratory-scale processes. The functional unit of this study was defined as 5 mL of treated wastewater by each micromixer. System boundaries were set from the use of raw materials for the manufacturing microfluidic devices and synthesis of Lac-Magnetite nanoparticles until the absorbance analysis of treated wastewater.

2.6.2 Life cycle inventory (LCI)

Data from the synthesis of Lac-magnetite and the process of wastewater treatment of each micromixer were measured on site. These data collection involved the determination of the relevant flows, use of reagents, emissions, wastes, and energy consumptions for this LCA study. Data concerning distribution of electricity and production of reagents were obtained from the Ecoinvent 3.6 database. Inventory report of this LCA study was mostly based on own laboratory experiments. **Table 1** shows the inventory report of raw materials, water consumption and energy required for the manufacturing of each micromixer and the corresponding operation process for wastewater treatment.

2.6.3 Impact assessment

Life cycle impact assessment (LCIA) aims to calculate the potential environmental and human health impacts associated with the manufacturing and operation of six micromixers for wastewater treatment. This LCIA was carried out with the aid of Ecoinvent 3.6 database. Characterization factors reported by the International Reference Life Cycle Data System (ILCD) method for LCIA were applied as impact assessment tools. In addition, eight impact categories were considered in this study: human toxicity non-cancer effects, human toxicity cancer effects, ecotoxicity freshwater, climate change total, resource depletion of minerals and metals, resource depletion of dissipated water, freshwater and terrestrial acidification, and photochemical ozone formation.

Regarding the assumptions, data concerning environmental impacts included the production of reagents necessary to synthesize the magnetite nanoparticles, i.e., the production of iron chlorides (II) and (III). However, environmental impact data to produce Tetramethylammonium Hydroxide (TMAH) has not been reported yet and therefore was neglected from the LCA analysis.

3. Results and discussion

3.1 Life cycle impact assessment of the manufacturing stage

The impact assessment was divided into two stages, the one related to raw materials and the manufacturing of micromixers, and the one that involved the operation in wastewater treatment. At the manufacturing stage, micromixers were analyzed based on the resources required for their fabrication and the number of PMMA layers to assemble them. For example, circular and triangular micromixers only required one PMMA layer, while one loop, two horizontal loops, and rectangular-3D micromixers were formed by two PMMA layers. Finally, the two vertical loops micromixer was formed by four or even more PMMA layers. Based on these features, the micromixers' manufacturing was analyzed individually in terms of environmental impacts. Alternatively, for the operation stage, each micromixer was analyzed based on its specific retention rate of Lac-magnetite in each work cycle. Finally, LCA results of both stages were added up to determine the total impact of each micromixer.

Figure 3 shows the detailed factors that, in the manufacturing stage of micromixers, impacted human health and global warming. These results showed that PMMA contributed with 55%, energy consumption with 44%, and other raw materials with only 1% of the total impact of the human toxicity, within the cancer effects category (see **Figure 3A**). Therefore, effects on human toxicity may be most likely associated with the use of PMMA for the manufacture of microfluidic devices. The selection of suitable materials to design micromixers with low costs and high manufacturability, is an important factor. New alternatives have been proposed to minimize environmental and human health impacts of PMMA. For instance, Wan et al. [17] demonstrated that PMMA used to fabricate microfluidic devices can be recycled multiple times preserving a high optical quality and their properties for biological experiments. Also, their results highlighted the importance of choosing appropriate parameters for the recycling process such as temperature, time, and pressure. Therefore, an alternative to reduce the impacts associated with the manufacture of micromixers in our case is by recycling the PMMA.

Figure 3B shows the impact assessment in climate change category. Energy consumption contributed with 53%, which can be explained by the energy consumed during the laser cutting process for the manufacture of micromixers. Overall, the results reflected that multiple layer micromixers showed the highest values on human toxicity and climate change categories, with up to 2 to 4 times increase in the values compared to two layers and single layer micromixers, respectively. This trend was also observed in other impact categories considered in this study. Although energy spent in the laser cutting had the highest contribution in the impact assessment, this technology reduced the micromixer manufacturing time compared to other wet chemical etching processes [22]. Also, this technique facilitated maintaining consistent dimensions and the appropriate device functionality due to its resolution and flexibility in terms of the variety of materials that can be handled [23]. Therefore, laser cutting offers significant benefits over other manufacturing techniques to achieve an accurate design of micromixers at very low cost.

Experimental tests determined that retention of Lac-magnetite nanoparticles was 87% for the two vertical loops micromixer, 80% for the one loop and the two horizontal loops micromixers, 40% for the triangular micromixer, and 0% for the rectangular-3D and circular micromixers. This analysis was carried out by measuring the amounts of Lac-magnetite bionanocompounds exiting the micromixer after wastewater treatment process. These nanoparticles remained attached to the walls of the micromixer in each work cycle. Based on retention information, the equivalent amount of Lac-magnetite held in each work cycle was estimated to calculate

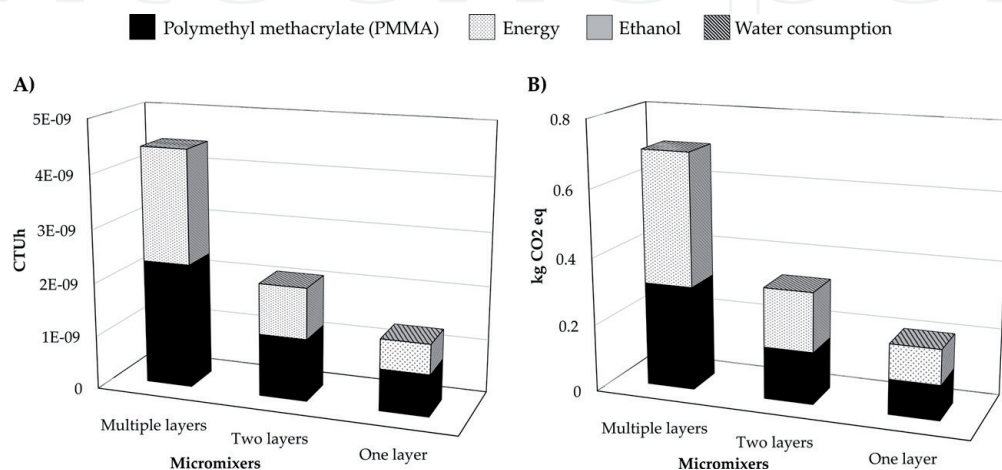


Figure 3. Impact assessment in manufacturing stage for: A) cancer effects category of human toxicity, B) climate change.

the corresponding environmental impacts. Therefore, we considered the operation stage of each micromixer from its use in the first cycle until the completion of a total of ten work cycles. The initial input of the Lac-magnetite bionanocompound was 5 mg during all the operation process in the wastewater treatment. Then, this amount was different for each microsystem and work cycle. The total amounts of Lac-magnetite per work cycle for each micromixer are summarized in **Table 2**.

3.2 Life cycle impact assessment of the operation stage

Figure 4 shows the impact assessment results for the wastewater treatment operation stage for all micromixer devices. The total impact of each micromixer, in this stage, was determined by the summation of all impacts during ten work cycles. **Figure 4** compiles the results of all evaluated impact categories for the six devices under study. Overall, the LCIA results showed that in the operation stage, circular and rectangular-3D micromixers presented 30% more impact than the other micromixers. This finding can be explained by the high retention of these devices and the Lac-magnetite amount required for each work cycle. Two vertical loops and multiple layers micromixers presented the lowest impact in all impact categories, due to their high retentions per work cycle. Impact assessment was measured in four general categories: human health, ecosystem quality, climate change and resource depletion.

Figure 4A–C show impacts on human toxicity, non-cancer effects category, human toxicity-cancer effects category, and photochemical ozone formation category, respectively. Results indicate that human toxicity impacts are mainly related to ABTS use. This is a chemical compound used to track the reaction kinetics of specific enzymes such as laccases [24]. In this study, ABTS is used in the enzymatic activity assay of the obtained Lac-magnetite bionanocompounds. Assessment of toxicological effects of ABTS emitted into the environment were considered by estimating a specific characterization factor, i.e., comparative toxic units (CTUh). This factor provides an estimate of increase morbidity for the human population per unit mass of an emitted chemical (cases per kilogram) by assuming equal weighting between cancer and non-cancer situations [25]. However, some studies have

<i>Micromixer</i>		<i>Two vertical loops</i>	<i>One loop</i>	<i>Two horizontal loops</i>	<i>Triangular</i>	<i>Circular</i>	<i>Rectangular-3D</i>
<i>Retention rate</i>		87%		80%	40%		0%
<i>Lac-magnetite amount (mg)</i>	<i>Cycle 1</i>	5		5	5		5
	<i>Cycle 2</i>	0.65		1	3		5
	<i>Cycle 3</i>	0.0845		0.2	1.8		5
	<i>Cycle 4</i>	0.0109		0.04	1.08		5
	<i>Cycle 5</i>	0.0014		0.008	0.648		5
	<i>Cycle 6</i>	0.00018		0.0016	0.3888		5
	<i>Cycle 7</i>	2.4E-05		0.00032	0.2332		5
	<i>Cycle 8</i>	3.1E-06		6.4E-05	0.1399		5
	<i>Cycle 9</i>	4.08E-07		1.28E-05	0.0839		5
	<i>Cycle 10</i>	5.3E-08		2.5E-06	0.0503		5

Table 2.
Lac-magnetite amount per work cycle for the six micromixer devices.

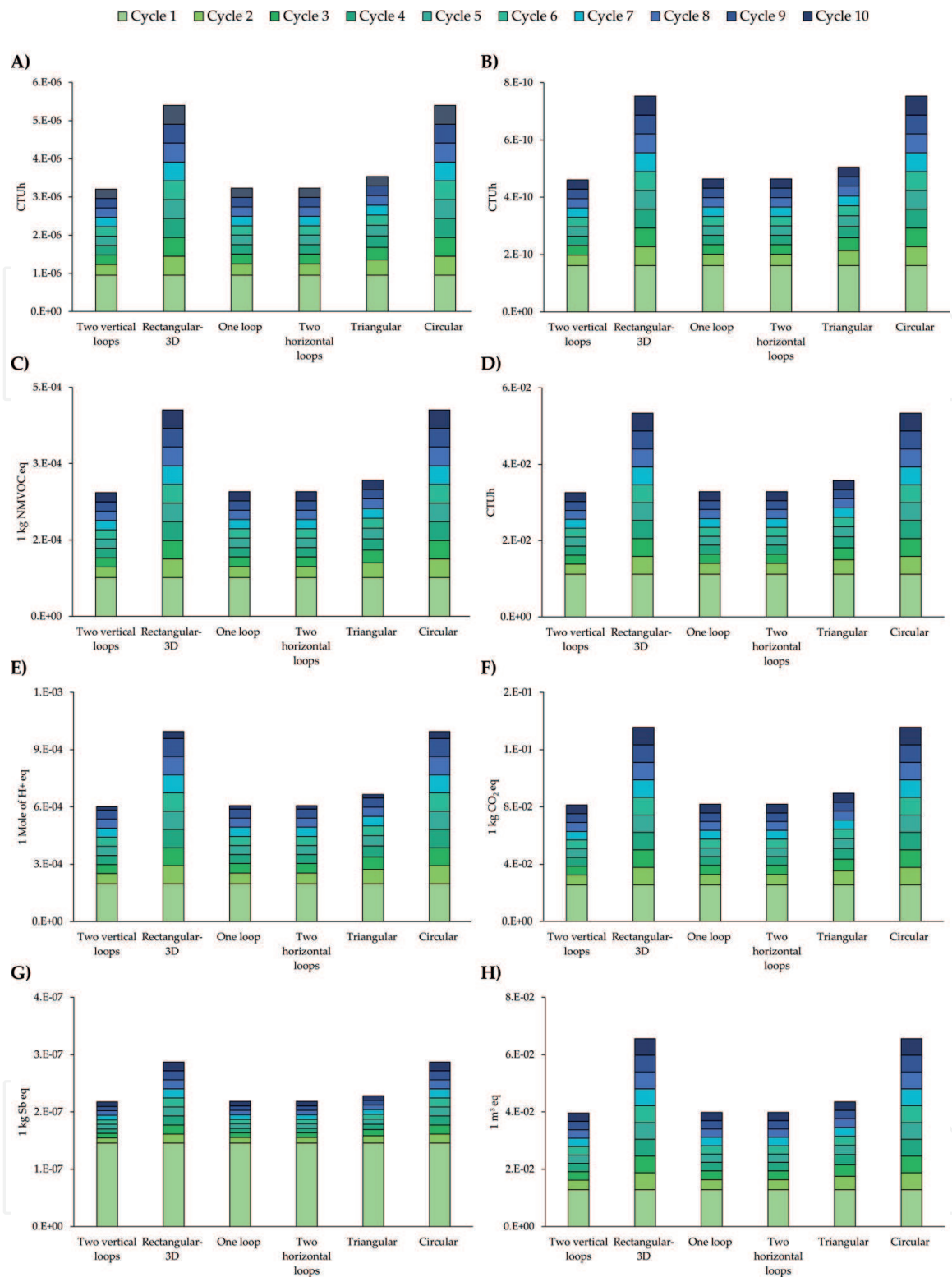


Figure 4. Impact assessment results for the operation stage: A) human toxicity, non-cancer effects, B) human toxicity, cancer effects, C) photochemical ozone formation, D) Ecotoxicity freshwater, E) freshwater and terrestrial acidification, F) climate change, G) resource depletion, minerals and metals, H) resource depletion, dissipated water.

proposed the calculation of human health effect factors for cancer and non-cancer effects via ingestion and inhalation exposure, respectively. Additionally, toxic effects models have been considered to determine impacts on human health per kilogram substance emitted [26]. These calculations have been developed through steps, such as environmental fate, exposure, and effects of chemicals, which implies a cause–effect chain that links emissions to impacts.

Regarding the ecosystem quality, **Figure 4D** and **E** present impacts on the ecotoxicity freshwater category and the freshwater and terrestrial acidification category. According to Aurisano et al. [27], assessing ecotoxicological impacts on freshwater ecosystems after chemical exposure is an important component of various environmental and chemical management frameworks. These impact categories were considered here because we needed to determine impacts associated with compounds from our process that potentially come into contact with aquatic organisms and human beings [28]. Results showed that ABTS had the highest impact contribution on these categories due to its potential impact on aquatic ecosystems. Many authors have agreed that freshwater acidification is mainly caused by protons resulting from the mineralization of nitrogen and sulfur deposition, while carbon dioxide is the main cause of (coastal) marine acidification [29, 30]. These environmental impacts directly compromised the operation stage of micromixers in wastewater treatment.

Figure 4F shows impacts on the climate change category. Emissions of CO₂ and other greenhouse gases (GHGs), aerosols, and ozone precursors are thought to be responsible for detrimental climate impact [31]. In this study, energy use in operation processes of micromixers had the highest contribution to this impact category, which agrees well with previous studies [32]. This energy along with the energy used during the life span of a micromixer comprise the life-cycle energy and emissions footprint. According to Yousefi et al. [33], in addition to the energy consumption issue, greenhouse gas (GHG) emission issues and an understanding of emissions in a production process based on the kilogram of carbon equivalent (CO₂eq) are also critical in any production process. Several studies have reported some greenhouse gas removal technologies that will be needed to balance residual emissions and meet the emission targets [34]. Overall, most of these technologies proposed involve carbon dioxide removal or conversion of a higher global warming potential (GWP) gas to a lower GWP gas [35]. However, some removal technologies require significant amounts of energy for both installation and operation. Therefore, it is necessary to continue investigating in this field to assess potential environmental tradeoffs, including those related to energy use and climate change.

Finally, **Figure 4G** and **H** show the impact assessment results for the resource depletion of minerals and metals category and resource depletion of dissipated water category, respectively. Results in these categories are mainly associated with the energy consumption due to the use of non-renewables such as fossil fuels. According to Klinglmair et al. [36], resources could be evaluated according to their depletion (consumption related to geological or natural reserve), scarcity (economic availability) and their criticality (a resource that is scarce and crucial for society). Hence, depletion refers to the decrease of the physical amount of a resource that is available for future human use [37]. Minerals and metals depletion are considered within the abiotic depletion potential (ADP) method, which is recommended by the ILCD handbook and the Product Environmental Footprint (PEF) as the best available practice for assessing resource depletion on a midpoint level [37, 38]. Therefore, here we considered this impact category to determine the potential impacts associated with resource use when operating wastewater treatment processes enabled by the developed micromixers. However, both environmental and human health impacts related to extraction or use, such as toxic emissions, are kept as separate environmental impact categories, and resource depletion directly impacting ecosystem health was disregarded in importance.

3.3 Total impact assessment

Figure 5 shows the impact assessment results of manufacturing and operation stages for each micromixer. The manufacturing stage had the highest contribution

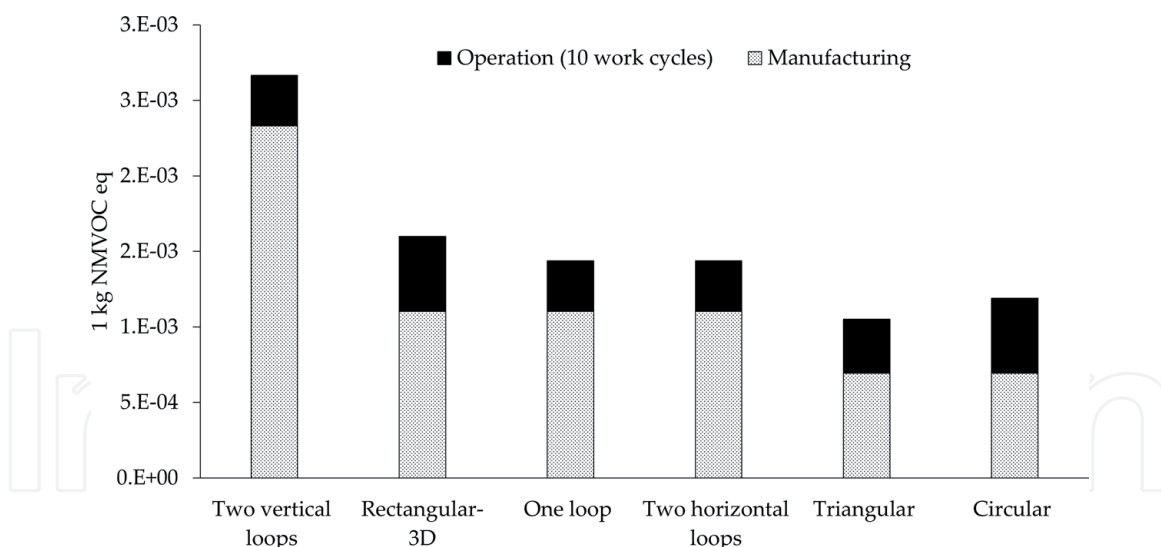


Figure 5.
Impact assessment of manufacturing and operation stages in the photochemical ozone formation category.

to the total impact of each micromixer in the photochemical ozone formation category, representing 87% in the two vertical loops micromixer, 69% in the rectangular-3D micromixer, 77% in the one loop and two horizontal loop micromixers, 66% in the triangular micromixer, and 58% the in circular micromixer. Similar results were also obtained in other impact categories due to the energy spent for laser cutting to manufacture the device in addition to the use of raw materials, such as PMMA. Regarding the operation stage, results showed that although ten work cycles for each micromixer were considered, this stage had the lowest contribution in all impact categories. This can be explained by the use of low impact raw materials in the enzyme activity assay, the preparation of artificial wastewater, and the micromixer operation.

Specifically, the two vertical loops micromixer presented, on average, 56% more impact in all categories than other micromixers, considering the manufacturing and operation stages. In contrast, the circular micromixer had the lowest impact in the manufacturing stage due to a significant reduction in the use of PMMA. Also, this micromixer had the highest impact during the operation stage due to its low retention of Lac-Magnetite, which leads to an increased requirement of the bionanocompound per cycle. However, total impact of circular micromixer is one of the lowest compared to other designs. This result showed that to calculate the impact assessment, it is necessary to consider all stages of a micromixer from its manufacture to its final operation.

4. Conclusions

Results from this study showed that six prototypes of micromixers for wastewater treatment can be analyzed in terms of impacts to human health and environment using the LCA methodology. This tool confirmed to be useful for this early research stage as it allows to identify potential impacts during the different phases required to implement these technologies.

According to the four general impacts categories considered in this study, we successfully identified the main flows that contributed to each one. The ABTS chemical for enzyme activity assays significantly contributed to human health and ecosystem quality categories. Assessment of potential toxicological effects of this compound on human health were determined in several impact categories including

human toxicity, cancer and non-cancer effects, and photochemical ozone production. Also, in terms of ecosystem quality, impacts on the ecotoxicity freshwater and the freshwater and terrestrial acidification categories were considered. Toxic effects of the ABTS were the highest compared to other raw materials during the operation stage mainly due to its release to aquatic ecosystems where it might eventually reach organisms and human beings. Moreover, energy use contributed to climate change and resource depletion categories. Emissions of CO₂ and other greenhouse gases were considered in the climate change category. Regarding the resource depletion category, results showed that the use of non-renewables such as fossil fuels to produce electricity was the major contributor to this category.

Multiple layers micromixers showed the highest impact while the one-layer ones the lowest. These results were associated directly with the manufacturing stage, where PMMA and energy used had the highest contribution to impacts on environmental and human health categories, respectively. Therefore, the manufacturing stage had the highest contribution to the total impact of each micromixer in all impact categories. Also, the operation stage depended directly on the retention of the active bionanonanocompounds within each micromixer, in addition to others raw materials necessary for wastewater treatment. Finally, impact assessment results of the manufacturing and operation stages determined the total impact of a micromixer during its work cycle.

This study represents a first step for the impact assessment on the environment and human health of the wastewater bioremediation treatment enables by low and high efficiency micromixers. Moreover, this work sets a starting point to further explore the potential of micromixers and the possible environmental concerns arising from their implementation in large-scale operations.

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