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TKI 'Kleinschalige Bioraffinage', WP9: Fatty acid and PHA production based on residues

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ACRRES - Wageningen UR january 2016

PPO-677

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ACRRES-Wageningen UR PPO nr. 677

PPO project number: 3750295700.

This study is part of the project 'Kleinschalige Bioraffinage' (TKI-AF-12040), and was co-financed by the 'Toeslag voor Topconsortia voor Kennis en Innovatie (TKI's), of the Dutch Ministry of Economic Affairs TKI-AgriFood.

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Content

1 Introduction

During peak production periods it can be interesting to harvest and silage seasonal organic by-product streams for the production of organic acids such as lactic acid and short-chain volatile fatty acids (VFA). The main project of which the research described in this report is a part, focuses on using organic acids as carbon and energy source of polyhydroxyalkanoate (PHA) accumulating bacteria (Salehizadeh & van Loosdrecht, 2004; Bengtsson et al, 2008; Cerrone et al, 2014), but there are also other options, such as conversion of VFAs to liquid biofuels (Steinbusch, 2010). Potentially interesting streams are organic household waste, roadside grass, and by-product streams from greenhouses such as tomato and cucumber leaves.

The experiments described in this report focus on the production of organic acids from ensiled meadow grass. The grass was ensiled during 10 weeks. At the start and after 2, 4, 6 and 10 weeks the ensiled grass was pressed into a cake and a juice fraction. Both fractions were analysed for dry matter content, pH, and energy input required for pressing. Furthermore, levels of short-chain FAs and lactic acid were measured. In this way, the feasibility of producing organic acids from ensiled grass can be evaluated.

2 Materials and methods

2.1 Ensiling

14 clean HDPE barrels with lids were filled with layers of meadow grass as a model substrate. The barrels were filled up to a packing density of ~0.45 g of fresh material/cm^3 (Hoedtke and Zeyner, 2011) by stomping and the exact weights of grass and empty barrels were registered. The empty volume per barrel was 150 L and they were made liquid- and air-tight by means of clamping rings. The meadow grass (3rd cut) originated from Kampen (the Netherlands), was sown five years earlier, grazed by sheep and fertilised with cattle slurry. The cut grass was 5 to 15 cm long had been harvested 3 to 4 hours before filling the barrels. The barrels were stored at 20 °C and checked five times a week for overpressure. If the lid was bulging, the clamping ring was released until gas was audibly released.

2.2 Pressing

Within three hours of filling, the contents of two barrels were pressed and analysed (t=0). Thereafter, three barrels at a time were analysed after 2, 4, 6 and 10 weeks (Table 1). At the start date, the fresh material was analysed in triplicate for dry matter content after drying overnight at 100 °C. The content of each barrel (silage) was pressed, resulting in a juice and a cake (fibre) fraction. The used press was a 6 inch Modular Screw Device, from Andritz Sprout Bauer. This single screw press is continuously fed and the cake plug that forms at the end is continuously pushed out. The effluent can exit through holes in the wall of the press. The ease at which the plug can be pressed out is controlled by 'fingers' at the end, which for the current experiment were screwed in to 1 mm from the screw. As the device uses 2,8 kW of power constantly, the energy requirement could be calculated by recording the time needed for pressing. Weight and dry matter (after drying at 105 °C) were determined in triplicate for each barrel before pressing ('silage'), and the press juices ('juice') and press cakes ('cake') after pressing. Aliquots of the juice, after measuring the pH, were stored at -20 °C, and when all juice fractions were collected, all were analysed in the same run.

2.3 VFA and lactic acid analyses

2.3.1 Preparation

Samples were centrifuged at 16.800×g for 15 minutes to facilitate the filtration process. After centrifugation the supernatant from the samples was filtered using a 0.45 µm filter. The filtrate was used to conduct the VFA and lactic acid analyses. Demineralised water was used to dilute the samples.

2.3.2 VFA analysis

Formic acid was added to the samples in a 1:1 volume ratio. A gas chromatograph (Hewlet Packard 5890) with a flame ionisation detector was used to measure concentrations of acetic acid (C2), propionic acid (C3), isobutyric acid (i-C4), n-butyric acid (n-C4), isovaleric acid (i-C5), n-valeric acid (n-C5), isocaproic acid (i-c6), n-caproic acid (n-c6) in the press juice.

2.3.3 Lactic acid analysis

The HPLC used an organic acids column OA-1000 (l=300 mm; id=6.5 mm; Alltech part nr.9046). Eluent is sulphuric acid (0.0025 N = 1.25 mM), flow = 0.6 mL/min, temperature= 60 °C. Components were detected by means of a Refractive Index detector (RI). Data were acquired and processed by Chromeleon v6.8, quantification was done by a three point calibration.

3 Results and Discussion

The packing densities of the grass in the 14 barrels were between 0.41 and 0.45 g/cm^3 (Table 1) (Hoedtke and Zeyner, 2011).

Table 1 Grass weight per barrel, packing density and analysis week

Barrel	Grass weight (kg)	Packing density (g of fresh material / cm^3)	Analysis week
1	63.5	0.42	10
2	64.0	0.43	2
3	65.0	0.43	0
4	64.5	0.43	6
5	63.5	0.42	2
7	62.0	0.41	0
8	63.0	0.42	6
9	66.5	0.44	4
10	62.5	0.42	4
11	63.5	0.42	2
12	67.5	0.45	6
13	67.5	0.45	10
15	68.0	0.45	4
16	67.5	0.45	10

At the start, around 43 % of the grass could be pressed into juice, while after two weeks this percentage increased to around 68 %, remaining more or less the same for the rest of the ten weeks (Figure 1).

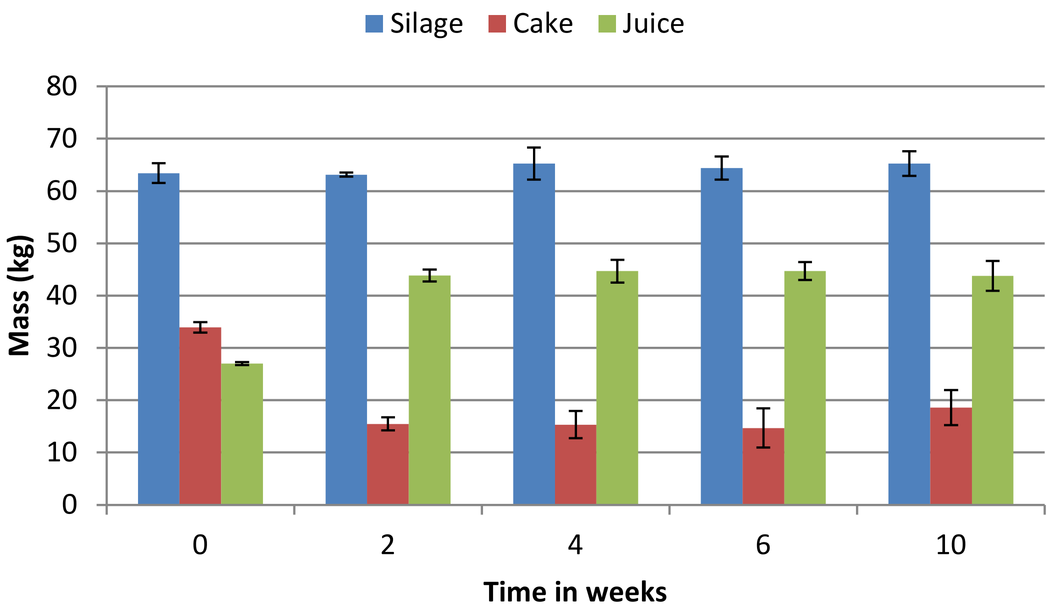

Figure 1 Mass (kg) of total barrel content (silage), cake fraction, and juice fraction, as a function of ensiling time. Error bars represent standard deviation (n=3, except n=2 for start date).

The pH of the press juice at the start of the experiment was 6.4 and after two weeks this had decreased to around 4.2 (Table 2). During the remainder of the experiment this did not change until week 10, when a small increase was found.

Table 2 pH in the press juice during the experiment with standard deviations (n=3, except n=2 for start date)

Ensiling time	pH
0	6.4 (±0.0)
2	4.2 (±0.0)
4	4.2 (±0.1)
6	4.2 (±0.2)
10	4.4 (±0.2)

The dry matter percentage of the fresh meadow grass at the start date was 15.6 (+/- 1.0) %, while those of cake and juice were 25.2 (±0.1) % and 3.5 (±0.1) % respectively (Figure 2). During the test period the dry matter percentage of the of cake and juice increased after the first two weeks and then stabilised at around 35 % and 5 % respectively.

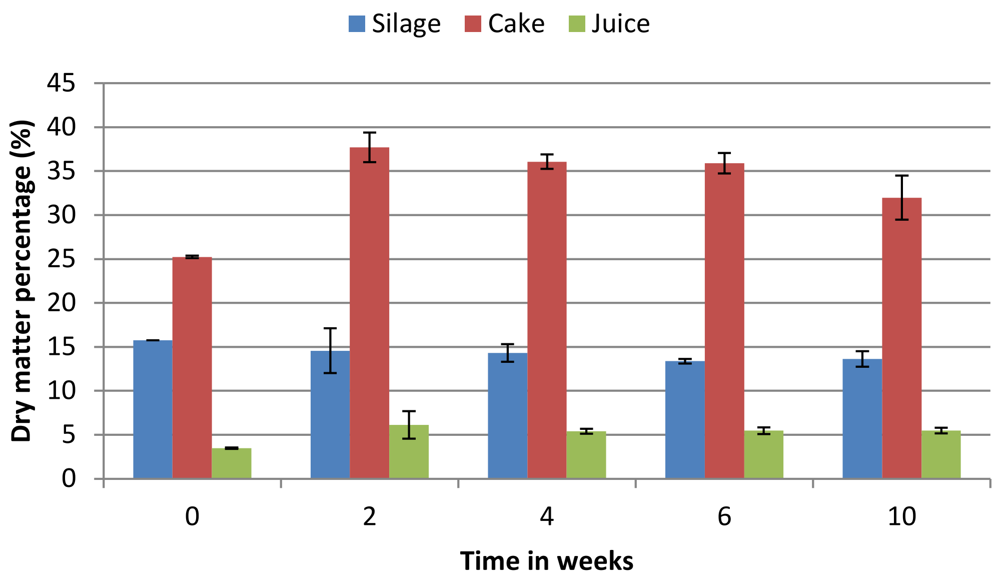

Figure 2 Dry matter percentage of total barrel content (silage), cake fraction, and juice fraction, as a function of ensiling time. Error bars represent standard deviation (n=3, except n=2 for start date).

The total amount of dry matter of the cake decreased and that of the juice increased after the first two weeks, after which they remained more or less constant (Figure 3). Clearly, the ensiling process leads to breakdown of the original material and increased compressibility, in turn leading to more dry matter in the juice fraction. It should be noted that any produced volatile FAs are not included in the dry matter analyses of the pressed fractions, as these evaporate with the water during drying.

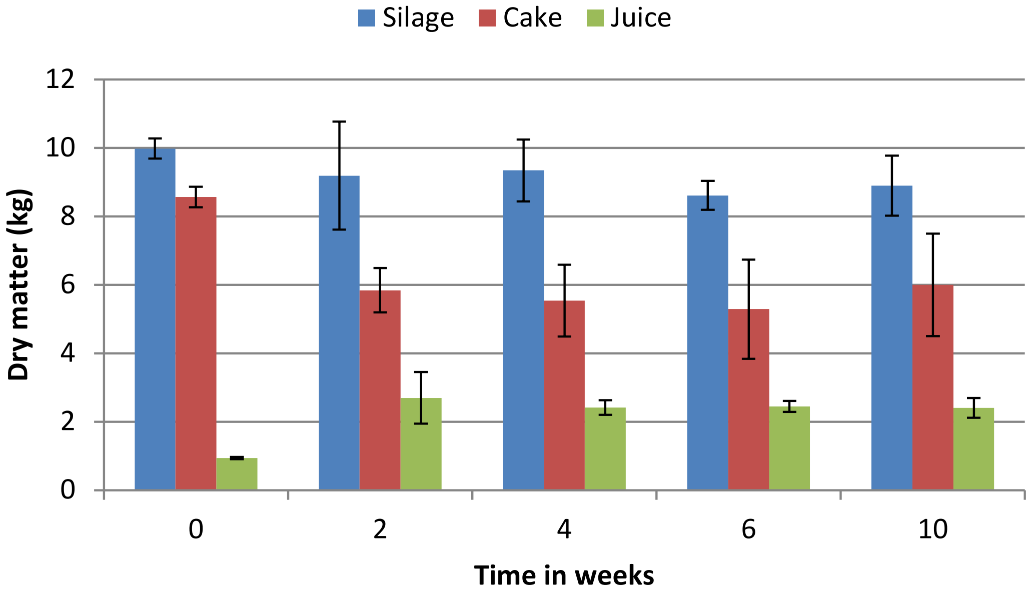

Figure 3 Dry matter (kg) of total barrel content (silage), cake fraction, and juice fraction, as a function of ensiling time. Error bars represent standard deviation (n=3, except n=2 for start date).

In the juice, lactic acid, n-butyric acid and acetic acid were found at maximum concentrations of 16.25 (±0.27), 6.71 (±0.87) and 2.70 (±0.19) g/L, respectively (Table 3).

Table 3 Maximum FA and lactic acid concentrations (with standard deviations) in the juice fraction with ensiling times at which maximum occurred.

Acid	Maximum concentration (g/L)	Ensiling time (weeks)
Acetic acid (C2)	2.70 (±0.19)	4
Propionic acid (C3)	0.42 (±0.11)	10
Isobutyric acid (i-C4)	0.09 (±0.02)	10
n-butyric acid (n-C4)	6.71 (±0.87)	10
Isovaleric acid (i-C5)	0.05 (±0.04)	10
n-valeric acid (n-C5)	0.04 (±0.04)	10
Isocaproic acid (i-C6)	0.00 (±0.00)	-
n-caproic acid (n-C6)	0.77 (±0.28)	10
Lactic acid	16.25 (±0.27)	2

Of the three main acids formed, n-butyric acid concentration showed the strongest increase in time, while after two weeks that of lactic acid decreased and acetic acid remained constant (Figure 4).

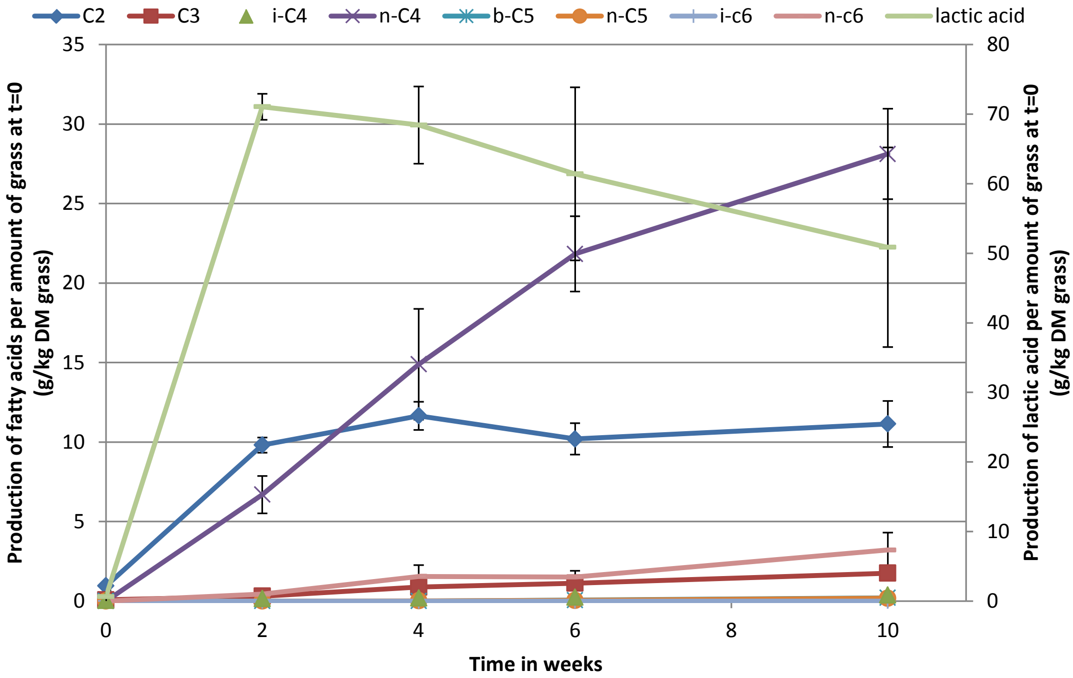

Figure 4 Amounts of FA and lactic acid in the juice fraction produced per amount of dry matter grass at t=0 as a function of ensiling time. Error bars represent standard deviation (n=3, except n=2 for start date). Lactic acid concentrations are plotted on the secondary y-axis.

Other fatty acids never reached concentrations higher than 0.77 (±0.28) g/L (Table 3), but slowly increased with time (except isocaproic acid, which was not detected). Butyric acid is produced by clostridia bacteria from sugars and/or lactic acid and is an unwanted FA in animal feed (Muck, 2006) but is suitable for the production of PHA (Chen et al, 2010). Usually lactic acid bacteria (LAB) will outcompete clostridia unless pH is not low enough (e.g. in spoiled areas in the silage for animal feed). For example, at a dry matter content of 15 %, as was found in the start material of this test, a pH above 4 will enhance the production of butyric acid. A higher dry matter content prevents growth of clostridia and can thereby prevent butyric acid formation (Leibensperger and Pitt, 1987). The pH in this experiment remained just above 4 and increasing butyric acid production from fermenting lactic acid would raise pH further. Towards the end of the experiment, pH seems to have increased slightly (Table 2) and lactic acid decreased, as the highest concentration was measured after two weeks (Table 3). The differences between lactic acid concentrations in the triplicate measurements increased during the ensiling process. Lactic acid, n-butyric acid and acetic acid were found at maximum concentrations of 71.1 (±1.9), 28.1 (±2.8) and 11.7 (±0.9) g/kg dry matter (of the grass at the start of the experiment). Other individual fatty acids never reached concentrations higher than 3.2 (±1.1) g/kg dry matter. After ten weeks, on average 45 g total FA/kg dry matter were produced. The total production of lactic acid was higher than the cumulative production of short-chain fatty acids (Figure 4). Johnson et al (2005) describe similar lactic acid concentrations during ensiling of perennial rye grass in 16 days. Haigh (1998) measured acid concentrations in grass silage of 82 g lactic acid/kg dry matter, 28 g acetic acid/kg dry matter, 13 g butyric acid/kg dry matter, 47 g total short-chain fatty acids/kg dry matter and 129 g total acids/kg dry matter. The results for lactic acid and total short-chain fatty acids are comparable, but in the current experiment the concentrations of butyric acid and acetic acid were different, which can be explained by growth of clostridia. Lactic acid as proportion of total acids was 0.64 in Haigh's research, which is higher than the ratio of 0.53 (51/96) measured at the end of the current experiment. A lactic acid content > 65 g/kg dry matter indicates well-fermented silage (for cattle feed purposes), while < 30 g/kg dry matter indicates poorly fermented silage. The lactic acid concentration in the current experiment decreased after two weeks, indicating that the abovementioned mechanism takes place towards the end of the ensiling period.

Cerrone et al (2014) reached maximum concentrations of 15.3 g total VFAs/L with 12.8 g butyric acid/L by anaerobic digestion of grass that had been ensiled for three months. This is twice the concentration found (6.7 g/L) at the end of the ensiling process in the current study (Table 3). They concentrated the mixture of VFAs to 732.5 g/L by salting out and liquid extraction with a 93.3 % yield of butyric acid. Dilutions of this mixture were successfully applied in two concentrations (1.69 and 3.38 g/L) for production of PHAs. Both the concentration steps and the production of PHA from the diluted mixture should thus be feasible with the press juice produced in the current trial. However, Cerrone et al (2014) did not discuss the formation of lactic acid and its possible influence on the process. Chakraborty et al (2009) found butyric and propionic acid are more effective carbon sources than lactic acid to maximise PHA production by *Ralstonia eutropha*. To produce more butyric acid instead of lactic acid for PHA production an elongation of the ensiling process (> ten weeks) seems beneficial, since conversion of lactic acid into butyric acid was increasing towards the end of the experiment.

The energy requirements for pressing the grass and silage were 84 (±10), 84 (±13), 72 (±8) and 63 (±8) kWh/1000 kg dry matter grass for 2, 4, 6 and 10 weeks respectively. Pressing required less energy with increasing ensiling time. When taking into account an electricity price of €0.10 per kWh, pressing of ensiled grass (ensiling time ten weeks) costs €6.30 per 1000 kg dry matter. From this amount of grass, 28.1 kg of butyric acid can be produced (Figure 4), resulting in an energy requirement cost of €0.22 per kg of butyric acid. This is an indicative number, as other cost factors are ignored, as well as the possibility of producing higher concentrations of butyric acid during longer ensilage, or using a combination of ensilage and anaerobic digestion as done by Cerrone et al (2014). Chakraborty et al (2009) mention an optimum productivity of 4.6 g/L PHA when fed with 5 g/L butyric acid. From 1000 kg of dry matter grass 25.9 kg of PHA could thus be produced and the electricity costs of pressing would be €0.24 per kg of PHA. As the current price of PHA is €4-5 per kg (de Hart et al, 2014), pressing represents 5-6 % of the total.

4 Conclusions

Ensiling of meadow grass for ten weeks in barrels and subsequent pressing of the silage into a juice and a cake fraction resulted in maximum concentrations of lactic, n-butyric and acetic acid in the juice of 16.25 (±0.27), 6.71 (±0.87) and 2.70 (±0.19) g/L respectively. Other fatty acids never exceeded concentrations higher than 0.77 (±0.28) g/L. During the ten weeks, lactic acid gradually decreased in concentration, while that of butyric acid continued to increase. On average 45 g total FA/kg dry matter had been produced at the end of the ensiling period. After two weeks a stable production of 68 % juice from pressing (as mass percentage of fresh grass at the start) was reached. The dry matter content of silage, cake and juice after two weeks remained stable at around 15 %, 35 % and 5 %, respectively. Increasing the ensiling time could result in higher concentrations of butyric acid, due to the conversion of lactic acid to butyric acid. Energy requirement costs for pressing amount to an estimated €0.22 per kg butyric acid. If butyric acid were to be used for production of PHA, energy requirement costs represent an estimated 5-6 % of the total market price of PHA.

5 Acknowledgments

The authors would like to thank Edwin Keijsers (WUR-FBR) for performing the press experiments and doing the dry matter analyses, Hardy Temmink (WUR-ETE) & Robert de Leeuw (WUR-ETE) for the FA and lactic acid analyses and providing additional information and Herman van Schooten (WUR-ASG) for providing information used for the experimental setup.

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