LIGNIN UTILIZATION FOR THE REMOVAL OF MICROPLASTIC PARTICLES FROM WATER
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UTILIZAREA LIGNINEI PENTRU ÎNDEPĂRTAREA PARTICULELOR DE MICROPLASTIC DIN APĂ

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ABSTRACT

The focus of the research was to evaluate the use of lignin from different sources as an agent for the removal of diverse types of microplastics when present in wastewater. Organosolv lignin was obtained from three different sources (Miscanthus sp., pine bark and solid anaerobic digestates from Organic Fraction of Municipal Solid Wastes) by an ethanol-based organosolv treatment carried out in a pressurized stirred-tank reactor. The lignins obtained were evaluated as an adsorbent for diverse types of microplastics: High-density polyethylene (HDPE), Polystyrene (PS), Expanded Polystyrene (EPS), and Polypropylene (PP). All lignins used had the capacity to capture plastic particles from all plastic types, but a differential absorbance potential was found both for plastic types and lignin samples. EPS was the least adsorb type for all lignin sources, with the remaining plastics presenting equivalent results. Pine bark lignin was the best adsorbent among the tested feedstocks, always presenting the best performance for all plastic types. The direct utilization of organosolv hydrolysates, avoiding lignin recovery presented a similar behaviour. These results open the possibility to develop new natural, plant-based, adsorbents for microplastic removal from contaminated wastewater.

REZUMAT

Scopul cercetării a fost evaluarea utilizării ligninei din diferite surse ca agent pentru îndepărtarea diferitelor tipuri de microplastic atunci când sunt prezente în apele uzate. Lignina a fost obținută prin metoda organosolv din trei surse diferite (Miscanthus sp., scoarță de pin și digestat anaerob solid din fracțiunea organică a deșeurilor solide municipale) printr-un tratament pe bază de etanol, realizat într-un reactor sub presiune cu agitare. Lignina obținută a fost evaluată ca adsorbant pentru diverse tipuri de microplastic: polietilenă de înaltă densitate (HDPE), polistiren (PS), polistiren expandat (EPS) și polipropilenă (PP). Toate tipurile de lignină au avut capacitatea de a capta particule de plastic din toate tipurile de plastic, dar a fost observant un potențial de absorbție diferențiat atât pentru tipurile de plastic, cât și pentru mostrele de lignină. EPS a fost tipul cel mai puțin captat pentru toate sursele de lignină, materialele plastice rămase prezentând rezultate echivalente. Lignina din scoarța de pin a fost cel mai bun adsorbant dintre materiile prime testate, prezentând cea mai bună performanță pentru toate tipurile de plastic. Utilizarea directă a hidrolizatelor de organosolv, evitând recuperarea ligninei a prezentat un comportament similar. Aceste rezultate deschid posibilitatea de a dezvolta noi absorbanți naturali, pe bază de plante, pentru îndepărtarea microplasticului din apele uzate contaminate.

INTRODUCTION

Plastic production has so far exceeded 348 million tonnes per year and despite the great efforts to reduce its use, the production is expected to double by 2035. Since it will take hundreds of years for some of the polymers in plastics to fully mineralize 3-5 hundreds of years for the majority of plastic materials), plastics cause serious pollution due to their cumulative and persistent properties (*Geyer et al., 2017*). (*Moharir & Kumar, 2019; Kyrikou & Briassoulis, 2007*).

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Plastics are made from various combinations of over 5,000 different polymers and other chemicals. In general, the origin of microplastic (MP) particles can be divided into two sources: primary and secondary. Primary microplastic particles are produced as such to be used in the manufacture of larger objects, or e.g. directly in cosmetic products such as facial scrubs and toothpaste, or in abrasive blasting (e.g. to remove varnish). Secondary microplastic particles are formed from the breakdown of larger plastic products (*Zhang & Zhang, 2021*).

Several definitions of Microplastics (MP) are present in the literature. Generally, polymer particles with a diameter of 1 μm to 1 mm are called microplastics (*Bayo et al., 2020; Kefer et al., 2021*), but the most common adopted definitions define MP as fragments of any type of plastic less than 5 mm in length (*Bergmann et al., 2015; Koelmans et al., 2019; Crawford & Quinn, 2016, 2016; Collignon et al., 2014, Morioka et al., 2023*).

This diversity in size, is followed by a diversity in shapes, such as microspheres, fragments, foil foam granules, and fibres, to name just a few. Most synthetic particles are plastic fibres (*Belioka and Achilias, 2023).*

As previously mentioned, the most common definition of microplastics is defined as particles with a longest diameter of less than 5 mm. Plastic particles fall into two categories: primary microplastics, which are developed for industrial use, and secondary microplastics, which are produced when plastic products and things break down. Practically speaking, the size range that has been established has been accepted as it is thought to be the range at which many biota species take food. On the other hand, nanoplastics, which fall under the conventional definition of microplastics, are described as plastic particles with a size between 0.001 and 0.1 μm (*Lusher et al, 2017)*.

In recent years, significant attention has been drawn to the widespread presence of microplastic particles in nature and the potential threat posed by their ingestion by living organisms and their accumulation over the trophic system (*Ziani et al, 2023*). However, reliable, easy, cost-effective and reproducible ways to minimize these constraints still remain an important issue to be solved. As plastics degradation, e.g. by **biological means** is a challenge, due to their **hydrophobic nature,** a number of methods have been developed to remove microplastics from water. These include i) filtration, ii) froth flotation, iii) microbial transformation/dissimilation; iv) electrostatic separators, v) microplastics aggregation; vi) biological aggregation and vii) use of organosilanes.

1. **Filtration is the** simplest method, although it is limited to the efficiency of the filtration process. Filter-based technologies such as biofilter (*Liu et al., 2020*), ultrafiltration (UF) (*Tadsuwan & Babel, 2022*), rapid sand filter (RSF) (*Sembiring et al., 2021*), among others, have achieved the best performance in removing microplastics. Among them, the RSF technology ensures quick and effective removal of microplastics.
2. **The froth flotation method** - is a physicochemical separation based on the differences in surface properties of materials. The principle behind this method is that hydrophobic plastics are picked up by air bubbles and rise to the surface, where they are collected and separated from hydrophilic plastics (*Wang et al., 2015; Crawford & Quinn, 2017; Kokkilic et al., 2022*).
3. **Microbial transformation/dissimilation –** consists on plastic decomposing by microorganisms, involving (a) microbial adhesion to the polymer's surface, (b) the polymer's use as a carbon source, and (c) polymer degradation. Besides being difficult, this process also takes a long time.
4. Electroseparation is another possibility, in which the recovery rate of microplastics is almost 99%, making it an effective and promising technique for density separation (*Felsing et al., 2018*).
5. **Aggregation, e.g., flocculation** using chemical or biological substances is one of the promising methods for plastic separation. In this process flocs interacted with microplastics through hydrogen bonding, van der Waals forces or electrostatic forces (*Duan and Gregory, 2003; Lapointe et al., 2020*).
6. **Biological agglomeration,** is another method of purifying wastewater from microplastics is the use of bioreactors. A bioreactor system removes microplastics mainly through microbial uptake and sludge aggregate formation. In particular, domestic activated sludge likely promoted the accumulation of microplastics in wastewater treatment plants. The deposit containing microplastics is removed during the subsequent secondary deposition process (*Jeong et al., 2016*).
7. **Treatment with organosilanes**. The interaction of the organic group of organosilanes with the surface of microplastics leads to their attachment to the surface of the microplastic being collected in agglomerates in the first stage of the fixation process. The disadvantage of this method is the need to remove organosilane residues from the water.

Although the methods described above are suitable for purifying water from plastic in the vicinity of the source of contamination, they also present some drawbacks as: high costs for reactors of complex systems, the need to perform additional operations to remove some of the added compounds from the treated water, the use of chemical substances, etc.

As alternative to the former processes, the use of lignin for microplastic extraction from water is starting to be discussed and investigated, as it could represent a natural and safe manner to clean wastewaters that does not involve additional equipment or special reactors/tanks for processing the wastewater.

It has been proven in many publications that lignin can be used as an absorbent of metal ions. The factor that is responsible for the sorption function of lignin, free phenolic hydroxyl group and abundant vacant ortho- or para-sites, is able to absorb the heavy metal ions (Gupta et al.,2021). Therefore, due to the hydrophobic nature of plastics (PE, HDPE), it is possible for plastic microparticles to attach to lignin particles and form micro agglomerates.

Lignin is a complex natural polymer that represents up to one-third of the lignocellulosic biomass content whose structure depends on the origin source and on the method of obtaining it. Lignin can be extracted from many lignocellulosic biomass residues and byproducts. In industry it is typically extracted using alkaline or sulphite-base processes, producing kraft lignin, or lignosulfonates, which are byproducts of the pulp and paper industry (Ekielski and Mishra, 2021). Other types of lignin are those obtained as biorefinery by-products, i.e. after steam explosion or acid hydrolysis pre-treatments (*Martins et al., 2022, Gosselink, 2011*), but the purity of those lignins is typically low. High pure lignins, i.e., more reactive, and containing a low carbohydrate and ash content, can be obtained through the pre-treatment of biomass with organic solvents, i.e., alcohols, organic acids, or ketones, called organosolv processes (*Zhang et al., 2016*). Organosolv covers a broad range of solvents but the most used is ethanol due to its low cost and low boiling point, which allow an easy recovery. Furthermore, it also allows an efficient delignification (*Carvalheiro et al., 2022*).

MATERIALS AND METHODS

Biomass feedstocks

Mischantus sp. biomass was purchased from Comgoed (NL) by TU Delft and distributed by the Dutch Organization for Applied Scientific Research (TNO) within the consortium of the Brisk 2 project. The feedstock was supplied as pellets and was stored in plastic containers at room temperature. Pine bark (Maritine Pine, *Pinus pinaster*) was kindly provided by a Portuguese processor (Alfarroxo, Figueira da Foz, Portugal). The feedstock was supplied as chips and was stored in plastic containers at room temperature. Anaerobic solid digestate obtained from the Anaerobic Digestion of the organic fraction of municipal solid wastes (OFMSW) was kindly provided by Tratolixo (Abrunheira, Portugal). The feedstock was supplied as a slurry, and upon reception it was dried at 80º C until constant weight, screened for the removal of plastics by visual inspection, and then stored at room temperature. Figure 1 presents their typical morphology. All materials were milled to pass a 4 mm screen before use.

Organosolv process and lignin recovery

Lignin was obtained by an organosolv extraction using a 2-liter stainless steel, pressurized reactor (Parr Instruments Company, Moline, IL, USA) (Figure 2). The organosolv process was carried out using a liquid-to-solid ratio (LSR) of 7 (for *Miscanthus sp.* and pine bark) and a LSR=3 for the digestate, dry basis. A solvent ethanol:water (50:50 w/w) solution was used. The process was run under non-isothermal conditions heating up to 190ºC followed by a rapid cooling to room temperature.

Miscanthus pellets Pine bark Digestate

Fig. 1 - Sources for obtaining lignin used for experiments

Fig. 2 - Laboratory equipment for extracting lignin

The liquid obtained from the process containing dissolved lignin was then treated to precipitate lignin using cold acidified water (distilled water brought to pH = 2 using H_2SO_4). After incubation (2 h, 30ºC, 150 rpm), the precipitated lignin was recovered after centrifugation in a benchtop centrifuge (at 5000 *g*, 20 min, room temperature).

After drying (45ºC, 48 h), the lignin thus obtained was intended to be used as a capturing agent for microplastics. Another approach was to use water contaminated with microplastics to precipitate lignin, analysing in the end both the precipitated lignin and the supernatant obtained.

Microplastic particles preparation

The types of plastic used were high-density polyethylene (HDPE), polypropylene (PP), polystyrene (PS) and expanded polystyrene (EPS), collected from waste commercial packaging materials. Large pieces of plastic were used to obtain microplastic by sanding the plastic pieces using sand paper with a medium grit P40 (ISO 6344). Plastic samples thus obtained were mixed with water at a 1:20 ratio (Figure 3).

HDPA EPS PS PP

Fig. 3 - Plastic samples used for experiments

Microplastic particles characterization

A Brightfield OPTIKA B-50 microscope equipped with camera was used to determine the size of the different types of microplastics. The plastic-water solution was mixed and samples were placed on microscope slides and then observed using the 40x objective, taking pictures. Reference size images were taken using a 100 μm wide object subjected to microscope observation (Figure 4). The images were processed using ImageJ.JS software version 11.

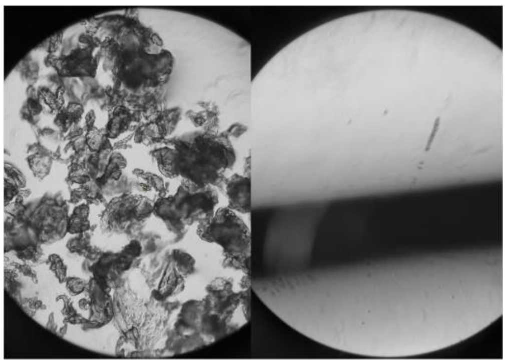

Fig. 4 - Plastic size analysis

Left – Microscopic view of the Microplastic water solution microscopic; right – reference size picture

Microplastics removal by lignin assays

To determine the possibility of removing microplastic from water using lignin, microscope slides were covered with 0.5 g of the three types of lignin obtained, dried, and weighed (Figure 5). The microscope slides were submerged in the plastic-water suspension (contained 1 g of plastic in 500 ml demineralized water), prepared for each type of plastic and left inside the mix for two hours, in circular containers with 60 mm diameter, next creating a water current at 50 rpm using a magnetic agitator, in order for the plastic particles to flow through the solution. The slides were then removed, weighed and let to dry.

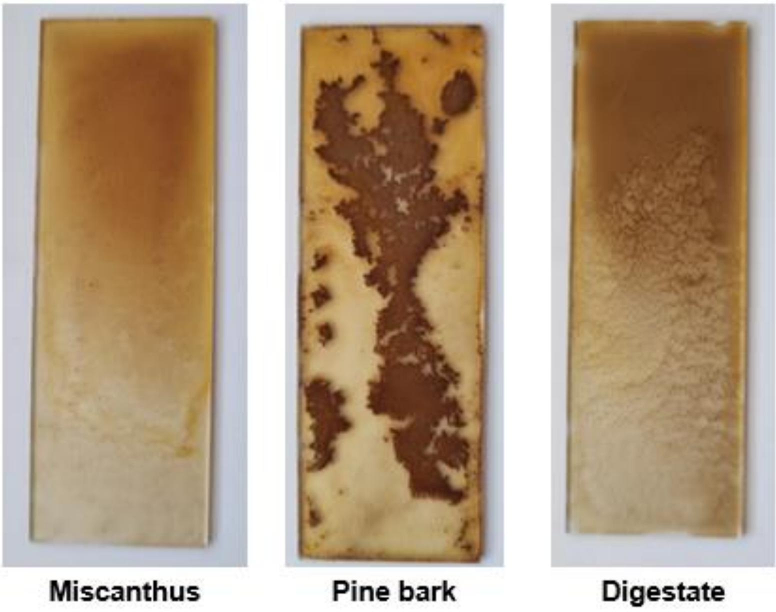

Fig. 5 - Slides covered with lignin samples

The dried slides were observed using the microscope to determine if the plastic particles adhered to the lignin particles for each type of plastic and each type of lignin separately.

A second test using the hydrolysate obtained directly from organosolv treatment which contained soluble lignin, was also carried in order to evaluate both lignin and plastic co-precipitation. For this method, 50 ml capacity falcons were used, adding 6.5 g of lignin solution (organosolv hydrolysate) to the falcon and 26 g of plastic containing-water 1:20 solution. The filled falcons (Figure 6) were set in the incubator at 30 ^oC for 2 h.

Fig. 6 - Falcons containing organosolv and plastic-water solution:
Left – before centrifugation; right – after centrifugation

After incubation, the suspension was centrifuged as described above. The supernatant) was removed and weighed and the falcon with pellet was dried in oven (45º C, 48 h) and then weighed.

RESULTS

Plastic particle size analysis

Using the particle size analysis software, 50 measurements were taken, the results being presented in Table 1.

Table 1

Plastic particle size analysis results

	Area	Mean	StdDev	Min	Max	Perim.	Angle	Circ.	Median	Skew	AR	Round	Solidity	Length
No.	μm²	μm²		μm	μm	μm	°		μm					μm
1	2634	118	37	55	202	546	-34	0	116	-0	0	0	NaN	546
2	1947	142	42	54	214	400	-37	0	151	-0	0	0	NaN	400
3	2451	137	28	102	195	506	-29	0	131	-0	0	0	NaN	506
4	1214	61	14	38	96	248	-49	0	58	-0	0	0	NaN	248
5	1947	104	33	38	200	404	-49	0	101	-0	0	0	NaN	404
6	2290	96	32	47	184	472	-40	0	94	-0	0	0	NaN	472
7	2588	26	27	0	170	538	-39	0	22	-0	0	0	NaN	538
8	2954	58	18	26	142	615	-140	0	51	-0	0	0	NaN	615
9	1351	87	22	51	136	278	-48	0	85	-0	0	0	NaN	278
10	2359	97	27	34	152	490	-116	0	100	-0	0	0	NaN	490
11	1351	93	39	37	186	276	-99	0	82	-0	0	0	NaN	276
12	412	101	18	68	132	84	-31	1	105	-0	0	0	NaN	84
13	2290	90	18	57	140	475	-118	0	85	-0	0	0	NaN	475
14	1443	83	32	44	166	298	-37	0	69	-0	0	0	NaN	298
15	2107	109	38	48	184	435	-66	0	104	-0	0	0	NaN	435
16	962	107	34	57	187	195	-152	0	103	-0	0	0	NaN	195
17	1305	110	39	51	187	268	-90	0	102	-0	0	0	NaN	268
18	802	110	32	59	211	163	-58	0	102	-0	0	0	NaN	163
19	412	119	20	86	163	82	-126	1	118	-0	0	0	NaN	82
20	389	138	32	87	199	77	-150	1	145	-0	0	0	NaN	77
21	802	111	39	55	204	163	-118	0	109	-0	0	0	NaN	163
22	2061	72	23	39	171	427	-56	0	63	-0	0	0	NaN	427
23	1534	95	28	59	185	315	-138	0	91	-0	0	0	NaN	315
24	1512	119	47	37	206	310	-9	0	113	-0	0	0	NaN	310
25	1008	168	20	119	204	206	3	0	170	-0	0	0	NaN	206
26	2267	89	23	51	153	467	-43	0	85	-0	0	0	NaN	467
27	2886	84	26	36	153	597	-74	0	83	-0	0	0	NaN	597
28	916	108	15	85	136	187	-90	0	102	-0	0	0	NaN	187
29	5451	64	29	34	187	1132	-163	0	53	-0	0	0	NaN	1132
30	4191	69	24	34	161	870	5	0	64	-0	0	0	NaN	870
31	916	132	26	68	184	185	-53	0	132	-0	0	0	NaN	185
32	847	73	16	51	117	172	2	0	70	-0	0	0	NaN	172
33	2863	84	26	35	136	595	-77	0	85	-0	0	0	NaN	595
34	2657	70	28	34	156	552	-142	0	61	-0	0	0	NaN	552
35	1672	102	32	51	187	345	2	0	100	-0	0	0	NaN	345
36	1351	101	37	34	187	278	-94	0	91	-0	0	0	NaN	278
37	1992	166	35	85	213	410	-138	0	172	-0	0	0	NaN	410
38	2473	95	35	39	187	512	-48	0	91	-0	0	0	NaN	512
39	1145	115	29	53	175	235	-27	0	113	-0	0	0	NaN	235
40	1328	135	32	68	203	272	10	0	134	-0	0	0	NaN	272
41	756	149	29	101	212	152	-13	0	150	-0	0	0	NaN	152
42	2176	100	36	39	181	449	129	0	98	-0	0	0	NaN	449
43	2107	141	32	74	204	434	-14	0	136	-0	0	0	NaN	434
44	1374	72	23	46	153	282	-40	0	70	-0	0	0	NaN	282
45	1328	86	24	51	149	272	-64	0	86	-0	0	0	NaN	272
46	2451	99	32	51	204	505	-62	0	91	-0	0	0	NaN	505
47	1099	121	32	60	185	225	-27	0	117	-0	0	0	NaN	225
48	1947	115	42	47	216	404	-174	0	114	-0	0	0	NaN	404
49	802	120	37	56	186	163	-152	0	114	-0	0	0	NaN	163
50	618	109	32	60	161	123	-39	1	107	-0	0	0	NaN	123

Regardless of the plastic type, it was found that the minimum length of plastic particles was 77 μm, the smallest total area of 389 μm^2 and the maximum length was 1132 μm with an area of 5451 μm. The average length was 362 μm with a total area of 1755 μm.

Plastic removal

The dried slides covered with lignin and then submerged in plastic-water solution were observed under the microscope and the images taken were analysed (Figure 7), showing that the smaller plastic particles adhered to the lignin particles, which could mean that there is good potential for using lignin as water cleaning agent for water contaminated with microplastic.

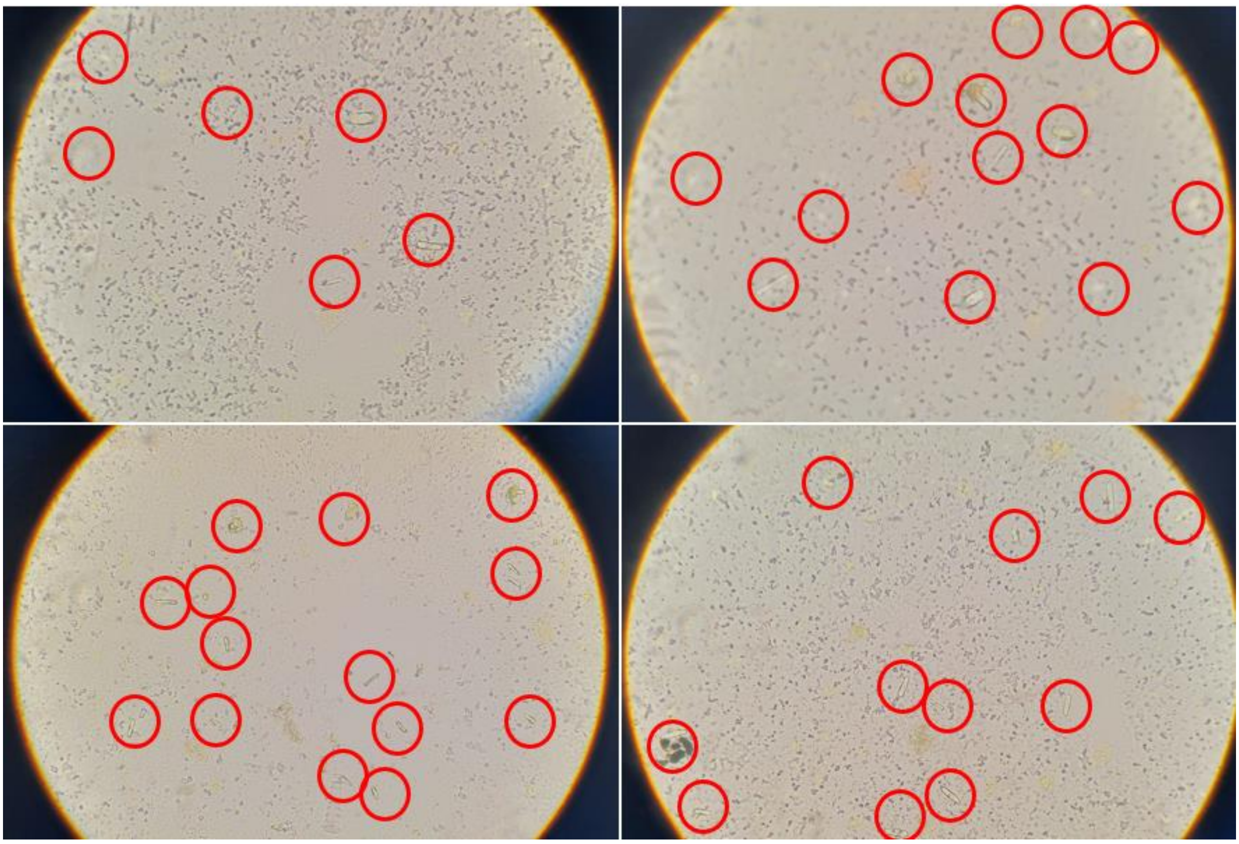

Fig. 7 - Plastic captured in lignin solution

The results from calculating the percentage of plastic removed from the plastic-water solution using lignin as a capture agent are shown in Figure 8.

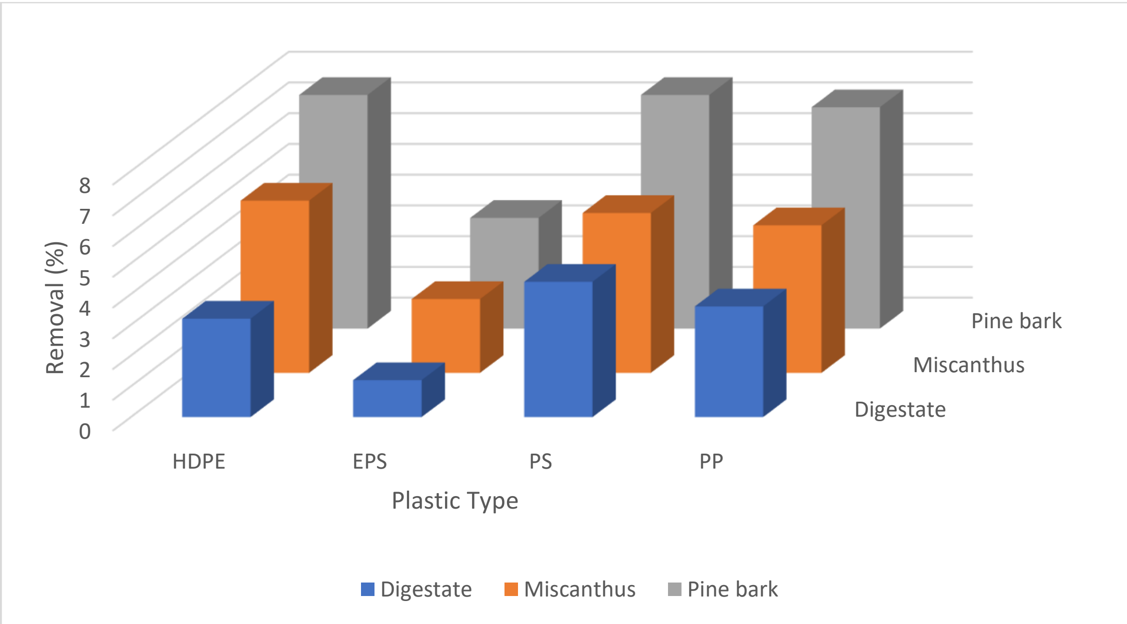

Fig. 8 - Microplastic removal as a function of plastic type and lignin origin

Analysing the data in Figure 8, it was found that the best results were obtained in the case of pine bark lignin and the weakest plastic removal was in the case of digestate lignin, for all the types of plastics used. Also, expanded PS adhered the least to the lignin surface for all types of lignin.

The results from calculating the amount of plastic captured in the lignin by using the plastic-water solution to precipitate the lignin are shown in Table 2 and figure 9.

Table 2

Results from calculating the amount of plastic captured in the lignin after precipitation

Lignin types	Plastic types	Falcon	Organosolv	Plastic water solution	Filled falcon	Supernatant	Falcon + precipitate	Plastic captured in the precipitated lignin	Percentage of plastic captured
		g	g	g	g	g	g	g	%
Miscanthus sp.	HDPA	13.76	6.51	26.05	46.32	26.13	20.19	0.076	5.80
	PS	13.93	6.51	26.05	46.49	26.09	20.40	0.036	2.80
	PS	13.75	6.5	26.04	46.29	26.11	20.18	0.069	5.29
	PP	13.75	6.62	26.04	46.41	26.11	20.30	0.075	5.75
Pine bark	HDPA	13.92	6.51	26.05	46.48	26.14	20.34	0.089	6.80
	PS	13.81	6.51	26.06	46.38	26.10	20.28	0.038	2.90
	PS	13.78	6.5	26.05	46.33	26.13	20.20	0.080	6.14
	PP	13.81	6.51	26.05	46.37	26.16	20.21	0.108	8.30
Digestate	HDPA	13.84	6.52	26.06	46.42	26.07	20.35	0.014	1.10
	PS	13.84	6.51	26.04	46.39	26.05	20.34	0.007	0.55
	PS	13.76	6.52	26.05	46.33	26.07	20.26	0.020	1.55
	PP	13.74	6.51	26.04	46.29	26.05	20.24	0.008	0.65

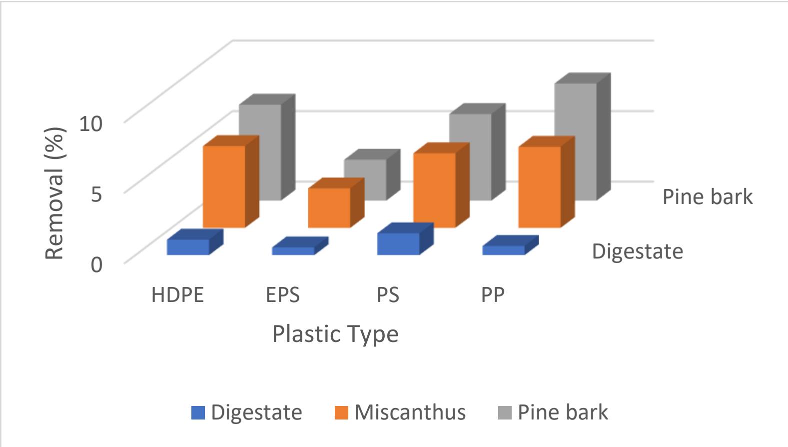

Fig. 9 - Microplastic removal from the lignin after precipitation

Analysing the data in Table 2 and Figure 9, it was found that the best results were obtained again in the case of pine bark and *Miscanthus sp.* lignin and the weakest plastic removal was in the case of digestate lignin, for all the types of plastics used. Also, expanded PS adhered the least to the lignin surface for all types of lignin. An explanation for which the percentages of plastic captured in the digestate lignin is even lower than in the case of lignin covered slides is because the digestate had a lower lignin content than *Miscanthus sp.* and pine bark, therefore, the organosolv used for precipitation had less lignin in which the plastic could be captured.

Overall, from the experiments performed to evaluate how plastic particles adhere to lignin, it was found that lignin had the capacity to capture plastic particles both in the solid lignin part after precipitation and centrifugation, but also in the supernatant obtained, leading to the conclusion that contaminated water can be used for lignin precipitation, reducing clean water consumption for obtaining lignin.

As microplastic removal using lignin has only been researched using lignin to form larger flocs that need to be subsequently removed (*Sacco et al., 2023*), the method proposed in this paper represents a novel approach for wastewater treatment and additional research is needed to further explore the effectiveness of lignin to capture microplastic particles from wastewater.

CONCLUSIONS

Microplastic poses a real threat to both humans and animals, because it can be easily ingested. Microplastic presents in wastewater or even water that is considered fresh is difficult to capture because of its size and floatability. The paper proposed a preliminary study on the possibility to use lignin as an agent for capturing microplastic particles from water by using two methods: the use of lignin as a passive filter for plastic contaminated water and the use of plastic contaminated water to precipitate lignin.

Lignin from three types of sources (*Miscanthus sp.*, pine bark and digestate) was used and the experiments were conducted using water contaminated with four types of plastic (high-density polyethylene, polypropylene, polystyrene and expanded polystyrene).

Both methods showed good preliminary results, plastic being captured in the lignin for both methods in all the samples examined, leading to the conclusion that lignin has potential for removing microplastic particles from wastewater.

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