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Energimyndighetens titel på projektet – svenska Fiberrejekt för produktion av ätlig svamp och aktivt kol	
Energimyndighetens titel på projektet – engelska Fiber rejects for production of edible fungi and activated charcoal	
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Preface

The work included in this project was carried out in a laboratory and a industrial-like environment. The project was financed by the RE:source program (2019) through the Swedish Energy Agency (50%) and the rest by industrial partners, namely Airgrinder AB, Hällnäs Handelsträdgård AB, Biosteam AB, SCA Obbola AB, and Hällnäs Handelsträgård AB. The research work was carried out successfully thanks to good collaborations with Mr Erik Bäcklund (Airgrinder AB) who contributed with maintenance work related to the cyclone dryer used to process fibre rejects, Lars Atterhem (Biosteam), and Mr Isacssion (Hällnäs Handelsträgård AB) who help during the pot-tray cultivation of oyster mushroom, Anders Kyösti and Hans Thorén (SCA Obbola) facilitated the fibre reject that was used for the whole work including analyses, sampling and delivering to SLU. The fibre rejects were processed at SLU Biomass Technology Center (BTC), help from Gunnar Kalén and Markus Segerström is greatly appreciated. Mushroom nutrition analyses were performed by SLU Husdjurens Utfodring och Vård Laboratoriet in Uppsala. Heavy metal and substrate analyses were performed by Eurofins AB, Lidköping. The work related to the production and characterization of activated carbon was done at the laboratories of the Department of Forest Biomaterials and Technology (SLU SBT) and the Department of Chemistry, Chemical-Biological Center (Umeå University). Help from professor Jyri-Pekka Mikkola (UmU) and Mr Van Minh Dinh is greatly appreciated. The project team thanks the support and advice from the supervisors at the Swedish Energy Agency. The work included in this report led to 2 scientific papers, one was published in the Journal of Environmental Science and Pollution Research (https://doi.org/10.1007/s13399-022-02618-7), and the other is a submitted manuscript under revision.

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Sammanfattning

I detta projekt utvärderades möjligheten att förbättra sammansättningen av fiberrejekt från industriell återvinning av returpapper genom en kombinerad torkning och ask avlägsnande process utförd i en cyklontork. Det bearbetade fiberrejektet användes som komponent i substrat för odling av ostronskivling, kungsmussling och shiitakesvamp. Det förbrukade substratet användes som råmaterial för produktion av aktivt kol. De främsta resultaten visar att: (1) Askhalten i fiberrejektet kan minskas med ~50 % (från 42 till 21 vikt-%). (2) Innehållet av potentiellt giftiga tungmetaller i fiberrejetket kan reduceras i stor utsträckning. (3) ostronskivling och kungsmussling växte bra i alla testade substrat gjorda av fiberrejekt. 4) shiitake lyckades inte växa på fiberrejekt substrat, flera tester gjordes med olika substrat sammansättningar, men inga ledde till fruktkroppar. 5) Förbrukat svampsubstrat kan användas som råvara för produktion av aktivt kol av jämförbar kvalitet med kommersiella produkter.

Summary

In this project, the possibility of improving the composition of fibre rejects from industrial recycling of waste paper was evaluated through a combined drying and deashing process performed in a cyclone Airgrinder. The processed fibre reject was used as a component in substrates for the cultivation of grey oyster, king oyster and shiitake mushroom. The spent substrate was used as raw material for the production of activated carbon. The main results show that: (1) The ash content of the fibre reject can be reduced by 50% (from 42 to 21 wt-%). (2) The content of potentially toxic heavy metals in the fibre reject can be reduced to a great extent. (3) grey and king oyster mushrooms grew well in all tested substrates made of fibre reject. 4) shiitake had problems growing on substrates containing fibre reject, several tests were made with different substrate formulations, but none led to fruit bodies. 5) Spent mushroom substrate can be used as raw material for the production of activated carbon of comparable quality to commercial products.

1. Background

Only in Sweden, approximately 30 Mt dry weight of biowastes are generated per year. Of this, approximately 5 Mt are variable biogenic wastes/or byproducts from wood processing, pulp & paper industry, and energy industry (ICCT 2014). Fibre rejects, generated during the recycling of waste paper, are a problematic material from the economic and environmental point of view. This type of waste is currently disposed of in landfill sites, which is costly, or used as a fuel within the paper mill which leads to energy losses due to its high moisture content (around 50-60 wt%). European pulp&paper industries produce significant quantities of biogenic wastes annually, SCA Obbola alone contributes with about 8000 tons of fibre rejects generated during the recycling of waste papers.

Among biowastes generated from industrial processes, fibre reject can be seen as an interesting resource for the development of a circular bio-economy. This waste can be classified into different types according to the composition, from coarse materials such as plastics and metals to cellulose fibre of low quality that is an interesting material for its use in substrates for the cultivation of white-rot fungi. The latter are mushrooms that can degrade and use cellulose, hemicellulose and lignin as carbon sources, i.e., they do not need (or need very little) sunlight to grow. After the cultivation period, a waste called "spent mushroom substrate (SMS)" is generated. The SMS is usually used as fuel (in some countries burned on the field) or sent to landfill sites, which leads to environmental issues.

This project was the continuation and improvement of the results obtained from a pre-project P42481-1 that was focused on finding possibilities of applying our concept "fibre rejects for a combined mushroom and fuel production". The work shown in this report is an evaluation of the effect that a process used to remove ash from raw fibre rejects has on the quality of mushroom fruit bodies when use in substrates. Instead of using the SMS as fuel, here we show how it can be converted into activated carbon of commercial quality. The activated carbon was characterized by its effectiveness during the removal of organic contaminants from water.

2. Implementation

The project has been performed successfully through the following activities:

2.1. Reduction of the amount of contaminant (ash) in the raw fibre rejects.

Fibre reject from industrial-scale recycling of waste paper was provided by SCA, Obbola. Approximately 200 Kg of fibre reject with an initial moisture content of approximately 60 % was processed in a pilot-scale cyclone "Airgrinder". The setup, Figure 1, is composed of a reverse-flow cyclone with tangential entry, i.e., a barrel mounted atop a conical body, which is used for combined fractionation and drying of the feedstock. Process drying air is drawn in by a side-channel air blower (150 kW) and is heated up with the aid of a biomass boiler with an integrated air-cooled heat exchanger. The effect of the boiler burner can be adjusted to obtain different air temperatures. Hot air from the outlet port of the blower was fed into the cyclone. The feeding system consists of a hopper and a belt conveyor resting on weighing pads connected to a digital indicator. The speed of the belt was adjusted to regulate the feeding rate. The material was lifted

from ground level to the cyclone's rotary valve feeder using a flighted belt conveyor. Thermocouples (type K) were used for the measurement of process temperatures and pitot tubes (Kimo Instrument Sweden AB) for the measurement of air velocity. A real-time data logging system (Intab PC-logger 31500-BT combined with EasyView 10) was used for the acquisition and recording of process variables. Once the feedstock enters the cyclone, particles entrained in the inlet-air flow in a helical pattern, starting at the top (cyclone inlet) and ending at the bottom (cone outlet). Coarse particles flow out of the bottom of the cyclone cone into a drum below, and a mixture of humid air and uncollected entrained fine particulate material formed an ascending spiral in the central core of the cyclone and then flow out through the gas-exit duct passing through a bag-filter where dust is collected.

Figure 1. Cyclone dryer "Airgrinder" used for processing of fibre reject.

The fraction collected in the cyclone filter was discarded due to its high ash content (approx. 83 %), and the fraction that flowed out of the cyclone cone was sieved to remove fine cellulose dust and used as such for the cultivation of edible fungi.

2.2. Cultivation of oyster and shiitake mushrooms.

Three types of white-rot fungi were used, i.e., two species of oyster mushroom (grey and king) and shiitake mushroom. For the sake of practicality, the work was done first on a micro-scale to find fibre reject-based substrate formulas that lead to the formation of fruit bodies. Substrate formulas that led to good results were used for tests on a laboratory scale and carried out using the bag technic. Finally, the substrate that led to the best results on a laboratory scale was tested on a pilot scale using the pot-tray technic.

The formulation of the substrates tested is shown in Table 1. Wheat bran and barley grain were added to all the substrates to promote productivity. Two substrates with 60 wt% and 80 wt% deashed fibre reject, i.e., DFR-60 and DFR-80, were produced. A conventional substrate formula based on birch (*Betula* spp.) sawdust (DFR-00) was used as a comparison. The substrate components were mixed on a dry basis using a ribbon mixer, and during the process, water was added to the DFR-00, DFR-60 and DFR-80 substrates to obtain mixtures with a moisture content of 65 wt%. A TESTO 0563-2062 pH meter for semi-solid substances was used for the measurement of the pH of the substrates. The pH of the DFR-00 was too low for mycelium growth, and therefore, was corrected by adding small amounts of calcium carbonate ($CaCO_3$). The pH of the substrates with 60 and 80 wt% fibre reject was too high for mycelium growth, and therefore, was adjusted by adding acetic acid (AA), malic acid (MA), oxalic acid (OA), or acid whey (AW) together with water to obtain mixtures with a moisture content of 65 wt% and a pH of approximately 6.5, Table 1. The acid whey was produced from sweet whey powder obtained from Norrmejerier, Umeå. It was reconstituted in water at 50 g/L and then inoculated with *Lactobacillus plantarum* strain LB14, thoroughly mixed and fermented at 37 °C until pH 4.

For experiments done on a micro-scale, substrates were packed into micro-boxes. For each substrate, 5 boxes were packed with 200g wet mass. For laboratory scale, substrates were packed into polypropylene breathing bags (Microsac). Bags were filled to obtain 10 blocks of 1 kg of each substrate type and sealed with a plastic clip. Pot-tray experiments were done using the substrate that led to the best results, according to what was found in the bag trials. Eight pot-trays were used for the experiments. Each tray was packed with 2.5 kg of substrate. Inoculation was carried out using commercial grain spawn from Svampkungen AB. Micro-boxes, bags and pot-trays were inoculated in a laminar flow cabinet under sterile conditions using a ratio of 2.5 wt% wet mass.

Table 1. Mushroom substrate formulations

Substrate ID [a]	Components of the initial substrate (g)							
	Deashed fibre reject	Birch sawdust	Wheat bran	Barley grain	$CaCO_3$	Acid	Water	pH [b]
DFR-00	0	280	35	35	3	0	650	6.51
DFR-60	210	70	35	35	0	0	650	7.10
DFR-80	280	0	35	35	0	0	650	7.73
DFR-60 + AA	210	70	35	35	0	13 (24%)	637	6.52
DFR-80 + AA	280	0	35	35	0	22.7(24%)	627.25	6.54
DFR-60 + AW	210	70	35	35	0	390 (50g/l)	260	6.49
DFR-80 + AW	280	0	35	35	0	650 (50 g/l)	0	6.56
DFR-60 + MA	210	70	35	35	0	1	650	6.49
DFR-80 + MA	280	0	35	35	0	3	650	6.56
DFR-60 + OW	210	70	35	35	0	1	650	6.51
DFR-80 + OW	280	0	35	35	0	3	650	6.60

[a] numbers in the substrate ID denote the amount of deashed fibre reject in wt% dry mass. [b] average value of three measurements

2.3. Evaluation of quality of fruit bodies grown on fibre rejects

Mushroom nutritional value was analysed at SLU, and the contents of potentially toxic heavy metals were analysed by Eurofins Food & Feed Testing AB.

2.4. Production of activated carbon using spent mushroom substrate (SMS)

The fibre reject-based SMS that led to the highest mushroom yield was used as raw material for the production of activated carbon. Birch wood-based spent substrate was used as a comparison. Blocks of SMS were ground in a hammer mill equipped with a 5 mm sieve and used for the experiments.

Spent substrate samples (50 g) were impregnated with an 8.6 M phosphoric acid (H_3PO_4) or an 8.6 M potassium hydroxide (KOH) solution in a weight ratio of 1 precursor : 3 acid / or base. The mixture was kneaded to obtain a homogeneous consistency and left to soak overnight at room temperature. The impregnated precursor was pyrolysed in a tubular stainless steel fixed-bed reactor (Figure 2) equipped with a thermocouple to measure the temperature of the sample.

Figure 2. The reactor used for the production of activated carbon

The reactor was heated externally by a muffle furnace. Nitrogen gas (1 L/min) was used to keep an inert atmosphere during the pyrolysis. The activated carbons were produced at different temperatures (from 500 °C to 900 °C). The temperature of the furnace was raised from room temperature up to the final treatment temperature at a rate of 10 °C/min and held for 1 h. After this, the muffle furnace was turned off, and the samples were allowed to cool down to room temperature under a nitrogen gas flow of 0.2 L/min. The chars left after the treatments were washed several times with hot water until neutral pH and finally dried overnight in an oven at 105 °C.

The specific surface area of each activated carbon was determined using a Tristar 3000 sorptometer (Micrometrics Instrument Corp., Norcross, GA, USA). Samples were also tested for their performance in the removal of organic contaminants from water.

2.5. Evaluation of technical solutions to scale up the project and raise the level of technology maturity (TRL)

The cost related to the processing of the fibre rejects was estimated using process parameters obtained from our experiments. Technical solutions are proposed based on our results and experience. Factors such as labour costs were not taken into account.

3. Results and discussion

3.1. Drying and ash removal from fibre rejects

The main goal of the combined cyclone-drying and ash removal process was to obtain a shelf-stable material and at the same time reduce the ash content and other sorts of contaminants, in other words, to increase the share of carbon sources available for mycelium growing. Fibre reject was processed using a feeding rate of 200 kg wet mass/hour. A mass balance showed that approximately 70 wt% dry mass of the fibre reject that was processed flowed out of the cone of the cyclone (so-called coarse fraction) and the remaining part was collected in the cyclone filter (so-called fine fraction). The latter had an ash content of approximately 80 wt% and was discarded. The coarse fraction was screen sieved to remove fine cellulose dust and remnants of contaminants to obtain a material suitable for use in mushroom substrates. Approximately 60 % dry mass of the raw fibre reject was left after the treatments. Table 2 shows that the combined processes, i.e., cyclone drying followed by screen sieving, were capable of reducing the moisture content from 57.8 to 2.4 wt% and the ash content from 39.2 to 21.5 wt%. Consequently, the concentration of potentially toxic heavy metals was considerably reduced (Table 2), meaning the ash removal process was capable of improving the quality of the fibre reject to a great extent.

Table 2. Substrates ash content, main elements, main ash forming and trace elements.

		Raw fibre reject	Deashed fibre rejects (DFR)	Relative change in content %
oisture content	wt% w.w.	57.8	2.4	-95.85
Ash content	wt% d.w.	39.2	21.5	-45.15
Sulfur S	wt% d.w.	0.1	0.1	0.00
Chlorine Cl	wt% d.w.	0.2	0.1	-50.00
Carbon C	wt% d.w.	33.6	39.8	18.45
Hydrogen H	wt% d.w.	3.9	4.7	20.51
Nitrogen N	wt% d.w.	0.3	0.3	0.00
Oxygen O (calc.)	wt% d.w.	22.7	33.5	47.58
Aluminium Al	mg/kg d.w.	8600.0	6200.0	-27.91
Antimony Sb	mg/kg d.w.	1.4	0.4	-71.43
Arsenic As	mg/kg d.w.	1.0	0.6	-40.00
Barium Ba	mg/kg d.w.	210.0	60.0	-71.43
Beryllium (Be)	mg/kg d.w.	0.1	0.1	0.00

Lead Pb	mg/kg d.w.	38.0	20.0	-47.37
Boron (B)	mg/kg d.w.	51.0	18.0	-64.71
Phosphorus P	mg/kg d.w.	180.0	150.0	-16.67
Iron (Fe)	mg/kg d.w.	4800.0	2800.0	-41.67
Cadmium Cd	mg/kg d.w.	1.5	0.5	-66.67
Calcium Ca	mg/kg d.w.	92000.0	62000.0	-32.61
Potassium K	mg/kg d.w.	390.0	340.0	-12.82
Cobalt Co	mg/kg d.w.	8.6	3.1	-63.95
Copper Cu	mg/kg d.w.	70.0	52.0	-25.71
Chromium Cr	mg/kg d.w.	14.0	0.2	-98.57
Mercury Hg	mg/kg d.w.	0.3	0.2	-33.33
Magnesium Mg	mg/kg d.w.	2500.0	2000.0	-20.00
Manganese Mn	mg/kg d.w.	140.0	98.0	-30.00
Molybdenum Mo	mg/kg d.w.	37.0	1.2	-96.76
Sodium Na	mg/kg d.w.	810.0	100.0	-87.65
Nickel Ni	mg/kg d.w.	310.0	11.0	-96.45
Tin Sn	mg/kg d.w.	12.0	4.9	-59.17
Titanium Ti	mg/kg d.w.	85.0	52.0	-38.82
Vanadium V	mg/kg d.w.	10.0	3.7	-63.00
Zinc Zn	mg/kg d.w.	190.0	130.0	-31.58
Silicium Si	mg/kg d.w.	32000.0	17000.0	-46.88

3.2. Cultivation of oyster and shiitake mushroom.

3.2.1. Cultivation in micro-scale

Experiments were done first in micro-boxes using shiitake and grey oyster mushrooms. Shiitake did not prosper on substrates based on fibre reject. Regardless of the substrate formulation, correction of the pH, and use of different supplements, the mycelium was not able to grow well and produce fruit bodies. Shiitake mushroom is known to be very difficult to cultivate on substrates made of materials other than clean hardwood. To check if completion between the fungi with microorganisms present in the substrate was the problem, pasteurization of fibre reject-based substrates was carried out at low (65 °C) and high (110 °C) temperatures. Using high pasteurization temperature to deactivate all kinds of adventitious organisms did not work. To check if contaminants (other than microorganisms) present in the reject fibres hinder the shiitake mycelium from producing fruit, the reject was washed several times with hot tap water and dried in an oven at 105 °C. Substrates made with the washed reject led to the same result, shiitake does not succeed on substrates made of fibre reject no matter what kind of reject or substrate formula is used.

Grey oyster, on the other hand, grew well on substrates made of fibre reject. Figure 3 shows a picture of the fruit bodies obtained from the substrates.

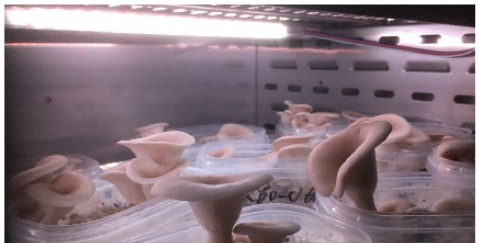

Figure 3. Micro-box experiments with grey oyster mushroom.

Mycelium of grey oyster grew faster on substrates made of fibre reject (DFR-60 and DFR-80) compared to the control birch wood-based substrate (DFR-00). Substrates with the addition of whey, oxalic acid and malic acid were colonized faster than the control. The colonisation time of substrates with acetic acid was slightly longer. Three flushes of fruit bodies were collected. When the accumulated biological efficiency of the first and second flush are compared, one can see that the fibre reject-based substrates resulted in comparable or higher production yields compared to the control substrate. The third flush was collected for most substrates, DFR-00 and DFR-60 + whey did not result in a third flush.

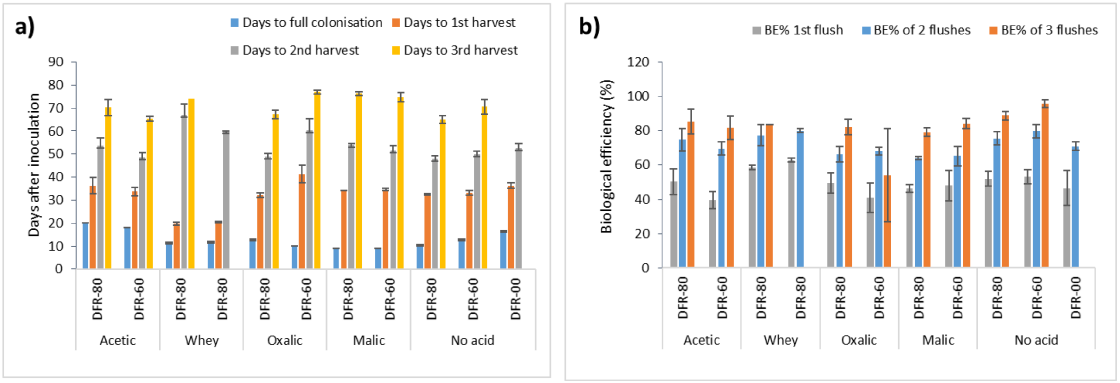

Figure 4. Days from inoculation to full colonisation, harvest of the first, second and third flush (a), and yield (b). The yield is expressed by Biological Efficiencies (BE%= fresh weight of fruit bodies*100/dry weight of initial substrate).

Substrates based on fibre reject with the addition of acid whey or acetic acid, as well as the corresponding substrates without pH correction (DFR-60 and DFR-80) and DFR-00 used as a control were chosen for cultivation on a laboratory scale using the bag technic. Substrates with oxalic and malic acid were left out because these acids are expensive and the differences in the mushroom yields obtained from these substrates compared to the others are not high enough to justify their use.

3.2.2. Cultivation on a laboratory scale.

For these experiments, grey oyster and king oyster mushroom were tested. Both species grew well in all studied substrates. Figure 5 shows a picture of the fruit bodies.

Figure 5. Bag experiments with grey oyster (left) and king oyster (right) mushrooms.

The time that it took for these two species to colonize 50 % and 100 % of the substrate block was longer for the king oyster, Fig 6. Comparing the colonisation times between substrates, one can see that both fungal species colonised the DFR-80 substrate faster than the other substrates. For both fungi, the addition of acid whey (DFR-80 + AW) prolonged slightly the colonisation of this substrate compared to the DFR-80, and the addition of acetic acid (DFR-80 + AA) led to a significantly longer colonisation time. For substrates made of a mix of fibre reject and birch wood (DFR-60, DFR-60 + AA and DFR-60 + AW) the colonisation times were similar to that of the control substrate. It was observed that the grey oyster and king oyster mycelium density in the substrates with acetic acid (DFR-80 + AA and DRF-60 + AA) was much higher than in the other substrates.

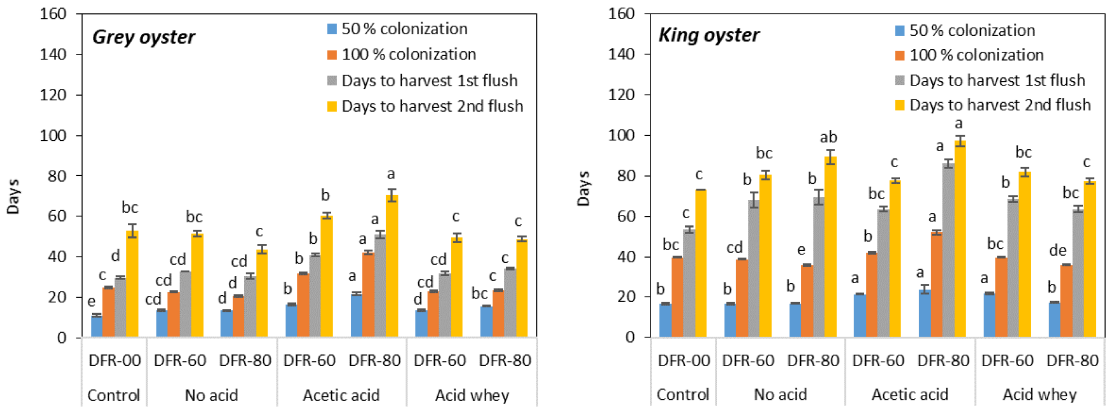

Fig. 6 Mycelium growth and days from inoculation to harvest of the first and second flush of grey oyster and king oyster. Different letters over the bars indicate statistically significant differences (according to the Tukey's multiple comparison test, $p < 0.05$) between substrates

The time required to produce the first and second flush of fruit bodies is shown in Fig. 6. For grey and king oysters, substrates with acetic acid led to a significant delay in the harvest of the first flush of fruit bodies. In general, for the other substrates containing fibre reject, the time for the harvest of the first flush of fruit bodies was comparable to or slightly longer than that of the control substrate (DFR-00). Depending on the substrate, the second flush of fruit bodies for both species was harvested between 11 and 20 days after the first flush, Fig. 6.

Differences that one can see between experiments in micro-boxes and the bags, for example, the longer colonisation and harvest time for substrates with acetic acid are probably due to the upscaling, but the relationship between results from the two is still the same.

The biological efficiency (BE) of the first and second flush of fruit bodies from the bag experiments is shown in Fig. 7. For the grey oyster, the DFR-80 + AA substrate led to the first flush highest BE with approximately 58% compared to 43% for the control substrate. For the other substrates, the BE was comparable to that of the control substrate with no significant differences. The maximum BE of the second flush of grey oyster grown on substrates containing fibre rejects was for the FFR-80 + AW with 27% and DFR-80 with 25%. For the other substrates, the BE values of the second flush ranged from approximately 14 to 20 depending on the substrate compared to 16 % for the control substrate. The substrate that led to the highest total BE of grey oyster was DFR-80 + AA with approximately 72% and DFR-80 + AW with approximately 68 % compared to 60% for the control substrate.

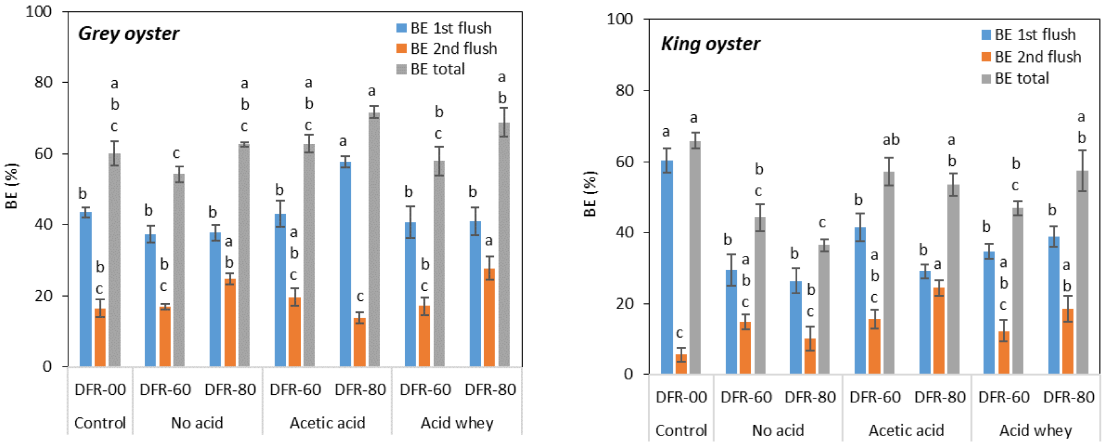

Fig. 7 Biological efficiency of the first and second flush of *grey oyster* and *king oyster*. Lack of letters in common indicates statistically significant differences (Tukey's t-test, $p < 0.05$) for comparisons of treatment means between different substrates.

For king oyster, the BE of the first flush of fruit bodies grown on substrates containing fibre rejects was lower than in the control substrate. The differences in the BE were not significant for substrates containing fibre reject. The values ranged from 29 to 41% depending on the substrate composition compared to approximately 60% for the control substrate. The BE of the second flush of king oyster fruit bodies was higher in the substrates based on fibre reject, with values from 10 to 24% depending on the substrate compared to approximately 5% for the control substrate. The total BE of the DFR-60 + AA, DFR-80 + AA and DFR-80 + AW were similar, with values from 53 to 57 % compared to 66 % for the control substrate

In the pre-project (P42481-1) grey oyster mushroom was grown on substrates made of raw fibre reject, i.e., as is produced in the paper mill without any kind of treatments. Comparing the BE of the first flush of mushrooms grown on the FR-80 substrate with the

ones grown on the deashed reject (DFR-80), one can see a clear improvement, from approximately 30% to 40%. By removing ash from the fibre reject and adjusting the pH of the substrate the BE was increased from 30% for FR-80 to 60% for DFR-80 + AA.

3.2.3. Nutritional values

The contents of ash and nutritional analysis of the first flush of grey oyster and *king oyster* fruit bodies grown on the studied substrates are shown in Table 3.

The ash contents of the grey oyster fruit bodies grown on the fibre reject substrates were slightly higher than that of the fruit bodies grown on the control substrate, with values between 4.77 and 5.57 % dw. The ash contents of king oyster fruit bodies grown on fibre reject substrates (6.00 – 5.20 % dw.) were higher than that of the fruit bodies grown on the control substrate (4.13 % dw).

Table 3. Ash content and nutritional analysis of the first flush of grey oyster and king oyster fruit bodies.

Substrate	Ash	Crude fibre	Crude fat	Crude protein
		(% dw.)		
Grey oyster				
FR00	4.77 ± 0.03 ^{a}	5.53 ± 0.17 ^{a}	1.55 ± 0.18 ^{ab}	15.8 ± 0.2 ^{a}
FR60	5.03 ± 0.12 ^{a}	4.53 ± 0.15 ^{b}	1.66 ± 0.18 ^{ab}	11.8 ± 0.3 ^{b}
FR80	4.87 ± 0.03 ^{a}	4.51 ± 0.26 ^{b}	0.92 ± 0.09 ^{b}	11.9 ± 0.4 ^{b}
FR60 + AA	5.17 ± 0.19 ^{a}	4.45 ± 0.17 ^{b}	1.27 ± 0.06 ^{ab}	15.0 ± 0.7 ^{a}
FR80 + AA	4.83 ± 0.03 ^{a}	5.18 ± 0.33 ^{ab}	1.64 ± 0.21 ^{ab}	14.2 ± 0.4 ^{ab}
FR60 + AW	5.30 ± 0.06 ^{a}	4.36 ± 0.09 ^{b}	1.81 ± 0.22 ^{a}	13.9 ± 0.3 ^{ab}
FR80 + AW	5.57 ± 0.38 ^{a}	4.35 ± 0.08 ^{b}	1.62 ± 0.20 ^{ab}	14.8 ± 1.1 ^{a}
King oyster				
FR00	4.13 ± 0.07 ^{c}	4.59 ± 0.13 ^{a}	1.71 ± 0.19 ^{a}	9.8 ± 0.3 ^{b}
FR60	5.67 ± 0.13 ^{ab}	4.29 ± 0.15 ^{a}	1.55 ± 0.12 ^{a}	11.9 ± 0.5 ^{ab}
FR80	5.60 ± 0.15 ^{ab}	4.33 ± 0.18 ^{a}	1.73 ± 0.15 ^{a}	10.3 ± 0.5 ^{ab}
FR60 + AA	5.33 ± 0.09 ^{ab}	4.30 ± 0.07 ^{a}	1.65 ± 0.07 ^{a}	11.8 ± 0.9 ^{ab}
FR80 + AA	6.00 ± 0.01 ^{a}	4.57 ± 0.17 ^{a}	1.93 ± 0.29 ^{a}	11.1 ± 0.3 ^{ab}
FR60 + AW	5.20 ± 0.26 ^{b}	4.28 ± 0.27 ^{a}	2.05 ± 0.03 ^{a}	10.7 ± 0.3 ^{ab}
FR80 + AW	5.67 ± 0.13 ^{ab}	4.87 ± 0.28 ^{a}	1.72 ± 0.04 ^{a}	12.7 ± 0.5 ^{a}

Values are given as mean ± standard error in dry weight, n = 3. Lack of letters in common indicates statistically significant differences (Tukey's t-test, $p < 0.05$) for comparisons of treatment means between different substrates.

For grey oyster grown on the fibre reject substrates, the contents of crude fibre (5.18-4.35 wt% dw.), crude fat (1.81-0.92 wt% dw.) and crude protein (14.9-11.8 wt% d.w) were slightly lower or comparable to the corresponding contents in those grown on the control substrate. For king oyser, the contents of crude fibre (4.86-4.28 wt% dw.), crude fat (2.05-1.54 wt% dw.) and crude protein (12.71-10.29 wt% dw.) were comparable to that of the fruit bodies grown on the control substrate.

3.2.4. Contents of potentially toxic elements

Fungi are known to absorb and bioaccumulate metals in the fruit bodies, one of the reasons there is interest in mycoremediation. Heavy metals are elements of relatively high density or high relative atomic weight. The correct term for them is not really heavy metals but potentially toxic elements. The most common toxic elements found in water and foodstuffs are lead (Pb), mercury (Hg), arsenic (As) and cadmium (Cd). The Food and Drug Administration (FDA) does not establish any limits for metals in foodstuffs. However, the European Commission (EC) regulation (2015/1006) set the reference up-limit values given in Table 4.

Table 4. Contents of potentially toxic elements in the first flush of grey oyster and king oyster fruit bodies.

	Arsenic (As)	Lead (Pb)	Cadmium (Cd)	Mercury (Hg)
Up-limit values set in the EC regulation (2015/1006)	0.30 µg/day per kg of human body	300 µg/kg mushroom (ww.)	200 µg/kg mushroom (ww.)	300 µg/kg fish (ww.)
Substrate ID	Values below are given in µg/kg (ww.)			
Grey oyster				
FR00	5.00 ± 0.00^{*d}	53.00 ± 5.13^{a}	65.67 ± 4.41^{a}	2.00 ± 0.00^{*c}
FR60	24.33 ± 0.67^{b}	39.67 ± 2.91^{b}	31.67 ± 0.88^{b}	4.67 ± 0.37^{b}
FR80	23.33 ± 0.67^{b}	16.67 ± 2.91^{c}	31.33 ± 3.53^{b}	4.93 ± 0.13^{ab}
FR60 + AA	17.67 ± 1.20^{c}	28.67 ± 1.76^{bc}	32.67 ± 2.91^{b}	3.63 ± 0.15^{b}
FR80 + AA	14.00 ± 0.58^{c}	37.67 ± 3.48^{b}	24.00 ± 1.53^{b}	3.90 ± 0.35^{b}
FR60 + AW	34.67 ± 1.76^{a}	22.33 ± 1.20^{c}	25.33 ± 3.18^{b}	4.93 ± 0.22^{ab}
FR80 + AW	34.00 ± 1.15^{a}	21.33 ± 1.20^{c}	22.33 ± 0.88^{b}	6.07 ± 0.47^{a}
King oyster				
FR00	5.00 ± 0.00^{*a}	24.33 ± 2.91^{a}	49.00 ± 1.73^{a}	2.00 ± 0.00^{*e}
FR60	6.23 ± 1.23^{a}	9.03 ± 0.62^{b}	34.33 ± 5.70^{b}	4.40 ± 0.55^{bcd}
FR80	6.10 ± 0.15^{a}	15.00 ± 1.53^{ab}	28.67 ± 4.91^{b}	6.00 ± 0.51^{ab}
FR60 + AA	5.00 ± 0.00^{*a}	19.00 ± 3.21^{ab}	26.67 ± 2.73^{b}	3.70 ± 0.10^{d}
FR80 + AA	5.00 ± 0.00^{*a}	16.00 ± 3.06^{ab}	31.67 ± 1.20^{b}	6.33 ± 0.41^{a}
FR60 + AW	5.07 ± 0.07^{a}	20.67 ± 2.40^{ab}	20.00 ± 0.58^{b}	4.00 ± 0.36^{cd}
FR80 + AW	6.60 ± 0.56^{a}	20.00 ± 2.65^{ab}	20.00 ± 1.00^{b}	5.60 ± 0.38^{abc}

$^{(*)}$ Below detection limits. Values are given as mean ± standard error in wet weight, n = 3. Lack of letters in common indicates statistically significant differences (Tukey's t-test, $p < 0.05$) for comparisons of treatment means between different substrates.

Results showed that grey oyster has a stronger ability to bioaccumulate As than king oyster. The levels of As in the fruit bodies from the latter were in most cases below the detection limit (5 µg/kg). The highest As level was about 35 µg per kg fresh oyster mushrooms, suggesting that an adult person of 50 kg body weight could eat as 420 g everyday without over the limit value set in EC regulations (0.3 µg per kg of human body). Clearly, the As content is far below the level risking human health and even much lower than previous reported content in oyster fruit bodies grown in raw fibre rejects (Grimm et al. 2021). The levels of Pb and Cd in the fruit bodies grown on substrates containing fibre rejects were lower than that of the fruit bodies from the control substrate.

The concentration of both metals was well below the limit values set in EC regulations. Hg concentrations in fruit bodies grown in fibre reject substrates were slightly higher than that of the fruit bodies from the control substrate, with a value below the detection limit (2 µg/kg). The concentration of Hg was in all cases well below the up-limit values set in EC regulations.

In the pre-project (project P42481-1) grey oyster mushroom was grown on substrates made of raw fibre reject, i.e., as is produced in the paper mill (see https://doi.org/10.1007/s12649-020-01311-y). Comparing the amounts of toxic heavy in the mushrooms grown on the FR-80 substrate with the ones grown on the deashed reject (DFR-80), one can see a clear reduction of the arsenic content by more than 50% (from 53.0±7 to 23.3±0.7 µg/kg (ww.)). For the other elements, some remain at approximately the same level and others increased: Pb from 10.1±5 to 16.7±3, Cd from 19±3 to 31±3, and Hg from 6.2±0.4 to 4.9±0.1.

3.2.5. Cultivation in industrial relavent environment (2.5 kg pot-tray technic)

The substrate based on fibre reject that led to the highest BE (FR80 + AA where grey oyster was cultivated) was chosen for this part of the work.

The days taken from inoculation to full colonization of the substrate and from inoculation to the harvest of the first and second flush were shorter compared to the results obtained from the experiments carried out using the bag technic. This difference is probably due to the geometry of the substrate. The pot-tray has a much larger surface area exposed to the environment compared to the bag, and this dissipates the heat produced by the mycelium during growing more effectively, which in turn, helps to increase the mycelium growing rate. Average values of these measurements, with standard error, are shown in figure 8.

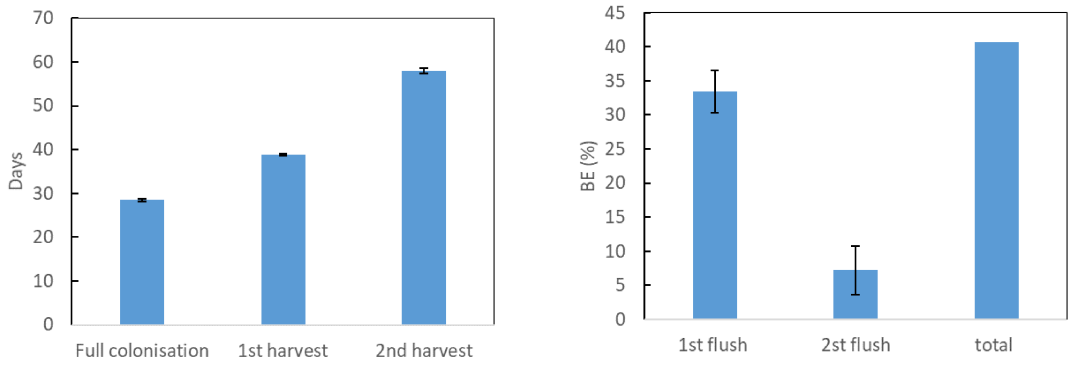

Figure 8. Days from inoculation to full colonization, harvest of the first and second flush of fruit bodies, and biological efficiency of the first and second flush.

The total BE was lower than the one obtained from bag experiments, but the pot-tray reduced the cultivation time to a great extent, meaning that this cultivation technology is better suited for industrial-scale cultivation in countries where labour costs are high.

3.3. Production of activated carbon from the fibre reject-based SMS.

The SMS from experiments that led to the highest BE (FR-80 + AA where grey oyster was cultivated) was chosen as raw material for the production of activated carbon. H_3PO_4 and KOH were used as activating agents. The SMS was impregnated using a weight ratio of 1 SMS : 3 acid/base. The activated carbons were characterized for their textural properties by BET analysis, and performance during the removal of model emerging organic pollutants from water.

3.3.1. Textural characteristics

Both activated carbons had a particle size between 50 and 100 µm. Textural characteristics are listed in Table 5. The specific surface area (S_{BET}) of the carbons increased with increasing pyrolysis temperature, and the average pore size decreased with increasing pyrolysis temperature.

Table 5. Textural properties of the activated carbons

	H_3PO_4			KOH		
	Pyrolysis temperature					
Parameters	500 °C	600 °C	700 °C	500 °C	600 °C	700 °C
S_{BET} (m^2/g)	302.3	375.9	396.5	61.4	81.4	199.4
External surface area (m^2/g)	247.2	279.4	281.9	41.1	43.6	95.4
Mesopore area (%)	81.8	74.3	71.1	66.9	53.6	47.8
Micropore area (m^2/g)	55.1	74.5	114.6	20.3	37.8	104.0
Micropore area (%)	18.2	19.8	28.9	33.1	46.4	52.2
Total pore volume (cm^3/g)	0.5	0.6	0.7	0.1	0.1	0.3
Micropore volume (cm^3/g)	0.0	0.0	0.1	0.0	0.0	0.1
Micropore volume (%)	5.8	6.3	7.7	8.3	13.2	16.6
Mesopore volume (cm^3/g)	0.5	0.6	0.7	0.1	0.1	0.3
Mesopore volume (%)	94.2	93.7	92.3	91.7	86.8	83.4
Average pore width (nm)	7.5	7.0	6.7	8.0	7.2	6.4

3.3.2. Removal of emerging organic pollutants from water

Model organic contaminants such as acetaminophen (common analgesic commercially known as paracetamol) and amoxicillin (antibiotic) usually found in urban wastewater were used to test the ability of the activated carbons produced from SMS to remove them from water. The concentrations of the drug solutions used here are well above the levels found in wastewater, however, these high concentrations are needed for the sake of characterisation of the carbons. Using ”normal” concentrations make this work impossible because the carbon removes all the drugs present in diluted solutions leaving nothing to measure. The saturation of the carbon is needed to find how much drug the carbon is capable to remove, and for this, high drug concentrations are needed. The concentration of the drugs in the original and depleted solutions was measured using a UV-Vis spectrometer at a λ_{max} of 243 nm (acetaminophen) and 228 (amoxicillin).

The effect of the sample dose on the removal of acetaminophen and amoxicillin from solutions of 200 mg/L is illustrated in Fig. 9. The per cent of removal and amount

of drug uptake and at equilibrium per unit of mass of activated carbon was determined according to equations 1 and 2, respectively.

$$\% \, Removal = 100.\frac{(C_i - C_e)}{C_i} \quad (1)$$

$$q_e = \frac{(C_i - C_e)}{m}.V \quad (2)$$

Where, C_i is the initial concentration of the drug in the solution (mg/l), C_e is the equilibrium concentration of the drug in the solution (mg/l), q_e is the drug adsorption capacity at equilibrium, in mg drug/g of the activated carbon, V is the volume of adsorbate solution (L) and m is the mass of activated carbon sample (g).

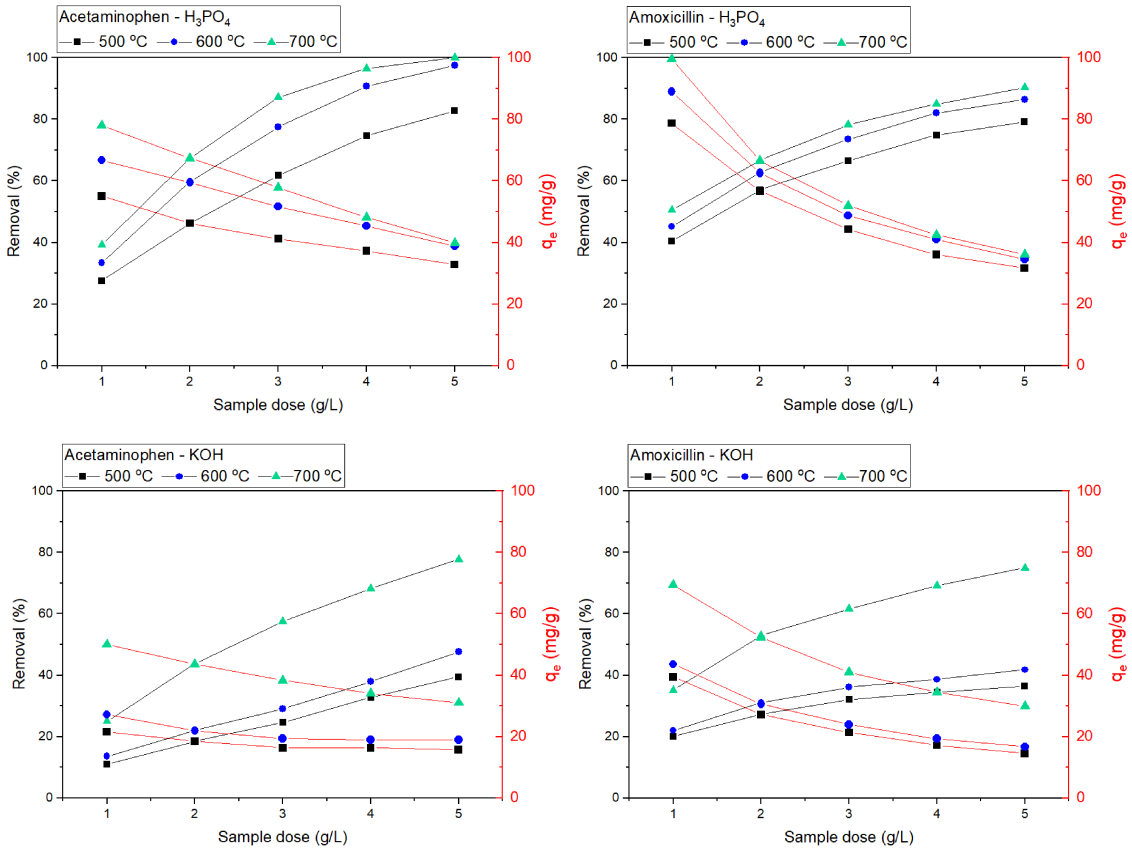

Fig. 9 Effect of the adsorbent dose on the equilibrium removal and adsorption capacity of acetaminophen and amoxicillin for the activated studied carbons using an initial drug concentration of 200 mg/l, pH 6.0, temperature of 22 °C, contact time of 6 h. Solid lines only to guide the eyes.

As expected, at a constant initial concentration of acetaminophen or amoxicillin, increasing the sample dose provides a greater surface area and a larger number of adsorption sites and hence the enhancement of the drug uptake but reduced adsorption capacity (q_e). Comparing the dosage curves (Fig. 9) with the surface area analysis of the activated carbons (Table 5), one can see that the highest efficiencies followed the highest surface areas. The activated carbons produced at 700 °C, especially those where H_3PO_4 was used as an activating agent, showed the best performances. Based on the adsorption

capacity (q_e), which decrease with the increase of the sample dose, the amount of activated carbon used for further adsorption studies was set to 2 g/L. Especially for the carbons made with KOH, the q_e differences between sample doses of 2 and 3 g/L is not high enough to justify the usage of sample doses higher than 2 g/L. The latter sample dose lead also to a reduction in the generation of waste. For further characterisation of the adsorbents only the activated carbons that showed the best performance, i.e., those pyrolysed at 700 °C.

3.3.3. Effect of the pH on the removal efficiency

The effect of the pH of the solution of contaminants in water is one of the factors that may affect the adsorption process. The optimum pH of the adsorbate depends on the chemical nature of the adsorbent, as well as the solubility of the organic substance and that pH. According to the results shown in Fig. 10, the highest q_e for both drugs was obtained at a slightly acid pH, but the differences in the adsorption capacity at acid or basic pH were minor. This reveals that electrostatic attraction between the studied adsorbents and adsorbates should not contribute largely to the adsorption process, since this mechanism of adsorption depends heavily on the pH. The results obtained here are in accordance with other research studies where was found that the percentage of removal of these drugs from water-based solutions does not depend on the pH [Saucier et al., 2017]. Further adsorption studies were carried out at pH 6, which gave the best results.

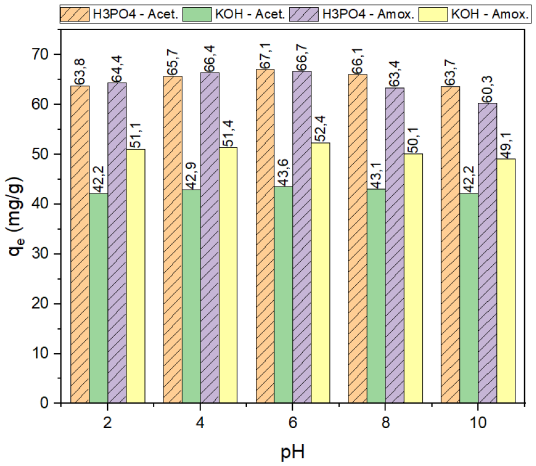

Fig. 10. Effect of the pH on the adsorption capacity at an initial drug concentration of 200 mg/l, sample dose of 2 g/L, temperature of 22 °C and contact time of 6 h for activated carbons produced at 700 °C.

3.3.4. Kinetic of adsorption

What follows is necessary to determine the time needed for the carbons to remove the contaminants from water and gives an idea of their effectiveness. The results from kinetic measurements were fitted using the nonlinear pseudo-first-order (Eq. 3), pseudo-second-order (Eq. 4) and general-order (Eq. 5) kinetic models. The software Origin Pro, 2021 was used for this work.

$$q_t = q_e.[1 - \exp(-k_1.t)] \quad (3)$$

$$q_t = \frac{k_2 . q_e^2 . t}{1 + q_e . k_2 . t} \quad (4)$$

$$q_t = \left(q_e - \frac{q_e}{[k_N . (q_e)^{n-1} . t . (n-1) + 1]^{1/(1-n)}} \right) \quad (5)$$

where ***t*** denotes the contact time (min); q_t, q_e are the adsorption capacities at time *t* and the equilibrium, respectively (mg/g); k_1 the pseudo-first-order rate constant (1/min); k_2 the pseudo-second-order rate constant (g/mg min); k_N the general-order constant rate [(g/mg)$^{n-1}$)/min], and **n** the dimensionless general-order adsorption rate.

Fig. 11 shows the kinetic measurements for the adsorption of acetaminophen and amoxicillin for the studied activated carbons at a representative initial drug concentration of 200 mg/l. The adsorption increased sharply at contact times of less than 10 min and slowed gradually as equilibrium was approached. The results indicate that for both activated carbons, equilibrium was attained for a contact time of between 20 min and 1 h.

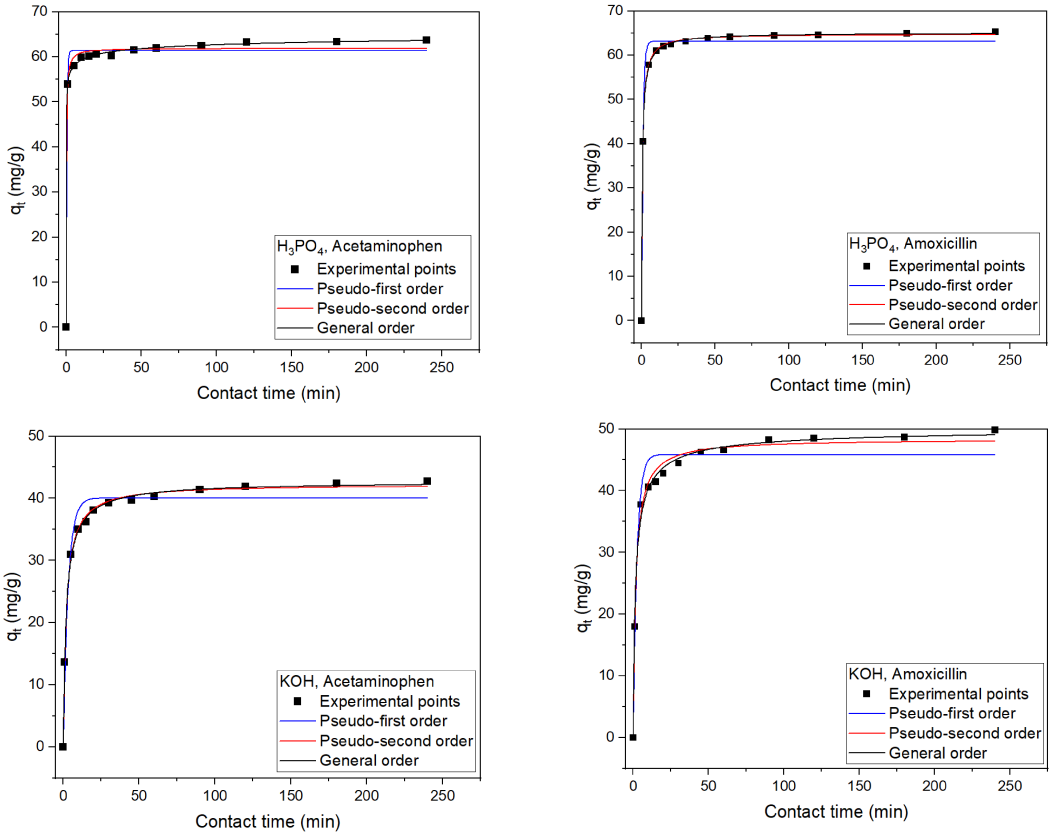

Fig. 7. Kinetic data for adsorption of acetaminophen and amoxicillin onto the activated carbons produced at 700 °C and comparison between experimental measurements and predictions of the pseudo-first-order, pseudo-second-order and general order models. Initial drug concentration of 200 mg/l, pH 6.0, temperature of 22 °C and adsorbent dose of 2 g/L.

The model fitting parameters obtained from the models are presented in Table 6. The suitability of each model was judged according to the adjusted determination coefficient

(R^2_{adj}) and the standard deviation of residues (SD) values. A low SD and a R^2_{adj} near 1 indicate a lower difference between the experimental and the theoretical adsorption capacity (q) given by the models. According to the results (Table 6), the general order kinetics model showed a better fitting of the experimental kinetic measurements, meaning the q_n foreseen by this model is closest to the q_t values experimentally measured. The best adsorption capacities were obtained for the carbons produced with H_3PO_4 as activating agent and led to values of 68 mg/g for acetaminophen and 65 mg/g for amoxicillin based on the general order model.

The general order kinetic model leads to different values for n (order of adsorption rate) when the concentration of the adsorbate is changed, which makes it difficult to compare the kinetic parameters of the model. Therefore, the initial sorption rate h_o (Eq. 6) is useful to evaluate the results given by the model (Ho, 2006).

$$h_o = k_N \,.\, q_e^n \qquad (6)$$

Where, $\mathbf{h_o}$ denotes the initial sorption rate (mg/g h), $\mathbf{k_n}$ is the rate constant [$(g/mg)^{n-1}$/min], $\mathbf{q_e}$ is the amount adsorbed at equilibrium (mg/g), and **n** is the order of the kinetic model.

Comparing the h_o values from the two activated carbons, one can see that the adsorption of amoxicillin, i.e., the removal of the drug from the solution, was faster. The activated carbon made with H_3PO_4 as an activation agent resulted in higher h_o values, however, this is not surprising considering the larger surface area of this carbon.

Table 6. Estimated parameters of the pseudo-first-order, pseudo-second and general order kinetic models for the adsorption of acetaminophen and amoxicillin

	H_3PO_4		KOH	
Model	Acetaminophen	Amoxicillin	Acetaminophen	Amoxicillin
Pseudo-first-order				
q_{e1} (mg/g)	61.35	63.12	40.03	45.83
k_1 (1/min)	2.1106	1.0073	0.3010	0.3886
R^2_{adj}	0.9903	0.988	0.9703	0.9571
SD (mg/g)	1.679	1.985	2.203	2.995
Pseudo-second-order				
q_{e2} (mg/g)	61.89	64.79	42.25	48.36
k_2 (g/mg min)	0.0175	0.0255	0.0115	0.01192
R^2_{adj}	0.9946	0.99975	0.9977	0.9923
SD (mg/g)	1.245	0.289	0.609	1.269
General order				
q_{en} (mg/g)	68.58	65.07	42.81	50.35
K_N [$(g/mg)^{n-1}$/min]	0.0130	0.0175	0.0063	0.00174
n	2.132	2.104	2.176	2.529
h_o (mg/g h)	106.74	114.47	22.37	35.18
R^2_{adj}	0.9995	0.9998	0.9979	0.9947
SD (mg/g)	0.373	0.236	0.581	1.049

3.3.5. Equilibrium adsorption isotherms (maximum adsorption capacity)

Adsorption isotherms were used to describe the relationship between the amount of adsorbate adsorbed by the activated carbon (q_e) and the concentration of adsorbate that remain in the solution at equilibrium (C_e). The adsorption parameters obtained from equilibrium models provide an idea of the adsorption mechanisms and affinity of the adsorbent for the adsorbate. The results from equilibrium measurements were fitted using the nonlinear Langmuir (Eq. 7), Freundlich (Eq. 8) and Sips (Eq. 9) models.

$$q_e = \frac{q_{max}.K_L.C_e}{1+K_L.C_e} \quad (7)$$

$$q_e = K_F.C_e^{1/n_F} \quad (8)$$

$$q_e = \frac{q_{max}.K_S.{C_e}^{1/n_S}}{1+K_S.{C_e}^{1/n_S}} \quad (9)$$

Where, **qe** denote the amount adsorbate adsorbed at the equilibrium (mg/g); $\boldsymbol{C_e}$ is the adsorbate concentration at equilibrium (mg/L); $\boldsymbol{q_{max}}$ is the maximum adsorption capacity of the adsorbent (mg/g); $\mathbf{K_L}$ and $\mathbf{K_S}$ are the Langmuir and Sips equilibrium constant (L/mg); $\mathbf{K_F}$ the Freundlich equilibrium constant [mg/g.(mg/L)$^{-1/n_F}$]; $\mathbf{n_F}$ and $\mathbf{n_S}$ are the dimensionless exponents of the Freundlich and Sips model, respectively.

The isotherms of adsorption (Fig. 8) were carried out at a temperature of 22 °C using the optimal pH conditions as described in section 3.3.3, and a contact time of 3h that according to the results from kinetic measurements is more than enough to reach the equilibrium. The estimated parameters obtained from the nonlinear regression of each model are shown in Table 7. The suitability of each model was judged using the R^2_{Adj} and SD values.

Regardless of the type of drug, isotherms of adsorption onto the activated carbon produced with H_3PO_4 were better described by the Freundlich model, and adsorption onto the activated carbon produced with KOH was better described by the Langmuir model.

The good agreement between the adsorption isotherms with the Freundlich model for the carbon produced with H_3PO_4 points to multilayer adsorption with heterogeneity in the energy of the adsorption sites. On the other hand, the good agreement between the adsorption isotherms with the Langmuir model for the carbon produced with KOH point to a process dominated by monolayer adsorption on the surface of the activated carbon particles as well as a finite number of energetically equivalent adsorption sites. Analysing the q_{max} values obtained from the Langmuir or sips isotherms, one can see that both carbons resulted in higher amoxicillin removal compared to acetaminophen (Table 7).

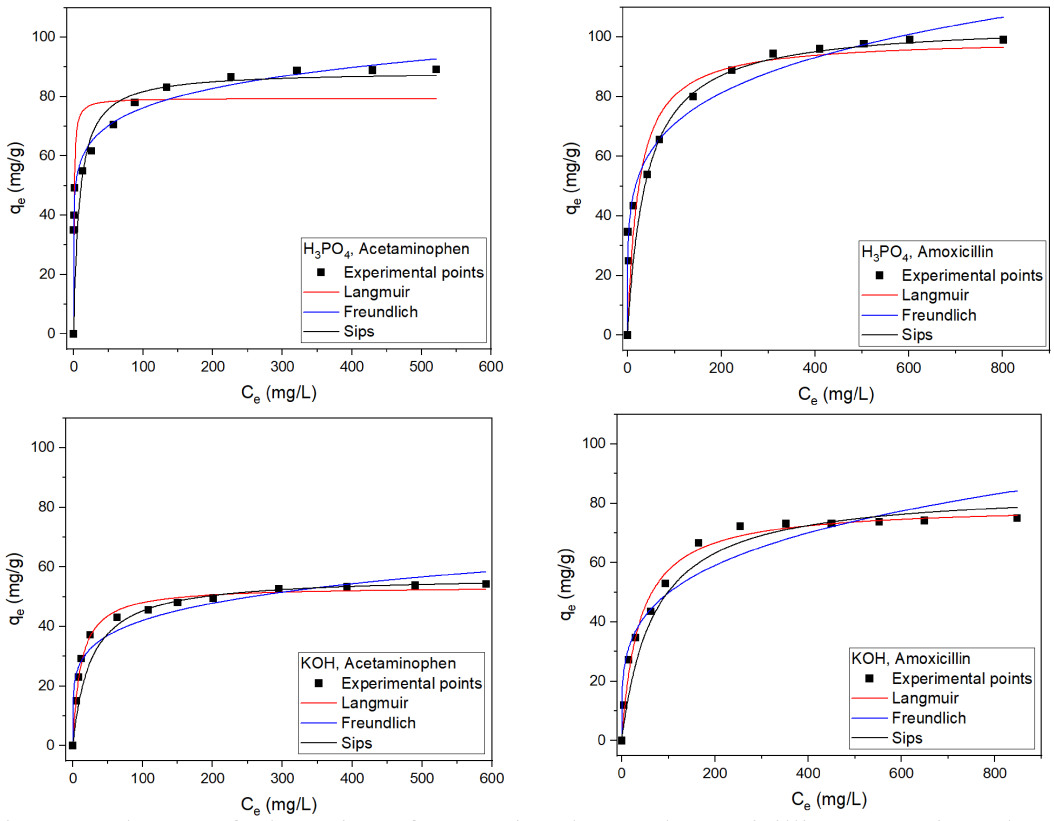

Fig. 8. Isotherms of adsorption of acetaminophen and amoxicillin onto activated carbons produced at 700 °C and comparison between the experimental data points and predictions of the Langmuir, Freundlich and Sips models. Conditions: pH 6.0, temperature of 22 °C, adsorbent dose of 2 g/L and contact time of 3 h.

Table 7. Estimated parameters of the Langmuir, Freundlich and Sips isotherm models for the adsorption of acetaminophen and amoxicillin

	H_3PO_4		KOH	
Model	Acetaminophen	Amoxicillin	Acetaminophen	Amoxicillin
Langmuir				
q_{max} (mg/g)	79.38	99.38	53.41	79.23
K_L (L/min)	1.38	0.0421	0.0881	0.0264
R^2_{adj}	0.708	0.866	0.989	0.987
SD (mg g^{-1})	14.61	12.15	1.721	2.917
Freundlich				
K_F (mg/g $(mg/L)^{-1/n_F}$)	44.28	28.71	17.93	16.08
n_F	8.47	5.093	5.409	4.074
R^2_{adj}	0.836	0.971	0.943	0.932
SD (mg/g)	10.97	5.59	4.069	6.835
Sips				
q_{max} (mg/g)	88.45	104.57	56.81	84.97
K_S (L/mg)	0.121	0.025	0.039	0.0145
N_S	1	1	1	1
R^2_{adj}	0.706	0.849	0.833	0.895
SD (mg/g)	5.032	5.93	3.25	4.46

The polar surface area of the amoxicillin molecule is 16.27 nm^2 and for the acetaminophen 4.93 nm^2. Based on the latter, the size of the acetaminophen molecule should lead to higher removal based on the average pore size given in Table 5. However, the hydrophilic-lipophilic balance for amoxicillin is 19.72 and for acetaminophen is 5.57, meaning that amoxicillin is more soluble in water compared to acetaminophen. This, in turn, increases the possibility for the carbon to interact with amoxicillin. The number of functional groups in the amoxicillin molecule that can bind to the surface of the carbon is also greater compared to acetaminophen, which may also explain why the removal of amoxicillin was higher than acetaminophen.

3.4. Production of activated carbon from birch wood-based SMS

Hardwood is the most common material used by mushroom growers around the world. Activated carbon produced from birch wood-based SMS was used as a comparison. Here is shown a summary of the results. A complete description of the results from the characterisation of these carbons can be found in a published open access paper (https://doi.org/10.1007/s13399-022-02618-7). Activated carbon was produced using SMS from the cultivation of oyster mushrooms. Samples were produced using an impregnation of 1 SMS : 2 H_3PO_4 (50%). The impregnated samples were pyrolysed at 700, 800 and 900 °C. The textural characteristics of each carbon are shown in Table 8. The surface areas obtained are much higher compared to the activated carbon made from fibre reject because the quality of birch wood-based SMS is higher.

Table 8. Textural properties of the biochars

Parameters	700 °C	800 °C	900 °C
SSA ($m^2\ g^{-1}$)	975	1031	1215
Mesopore surface area ($m^2\ g^{-1}$)	735	809	1000
Mesopore area (%)	75.38	78.47	82.30
Micropore area ($m^2\ g^{-1}$)	240	223	215
Micropore area (%)	24.62	21.63	17.70
Total pore volume ($cm^3\ g^{-1}$)	0.7086	0.7163	0.8357
Micropore volume ($cm^3\ g^{-1}$)	0.1199	0.1104	0.1048
Micropore volume (%)	16.92	15.41	12.54
Mesopore volume ($cm^3\ g^{-1}$)	0.5887	0.6059	0.7309
Mesopore volume (%)	83.08	84.59	87.46
Average pore size (nm)	2.906	2.777	2.751

The carbons were used to remove acetaminophen from water using the same methods described previously. The results are shown in Table 9.

The equilibrium of adsorption data was well represented by the Liu isotherm model, attaining a maximum adsorption capacity of 236.8 mg/g. The quality of the activated carbon produced here is comparable to or higher than commercial products, meaning that SMS can be used for the production of valuable materials instead of fuel.

Table 9. Equilibrium parameters of acetaminophen onto the activated carbon samples.

Model	Samples		
	700 °C	800 °C	900 °C
Langmuir			
Q_{max} (mg g^{-1})	163.6	185.3	231.0
k_L (L mg^{-1})	0.09581	0.1399	0.3029
R^2_{adj}	0.9967	0.9766	0.9901
SD (mg g^{-1})^2	3.563	9.481	7.943
Freundlich			
k_F ((mg g^{-1})(mg L^{-1})$^{-1/nF}$)	40.98	72.62	102.0
n_F (dimensionless)	4.246	6.155	6.854
R^2_{adj}	0.8747	0.9482	0.9239
SD (mg g^{-1})^2	22.03	14.11	21.99
Liu			
Q_{max} (mg g^{-1})	161.4	205.7	236.8
Kg (L mg^{-1})	0.1012	0.1068	0.2817
n_L (dimensionless)	1.066	0.6186	0.8187
R^2_{adj}	0.9968	0.9954	0.9922
SD (mg g^{-1})^2	3.540	4.224	7.042

It is well known that white-rot fungi degrade the substrate (wood) during the cultivation period. To study the effect of the degradation of the substrate caused by the fungi on the biochar properties, the initial substrate was used as a comparison. The impregnation was carried out in one step using a weight ratio of 1 SMS : 3 H_3PO_4, pyrolysis temperatures of 500, 700 and 900 °C, a heating rate of 10 °C/min and a treatment time of 1 h. It was found that SMS led to higher surface area at lower pyrolysis temperature. Table 10 shows the specific surface area (BET) for the SMS based activated biochars was 1325 m^2/g (500 °C), 1223 m^2/g (700 °C), and 1073 m^2/g (900 °C). The BET surface areas for the biochars produced from the initial substrate were 1214 m^2/g (500 °C), 1079 m^2/g (700 °C), and 1002 m^2/g (900 °C).

Table 10. Textural properties of the biochars

Sample code	Sample name	S_{BET} (m^2/g)	Pore volume (cm^3/g)	Pore size (nm)
BIRCH500	Birch Subs. 500	1214	1.28	4.20
BIRCH500SMS	Birch SMS 500	1325	1.39	4.19
BIRCH700	Birch Subs. 700	1079	1.03	3.82
BIRCH700SMS	Birch SMS 700	1223	1.14	3.74
BIRCH900	Birch Subs. 900	1002	0.96	3.84
BIRCH900SMS	Birch SMS 900	1073	1.01	3.79
500FR60	FR60 Sub. 500	656	1.38	8.44
500FR60SMS	FR60 SMS 500	615	1.17	7.61
900FR60	FR60 Sub. 900	931	1.03	4.43
900FR60SMS	FR60 SMS 900	515	0.61	4.72
500FR80	FR80 Sub. 500	621	1.12	7.24
50080SMS	FR80 SMS 500	474	0.85	7.18
900FR80	FR80 Sub. 900	769	0.91	4.73
90080SMS	FR80 SMS 900	598	0.83	5.59

The main difference between the SMS and the initial substrate lies in the content of lignin and hemicellulose, 23.26% and 16.07% for SMS versus 18.29% and 6.51% for the initial substrate. Thermal degradation of lignin and hemicellulose in N_2 atmosphere occurs at temperatures between 320-410 °C and around 290 °C, respectively. The differences in the contents of these two are probably the cause of the differences in the surface areas. Apart from this, the SMS has a structure that is more porous compared to the initial substrate because of the partial decay of the cell wall caused by the fungus, and that probably improved the contact and reaction between the activating agent (H_3PO_4) and the precursor and led to higher surface areas, but this is part of ongoing work and we do not know exactly the cause of these differences.

Activated carbons produced from fibre reject-based SMS were also compared to the original substrate. For these carbons, it was found that the original substrates (before cultivation) led to carbons with higher surface area compared to the ones obtained from the corresponding SMS. What probably happened here is that the fibre reject has a very small particle size, i.e., a very high surface area that is in contact with the mycelium, and thereby, it is degraded to a greater extent compared to wood sawdust. Then, heavily degraded substrate leads to a carbon of acceptable quality, but lower quality than the ones produced from the original.

3.5. Evaluation of the costs related to the processes used in this work

What follows is a simple calculation of the costs based on our results and the equipment used by us. Factors such as labour costs were not included.

Calculation made based on the FR80+AA substrate, which is the one that gave the best biological efficiency:

1. Total BE for grey oyster = 71.6 % = 716 g fruit/800 g reject

2. Total BE for king oyster = 53.5 % = 535 g fruit/800 g reject

3. Price grey oyster = 200 g, 28 SEK = ~140 SEK/kg (ICA)

4. Price king oyster = 450 g, 159 SEK = ~350 SEK/kg (Svampkungen.se)

5. Cost for processing of the fibre reject: the setup used for this work only consumes electricity for the cyclone blower (150 kW/h), the energy to heat the process air is from a biomass boiler and the fuel can be SMS that cost nothing.

The cost of electricity in Sweden changes depending on the region, in south Sweden, it is twice as expensive.

According to e.on (https://www.eon.se/el/elpriser/aktuella), the average spot price exclusive VAT, surcharges and certificates on 1-28 April 2022 was:

Area 1, Northern Sweden: 54.37 cents/kWh = 0.5437 SEK/kWh

Area 2, Northern cental Sweden: 55.18 cents/kWh = 0.5518 SEK/kWh

Area 3, Southern central Sweden: 88.18 cents/kWh = 0.8818 SEK/kWh

Area 4, Southern Sweden: 106.59 cents/kWh = 1.0659 SEK/kWh

The cost of processing 100kg of raw reject (it takes 30 minutes/100kg) =

Area 1, Northern Sweden:

54.37 cents/kWh = 0.5437 SEK/kWh * 0.5 h * 150 kWh = 40.77 SEK

Area 2, Northern cental Sweden:

55.18 cents/kWh = 0.5518 SEK/kWh * 0.5 h * 150 kWh = 41.38 SEK

Area 3, Southern central Sweden:

88.18 cents/kWh = 0.8818 SEK/kWh * 0.5 h * 150 kWh = 66.14 SEK

Area 4, Southern Sweden:

106.59 cents/kWh = 1.0659 SEK/kWh * 0.5 h * 150 kWh = 79.94 SEK

From 100 kg raw reject 60 kg are left to be used for the cultivation of mushrooms, then:

Grey oyster = 0.8 kg reject ---> 716 g mushroom

60 kg reject (100 kg raw) ---> 53.7 kg mushroom * 140 SEK/kg = 7 518 SEK

King oyster = 0.8 kg reject ---> 535 g mushroom

60 kg reject (100 kg raw) ---> 40.2 kg mushroom * 350 SEK/kg = 14 043 SEK

If the SMS is used for the production of activated carbon, probably this can lead to some profit, but for us is difficult to estimate this based on what we did on a laboratory scale.

As said, here we did not include labour costs, but what one can see from this simple calculation is that some degree of automatisation is needed to make this business profitable in Sweden or any other country where labour costs are high.

Publications in academic journals produced during this project

1. Grimm, A., Eilertsen, L., Chen, F. *et al.* Cultivation of *Pleurotus ostreatus* Mushroom on Substrates Made of Cellulose Fibre Rejects: Product Quality and Spent Substrate Fuel Properties. *Waste Biomass Valor* **12,** 4331–4340 (2021). https://doi.org/10.1007/s12649-020-01311-y

2. Grimm, A., dos Reis, G.S., Dinh, V.M. *et al.* Hardwood spent mushroom substrate–based activated biochar as a sustainable bioresource for removal of emerging pollutants from wastewater. *Biomass Conv. Bioref.* (2022). https://doi.org/10.1007/s13399-022-02618-7

3. Alejandro Grimm [1,*], Feng Chen [1], Glaydson Simões dos Reis [1], Van Minh Dinh[2], Santosh Govind Khokarale[2], Jyri-Pekka Mikkola[2,3], Malin Hultberg [4], Shaojun Xiong.

Utilization of cellulose fibre rejects from the recycling of waste paper for cultivation of *Pleurotus* spp. mushrooms and production of activated carbon from spent substrate. Manuscript.

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Saucier C, Karthickeyan P, Ranjithkumar V, Lima EC, dos Reis GS, de Brum IAS (2017) Efficient removal of amoxicillin and paracetamol from aqueous solutions using magnetic-activated carbon. Environ Sci Pollut Res Int 24:5918–5932

Appendix

- Administrativ bilaga till slut rapport