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| Energimyndighetens titel på projektet – svenska<br>En ny utvecklad teknik för matsvampproduktion på återvunnet fiberrejekt samt att valorisera förbrukat svampsubstrat för värme och elproduktion       |  |
| Energimyndighetens titel på projektet – engelska<br>A new approach for edible mushroom production on recycled fibre rejects and the use of spent mushroom substrate for heat and electricity production |  |
| Universitet/högskola/företag<br>Sveriges lantbruksuniversitet SLU   | Avdelning/institution<br>Institutionen för skogens biomaterial och teknologi |
| Adress<br>SLU SBT, 901 83 Umeå  |  |
| Namn på projektledare<br>Shaojun Xiong  |  |
| Namn på ev övriga projektdeltagare<br>Biosteam AB, Valutec AB, SCA Obbola, SCA Munksund, Svampkungen AB and Swedfungi AB  |  |
| Nyckelord: 5-7 st<br>edible mushroom, waste paper, heavy metal, SMS, biofuel, combustion  |  |

## Preface

This project was a pilot study (“förprojekt”), 50% financed by RE:source program (2016) through Swedish Energy Agency and 50% by industrial partners, namely Biosteam AB, Valutec AB, SCA Obbola, SCA Munksund and Svampkungen AB.

The project has been carried out successfully in collaborations with the industrial partners. Mr Lars Atterhem, Biosteam, has a contribution in energy balance analysis and together with Robert Larsson, Valutec, in solutions of pasteurization and process integration. Anders Kyösti and Hans Thorén, SCA, contributed to the data of fiber reject streams and analyses, sampling and delivering the fiber rejects to SLU. Johnny Carlsson, Svampkungen, helped with mycelia. In addition, the bag-cultivation of oyster mushrooms was performed by renting an industrial mushroom growing chamber of Swedfungi AB in Ånäset, Västerbotten, thanks to Mr Huang.

Mushroom nutrition analyses were performed by SLU Husdjurens Utfodring och Vård Laboratoriet in Uppsala. Heavy metal and fuel analyses were performed by Eurofins in Lidköping.

Lill Eilertsen was the one working practically with the implementation of the project at SLU, which makes the project an efficient going. The fibre rejects were handled in SLU Biomass Technology Center (BTC) and many thanks to Gunnar Kalén and Markus Segerström. The project team thanks the support and advices from the supervisors at energimyndigheten.

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## Sammanfattning

I det här projektet studerades möjligheterna att utveckla en integrerad industriedja med fiberrejekt till kombinerad svamp- och bränsleproduktion – fiberrejekt som substrat till svampodling och använt odlingssubstrat för att producera biobränsle. De främsta resultaten visar att: (1) Fiberrejekt från returpapper fungerar bra som råvara för odlingssubstrat. Av denna råvara finns så mycket som 2100 ton/år (torrvikt) tillgängligt enbart från SCA Obbola; (2) Ostronskivling växer bra och ger bra skörd när den odlas i det utvalda fiberrejektsubstratet. Skörden var så hög som 469 g färsk svamp per kg substrat (torrvikt); (3) Den odlade svampen har ett bra näringsinnehåll och inga problem med främmande ämnen (tungmetaller); (4) Det förbrukade odlingssubstratet kan passa som bränsle för direkt förbränning, men samförbränning rekommenderas företrädesvis. Våra resultat visar på lovande möjligheter till att utveckla en integrerad process med kombinerad svamp- och bränsleproduktion genom att använda recirkulerat fiberrejekt från skogsindustrin.

## Summary

This project studied possibilities of developing an integrated industrial chain using fibre rejects for a combined mushroom and fuel production in Sweden - fibre rejects as growing substrates for mushroom production and recycled spent mushroom substrates (SMS) as biofuel. Major results include: (1) fibre reject from waste paper is identified as suitable feedstock for immediate use for mushroom substrates, and the resource is as much as 2100 tons of dry mass from SCA Obbola alone annually; (2) Oyster mushrooms grow and fruit well on the selected reject substrate and the yield was as high as 469 g fresh weight per kg dry substrate; (3) Produced oyster mushrooms have good nutrition and no problems of heavy metals; (4) The SMS can be good fuels for direct combustion but co-firing is preferably recommended. Our results show not only new knowledge but also a promising possibility and potential to develop a process integration of combined mushroom and fuel production using recycled fibre rejects from forest industries.

## Background

Sweden generates around 26-30 million tons (Mt)/year dry weight of biowastes, including 5 Mt variable biogenic wastes/byproducts (from wood processing, pulp & paper (fibre rejects), etc.) (ICCT 2014). Among others, fibre rejects, currently

considered as wastes from pulp & paper industries (Mäkelä et al. 2014), are a problematic material in environmental point of view. European pulp&paper industries produces around 11 Mt of wastes annually (Monte et al. 2009), SCA Obbola alone contributes about 8000 tons of fibre rejects annually.

Many biowastes can be good resources for a bioeconomy development. Fibre rejects as well as other waste biomass may contain components (Bajpai 2015) that can be converted to high value-added bio-based products such as edible mushrooms. Based on our previous research, the concept “discarded biomass for a combined mushroom and biofuel production” as well as key technologies suitable for Sweden have been developed, thanks to the financing from VINNOVA and Energimyndigheten. It is expected that our innovation in technologies may largely reduce both costs and environmental problems regarding mushroom production using forest residues in Sweden. These emphasize a necessity to investigate possibilities of using fibre rejects for mushroom and biofuel production.

The project P42481-1 was a “förprojekt” to study possibilities of applying our concept “fibre rejects for a combined mushroom and fuel production” – fibre rejects to be used as initial substrates for mushroom production and recycled spent mushroom substrates (SMS) as biofuel. Major goals were to: (1) identify suitable fibre rejects assortments for growing substrates, (2) analyse mushroom quality and (3) evaluate the possibilities of recycling SMS as fuels.

## Implementation

The project has been performed successfully through following activities:

### *1. Investigation of the quantities and quality of the fibre rejects from different processes.*

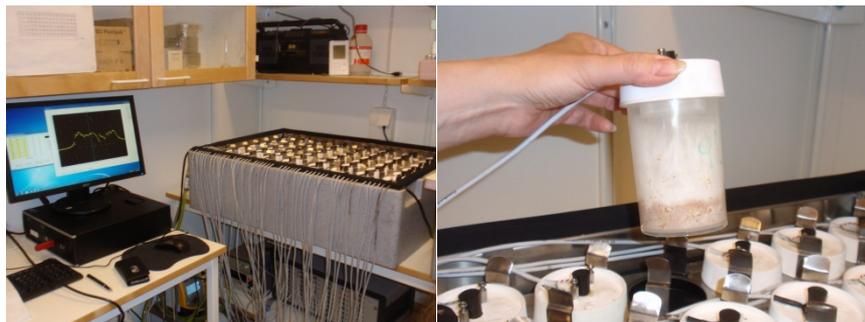
The processes generating fibre rejects were identified through study tours and discussions with experts from SCA. The data on the quantities of fibre rejects were provided by SCA. The quality of fibre rejects is determined by chemical analyses by this project and also some data from SCA report 2010 – 2015.

### *2. Verification of mushroom cultivation on substrates of fibre reject mixtures*

Shiitake (*Lentinula edodes*) and oyster mushroom (*Pleurotus ostreatus*) were used as target species. Table 1 is experimental design and substrate recipes. Two experiments for each species were carried out to examine both mycelia growth using respicond equipment (Figure 1) and mushroom fruitification/yield using bag cultivation technique (Figure 2). This work package was carried out by SLU team, in collaboration with all industrial partners.

**Table 1.** Ingredients of substrates, wt% based on dry mass.

| Treatment<br>(Fibre reject/birch) | Fibre rejects<br>(waste paper) | Birch sawdust | Wheat bran |
|-----------------------------------|--------------------------------|---------------|------------|
| 80/0                              | 80                             | 0             | 20         |
| 60/20                             | 60                             | 20            | 20         |
| 40/40                             | 40                             | 40            | 20         |
| 0/80                              | 0                              | 80            | 20         |



**Figure 1.** Instrument Respicond (<http://www.respicond.com/>) to measure mycelia respiration of shiitake and oyster mushrooms. Four replicates of each treatment.



**Figure 2.** Bag cultivation of the oyster mushroom. On left: mycelia colonization on day; on right: fruitification (28 days after inoculation) in a climate chamber. Each bag weighs 1 kg wet mass with moisture content of 60% (wet based). Twenty bags for each substrate mixture.

### *3. Evaluation of quality on mushroom cultivated on fibre rejects*

Oyster mushrooms are concerned only. Mushroom nutrition and heavy metal were analysed by SLU and Eurofins respectively.

### *4. Characteristics of the SMS as biofuels*

The initial and spent mushroom substrates as well as pure fibre rejects (waste paper) were analysed for their fuel properties. The analyses were conducted by SLU in collaboration with Eurofins according to EU standard methods.

### *5. Assessment of energy balance when recycling SMS for energy production*

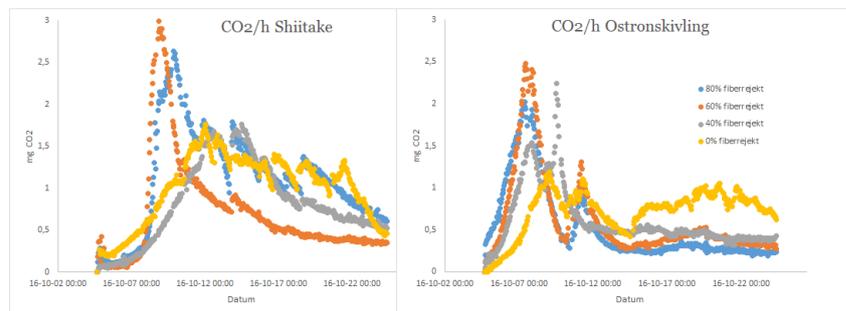
A preliminary and general mass and energy balance was performed by SLU and Biosteam AB in this pilot study stage. The process-wise estimates of mass and energy balance were not included in this study; because industrial process settings were not ready. It is hoped to make a full scale analysis at next stage.

## Result

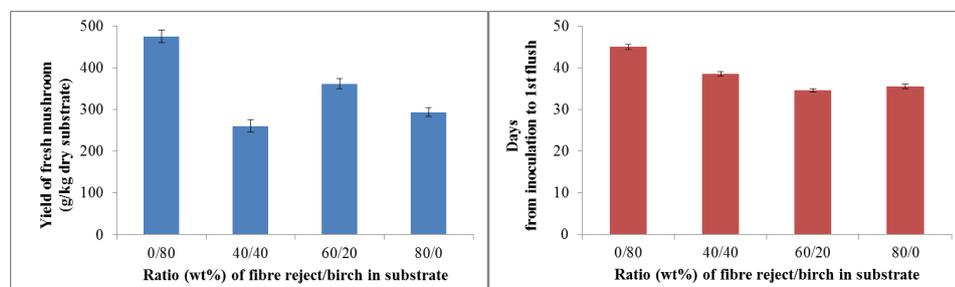
Major results are as follows:

- Among the five potential fibre reject streams preliminarily selected from SCA Obbola, the one from birch residues and the one recycled papers are identified as the best model feedstock to be immediately used for oyster mushroom growing substrates. The birch stream is mostly generated from tops/thinnings but the quantity is unknown. The waste paper stream amounted to 8100 tons in 2014 and 6800 tons (35% DM) in 2015 (SCA 2015). It is estimated that a similar quantity of this stream would be available in SCA Munksund Piteå. Today the fibre rejects from waste paper stream is used as boiler fuel mixed with other biomass (barks etc) for SCA's heat and power production.

The fresh softwood reject has a great potential as a mushroom growing substrate and it is available in large quantities, but pretreatment technologies need refining to give the mycelium best possible conditions to establish and grow well. Chemical analysis of biosludge and bark based rejects suggests that they are not suitable as substrate for the tested mushroom species. Whether biosludge and bark based rejects can be used for other mushroom species needs to be evaluated.
- Mycelia of both shiitake and oyster mushrooms can colonize and grow on all four studied substrates with different mixtures of fibre rejects (waste paper) and birch residues (Table 1, Figure 3), according to measurement of respiration ( $\text{CO}_2$  mg/hour). The mycelial growth shows a positive correlation with the ratio of the fibre rejects, probably because the reject is mostly composed of cellulose while fresh birch contains 20% lignin.



**Figure 3.** Mycelia respiration of shiitake (on left) and oyster (on right) mushrooms during colonization/incubation.



**Figure 4.** Yield of fresh oyster mushroom (containing 90% moisture) and days from inoculation to 1<sup>st</sup> flush. The data of yield for treatment 0/80 was from previous experiment for reference.

The bag cultivation concerned three substrates in different ratio of fibre reject (40/40, 60/20 and 80/0) only, each has 20 bags as replicates. The oyster mushrooms fruit on all three studied substrates. The first flush of the mushrooms was harvested between 34 and 40 days after inoculation, and the average yields of the first flush were between 261 g and 361 g fresh weight per kg dry substrate (Figure 4).

The highest and lowest yield was 469 g, (treatment 60/20) and 168 g (treatment 40/40) fresh weight per kg dry substrate, respectively. The treatment 60/20 gave a higher yield, consisting to our previous pilot experiment. In this study, considering recycling of SMS as fuel, only one flush harvest was tested.

Although shiitake showed good respiration in the respicond, it had problems colonising the substrates mixed with the recycled paper rejects in bag-cultivation. Mycelia died off a couple of weeks after inoculation. The reason remains unknown.

- Food analysis (Table 2) shows that oyster mushrooms grown on the waste paper rejects, from the bag cultivation, have a comparable quality to those commercial products grown on hardwood (USDA database 2016).

**Table 2.** Nutrition in oyster mushroom from this study in comparison with reference data of USDA\*. Data refer mean and standard error in brackets.

| Substrate           | Crude protein | Crude fibre  | Crude fat    | Ca           | K             | Mg           | Na           | P             | S            |
|---------------------|---------------|--------------|--------------|--------------|---------------|--------------|--------------|---------------|--------------|
|                     | wt% DM        |              |              | g/kg DM      |               |              |              |               |              |
| Hardwood*           | 30.0          | -            | 3.7          | 0.3          | 38.2          | 1.6          | 1.6          | 10.9          | -            |
| Fibre rejects/birch |               |              |              |              |               |              |              |               |              |
| 40/40               | 25.2<br>(1.6) | 9.8<br>(0.5) | 3.2<br>(0.1) | 0.6<br>(0.0) | 36.2<br>(1.3) | 1.8<br>(0.1) | 1.4<br>(0.0) | 10.2<br>(0.5) | 2.1<br>(0.1) |
| 60/20               | 22.3<br>(0.3) | 9.0<br>(1.0) | 3.0<br>(0.3) | 0.3<br>(0.0) | 29.3<br>(0.6) | 1.8<br>(0.0) | 1.2<br>(0.2) | 10.4<br>(0.1) | 1.9<br>(0.0) |
| 80/0                | 23.7<br>(0.9) | 8.1<br>(0.9) | 3.5<br>(0.0) | 0.8<br>(0.4) | 29.6<br>(0.4) | 2.0<br>(0.0) | 3.1<br>(0.1) | 10.5<br>(0.1) | 2.0<br>(0.1) |

The concentration of crude protein is 22.3-25.2 (wt% DM), which can be compared to 13% and 25% for wheat and milk, respectively (Croan 2004). In addition, contents of crude fat (3.0-3.5%), crude fibre (8.1 – 9.8) and major minerals (Ca, K, Mg, Na, and P) are also at good levels for nutritional food.

The contents of As, Pb and Hg became higher in the oyster mushrooms grown on waste-paper rejects than that on pure birch (Table 3), suggesting a trend of enrichment of heavy metals by the mushroom. However the cadmium (Cd) tended a decrease and tin (Sn) had no change after the cultivation. It is confirmed that concentrations of heavy metals (Pb, Cd, Hg, Sn) in the oyster mushrooms grown on paper rejects are far below the EU limit values for food products.

The arsenic (As) content was on average of 26.5 – 53.0 µg per kg of wet mushrooms depending on substrates (Table 3), which is comparable to that of rice products (Livsmedelsverket 2015). A recommendation on maximum daily intake of such fresh mushroom has to be considered, due to a new regulation on acceptable daily intake of As from rice. New up-limit of acceptable daily intake of As is 0.15 µg per

kg of human body weight in Sweden (Livsmedelsverket 2015), while it is 0.30  $\mu\text{g}$  in EU (EC Regulation 2015/1006). This suggests a Swedish person of 75 kg body weight shall not eat more than 210 g of this fresh oyster mushroom every day, if the substrate is composed of the highest ratio of fibre reject (80%). It is understandable that even 210 g of mushroom is usually more than enough for a daily intake. The arsenic in mushrooms can be tracked back to the substrate of recycled paper rejects, but its initial origin and possibilities of further reduction remain to be studied. Also the fibre reject ratio of 60/20 gave a much lower content of As in the mushrooms than other two ratios, which could be explained the uptake of As was “diluted” by higher yield of mushrooms (Figure 4).

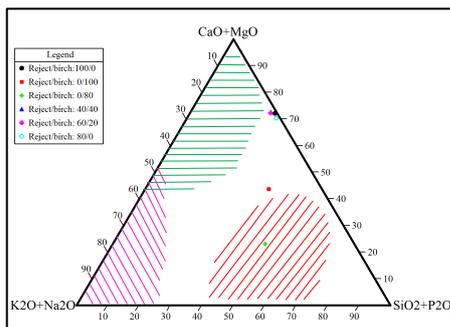
**Table 3.** Heavy metals in fresh oyster mushroom (90% moisture) grown on different fibre rejects (waste paper). Data are means  $\pm$  standard error (se).

| Metal        | Unit                    | Ratio of fibre reject/birch in substrate |                   |                    |                   | Up-limit value by EC Regulation (2015/1006)   |
|--------------|-------------------------|--|-------------------|--------------------|-------------------|---|
|              |                         | 0/100                                    | 40/40             | 60/20              | 80/0              |   |
| Arsenic (As) | $\mu\text{g}/\text{kg}$ | 7.4                                      | 48.0<br>$\pm 9.5$ | 26.5<br>$\pm 14.0$ | 53.0<br>$\pm 7.0$ | 0.30 $\mu\text{g}/\text{day}$ per kg human body weight in EU, but 0.15 $\mu\text{g}/\text{day}$ in Sweden, based on rice products (Livsmedelsverket 2015) |
| Cadmium (Cd) | $\mu\text{g}/\text{kg}$ | 64.0                                     | 20.5<br>$\pm 0.5$ | 28.5<br>$\pm 5.0$  | 19.0<br>$\pm 3.0$ | 200 $\mu\text{g}$ per kg wet mushroom   |
| Lead (Pb)    | $\mu\text{g}/\text{kg}$ | 5.9                                      | 7.7<br>$\pm 4.3$  | 5.3<br>$\pm 3.9$   | 10.1<br>$\pm 5.0$ | 300 $\mu\text{g}$ per kg wet mushroom   |
| Mercury (Hg) | $\mu\text{g}/\text{kg}$ | 4.0                                      | 5.0<br>$\pm 0.6$  | 4.9<br>$\pm 0.8$   | 6.2<br>$\pm 0.4$  | 500-1000 $\mu\text{g}$ per kg wet fish  |
| Tin (Sn)     | $\mu\text{g}/\text{kg}$ | 5.4                                      | 5.4<br>$\pm 0.1$  | 5.4<br>$\pm 0.0$   | 5.4<br>$\pm 0.0$  | 200 000 $\mu\text{g}$ per kg canned food  |

4. Fuel analysis indicate that the calorific value of fibre-reject SMS (reject/birch: 40/40, 40/60, and 80/0) is 8 -13 MJ/kg DM, about 50-60% of the energy of the pure birch (“0/100”, Table 4). Based on ash-transformation model proposed by Boström (2012), the risks of slagging and corrosion should be minor in the combustion (Figure 5), thanks to a high content of calcium, but particle emission control may have to be considered. The high ash content (up to 50 wt%) suggests that SMS are more suitable as a co-combustion fuel with biomass that benefits from its high Ca content (9-14%), according to a fuel design approach.

**Table 4.** Fuel properties of SMS vs. pure fiber reject and/or pure birch

| Item                               | Unit     | SMS                                      |       |       | Raw   |       |
|------------------------------------|----------|--|-------|-------|-------|-------|
|                                    |          | Ratio of fibre reject/birch in substrate |       |       | 100/0 | 0/100 |
|                                    |          | 40/40                                    | 60/20 | 80/0  |       |       |
| Gross calorific value              | MJ/kg DM | 10.35                                    | 12.65 | 8.02  | 9.6   | 20.67 |
| Effective calorific value          | MJ/kg DM | 9.56                                     | 11.70 | 7.36  | 8.92  | 19.26 |
| Effective calorific value ash-free | MJ/kg DM | 16.4                                     | 17.20 | 15.74 | 18.22 | 19.41 |
| Ash (550°C)                        | % DM     | 41.7                                     | 32.0  | 53.3  | 51.0  | 0.7   |
| Sulphur, S (1350°C)                | % DM     | 0.11                                     | 0.08  | 0.09  | 0.07  | 0.02  |
| Carbon, C, (1050°C)                | % DM     | 30.8                                     | 35.9  | 26.4  | 29.6  | 51.0  |
| Hydrogen, H (1050°C)               | % DM     | 3.7                                      | 4.3   | 3.1   | 3.1   | 6.5   |
| Nitrogen, N (1050°C)               | % DM     | 0.67                                     | 0.62  | 0.53  | 0.23  | 0.18  |
| Oxygen, O (calculated)             | % DM     | 22.9                                     | 27.1  | 16.6  | 15.9  | 41.6  |
| Chlorine, Cl                       | % DM     | 0.09                                     | 0.09  | 0.05  | 0.06  | 0.01  |



**Figure 5.** Prediction of ash transformation based on Boströms model (Boström et al. 2012). SMS mixed with fibre rejects seems to be far from slagging (red shading) and potassium-related erosion (pink shading) areas.

The concentrations of heavy metals in SMS were studied for two treatments of high fibre reject ratios (60 and 80%). They did not show in general significant differences between that before and after the cultivation (Table 5), although the fractions for 3 of the 5 metals tended to be lower after the cultivation.

**Table 5.** Heavy metals in the substrate before and after cultivation

| Metal        | Unit     | Before cultivation                               |      | After cultivation |           |
|--------------|----------|--|------|-------------------|-----------|
|              |          | Ratio of fibre reject/birch in initial substrate |      |                   |           |
|              |          | 60/20  | 80/0 | 60/20             | 80/0      |
| Arsenic (As) | mg/kg DM | 0.39   | 0.69 | 0.43±0.03         | 0.63±0.04 |
| Cadmium (Cd) | mg/kg DM | 0.42   | 0.37 | 0.35±0.01         | 0.35±0.05 |
| Lead (Pb)    | mg/kg DM | 13.0   | 23.0 | 11.5±0.5          | 19.5±0.5  |
| Mercury (Hg) | mg/kg DM | 0.03   | 0.04 | 0.02±0.00         | 0.04±0.00 |
| Tin (Sn)     | mg/kg DM | 2.4  | 3.0  | 2.9±0.1           | 3.9±0.6   |

5. Table 6 shows a preliminary and general estimates of mass and energy balance of before and after mushroom cultivation. After harvesting the first flush oyster mushroom, 80% of original substrate dry mass, containing about 53 – 80% original energy, is left as SMS in this study. However, it remains to be further understood on the reasons why there is a big difference in energy loss between substrates.

**Table 6.** Energy balance of substrate before and after mushroom cultivation, based on effective calorific value (LHV) and 1 kg dry mass of initial substrate

|   | Ratio of fibre reject/birch |       |       |
|---|-----------------------------|-------|-------|
|   | 40/40                       | 60/20 | 80/0  |
| Energy content in initial substrate, before cultivation, MJ/kg DM               | 14.72                       | 12.43 | 11.22 |
| Mass of spent mushroom substrate, after harvest of 1 <sup>st</sup> flush, kg DM | 0.78                        | 0.81  | 0.79  |
| Energy in remained SMS, MJ  | 7.45                        | 9.48  | 5.81  |
| Energy in harvested mushrooms, MJ   | 0.36                        | 0.50  | 0.40  |
| Energy loss during the cultivation, % of initial substrate                      | 46.94                       | 19.74 | 44.57 |

## Discussion

Without a doubt, this study proves that our innovative concept “fibre rejects for both mushroom and biofuel” is possible. Fibre rejects/waste papers, in addition to birch residues, are identified as suitable ingredients in oyster mushroom cultivation substrates. The mushrooms grown on the studied fibre/birch mixed substrate show a good nutritional values and no risk of heavy metals, although people need to keep in mind the arsenic issue in the same ways as for eating rice products. As long as not “over-dose” of daily intake, for example, at < 210 g for a human of 75 kg body weight, people shall not be poisoned by arsenic according to the medical evidences. The fuel characterization indicates that the SMS shall have no risks of slagging and corrosion when direct combusting. Preferably, this SMS shall be used for co-firing with other biomass that needs Ca addition for a good combustion. The heavy metals in the SMS were found in similar levels as those in original fibre rejects that was used as boiler fuel today, suggesting existing rules to control heavy metal in fibre rejects can be similarly applied to SMS burning.

The results show a considerable potential of the concept “combined mushroom and biofuel production”. Although an optimization of substrate composition and processes remain to be done, the yield of 360 g fresh mushroom per kg dry substrate suggests a market value of 113 million SEK, if all 2100 tons dry mass of waste paper from SCA Obbola would be used as the mushroom substrate and suppose the market price of oyster mushroom is 150 kr/kg. Additionally, the spent substrate will be used as cheap fuel for energy production.

Produced mushrooms can even be used as feedstocks for producing even higher value-added bioproducts, in addition to being food and feed. Researchers have confirmed that mushrooms contain substances (e.g. lectin) that may reduce the risk of cancer (Wong et al. 2009), boost the immune system and reduce the risk of coronary heart disease (Stamets 2000, Sánchez 2010, Hoyle 2016).

Future work shall focus on technical development of system solutions and economic life cycle assessment, which are necessary for industrial demonstration and commercialisation. The system solutions shall be holistic and covering all processes from feedstock handling, substrate preparation, pasteurization, mushroom cultivation, SMS recycling and combustion. Also, industrial tests shall be preceded, such as real combustion tests of SMS.

It shall be kept in mind that both substrate recipes and experiment settings were not optimized in this pilot study. Future project must include an optimisation and understanding of factors that may influence both mushroom production and fuel characteristics. There is also a necessity to perform both economic and environmental life cycle assessments.

## Publication list

No publication yet.

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## Appendix

- Administrativ bilaga till slut rapport