# Impact of digestate fractions recirculation in continuous stirred tank reactor for anaerobic digestion of wheat straw

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## Abstract
The impact of recycling different digestate fractions during anaerobic digestion of wheat straw was investigated in single-stage continuous stirred tank processes. The organic loading rate (OLR) was set at 2 g VS/ (L·d) and the solid retention time (SRT) at 40 days. The results showed relatively high methane yields and process stability with digestate recycling. Batch digestion showed an average methane yield of 259 ± 13 ml $CH_4$/g VS which was comparable to the reactor methane yield with digestate recycle (240 to 248 ml $CH_4$/g VS added). Without digestate recycling, the methane yield ranged from 133 to 207 ml $CH_4$/g VS added. Nutrient recycling led to higher nutrient content in the reactors rendering process stability and high performance.

**Keywords**: Anaerobic digestion, Digestate, Recirculation, Wheat straw, Nutrients

## INTRODUCTION
Biogas production through anaerobic digestion (AD) has gained much attention in recent years primarily due to its positive energy balance. Straw is an abundant agricultural residue but its conversion to biogas has been limited by its hydrolysis (Zhao et al., 2010). Many studies on pre-treatment have been performed to improve the anaerobic biodegradability of straw (Fernandes et al., 2009; Zhao et al., 2010). Another drawback of straw as feedstock for biogas production is its poor content of both macro-and micro-nutrients. Nutrients are needed by anaerobic microorganisms for physiological and enzymatic reactions (Nges et al., 2012; Scherer et al., 2009; Takashima et al., 2011). Studies have demonstrated that the hydrolysis step and high carbon to nitrogen (C/N) ratio were the main causes of poor process performance in the AD of straw (Lei et al., 2010). Usually, the seed sludge in the anaerobic digestion process provide both buffering and nutrients to the process. However, overtime, these nutrients as well as buffering will be washed out. Therefore, there is need to continuously replace these nutrients, especially in nutrient deficient feedstock such as straw.
The concept investigated in the presence study is that the performance of the continuously stirred reactor (CSTR) can be improved by recycling nutrients as well as bacterial biomass back into the reactors. Three scenarios were compared, one without recycling of digestate serving as a reference control while in the other two, the supernatant after centrifugation and after filtration were recycled to the reactors.

## MATERIALS AND METHODS
### Substrate and inoculums.
The substrate was pre-dried wheat straw bought from an animal store (Zoobutik, Malmö, Sweden), milled to <3mm with the aid of a homogenizer (Grindomix 200, Retsch, Germany). Inoculum was collected from an active biogas plant (Källby, Lund, Sweden). Characteristics of substrate and inoculums are presented in table 1.

### Process operation

*Biochemical methane potential test*

The specific methane yield of straw was studied in batch test using a novel automatic methane potential test system (AMPTS II) (Bioprocess Control AB, Sweden). The test was conducted under mesophilic conditions and the inoculum to substrate ratio was set at 2:1.

*Reactor experiments*

The experimental set-up consisted of six wall-jacketed, 4-L CSTRs in glass with a 3-L active volume. The reactors were initially charged with 3 L inoculums and maintained at 37 °C ± 1°C. Mixing was set as 1.7 Hz using agitators (EURO-ST D, Germany). The organic loading rate (OLR) was set at 2 g VS/L.d and the solid retention time (SRT) was set at 40 days. Feeding was performed manually, once a day, seven days a week. Reference reactors in duplicate were fed with water diluted straw only denoted NR. For the second scenario, the digestate from process was centrifuge at 1700 *x g* for 15 minutes and the supernatant was used to dilute the straw i.e. recovery of soluble nutrients denoted RSN. In the third scenario, the digestate was filtered through a 0.5 mm mesh and slurry fraction was mixed with straw i.e. 'nutrients and microbes' recovery denoted RNM. In some instances, water was added to complete the feed amounts.

*Analytical methods*

Total solids (TS), volatile solids (VS) and pH were conducted according to standard methods (APHA, 2005). Alkalinity, gas volume and methane content were determined as previously reported elsewhere (Nges et al., 2012). Volatile fatty acids (VFAs) were determined by HPLC (Crespo et al., 2012). Macro and micronutrients were determined using ICP-OES and ICP-MS by LMI AB (Helsingborg, Sweden) as described in (Nges et al., 2012).

**Table 1.** Characteristics of straw, inoculums and nutrients concentrations

| | **Straw** | **Inoculum** | **RSN** | **RNM** | **NR** |
|---|---|---|---|---|---|
| **C:N ratio** | 55 | 5 | 13 | 12 | 18 |
| | | | **% (ww)** | | |
| **TS** | 90.10 | 3.94 | 4.36 | 5.28 | 4.78 |
| **VS** | 88.20 | 2.49 | 3.66 | 4.27 | 4.22 |
| | | | **mg /kg ww** | | |
| **N** | 8120 | 2300 | 1340 | 1720 | 1060 |
| **P** | 800 | 1100 | 430 | 790 | 380 |
| **S** | 540 | 260 | 130 | 200 | 130 |
| **Fe** | 84 | 1400 | 400 | 960 | 400 |
| **Ni** | 0.41 | 0.45 | 0.81 | 0.85 | 0.48 |
| **Mo** | 0.20 | 0.15 | 0.20 | 0.26 | 0.16 |
| **Co** | 0.20 | 0.11 | 0.80 | 0.15 | 0.85 |

## PRELIMINARY RESULTS

**Feedstock characterisation and nutrient content**

Table 1 shows the characteristic of the straw, inoculums and the concentrations of nutrients in the different processes. It can be observed that straw has a very high C/N ratio (55) where Soluble nitrogen i.e. ammonium nitrogen ($NH_4$-N) was 454, 674 and 256 mg/kg for RSN, RNM and NR respectively (result not shown). Straw is also poor in nutrients such as phosphorous (P), iron (Fe) and nickel (Ni) as compared to the inoculums. However, molybdenum (Mo) and cobalt (Co) were much higher in straw as compared to the inoculums. All nutrients were higher in the processes with recirculation of digestate as compared to that without recirculation. The VS in the digestate (effluent) was also higher for the NR process as compared to RSN though in the same range as the RNM process.

**Biogas/methane production**

Biogas production, methane yield and methane content were used to evaluate process performance. Figure 1 shows the variation in biogas production and methane yields during 56 days continuous operation (Figure 1a & b). The methane content and pH are also shown (figures 1c & d). From day 18 to day 40 for NR and from day 18 to day 55 for RSN/RNM the processes seemed to show a stable biogas/methane production with the methane yield fluctuating between 207 and 248 ml/gVS. The biochemical methane potential obtained in batch test was higher, i.e. 259 ± 13. The NR processes showed the lowest methane yield during this period and the methane yield for the NR further decreased to 133 ml/gVS on day 56. The methane content in biogas showed the same trend in all processes, however, the methane content for the RSN and RNM remained above 50% while the values went below 50% for the NR process after 50 days of operation.

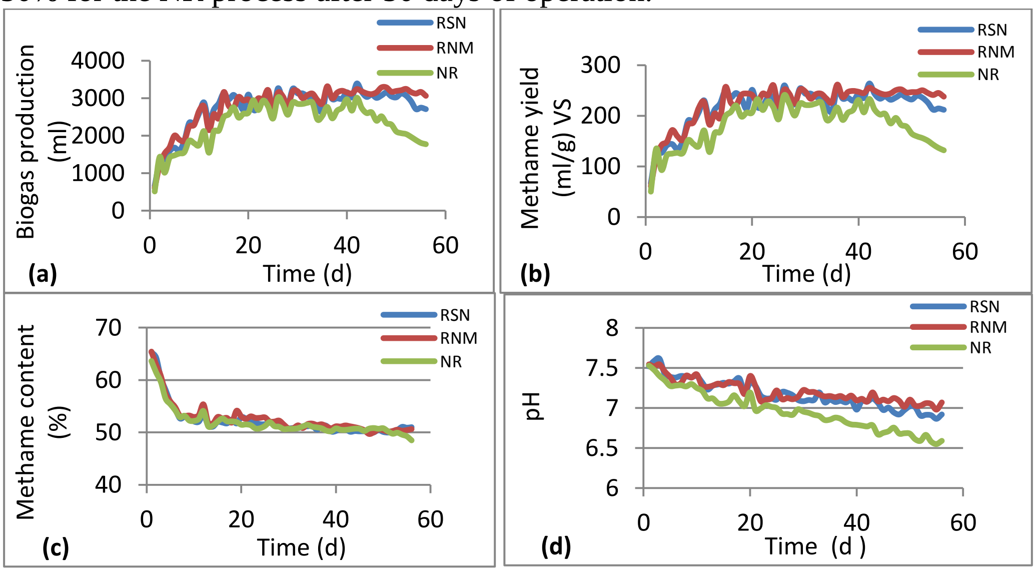


**Figure 1.** Biogas production, methane yield, methane content and pH variation

**Variation in pH, VFAs and VFAs to alkalinity ratio**

Process stability was studied in terms of pH, VFAs and the VFAs/alkalinity ratio. Figure 1d shows a gradual decrease in pH for all processes but the NR process showed a drastic decline in pH with values going below neutral from day 24. In fact, on day 56, the pH of the RN process was on average 6.5. However, there was hardly any VFAs accumulation. The prominent VFA was acetic acid where the following averages were recorded (day 56); 40 mg/l, 35 mg/l and 200 mg/l for processes RSN, RNM and NR respectively. The average alkalinity values were 4710 mg/L, 5690 mg/L and 3220 mg/L for RSN, RNM and RN respectively. The above values gave VFAs/alkalinity ratios ranging from 0.008 to 0.06.

## Discussion

The present study demonstrated that recirculation of anaerobic digestate fractions can lead to stable operation and relatively high methane yield as compared to the process without digestate recirculation. These can attributed to better buffering rendered by the recycled digestate fraction as well as the nutrients such as P, Fe and Ni. Lei et al. (2010) concluded that addition of P to rice straw significantly improved the methane production while Fe and Ni are important cofactors in enzymes directly linked to methanogesis as well as hydrolysis (Takashima et al., 2011). The poor levels of these nutrients were also reflected in the low pH of the NR process as well as the poor methane content. It should be noted that though the pH of the NR process was below 6.8, there were no VFAs accumulation. In fact, the VFAs/alkalinity ratio was less than 0.06. It has been reported in some studies that stability in anaerobic digestion processes can be judged on the VFAs/alkalinity ratio with values below 0.5 indicating stable processes (Nges et al, 2012). The low levels of VFAs though with poor methane production could be explained by the recalcitrance nature of straw to hydrolysis (slow release of sugars from lignocellulosic network). The stability and performance in the present study (RSN and RNM) was short-live, i.e. after 50 days of operation all processes showed a decline in stability and process performance. This was probably due to the continual dilution of the nutrients (with water) thereby rendering the processes less productive. The poor nutrient content of straw as well as the high C/N ratio indicated that for a sustained and viable process, under the present operational conditions, both macro and micronutrients or better still co-digestion of straw with a nutrient rich co-substrate should be implemented.

## CONCLUSIONS

The present study showed that recirculation of digestate in anaerobic CSTR digestion could lead to stable operation and relatively high methane yields over a certain period but the effect could not be sustained due to increasingly dilution of important nutrients. Co-digestion of straw with nitrogen-rich substrate or macro and micronutrient supplementation could be viable alternatives to overcome these nutrient deficiencies and hence improve process performance and stability.

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