CHALLENGES TO ANALYSE MICROBES IN WOOD CHIP STOCK PILES

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ABSTRACT

The wood material is practically always pre-treated when used in energy production. Typical pre-treatment is chipping the wood in wood chips. Wood chip storage times vary from one day up to one year. There are many factors that are influencing in microbial activity in the wood chip storage. In this article the focus is in describing the challenges to analyse microbial population in wood chip storage piles. Furthermore, some results of microbial population in "fresh" and "old" storage are introduced. The learnings and results are based on the experimental research "*Inhibition of microbial activities in the pile of energy wood chips during the storage*" carried out in JAMK University of Applied Sciences in 2013-2015 and funded by the Foundation for Research of Natural Resources in Finland.

In this article the focus is in challenges of microbial analyses of wood chip storage. One challenge was that there is no standard for the microbial sampling of wood chip microbes. In this research methods to take sample was developed. The microbe analyses themselves are time consuming and sensitive. Besides, it is important to manage contamination risk properly. Furthermore, many dublicates are needed. In quantitative analyses the scale of dilutions is large. Sampling and analysing are very much work and the results of the experiments are not necessary transferrable in different piles and different operational environment.

In this experimental research, it was recognised that in the wood chip storages the number of colony formatting units correlates with the storage duration of wood chips. In the storage piles, there are present microbes, such as *Aspergillus fumigatus* and actinobacteria that are safety risk for people working with wood chips.

Keywords: wood chips, stock piles, storage, microbial activity, wood energy

1 PURPOSE OF THE WORK

Finland has challenging targets to increase the use of renewable energy up to 38 % by 2020. According to the Finnish national renewable energy action plan, the most of the target is expected to be fulfilled with wood energy.[1] Therefore, the massive logistic chains are required. The wood material is practically always pre-treated when used in energy production. Typical pre-treatment is chipping the wood in wood chips.

In general, the round wood maintains heat value better than processed, e.g. chipped wood. The supply chains are various due to establishment of supply chain and the conditions in the combustion site. Wood chip storage times vary from one day up to one year.[2-8] Therefore, there is a great need to understand better the conditions. This information is essential when aiming to control the physical and chemical phenomenon in the storage piles. In this article the conditions in wood chip storage are shortly described and the challenges of analysing the conditions are represented.

The article bases on the experimental study "*Inhibition of microbial activities in the pile of energy wood chips during the storage*" carried out in JAMK University of Applied Sciences in 2013-2015 and funded by the Foundation for Research of Natural Resources in Finland. The project aimed to recognise chemical, physical or biological mean or means to inhibit the microbial activity in the storage piles. The large number of means were considered (e.g. preserving substance, storage method, chemical treatment), such as preventive, insulating, inhibiting, controlling means to decrease (energy, material) losses and increase safety during the storage.

The interventions that inhibit microbial activity require cross-sectoral approach. The experimental study benefitted a large variety of knowledge areas. It also considered that the mean(s) should not harm the combustion in the boiler, not to form unsafe emissions or particles and to be economic feasible solution. Some inhibiting means were found. Some means are further developed in BIOPOOLI project. Furthermore, one mean is under IPR process.

2 APPROACH

There are a large number of challenges in wood chip storage due to microbial activity. Like as Basidiomycota fungus also microbes (mold, yeast and bacterial) are utilized the woody material as

source of food. Therefore, the microbial activity causes decomposition of wood material and the mass as well as the energy content of the solid biomass decreases. It is known[9,10,11] that the microbes have a large variation of gaseous metabolites, such as carbon dioxide (CO_2), methane (CH_4), dinitrogen oxide (N_2O), terpenes. Some of the metabolites are green-house-gases and accelerate climate change and some are health issue. People working in supply chain are exposed to gas emissions as well as to the spores of fungus and other microbes. The endogenous microbial activity raises the temperature in storage. This sometimes leads to dangerous fires due to self-ignition and forming again harmful gas emissions. Based on the above mentioned reason the inhibition is economic, social and environmental issue.

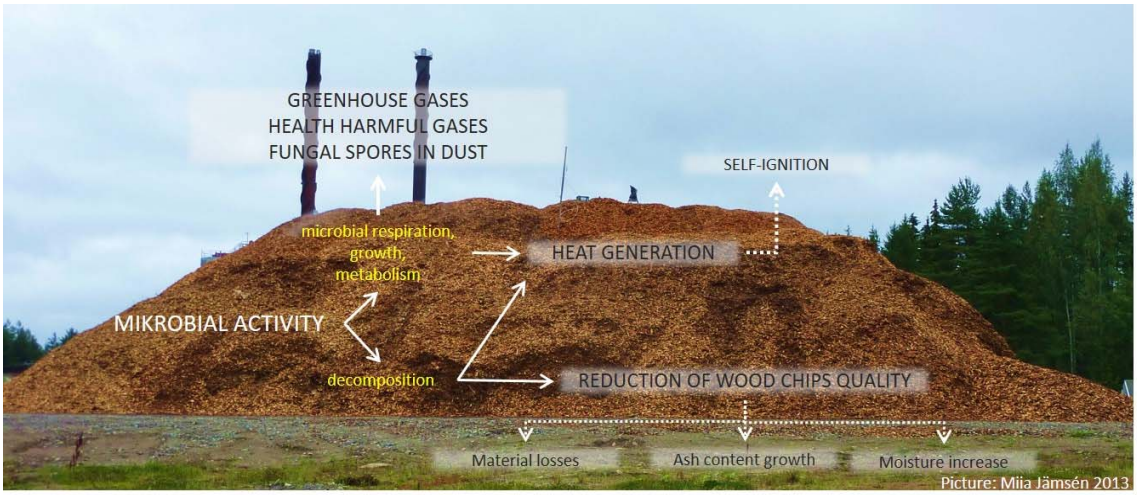

Figure 1: Connections of combined microbial activity effects of in wood chip storage. (Jämsén 2013)

There are many factors that are influencing in microbial activity in the wood chip storage.[2-3,6,9-11] First of all, it depends on the woody material that is stored. The moisture content, physical characteristics as well as chemical compound content varies in the chips. The differences depend for examples following factors:

- Wood species
- Age of chipped wood (young/ old)
- Growing environment of wood
- Part of the wood (whole tree, stems, stumps, branches)
- Season of cutting of wood
- Wood storage nature and time before chipping
- Wood moisture when chipped

Furthermore, the wood processing technology and equipment as well as operational practices and conditions all influence in the physical structure of the wood chip. The storage form and formation process, the pile structure (size, shape, density, coverage, ground) and formation process and its conditions has influence in the chemical conditions and biological environment (e.g. existing dominant microbes, minor populations) in the pile. During the storage the pile is exposed to the rain, wind, radiation and the temperature. These factors depend on the climate, seasons and weather as well as the storage time and the location of the pile.

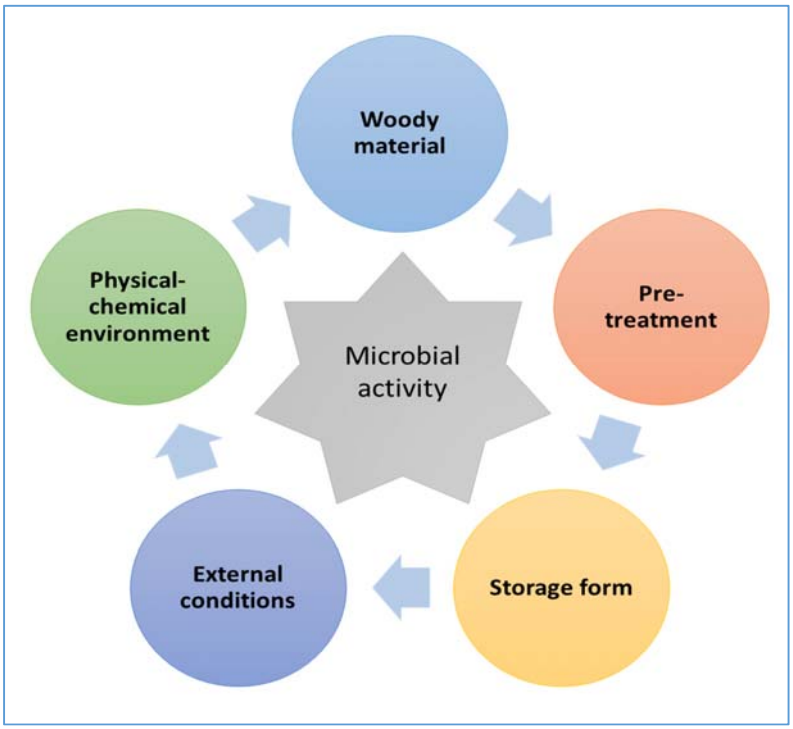

Figure 2: There are a large number of factors that influence in the microbial activity as well as in the quality and quantity of microbial flora in the wood chips storage piles. (Knuuttila 2016)

3 SCIENTIFIC INNOVATION AND RELEVANCE

In this experimental research the aim in year 2013 was to identify the microbes (molds, yeasts, bacteria) that are in important role in terms of decomposition of the material.

The first step was to take samples. There is no existing standard for sampling microbes of wood chip storage. Therefore, the following challenges were faced:

- What is suitable sampling equipment?
- How to avoid contamination of microbe samples? (risk in site, risk in laboratory)
- What is sufficient number of dublicates in sampling?
- What is proper reference samples?
- How to take representative sample out of 10 000 m^3 storage?

The samples were taken from three different storage sites in central parts of Finland. Two of these were “old” wood chips pile harvested 1-2 years ago and one of them was a “fresh” wood chips pile that was harvested less than 3 months ago. The samples of “old” piles were taken from three different layers of wood chips pile. The sampling was carried out according to exist biofuels solid standard (SFS-EN 14778, SFS-EN14789). The large number of duplicates were researched to ensure proper qualitative and quantitative analyses. The contamination risk of sampling and analyses were managed by fast pretreatment of samples in as aseptic conditions as possible. The analyses were carried out in fast time frame, preferable during sampling day.

After sampling the second step was to isolate the microbes from chips, cultivate the microbes and carry out qualitative and quantitative analyses of microbe population. Before the cultivation microbes were isolated to peptone water. The isolated molds, yeasts and bacteria of wood chips were incubated on different culture media at 25 °C for about 7 days and actinobacteria for 14 days. In every phase the contamination risk had to be managed carefully.

Qualitative analyses of microbes are based on professional skills of researcher. In this experimental study the researched was using light microscope (objective 10-100x) and camera to document the findings for further studies. In quantitative analyses the colony formatting units were calculated. Because

of large number of colony forming units the samples were serially diluted. The concentrations varied in range of $1x10^0$ to $1x10^8$ depending on storage age of wood chips.

In 2013 the important phase of experimental research was carried out. The sampling method was developed and the isolation method for wood chip storage microbes was selected. These both steps were essential for further phases of research to observe the microbial activity in the piles and to develop and test inhibition methods of microbial activity.

4 RESULTS

In this article the focus is in describing the challenges to analyse microbial population in wood chip storage piles. Furthermore, some results of microbial population in "fresh" and "old" storage are introduced.

In practise there is no existing standard for sampling microbes of wood chip storage. Methods used for sampling microbes in wood chip storage piles in this experimental studies were selected by testing method suitability from number of existing methods during year 2013. Thus, the research was able to continue to the next phase to identify and to quantify microbial flora of wood chip storage. This research did not consider the large Basidiomycota fungus growing on the pile. In general, this identification and quantification process had many challenges to face.

For Basidiomycota fungus there is existing analysing method, for instance method for wood-decay fungi. But in this study, the focus was to isolate molds, yeasts and bacteria from wood chips. Basidiomycota fungus were observed only visually. The different kind of sampling methods were tested. It was important to find right culture medium, which respond to different environments of wood chips pile. Furthermore, the microbes should be able to identify. In this experimental study, 3-4 suitable media were used.

Some results of microbe analyses for year 2013 are introduced in table 1. In "old" pile the number of microbes is remarkable greater than in "fresh" piles. The middle layers of the piles were the most beneficial (and most reactive) environment for microbes. The physical-chemical environment more than a meter from the surface of pile is favorable due to gaseous, temperature and moisture conditions. In general, bacteria growth is the most active over the life span of combustion process. In this experimental research it was also noted that there is larger portion of yeasts present when storage of wood chips pile was "fresh". The portion of yeasts decreased when the storage time of wood chips is longer. Counterwise, the portion of the molds increase when the piles get older. The results also provided information about health risk factors. In the "old" piles it was observed from one to 10 000 millions colony forming units per gram (mil. cfu/g) *Aspergillus fumigatus* mold or actinobacteria, which both were one of the dominant microbe species. It is known[12,13] that in indoor air depending on species, over 0,01 mil. cfu/g mold or 0,0001 mil. cfu/g actinobacteria expose people to harmful disease. The storage duration correlates with the number of harmful bacteria in wood chip storage and increases the risk to be exposed to harmful bacteria.

Table 1: Total microbial content (mil. cfu/g dry matter) of the "fresh" and "old" wood chips piles. Sampling depth of the "old" wood chips pile: surface layer (≤ 0,4 m), middle layer (≥ 1,0 ± 0,2m), inner layer (≥ 4,0 ± 1,0m). Harvested time: "fresh" < 3 moths and "old" 1-2 years ago.

Storage	Moisture (%)	Mold (mil. cfu / g)	Yeast (mil. cfu / g)	Bakterial (mil. cfu / g)
"Fresh" wood chips pile	39,9	0,27	0,87	3
Surface layer of "old" wood chips pile	64,7	166	86	3400
Middle layer of "old" wood chips pile	60,9	6600	5100	12000
Inner layer of "old" wood chips pile	46,1	4	1	25

5 CONCLUSIONS

In Europe wood chips are widely used in energy production. Therefore, the microbial activity in wood chip storage piles has importance in economic and environmental terms. The microbial activity is also health issue. However, microbial activity of wood chips storage has not raised much interest by now. The inhibition and managing the process is challenging topic and it requires cross-sectoral research and experiments.

Woody material, pre-treatment, storage conditions and external conditions all influence in the microbial activity due to impact on physical-chemical environment of storage (moisture, temperature, oxygen content, chemical content). In this experimental research the focus was in molds, yeasts and bacteria. Basidiomycota fungus were excluded. In this experimental research, it was recognised that in the wood chip storages the number of colony formatting units correlates with the storage duration of wood chips. In the storage piles, there are present microbes, such as *Aspergillus fumigatus* and actinobacteria that are safety risk for people working with wood chips.

In this article the focus is in challenges of microbial analyses of wood chip storage. To take the representative and uncontaminated sample itself is a challenge. There is no equipment in the markets and it is difficult to get samples that would be representative and uncontaminated. In this work the samples were taken from real life storage piles from different areas in central parts of Finland.

One challenge was that there is no standard for the microbial sampling of wood chip microbes. The seeking for the suitable one takes time, innovativeness and nerves. The microbe analyses themselves are time consuming and sensitive. Besides, it is important to manage contamination risk properly. Furthermore, many dublicates are needed. In quantitative analyses the scale of dilutions is large.

Sampling and analysing are very much work and the results of the experiments are not necessary transferrable in different piles and different operational environment. What are the results from 100 storages? What are the results in next year (variations in woody material, weather)? How to take results from laboratory scale to pilot and real life scale?

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