Results and discussion

Discussion

Physico-chemical composition

The fish waste contains 46.6% and food waste 14.0% dry matter. The pH was 6.70 and 5.89 respectively. Ntot content in the food waste was 0.3% and 2.6% in fish waste.

The density and conductivity in food and fish waste was similar. At the same time, the content of ash was higher in fish (3.2%) than in food waste (0.6). The food and fish waste contains similar amount of K, 202.3 and 176.2 mg/100g respectively.

Compare to the food waste, the fish waste contained remarkably higher amount of P 80.8 and 900 mg/100g and Ca 30.7 and 1064.3 mg/100g respectively. Thus, the nutrient content of the different final BBF, mainly N and P, largely depends on the proportion of both food and fish waste in it. In the case of BBF made from fish waste, the proportion of P is likely to be much higher than N and K.

The fish sludge dry matter content was very low (0,5%). The total N content was also low (0.02%). The pH was 5.35. Similar pH (5.80) in fish sludge was also found by Broad et al (2017). In addition, the content of P, K, Ca, Mg, S was 33.9, 8.3, 35.6, 7.1, 12.5 mg/100g respectively. The results were similar to the finding by Broad et al (2017).

The tree leaves (a mixture of different deciduous leaves) and common reed (*Phragmites australis*) will use as a bulking agents and carbon sources that mixed with the fermented material. It has been found, that the leaf litter chemistry is influenced by climate, species as well as by soil nutrient content (Suseela and Tharayil, 2018). Simon et al. (2018) found in higher C/N ratio on *Betula pendula* and higher total N *Tilia cordata* and *Tilia platyphyllus* leaf litter. Depending on the species, the C/N ratio ranged from 20 to100 and the N content ranged from 5 to 30 mg/g dry weight. In our case, the leaf litter consisted of a mixture of leaves from different species of trees. The litter contains 1.0% total N, 0,7% total K and 0,13% total P. The leaf litter had quite low C/N ratio (26.2) thus, in soil this material decomposes rapidly.

In the previous study (Hu, et al, 2020) had found that the P. *australis* straw had an average 90% of dry matter and its bulk density was 83 kg/m^3. The content of total P and K were found 2.82 and 32.95 g/kg respectively. The content of total carbon was 45% and nitrogen 1.07%, thus, the C/N ratio remained above 40. At the same time Toumpeli et al. (2013) found, that the C/N ratio for matured *P. australis* was evean 411.7. The *P. Australis* results are not yet available.

Contaminants

The content of heavy metals in fish sludge, fish- and food waste was low and did not exceed the threshold limits, which is set out in Estonian legislation on biodegradable compost (RT1, 2013) and in Quality Manual of the European Quality Assurance Scheme for Compost and Digestate (ECN-QAS Quality Manual, 2018). As food waste was of plant origin, pesticide residues were also analysed mainly N and Ped. None of the 76 analysed pesticide residues (Annex 1) in food waste were found.

Microbiology

The side-streams (fish waste, food waste and fish sludge) does not contain *Salmonella* bacteria and had E. Coli bacteria in 1.0 g side-stream materials less than 1 x 10^1 CFU.

All concentrations are calculated according to dry weight of samples (fish waste, food waste, fish mud, leaf litter and common reed straw). The relative abundances of each microbial group were calculated according percentage from total PLFAs. The highest concentration of total PLFAs was wound in fish sludge (8909.03 nmol/g) followed by leaf litter (3424.27 nmol/g) and fish waste (715.60 nmol/g). The lowest total concentration of PLFAs was found in food waste (95.826 nmol/g) and common reed straw (369.83 nmol/g).

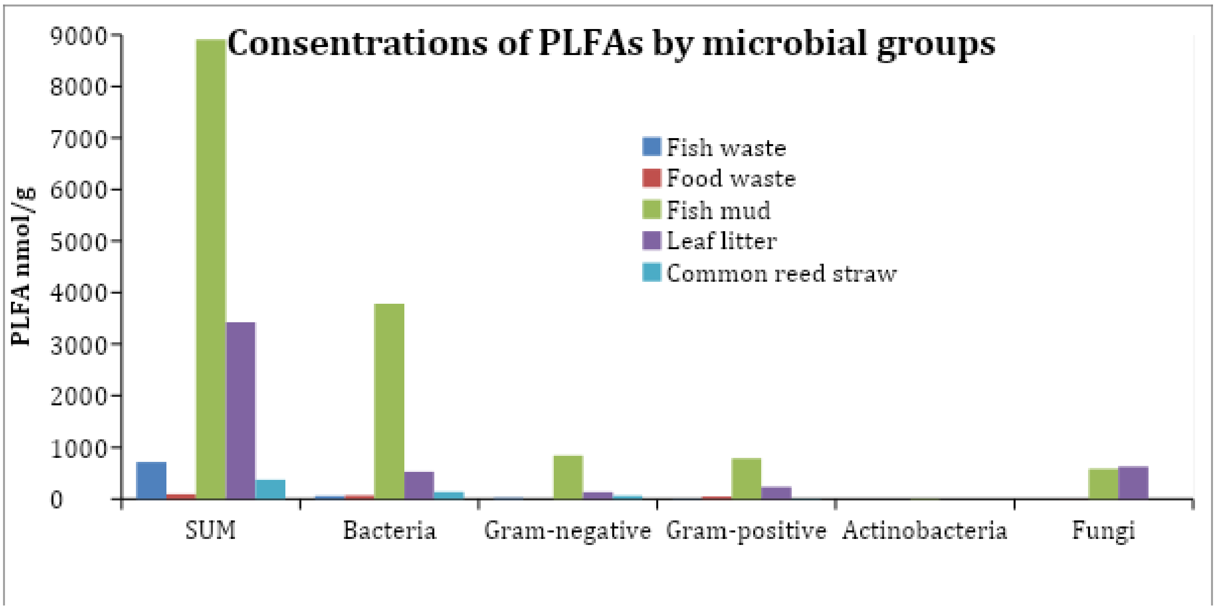
Regardless of relatively high level of total PLFA in fish waste, most of determined fatty acids are part of fish not from microbial community composition because fishes are rich in many fatty acids (Kandyliari *et al.* 2020). Only 8.5 % of total PLFAs could determine part of microbial biomass.

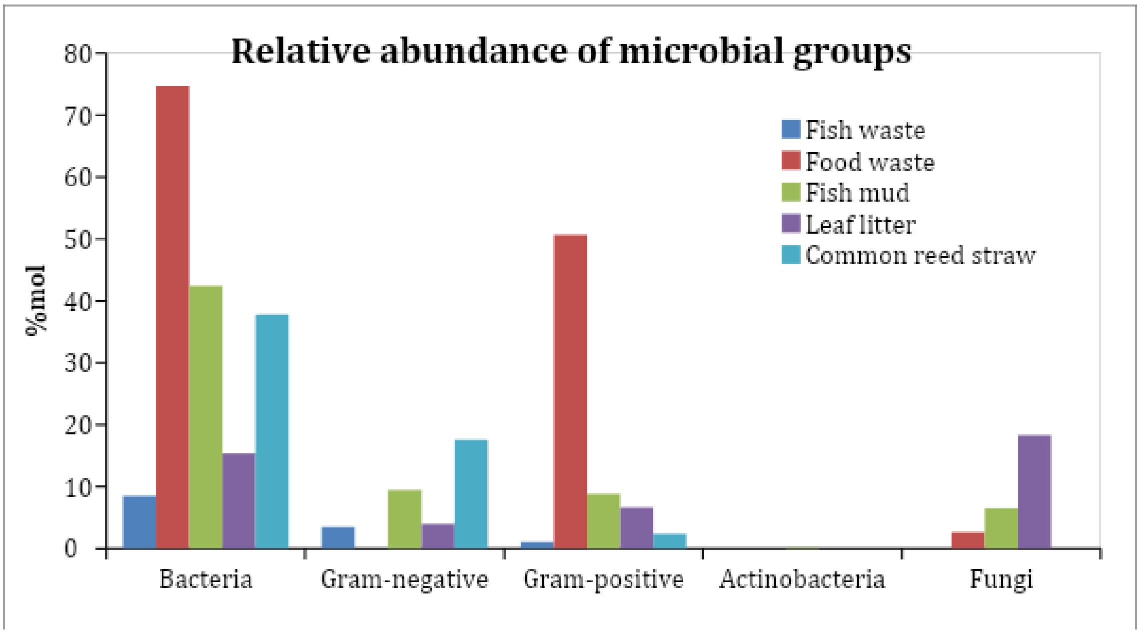
Overall, different microbial groups depending on substrate nature and due to that react differently in substrate utilization. Gram-negative bacteria are small cells and are sensitive to stress conditions. They usually prefer easily available, low molecular weight organic substances and prefer high C availability (Ahmed *et al.* 2018, Xu *et al.* 2020). For example, straw addition increases the abundances of Gram-negative bacteria (Aciego Pietri and Brookes, 2009). But Gram-negative bacteria are sensitive to different stress factor e.g. in metal polluted sites (Fostegard *et al.* 1993; Akerblom *et al.* 2007), lower pH (Xu et al. 2020), drought (Ahmed *et al.* 2018; Sun et al. 2020). In addition, negative correlation between Gram-negative bacteria and P has been identified (Mann *et al.* 2019). Some of the Gram-negative bacteria are anaerobic (Pannetieri *et al.* 2020). According to our results, the highest value of Gram-negative bacteria was found in fish sludge (844.38 nmol/g) where the total PLFA was also the highest. Still the relative abundance of Gram-negative bacteria was in common reed straw (17.59%) what is in accordance with previous studies where straws contain high availability of C (Ahmed *et al.* 2018; Xu et al. 2020).

Gram-positive bacteria are large with thicker cell walls. Due to that, they are more tolerant to water stress (Andersen and Petersen 2009) and different metals (Akerblom *et al.* 2007; Aciego Pietri and Brookes, 2009). Usually they degrade more complex compounds than gram-negative bacteria (Bird *et al.* 2011). The highest value of Gram-positive bacteria was also found in fish mud (787.77 nmol/g) but relative abundance was the highest in food waste (50.70%). High abundance of Gram-positive bacteria in food waste could be due to relatively high content of different contaminants (e.g. Na, Cr, Ni, Zn, Cu). Gram-positive bacteria are more tolerant to stress and due to that more abundant in food waste.

Fungi are dominant in habitats with recalcitrant organic matters due to the ability to degraded complex components during early stages of decomposition (Arcand *et al.* 2016). The highest concentration of fungi was found in fish sludge (583.33 nmol/g) followed by leaf litter (626.14 nmol/g). Still, the relative abundance of fungi was the highest in leaf litter (18.28%) indicating the suitable material for fungi. No fungi were found in fish waste () and common reed straw.

Actinobacteria are major decomposers of complex polymers in soils (Xu *et al.* 2020). Results showed minor amount of *Actinobacteria* only in fish mud (0.16%) indicating the inappropriate habitat for *Actinobacteria* in all site-streams.


Consentrations of PLFAs by microbial groups
PLFA nmol/g
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Fish waste
Food waste
Fish mud
Leaf litter
Common reed straw
SUM
Bacteria
Gram-negative
Gram-positive
Actinobacteria
Fungi


Relative abundance of microbial groups
%mol
80
70
60
50
40
30
20
10
0
Fish waste
Food waste
Fish mud
Leaf litter
Common reed straw
Bacteria
Gram-negative
Gram-positive
Actinobacteria
Fungi

1.1.1 Fish waste from M.V. Wool AS

Physico-chemical composition

Parameters	Unit	Fish waste
Dry matter	%	46.6
Organic matter	%	43.4
Density	kg/m^3	1123
Ash	%	3.2
conductivity	mS/cm	1.7
P	mg/100 g	900
pH		6.7
Chlorides	mg/100 g	32.6
S	mg/100 g	34.1
K	mg/100 g	176.2
Ca	mg/100 g	1064.3
Mg	mg/100 g	28.8
Fe	mg/kg	103.7
Na	mg/100 g	72.6
Protein	%	16.25
Organic and mineral nitrogen	%	2.6

Contaminants

Parameters	Unit	Fish waste
Hg	mg/kg	0
Cd	mg/kg	0
Cr	mg/kg	0.072
Ni	mg/kg	0.033
Pb	mg/kg	0
Zn	mg/kg	9.51
Cu	mg/kg	0.16

Microbiology

Parameters	Unit	Fish waste
E. coli	CFU/1.0 g	< 1 x 101
Salmonella	CFU/25 g	0
Total PLFAs	Nmol/g per DW soil	715.601
Bacteria	Nmol/g per DW soil	60.947
Gram-negative bacteria	Nmol/g per DW soil	25.288
Gram-positive bacteria	Nmol/g per DW soil	7.812
Actinobacteria	Nmol/g per DW soil	0
Saprotrophic fungi	Nmol/g per DW soil	0
Total PLFAs	%nmol	100
Bacteria	%nmol	8.517
Gram-negative bacteria	%nmol	3.534

Gram-positive bacteria	%nmol	1.092
Actinobacteria	%nmol	0
Saprotrophic fungi	%nmol	0

Other

No other analysis of the side-stream was performed

1.1.2 Food waste from HORECA

Physico-chemical composition

Parameters	Unit	Food waste
Dry matter	%	14
Organic matter	%	13.4
Density	kg/m^3	1124
Ash	%	0.6
conductivity	mS/cm	1.6
P	mg/100 g	80.8
pH		5.89
Chlorides	mg/100 g	11.5
S	mg/100 g	70
K	mg/100 g	202.3
Ca	mg/100 g	30.7
Mg	mg/100 g	21.6
Na	mg/100 g	79.1
Fe	mg/kg	16.78
Protein	%	1.875
N_{tot}	%	0.3

Contaminants

Parameters	Unit	Food waste
Hg	mg/kg	0
Cd	mg/kg	0.008
Cr	mg/kg	0.061
Ni	mg/kg	0.036
Pb	mg/kg	0
Zn	mg/kg	2.16
Cu	mg/kg	0.24
Pesticide residues (76 different residues)	Annex 1	0

Annex 1.

List of pesticide residues analysed from samples of animal and plant origin method PM03:2017 (SANTE/12682/2019, (EU) 2017/644)

Pesticide residue	LOQ mg/kg
Acephate	0.01
Aldrin and Dieldrin (Aldrin and dieldrin combined expressed as dieldrin)	0.005
Aldrin	0.005
Dieldrin	0.005
Azinphos-methyl	0.008
Bromophos-ethyl	0.004
Bromophos-methyl	0.004
Chlorfenvinphos	0.004
Chlorpyrifos	0.004
Chlorpyrifos-methyl	0.008
Cypermethrin (sum of isomers)	0.01
DDT (sum of p,p´-DDT, o,p´-DDT, p-p´-DDE and p,p´-TDE (DDD) expressed as DDT)	0.001
p,p'-DDD	0.001
p,p'-DDE	0.001
o,p-DDT	0.001
p,p`-DDT	0.001
Deltamethrin (cis-deltamethrin)	0.01
Diazinon	0.008
Dichlorvos	0.004
Dimethoate	0.01
Disulfoton (sum of disulfoton, disulfuton sulfoxide and disulfoton sulfone expressed as disulfoton	0.01
Disulfoton	0.01
Disulforton sulfoxide	0.01
Disulfoton sulfone	0.01
Endrin	0.005
Ethion	0.004
Etrimphos	0.01
Fenamiphos (sum of fenamiphos, fenamiphos sulfoxide, fenamiphos sulfone)	0.01
Fenamiphos	0.01
Fenamiphos sulfoxide	0.01
Fenamiphos sulfone	0.01
Fenitrothion	0.004
Formothion	0.01
Heptachlor (sum of heptachlor and heptachlor epoxide expressed as heptachlor)	0.001

Heptachlor	0.001
Heptenophos	0.004
Cis-Heptachlorepoxide	0.001
Trans-heptachlorepoxide	0.001
Hexachlorobenzene (HCB)	0.001
Hexachlorocyclohexane (HCH), alpha-isomer	0.001
Hexachlorocyclohexane (HCH), beta-isomer	0.001
Hexachlorocyclohexane (HCH), delta-isomer	0.001
Lindane (Gamma-isomer of hexachlorocyclohexane (HCH))	0.001
Malathion (sum of malathion and malaoxon expressed as malathion)	0.004
Malathion	0.004
Malaoxon	0.004
Methacrifos	0.004
Methamidophos	0.01
Methidathion	0.004
Monocrotophos	0.01
Omethoate	0.01
Parathion -ethyl	0.004
Parathion methyl (sum of parathion-methyl and paraoxon-methyl expressed as parathion-methyl)	0.01
Parathion-methyl	0.01
Paraoxon-methyl	0.01
Permethrin (sum of isomers)	0.01
Phorate (sum of phorate, its oxygen analogue and their sulfones expressed as phorate)	0.01
Phorate	0.01
Phorate sulfone	0.01
Phorate oxon sulfone	0.01
Phorate oxon	0.01
Phosalone	0.008
Phosmet (phosmet and phosmet oxon expressed as phosmet)	0.01
Phosmet	0.01
Phosmet oxon	0.01
Phosphamidon	0.004
Phoxim	0.01
Pirimiphos-methyl	0.01
Profenofos	0.01
PCB (sum of PCB28, PCB52, PCB101, PCB138, PCB153, PCB180)	10 ng/g wet weight

PCB28	10 ng/g wet weight
PCB52	10 ng/g wet weight
PCB101	10 ng/g wet weight

PCB138	10 ng/g wet weight
PCB153	10 ng/g wet weight
PCB180	10 ng/g wet weight

Triazophos	0.01

LOQ – limit of quantification

Microbiology

Parameters	Unit	Food waste
E. coli	CFU/1.0 g	< 1 x 101
Salmonella	CFU/25 g	0
Total PLFAs	Nmol/g per DW soil	95.826
Bacteria	Nmol/g per DW soil	71.580
Gram-negative bacteria	Nmol/g per DW soil	0
Gram-positive bacteria	Nmol/g per DW soil	48.579
Actinobacteria	Nmol/g per DW soil	0
Saprotrophic fungi	Nmol/g per DW soil	2.565
Total PLFAs	%nmol	100
Bacteria	%nmol	74.698
Gram-negative bacteria	%nmol	0
Gram-positive bacteria	%nmol	50.696
Actinobacteria	%nmol	0
Saprotrophic fungi	%nmol	2.677

Other

No other analysis of the side-stream was performed

1.1.3 Fish sludge from Ösel Harvest OÜ

Physico-chemical composition

Parameters	Unit	Fish sludge
Dry matter	%	0.5
Organic matter	%	0.3
Density	kg/m^3	1008
Ash	%	0.2
Conductivity	mS/cm	3.1
P	mg/100 g	33.9
pH		5.35
Chlorides	mg/100 g	36
S	mg/100 g	12.5

K	mg/100 g	8.3
Ca	mg/100 g	35.6
Mg	mg/100 g	7.1
Na	mg/100 g	21.6
Protein	%	0.125
Organic and mineral nitrogen	%	0.02

Contaminants

Parameters	Unit	Fish sludge
Hg	mg/kg	0
Cd	mg/kg	< 0.006
Cr	mg/kg	0.05
Ni	mg/kg	< 0.023
Pb	mg/kg	0
Fe	mg/kg	22.8
Zn	mg/kg	0.46
Cu	mg/kg	0.16

Microbiology

Parameters	Unit	Fish sludge
E. coli	CFU/1.0 g	< 1 x 10^1
Salmonella	CFU/25 g	0
Total PLFAs	Nmol/g per DW soil	8909.026
Bacteria	Nmol/g per DW soil	3783.867
Gram-negative bacteria	Nmol/g per DW soil	844.382
Gram-positive bacteria	Nmol/g per DW soil	787.766
Actinobacteria	Nmol/g per DW soil	14.650
Saprotrophic fungi	Nmol/g per DW soil	583.325
Total PLFAs	%nmol	100
Bacteria	%nmol	42.472
Gram-negative bacteria	%nmol	9.478
Gram-positive bacteria	%nmol	8.842
Actinobacteria	%nmol	0.164
Saprotrophic fungi	%nmol	6.548

Other

No other analysis of the side-stream was performed

1.1.4 Garden waste (tree leaves) from Eesti Jäätmeringluse OÜ

Physico-chemical composition

Parameters	Unit	
N_{tot}	%	1.00
C_{tot}	%	26.23
P_{tot}	%	0.13
K_{tot}	%	0.7
Dry matter	%	49.4
C/N		26.23

Contaminants

Not analyzed

Microbiology

Parameters	Unit	Tree leaves
Total PLFAs	Nmol/g per DW soil	3424.272
Bacteria	Nmol/g per DW soil	526.364
Gram-negative bacteria	Nmol/g per DW soil	136.304
Gram-positive bacteria	Nmol/g per DW soil	227.995
Actinobacteria	Nmol/g per DW soil	0
Saprotrophic fungi	Nmol/g per DW soil	626.138
Total PLFAs	%nmol	100
Bacteria	%nmol	42.472
Gram-negative bacteria	%nmol	3.981
Gram-positive bacteria	%nmol	6.658
Actinobacteria	%nmol	0
Saprotrophic fungi	%nmol	18.285

Other

No other analysis of the side-stream was performed

1.1.5 Common reed *(Phragmites australis)* straw from Suckõrs, Sutu OÜ

Physico-chemical composition

The results are not yet available

Parameters	Unit	
N_{tot}	%	
C_{tot}	%	
P_{tot}	%	
K_{tot}	%	
Dry matter	%	
C/N		

Contaminants

Not analyzed

Microbiology

Parameters	Unit	Common reed straw
Total PLFAs	Nmol/g per DW soil	369.833
Bacteria	Nmol/g per DW soil	139.712
Gram-negative bacteria	Nmol/g per DW soil	65.070
Gram-positive bacteria	Nmol/g per DW soil	8.947
Actinobacteria	Nmol/g per DW soil	0
Saprotrophic fungi	Nmol/g per DW soil	0
Total PLFAs	%nmol	100
Bacteria	%nmol	37.777
Gram-negative bacteria	%nmol	9.478
Gram-positive bacteria	%nmol	8.842
Actinobacteria	%nmol	0
Saprotrophic fungi	%nmol	0

Other

No other analysis of the side-stream was performed

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