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# Potential Nutrient Recovery in a Green Biorefinery for Production of Feed, Fuel and Fertilizer for Organic Farming

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## Abstract

Nutrient recovery from organic green biomass after processing in a green biorefinery concept for the production of protein feed, biogas and fertilizer for organic farming was investigated. Mass balances in terms of wet weight, TS, VS, C, N, P, K and S were evaluated for processing red clover and clover grass into protein concentrate, press cake and brown juice according to the green biorefinery concept. Depending on the biomass, between 60 and 79% of C and between 52 and 63% of N, P and S in the fresh biomass were found in the press cake, while only 6–10% of C, 8–15% of N, but up to 27% of P and up to 26% of S were found in the brown juice. In

contrast, less than 45% of K was transferred to the press cake and 19–31% of K ended up in the brown juice. Moreover, nutrient recovery in the digestate after anaerobic co-digestion of press cake and brown juice produced from clover grass in a pilot-scale trial was assessed in a bench-scale biogas reactor. The analysis of the digestate from the AD process revealed that 56% of the influent total-C was converted into biogas and that the share of ammonia-N of total-N was increased from 9.4 to 43% during the biogas process. Therefore, the proportion of plant available N was improved in the digestate. The digestate to be applied as organic fertilizer presented a C:N ratio of 7 and a nutrients N:P:K ratio of 4:1:12.

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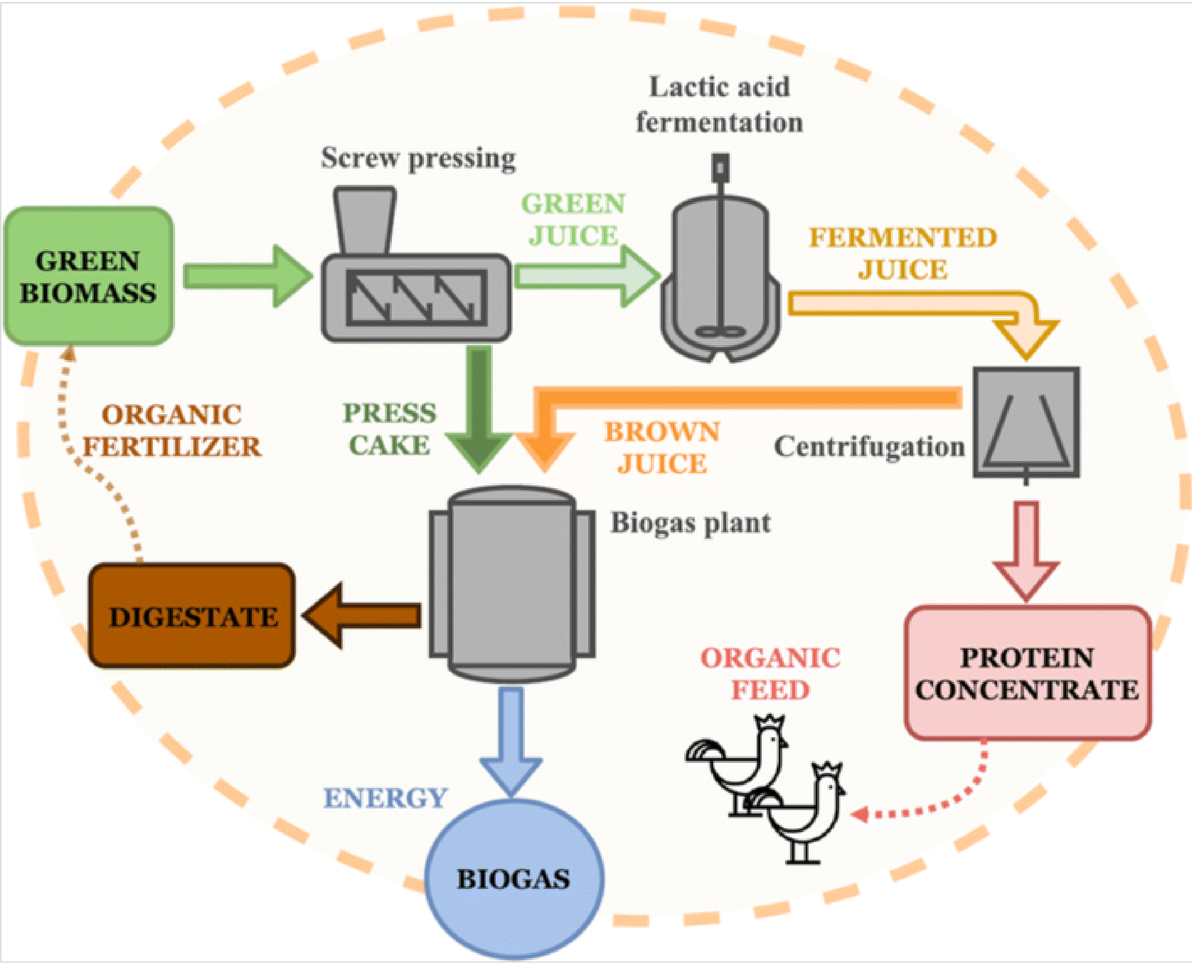
## Graphic Abstract




## Keywords

Forage grasses
Legumes
Nitrogen
Ammonia
Biogas
Digestate

## Statement of Novelty

The extraction of proteins from green biomass within a green biorefinery concept might be a promising alternative to the actual lack of protein-rich feeds. The distribution of nutrients in the different fractions produced along the process (protein concentrate, press cake and brown juice) is the aim of this study. More specifically, the proportion of nutrients remaining in the residual streams after the extraction of proteins, and the potential use of those streams for the production biogas and a nutrient-rich digestate, which could be applied as fertilizer. The distribution of nutrients along a biorefinery process is crucial for improving the use and recovery of nutrients in the final product streams.

## Introduction

Nowadays, the organic farming sector is developing rapidly in order to meet the increasing demand for organic products. However, the availability of organic protein feed for monogastric animals and organic fertilizer is often a limiting factor for the further development of the organic farming sector. The production of organic protein-rich feed for monogastric animals, of biogas and of organic fertilizer based on regionally grown grass biomass for organic farming is the main objective of a green biorefinery platform called Organofinery. The Organofinery concept includes extraction of a protein concentrate from green biomass (i.e. red clover or clover grass), conversion of the residual streams (press cake from screw press and brown juice after protein precipitation) into biogas by anaerobic digestion (AD), and recovery of the nutrients in the digestate to be used as fertilizer in organic farming [1].

The recovery of organic fertilizer may overcome lower crop yields in organic farming systems compared with conventional farming due to limited availability of organic fertilizers arisen from lower livestock density and restricted animal fodder acquisition [2].

During the anaerobic digestion process, organic matter is converted into energy-rich biogas and plant nutrient-rich digestate [3]. Indeed, the digestate presents a higher proportion of $NH_4^+$-N readily available for the plants than the feedstock [3]. This is a consequence of the mineralization of the organic N in the feedstock, mainly proteins into inorganic nitrogen i.e. $NH_4^+$-N [4, 5]. It is worth mentioning that in the case of feedstock with high N levels like animal slurry or manure, inhibition of the anaerobic digestion can occur, especially in thermophilic reactors due to overly free ammonia [4]. Besides, P and K are also essential macronutrients for the plants growth and function and these are required in relatively large quantities by plants [6]. P and K are usually preserved during the anaerobic digestion process [7] and actually, they might be concentrated in the digestate together with recalcitrant compounds [8]. Apart from readily available plant nutrients, the addition of recalcitrant organic matter to the soil might be beneficial by making the soil less erodible, easier to plough and enhancing the retention of nutrients [9].

The utilization of the digestate from a biogas plant as fertilizer in plant production contributes recycling the nutrients and replacing the application of mineral fertilizers [7] while reducing the greenhouse gas emissions derived from the production of synthetic fertilizers [10]. Accordingly, the overall sustainability of the biogas production is improved. Nevertheless, the organic composition and plant nutrients content in the digestate highly depends on the feedstock composition [3, 9, 11, 12] but also on the management of the AD process [3, 11]. Therefore, it is difficult to assess the fertilizer value of the digestates due to the wide variety of feedstock used for biogas production [7].

Up to date, several works have discussed different aspects regarding the fertilizer value of the digestates produced after the anaerobic co-digestion of animal slurries or manures together with energy crops or wastes from food and agricultural industries [3, 7, 10, 11, 13]. For instance, [13] proposed the use of perennial crops like forage grasses and legumes as valuable alternative co-digestion substrates for biogas production and further utilization of the digestate as N fertilizer. Despite the broad utilization of animal slurries and manure in biogas plants, manure from organic farming is a limited resource, so that the development of an anaerobic digestion process without manure addition is needed [14]. Co-digestion of the press cake and brown juice produced in a green biorefinery after the extraction of proteins might be a good alternative to the utilization of manure in biogas plants and might help overcoming the nutrient deficiencies restricting organic farming.

While the optimization of the protein extraction and the biogas process of the Organofinery concept have been described in detail in [1] and [14], the focus of the present study was on the fate of nutrients throughout the Organofinery concept using red clover and clover grass as feedstock. This includes the distribution of nutrients in the different fractions resulting from the screw pressing and the protein precipitation and in the digestate from the anerobic co-digestion of press cake and brown juice to evaluate its application as organic fertilizer.

# Materials and Methods

## Biomass and Unit Processes Used for the Green Biorefinery

### Small-Scale Biorefining Trial

For the initial small-scale investigation of the biorefinery, red clover (*Trifolium pratense*) was harvested in May 2014 at Vamdrup, Denmark (55°25′48.1″ N, 09°17′14.5″ E) and clover grass (a mixture of *Trifolium pratense* and *Lolium multiflorum*) was harvested in May 2014 at Orten, Denmark (55°39′49.9″ N, 08°25′59.6″ E). As first step of the green biorefinery, the mechanical fractionation, 62 kg of red clover and 97 kg of clover grass were processed within 12 h after harvest in a small-scale Vincent CP4 screw press (Vincent Corporation, FL, USA) into green juice and press cake. Right after, the green juice was fermented using lactic acid bacteria in order to lower its pH, triggering the precipitation of proteins according to the method developed by [15] for the extraction of functional proteins from the plant material. According to this method, the green juice was inoculated with a pure culture of *Lactobacilus salivarius*. The lactic acid fermentation was carried out overnight at 37 °C. At the end of the fermentation, the pH had dropped to around 4.1–4.3 and the fermented green juice was centrifuged (Sorvall™ RC 6 Plus Centrifuge, Thermo Scientific, USA) in order to separate the solid residue containing the proteins (i.e. protein concentrate) from the residual liquid containing lactic acid, left sugars and other compounds (i.e. brown juice).

### Pilot-Scale Biorefining Trial

The pilot-scale trial of the Organofinery concept was performed at the Research Centre Foulum of Aarhus University, Tjele, Denmark processing about 7.5 t of clover grass, a mixture of clover and grass. The freshly harvested clover grass was continuously fed into the screw press resulting in the continuous production of press cake and green juice. The green juice was inoculated with approx. 6% (v/v) of a *Lactobacillus salivarius* culture, previously grown in green juice. The temperature was kept around 38 °C with a heat exchanger and the fermentation was carried out overnight to a final pH of 3.9. Protein separation from the green juice was in this case performed in a decanter centrifuge (Alfa Laval, Nakskov, Denmark).

Samples of the in- and output of the different unit processes of the green biorefining (i.e. clover grass, press cake, brown juice and protein concentrate) were analyzed in terms of total solids (TS) and volatile solids (VS), C-content and the nutrients N, P, K and S. A full mass balance of these compounds was made over the whole biorefinery based on the concentration, analyses and mass flow data of the unit processes in small-scale. For the pilot-scale trial, uncertainties of the mass flow data of the unit processes did not allow performing a full mass balance over the whole biorefinery at such scale.

## Bench-Scale Reactor Experiment for Co-digestion of Press Cake and Brown Juice

Anaerobic co-digestion of the residual streams from the protein extraction of the green biorefinery i.e. press cake and brown juice was studied in a bench-scale semi-continuous stirred tank reactor (CSTR) setup. Press cake and brown juice produced from clover grass in the pilot-scale trial were used as feedstock for the anaerobic co-digestion experiment.

A stainless-steel reactor with 3 L working volume was utilized for the AD experiment. The temperature inside the reactor was kept at 37 ± 2 °C by a water jacket, connected to a temperature-controlled water bath. Agitation was carried out with a mechanical stirrer at 100 rpm. For start-up, the reactor was filled with 200 g press cake and 2500 mL digestate from Hashøj biogas plant (West Zealand, Denmark), which is treating a mixture of cow and pig manure and up to 25% of industrial organic waste. After 10 days, the reactors were fed manually once per day during weekdays with a mixture of 46 g of press cake and 102 g of brown juice, corresponding to 10 g VS and 2 g VS from the press cake and brown juice, respectively. The proportion of press cake and brown juice in the feeding mixture was chosen according to the VS ratio of these fractions leaving the biorefinery i.e. from one ton of clover grass (171 kg of VS) 133 kg of VS of press cake, leaving the mechanical fractionation was mixed with 19 kg of VS of brown juice from the protein precipitation. Accordingly, press cake and brown juice provided 83% and 17% of the VS in the feeding mixture, respectively. The anaerobic co-digestion of the press cake and brown juice was monitored for 74 days.

The reactor effluent (digestate) accounted for approx. 150 mL per day and was collected and analyzed twice per week in terms of TS, VS, and pH. C-content and nutrients N, $NH_4^+$, P, K and S were analyzed in the digestate after 35 days of reactor operation. Biogas production was quantified every day by water displacement. Biogas composition was analyzed twice per week by gas chromatography.

## Chemical Analysis

Analyses of TS, VS, C, N, P, K, S were performed in duplicates for each sample of fresh crop, protein concentrate, brown juice, press cake and digestate. The total solids (TS) content was measured by drying the samples at 105° to constant weight and the volatile solids (VS) content was determined by burning the samples at 550 °C for 3 h in accordance to [16]. The C content was analyzed using flash combustion in a FLASH 2000 HT Elemental Analyzer (Thermo Scientific). The N content was measured by Total Kjeldahl Nitrogen procedure according to APHA Standard Methods (2005). The $NH_4^+$ content was determined by Kjeldahl Nitrogen procedure in the digestate while the content in the brown juice was measured with a commercial kit, LCK-305 (1–12 mg/l range) purchased from Hach Lange. The content of S, P and K was determined using Inductively Coupled Plasma-Optical Emission Spectroscopy (ICP-OES).

The composition of the biogas was analyzed using a gas chromatograph (SRI GC model 310) equipped with a Porapak Q column (182.88 cm length and 2.1 mm i.d.). Nitrogen was used as carrier gas with a pressure of 196 kPa. The injector and detector temperatures were 80 °C; the temperature of the oven was constant on 80 °C. As standard gas, a mixture of 30% $CH_4$ and 70% $N_2$ was utilized.

## Mass Balances

Mass balances were performed in terms of wet weight (ww), TS, VS, C, N, S, P and K for (a) the green biorefining process carried out with red clover and clover grass in small-scale and (b) the anaerobic co-digestion of the press cake and brown juice produced in the pilot-scale trial. The mass balance for the green biorefining process was based on mass flows of each fraction processed from red clover and clover grass and on concentration of the respective compounds in the different fractions. The mass balance for the anaerobic co-digestion process was based on the mass flow and the concentration of the different compounds of in- and output of the lab-scale reactor after reaching steady-state operation.

Separation efficiencies of the mechanical fractionation and the protein precipitation of the biorefinery were calculated according to the following equations, where $m_{ww,\ fraction}$ refers to the wet weight of each fraction (kg) and $C_x$ refers to the concentration of the different compounds analyzed (i.e. TS, VS, C, N, S, P or K) in each fraction (g $kg^{-1}$):

$$Separation\ efficiency, wet\ weight\ (ww, \%) = m_{ww,fraction}/m_{ww,biomass\ input} \times 100 \quad 1$$

$$Separation\ efficiency,\ compound\ X\ (C_x, \%) = C_x x m_{ww,fraction}/C_x x m_{ww,biomass\ input} \times 100 \quad 2$$

Recoveries of the different compounds in the digestate of the anaerobic co-digestion of press cake (PC) and brown juice (BJ) were calculated based on the average daily amount of PC and BJ fed to the reactor, on the average daily amount of digestate collected as effluent ($m_{eff}$), and on the concentration $C_x$ of each compound in the different in- and output fractions as follows:

$$Recovery\ in\ digestate,\ compound\ X\ (C_x, \%) = C_{x,eff} \times m_{eff}/\ (C_{x,PC} \times m_{PC,input} + C_{x,BJ} \times m_{BJ,in}$$

# Results

## Nutrient Recovery Throughout the Organofinery Process

The concentration of nutrients in the fresh biomass, press cake, brown juice and protein concentrate from small- and pilot-scale processing of red clover and clover grass is presented in Table 1.

**Table 1** In Table 1, row number 2, 7 and 12 are sub-headers so they should have the same format and not different background colors (white and green). Is it possible to change them to the same?

TS, VS, C- and nutrient concentrations in the different fractions produced from red clover and clover grass in the Organofinery process

| Fraction | TS (g $kg^{-1}$) | VS (g $kg^{-1}$) | C (g $kg^{-1}$) | $N_{tot}$ (g $kg^{-1}$) | $NH_4^+$ (% $N_{tot}$) | C:N | P (g $kg^{-1}$) | K (g $kg^{-1}$) | S (g $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| *Red clover—used for small-scale processing* | | | | | | | | | |
| Standard deviation in brackets | | | | | | | | | |
| *n.d.* not determined, < *dl* below detection limit | | | | | | | | | |
| [a]Estimated based on the C content from the fresh clover grass and press cake processed in lab scale | | | | | | | | | |

| Fraction | TS (g kg$^{-1}$) | VS (g kg$^{-1}$) | C (g kg$^{-1}$) | $N_{tot}$ (g kg$^{-1}$) | $NH_4^+$ (%$N_{tot}$) | C:N | P (g kg$^{-1}$) | K (g kg$^{-1}$) | S (g kg$^{-1}$) |
|---|---|---|---|---|---|---|---|---|---|
| Fresh biomass | 163.6 (9.7) | 144.5 (9.2) | 71.0 (0.4) | 5.3 (0.2) | n.d | 13 | 0.45 (0.01) | 4.6 (0.2) | 0.25 (0.00) |
| Press cake | 236.4 (3.4) | 217.7 (0.9) | 106.1 (0.1) | 7.0 (0.2) | n.d | 15 | 0.59 (0.03) | 5.0 (0.3) | 0.32 (0.02) |
| Brown juice | 24.6 (0.1) | 18.8 (0.1) | 8.5 (0.02) | 0.8 (0.1) | 14.0 (2.5) | 11 | 0.13 (0.02) | 1.6 (0.2) | 0.03 (0.01) |
| Protein concentrate | 191.3 (1.4) | 178.2 (1.3) | 96.5 (0.1) | 12.0 (1.5) | 0.6 (0.1) | 8 | 0.47 (0.01) | 4.0 (0.3) | 0.58 (0.00) |
| *Clover grass—used for small-scale processing* | | | | | | | | | |
| Fresh biomass | 186.8 (6.8) | 170.6 (6.0) | 80.4 (0.05) | 3.1 (0.1) | n.d | 26 | 0.40 (0.01) | 5.5 (0.1) | 0.23 (0.01) |
| Press cake | 355.6 (25.1) | 338.0 (26.0) | 160.0 (0.8) | 4.9 (0.9) | n.d | 33 | 0.57 (0.05) | 5.5 (0.3) | 0.32 (0.03) |
| Brown juice | 43.8 (0.4) | 32.9 (0.3) | 14.5 (0.1) | 0.8 (0.0) | 9.4 (2.7) | 18 | 0.19 (0.03) | 3.0 (0.5) | 0.11 (0.00) |
| Protein concentrate | 206.1 (1.6) | 191.1 (1.8) | 104.0 (0.2) | 13.2 (0.6) | 0.8 (0.1) | 8 | 0.82 (0.00) | 4.2 (0.4) | 0.75 (0.01) |
| *Clover grass—used for pilot-scale processing* | | | | | | | | | |
| Fresh biomass | 180.4 (27.1) | 144.4 (20.2) | 80.8[a] | 5.8 (0.3) | n.d | 13 | 0.47 (0.1) | 6.5 (0.3) | 0.33 (0.01) |
| Press cake | 263.9 (15.9) | 215.0 (18.0) | 118.7[a] | 6.9 (0.7) | n.d | 18 | 0.53 (0.00) | 5.0 (0.0) | 0.40 (0.04) |
| Brown juice | 29.0 (6.9) | 19.8 (4.5) | 9.4 (0.2) | 0.6 (0.02) | < dl | 15 | 0.15 (0.01) | 2.6 (0.0) | 0.08 (0.00) |

Standard deviation in brackets

*n.d.* not determined, < *dl* below detection limit

[a]Estimated based on the C content from the fresh clover grass and press cake processed in lab scale

The resulting mass balance for the small-scale fractionation of red clover and clover grass into press cake and green juice, and subsequent separation of the green juice into protein concentrate and brown juice according to the Organofinery process is shown in Figs. 1 and 2, respectively. The processing of red clover biomass resulted in 40.1% (ww) press cake, 53.2% (ww) brown juice and 6.7% (ww) protein concentrate. Likewise, the processing of clover grass biomass resulted in 39.5% (ww) press cake, 56.8% (ww) brown juice and 3.7% (ww) protein concentrate.

**Fig. 1**

Mass balance for the Organofinery process of red clover into press cake, brown juice and protein concentrate based on small-scale processing by fractionation and fermentation

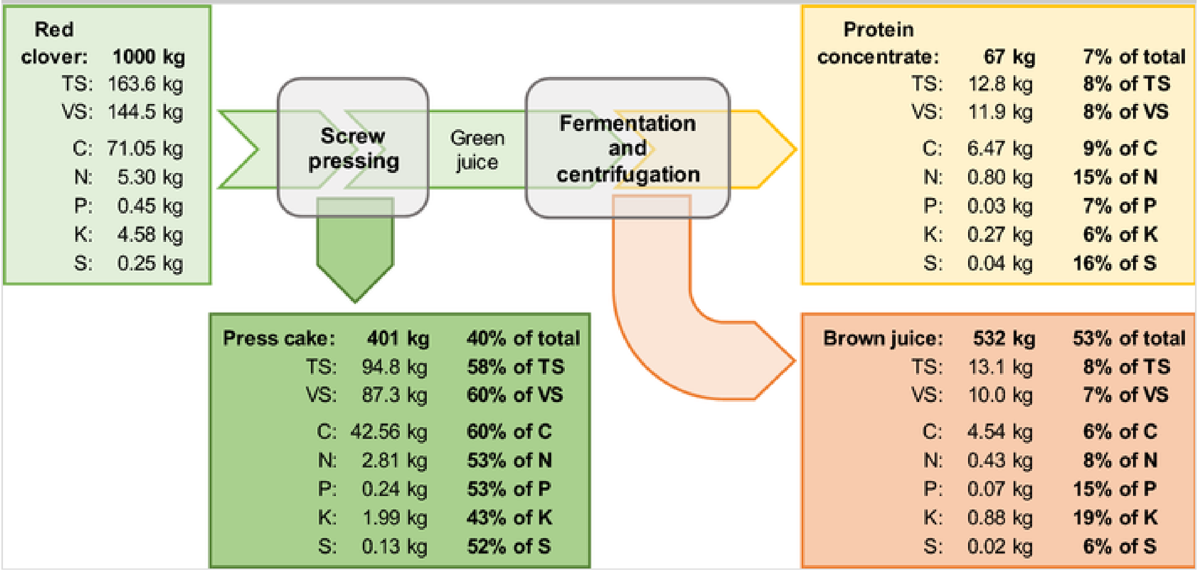
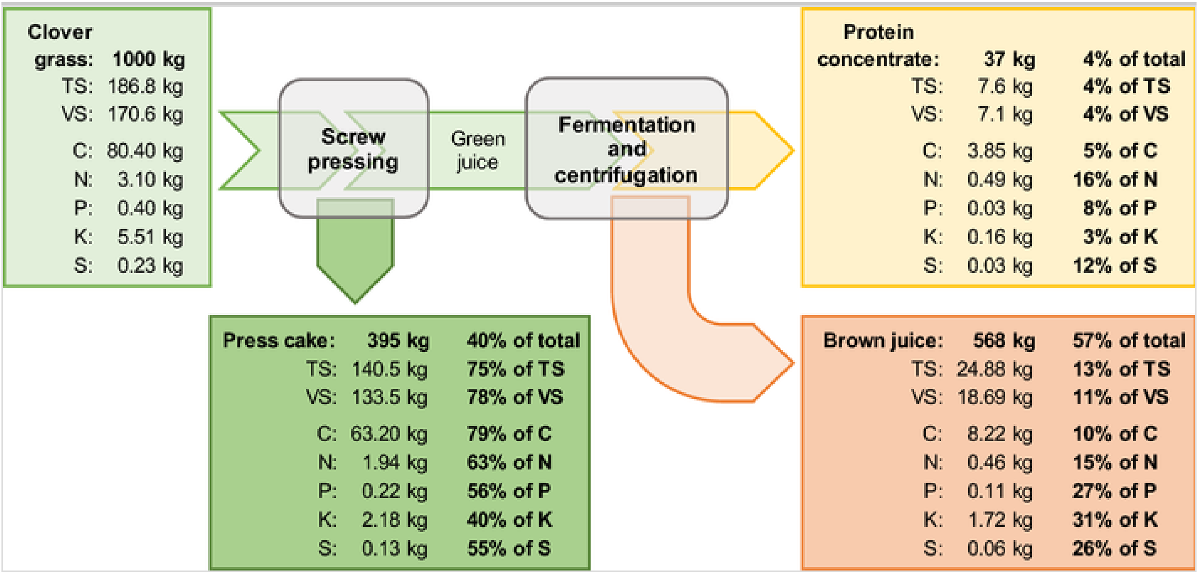



**Fig. 2**

Mass balance for the Organofinery process of clover grass into press cake, brown juice and protein concentrate based on small-scale processing by fractionation and fermentation




For the processing of red clover, the mass balance of C, N, P, K and S based on the calculations in Eq. 2 showed that the sum of output was significantly lower than the input (Fig. 1). In contrast, for the processing of clover grass, the recovery of the different nutrients in the output fractions was generally higher than 90% except for K, where it was 74%. It can be noted for the mass balance of both biomass feedstocks that the major part of N, P, K and S are bound to the solids TS, which mainly leaves in the press cake from the first fractionation. After fermentation and protein precipitation from the green juice, only a small part of C, P and K is found in the protein concentrate while the fraction of N and S is higher. Still, the N recovery in the protein concentrate of 15–16%, which is directly correlated to the protein recovery, is rather low and should be subject of improvement of the fractionation processes of the biorefinery. For K, it can be noted that its fraction is relatively high in the brown juice.

## Anaerobic Co-digestion of Press Cake and Brown Juice

The methane yield obtained during reactor operation of co-digestion of press cake and brown juice produced from clover grass in the pilot-scale trial is shown in Fig. 3. During start-up of the anaerobic digestion process (day 0–33), process instabilities due to irregular pumping of the influent were noted, leading to high fluctuations of the methane yield. After this adaptation period, the anaerobic co-digestion reactor performed stable for two consecutive hydraulic retention times (HRTs). On average, the anaerobic co-digestion of press cake and brown juice resulted in a methane yield of 238 ± 73 mL $CH_4$ g $VS^{-1}$ and a methane content of 53 ± 3% (Table 2). Based on the COD:VS ratio of the feeding mixture of 1.4, a theoretical maximum methane yield of 490 mL $CH_4$ g $VS^{-1}$ was calculated using the Buswell equation [17]. Accordingly, the anaerobic co-digestion of press cake and brown juice showed 48% conversion of the influent organic matter into biogas.

**Fig. 3**

Methane yield (mL $CH_4$ g $VS^{-1}$) for the anaerobic reactor experiment with co-digestion of press cake and brown juice. Horizontal line represents the average methane yield for the corresponding period

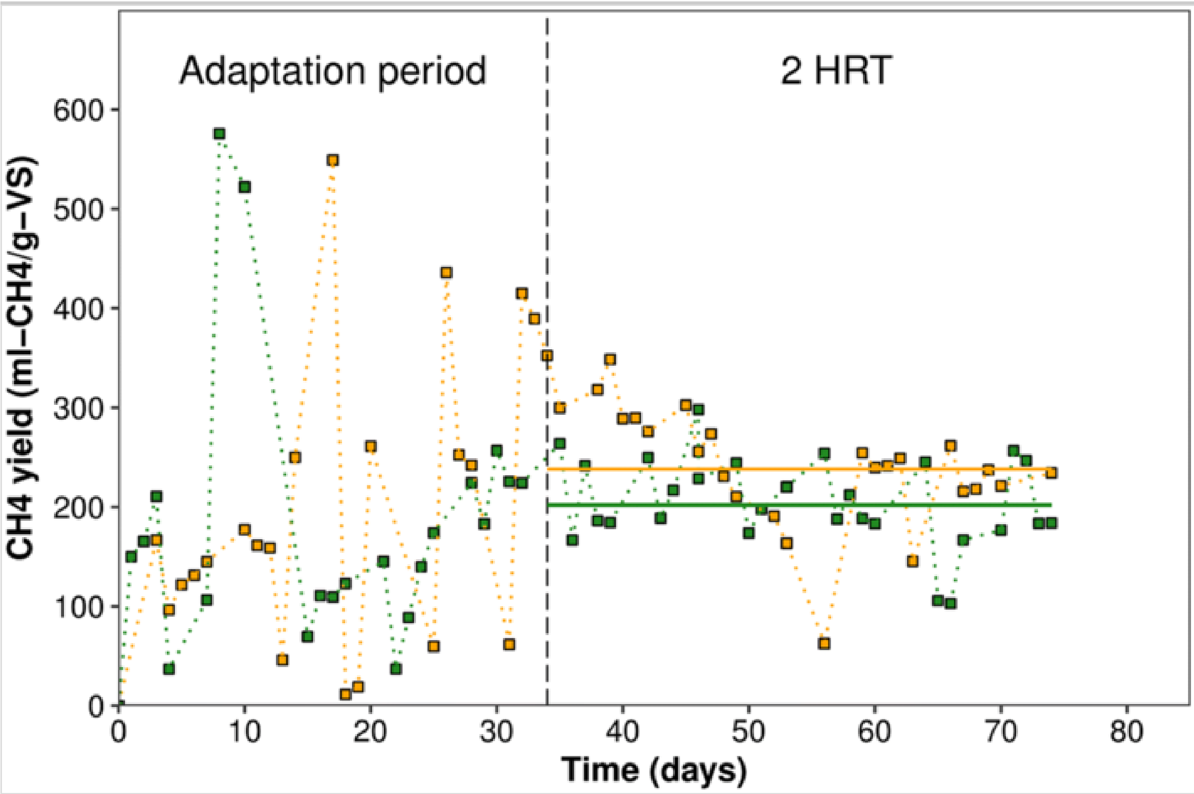



**Table 2** The format of Table 2 is not the correct one and should be change. Please see attachment

H, TS, VS, C- and nutrient concentrations in the influent and effluent (digestate) of the anaerobic co-digestion o juice produced from clover grass in the pilot-scale trial and operational parameters for the reactor performance

| **In-/effluent** | **pH** | **TS (g $kg^{-1}$)** | **VS (g $kg^{-1}$)** | **C (g $kg^{-1}$)** | **$N_{tot}$ (g $kg^{-1}$)** | **$NH_4^+$ (%$N_{tot}$)** | **C:N** | **P (g $kg^{-1}$)** |
|---|---|---|---|---|---|---|---|---|
| Anaerobic co-digestion of press cake and brown juice from clover grass in pilot-scale trial | | | | | | | | |
| Influent | 4.3 (0.1) | 99.7 (7.0) | 80.5 (7.0) | 43.4 | 2.5 | 9.4 (2.7)[a] | 17.0 | 0.26 |

[a]In the BJ; Standard deviation in brackets

| In-/effluent | pH | TS (g $kg^{-1}$) | VS (g $kg^{-1}$) | C (g $kg^{-1}$) | $N_{tot}$ (g $kg^{-1}$) | $NH_4^+$ (% $N_{tot}$) | C:N | P (g $kg^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| Digestate | 7.7 (0.3) | 51.0 (7.4) | 34.6 (6.5) | 19.1 (0.4) | 2.6 (0.3) | 42.9 (5.2) | 7.3 | 0.34 (0.06) |
| **HRT** | **OLR** | **TS removal** | **VS removal** | **$CH_4$ yield** | **$CH_4$ content** | | | |
| **Day** | **g VS $L^{-1}day^{-1}$** | **%** | **%** | **mL $CH_4$ g VS $^{-1}$** | **%** | | | |
| Operation and performance parameters of the anaerobic co-digestion process | | | | | | | | |
| 20.3 (3.5) | 3.8 (0.9) | 45.9 (8.1) | 53.7 (8.5) | 237.6 (73.3) | 52.6 (3.1) | | | |

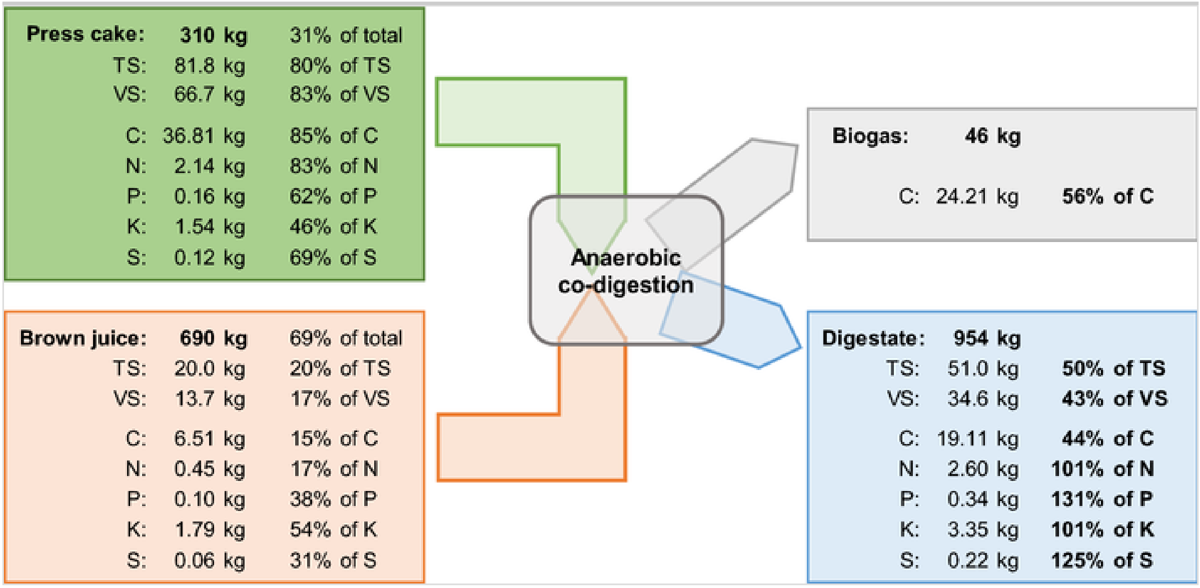
[a]In the BJ; Standard deviation in brackets

## Fate of Nutrients During Anaerobic Co-Digestion of Press Cake and Brown Juice

The concentration of TS, VS and nutrients in the influent and effluent together with the operation and performance parameters of the biogas reactor are shown in Table 2. The resulting mass balance of C, N, P, K and S for the anaerobic co-digestion per 1000 kg of feedstock of press cake and brown juice produced from clover grass in the pilot-scale trial is shown in Fig. 4. The TS, VS and nutrient concentration in the feed resulted from blending press cake and brown juice in a ratio of 31% (ww) and 69% (ww), equivalent to a VS ratio of 83% of VS from the press cake and 17% of VS from the brown juice. Consequently, the press cake provided the highest share of most nutrients to the feeding mixture, i.e. 85% of C, 83% of N, 62% of P, and 69% of S. Only for K, the share was higher from the brown juice (54%). The C:N ratio of 17 in the influent is at the lower end of the ideal ratio for anaerobic digestion, which should be in a range between 13 and 30 to avoid process failure [18]. During the AD process, the TS and VS content decreased from 102 to 51 g $kg^{-1}$ and from 80 to 34.6 g $kg^{-1}$, respectively, equivalent to a TS removal of 46% and a VS removal of 54%. The carbon content decreased accordingly from 43.3 to 19.1 g $kg^{-1}$, equivalent to a reduction by 56%. This decrease is accounted as conversion of carbon of the organic matter in the influent into biogas. The C:N ratio of 17 in the influent decreased to 7.3 in the digestate while the ratio of $NH_4^+$ to total-N increased from approx. 9.4% in the influent based on the value of the brown juice to 43% in the digestate. Accordingly, the C:$N_{org}$ changed from 19.2 to 20.0 in the influent to 12.8 in the digestate. The concentration of total-N, P, K and S remained rather constant at around 2.5, 0.3, 3.3 and 0.2 g $kg^{-1}$, respectively. The slightly higher concentration of P and S in the digestate compared to the influent was attributed measuring errors and the possibility that these nutrients partly originated from the inoculum added to the reactor during start-up.

**Fig. 4**

Mass balance for anaerobic co-digestion of press cake and brown juice for the production of biogas and digestate




# Discussion

## Nutrients Recovery Throughout the Organofinery Process

The mass balances for processing red clover and clover grass into press cake, brown juice and protein reveal generally quite similar nutrient recovery in these 3 fractions except for total-N, which has 10% higher recovery in the press cake of clover grass compared to red clover. For both biomass types, only 3–16% of the nutrients are removed in the protein concentrate while most of the nutrients, i.e. 66–89% of C, 61–78% of N, 68–83% of P, 62–71% of K and 58–81% of S are recovered in the press cake and brown juice, which could potentially be recirculated back to the fields as digestate fertilizer.

For both red clover and clover grass, the Organofinery processing resulted in concentrating the nutrients in the press cake due to removal of water from the fresh biomass by the screw pressing. Indeed, more than half of C, N and P present in the fresh biomass was left in the press cake. The press cake mainly contains lignocellulosic fibers from the fresh biomass, in which C is the structural component. N is an important component of proteins and is part of the chlorophyll as well. The high proportion of N left in the press cake is mainly because of remaining fiber-bound proteins. P is the structural component of DNA and RNA but is also present in the phospholipids constituting the cell membranes and thus, a high proportion of P also remains in the press cake. K is naturally dissolved as it does not belong to the structural plant components [5]. Therefore, a higher proportion of K was transferred to the green juice after screw pressing (57–60%) and further into the brown juice after extraction of proteins (19–31%). S is a key component of proteins in sulphur amino acids (i.e. cysteine and methionine), and thus, the recovery of S follows the recovery of N in the different fractions with a relatively high recovery in the protein concentrate; i.e. 15% of N and 16% of S were recovered in red clover protein concentrate while 16% of N and 12% of S were recovered in protein concentrate from clover grass.

Nutrient recovery in the brown juice was relatively low, except for K. In particular, the nutrient recovery in the brown juice from red clover was low with a recovery of C, N and S below 10%; and generally, the nutrient recovery in the brown juice processed from red clover was only half of the recovery found in the brown juice deriving from clover grass. The brown juice contained mainly lactic acid and minor amounts of other organic acids, free sugars (i.e. glucose and fructose), some nitrogen and other nutrients [14]. In contrast to the press cake, the brown juice contains mainly dissolved organic matter and nutrients. Previously, the brown juice produced as side stream from the production of green pellets from grasses, clover or alfalfa was applied on the fields as fertilizer [19, 20]. However, its application was limited to specific periods during the year or even banned in some countries due to the risk of groundwater contamination with nitrate. In case of the Organofinery process, the C/N ratio in the brown juice is still relatively high (11–18) and only a minor proportion of the N (9–14% of N) in the brown juices is readily available for the plants as ammonia [2]. reported that the availability of N in organic fertilizers with C:N ratios greater than 15 could be restricted by N immobilization in soil leading to a lower bioavailability of mineral N (i.e. $NH_4^+$) for microbial protein synthesis. The relatively low nutrient concentration in the brown juice, on the other hand, limits the feasibility of applying a sufficient amount of fertilizer per area. This would argue for application of the brown juice together with the press cake as fertilizer, which would furthermore support the co-digestion treatment of press cake and brown juice in a biogas plant for the production of biogas and a suitable fertilizer product.

## Anaerobic Co-digestion of Press Cake and Brown Juice

In the final phase of stable process operation, the co-digestion of press cake and brown juice in a 83%:17% ratio based on VS resulted in methane yields of 238 mL $CH_4$ g VS $^{-1}$ on average. Previously, anaerobic batch experiments co-digesting press cake and brown juice showed higher methane yields (346–364 mL $CH_4$ g VS $^{-1}$) when the feedstock mixture had about 15% of the VS provided by the brown juice [14]. The significantly lower methane yields in the reactor experiment are attributed to the lower HRT (20 days) and a higher organic loading rate (OLR) (3.8 g VS $L^{-1}day^{-1}$) in the reactor operation compared to the batch experiment (yield after 42 days and for a substrate/inoculum ratio of 1.6 and 0.5 based on VS, respectively). In comparison, higher methane yields (up to 384 mL $CH_4$ g VS $^{-1}$) were obtained when co-digesting manure with oilseed radish in a biogas reactor at significantly lower organic loading rate of 1 g VS $L^{-1}$ $day^{-1}$ [21].

## Fate of Nutrients During Anaerobic Co-digestion of Press Cake and Brown Juice

Carbon analysis in the influent and the digestate of the anaerobic co-digestion of press cake and brown juice revealed a conversion of 56% of C into biogas, which is in agreement with the VS removal of about 54% from VS analysis in the in- and effluent (Table 2 and Fig. 4). Nutrient concentration in the digestate was very similar to the concentration in the influent. P and K are usually preserved during the anaerobic digestion [7], although some precipitation processes of P could lead to a loss of P [5]. The organic matter degradation during anaerobic digestion may result in the formation of sulfate, which is further converted into hydrogen sulfide ($H_2S$) under anaerobic conditions [5]. According to [5], less than 50% of S leaves the anaerobic reactor in the digestate as part of the S leaves the anaerobic reactor within the biogas in the form of hydrogen sulfide. Therefore, the higher concentration of P and S in the digestate may be attributed to analysis errors and/or indicate that the inoculum utilized during start-up of the reactor, provided a considerable amount of these nutrients to the process.

The C:N ratio in the digestate of 7.3 was much lower compared to the feeding mixture (C:N of 17) due to the lower C content in the digestate resulting from the conversion of C into methane [3, 9, 22]. Müller-Stöver [13] reported C:N ratios of 3.9–5.2 in the digestate from batch co-digestion of slurry with several perennial crops including red clover and grass, which is slightly lower than in the present study, indicating a lower C-conversion.

The main difference between digestate and influent of the anaerobic digestion process with respect to fertilizer value was the increase of $NH_4$ + from approx. 9.4% to 42.6% of total-N due to mineralization of N-containing organic matter (mainly proteins) during the AD process. In comparison, digestates produced from a mixture of cattle slurry and maize presented a proportion of $NH_4^+$ (46–50% of total N) and a C:N ratio (11.1) which are similar to the digestate produced in the present study, despite higher C and N content in the influent [23]. The digestate produced from the co-digestion of press cake and brown juice falls within the range of a study of [3], who characterized 20 different digestates from anaerobic digestion of a broad variety of substrates under mesophilic and thermophilic conditions. The digestates presented DM content between 1.1 and 7.4%, total N content between 2.6 and 7.6 g $kg^{-1}$ and $NH_4^+$ content between 1.9 and 5.3 g $kg^{-1}$. Accordingly, [2] reviewed biogas residues produced from plant biomass, containing generally between 2 and 3 kg $m^{-3}$ N, between 35 and 60% of total N as $NH_4^+$ and C:N ratios of 5–8, which agrees with the present study. Slightly higher P and K contents of 0.9–1.1 g $kg^{-1}$ and 4.0–4.5 g $kg^{-1}$, respectively, were reported for the effluents from two biogas plants in Germany dedicated to energy crops, [22]. In another study evaluating the fertilizer value of six different digestates, a P content between 0.3 and 0.7 g $kg^{-1}$ and a K content between 0.1 and 4.5 g $kg^{-1}$ were found, which is similar to our results except for a considerably higher content of C and N in the six digestates compared to ours [7],

Overall, the digestate of the co-digestion of press cake and brown juice from the Organofinery process shows very similar nutrient contents to digestates from anaerobic digestion of slurries and other biomass resources. Nevertheless, it has to be noted that several authors have reported a large variability in terms of chemical and nutrient composition in digestates [3, 22] and that the digestate composition and stability highly depends on the specific raw material composition [11, 12]. Accordingly, it is difficult to make general statements about the fertilizer value of digestates and regular analysis of the nutrient content in the digestates should be performed [22].

## Fertilizer Value of the Final Digestate of the Organofinery Process Scheme

Of all nutrients, N is needed in the largest quantities since N is crucial for plant growth [6]. In organic fertilizers, the content of total-N and of mineral-N ($NH_4^+$) are key factors determining the N availability, especially in the short term [2]. Accordingly, the C:N ratio should be evaluated in the digestate. In general, soil application of digestates with C:N ratios above 18 might provoke N immobilization according to [9]; while [2] established a threshold of 15. In any case, the C:N ratio in the digestate produced after the anaerobic co-digestion of press cake and brown juice was about 7, indicating low risk for N immobilization upon application in the fields. In comparison, applying digestates from co-digestion of slurry with several perennial crops including red clover and grass with C:N ratios of 3.9–5.2, resulted in an increased $NH_4^+$ content in the soils [13]. On the contrary, soil application of digestates with a high C:N ratio (between 19 and 28) required additional mineral fertilization in order to compensate for the N immobilization [23].

On the other hand, the availability of N also depends on the stability of the remaining organic matter in the digestate [2]. The addition of easily degradable organic matter to the soil would favour the growth of microbial population, which immobilises the inorganic N [11]. However, it is most likely that most easily degradable organic matter has been removed during the anaerobic co-digestion of the press cake and brown juice (54% of VS removal). Therefore, the digestate is rather rich in recalcitrant organic matter such as lignocellulose and thus, stable. The presence of recalcitrant organic matter in the digestates has previously been associated with a reduced microbial activity and oxygen consumption causing less $N_2O$ emissions, which is a strong greenhouse gas [9]. Furthermore, the presence of organic material in the digestates has been related to an improved N availability in the soil top layer compared to mineral fertilizers that are easily washed out, especially in sandy soils [7].

The N:P:K ratio, based on inorganic N in the digestate from the co-digestion was 4:1:12, which is similar to the N:P:K ratios of some digestates previously evaluated [7]. Indeed, the N:P:K ratio in the digestate is also similar to the N:P:K ratio in the mineral fertilizer (i.e. 4:1:6) utilized in that study, except for a lower proportion of K. The relatively high K content in the digestate might be beneficial upon fertilization of sandy soils, which generally present limited amounts of K and other nutrients because of the nutrient washing out resulting from the lack of binding sites [7, 24]. However, the concentration of plant macronutrients in the digestate might be low for practical reasons. For instance, digestate application to supply 120 kg-N $ha^{-1}$ would require almost 50 tons $ha^{-1}$ of digestate implying large transportation costs. Alternatively, solid–liquid separation of the digestate could be performed in order to transport only the solid fraction and to facilitate its utilization as fertilizer [10, 22, 23]. The cost–benefit of this solid–liquid separation depends to a high degree on the separation equipment used, leading to different separation efficiencies and which utilization of the liquid digestate would be possible.

## Conclusions

Overall, the following main conclusions can be drawn from investigating the recovery of nutrients from red clover and clover grass biomass after extraction of a protein concentrate for animal feeding and anaerobic digestion of press cake and brown juice within a green biorefinery concept:

- In the investigated green biorefinery scheme less than 10% of C, P and K and about 15% of N and S are extracted into the protein concentrate. Consequently 85% of N and S and more than 90% of C, P and K are recovered in the residual press cake and brown juice.

- More than 50% of C, N, P, and S of the fresh biomass are left in the press cake after the first screw pressing; only K is to a larger part transferred into the liquid green juice fraction.

- The TS, VS, C, P and S concentration in the blend of press cake and brown juice as it is leaving the biorefinery is 54–56% of the concentration in the fresh biomass, only N and K have been reduced to 43% and 51%, respectively.

- The residual press cake and brown juice blend is still rich in nutrients and has a suitable C:N ratio to use this solely as co-digestion feed in a biogas process as part of the biorefinery concept; thus making it independent of other co-substrates. The brown juice provides high water content and easily degradable organic matter to the feeding mixture, favoring the treatment in a continuous stirred tank reactor (CSTR) and higher biogas yields compared to AD treatment of the press cake alone.

- The AD process results in a digestate with an N:P:K ratio similar to mineral fertilizer and an improved fertilizer value because of a reduced content of organic matter, a lower C:N ratio and higher share of ammonia-N of total-N. Consequently, the final digestate product from the biorefinery presents a higher proportion of plant-available N and a lower risk for microbial immobilization of nutrients compared to the fresh biomass.

- Further research is needed for investigating the possibilities for increasing the concentration of nutrients in the digestate by e.g. modifying the configuration of the AD reactor and the application of the digestate to the fields.

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