

# Feather waste recycle for Biogas production

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## Abstract

Feather is produced in large amounts as waste in poultry slaughterhouses. Only 60-70% of the poultry slaughterhouse products is edible for human. 15-20% of the slaughterhouse by-products contains keratin; from this the proportion of feather is 7-9% with 50-70% moisture content. This means more million tons annually worldwide (Papadopoulus 1985, Williams et al. 1991, Hegedűs et al. 1998). The high protein content of poultry feather makes it an excellent raw material for biogas production. Dried feather contains 91% proteins and with 0.2 methane potential ( $\text{m}^3 \text{ kg}^{-1} \text{ VS}_{\text{added}}$ ) and 0.05 methane potential ( $\text{m}^3 \text{ kg}^{-1} \text{ wet weight}$ ) (Salminen & Rintala 2002). The keratin-content of feather can be difficultly digested, so physical, chemical and/or biological pre-treatment are needed in practice, which have to be set according to the utilization method. Feather was enzymatic degraded, and then digested in anaerobic bioreactors in different ratios. Cattle slurry was used as control without feather. The bioreactor system (4 digesters with 6 litre volume) was controlled by ACE SCADA software running on

Linux platforms which granted pre-programmed measurement and points of intervention for pH, temperature, CH<sub>4</sub>, CO<sub>2</sub>, O<sub>2</sub>. The produced biogas flows through a safety gas-washer bottle and a cryogenic inventory. Following this the produced biogas was switched, so the producing line continues through a doubled valve-system or went directly to the output pipe. The gas-washer bottle was utilized to remove the organic acid while the cryogenic instalment to remove water. The content of the gas mixture was monitored with custom created gas-analyser and with MX42A gas-analyser (H<sub>2</sub>S, NH<sub>3</sub>) basis of absorbance measurement. The data transfer was achieved with serial RS232 port.

Our main objectives were to determine the effect of enzymatic pre-treatment, methane potential of feather waste and the most effective treatment ratios. In case of reproduction Student's t-test and variance analysis with Tukey's test were applied to examine significant differences between the control and different treatments. The effect of treatments was expressed in the percentage of the controls. Recycle of the slaughterhouse feather and different agricultural wastes and by-products can solve three main problems: disposal of harmful materials, producing of renewable energy and soil nutrient. Feather waste - this difficultly disintegrating material produced in large amount - recycling with anaerobic digestion provides an environmentally friendly way of utilization.

### **Keywords**

poultry feather, waste, recycle, biogas production, keratinolytic bacteria

## **1. Introduction**

The feather protein (keratin) is insoluble, respectively with high structural stability and capability of resistance for proteolytic microbes because of high degree of cross-linking

by cysteine disulphide, hydrogen bonding, and hydrophobic interactions (Kunert 1973, Kaluzewska et al. 1991, Friedrich & Antranikian 1996). Physical, chemical and/or biological pre-treatment are needed in practice, which has to be set according to the utilization method (Hegedűs et al. 1998; Perei et al. 2004; Bíró et al. 2008). The biodegradation of keratin occurs for proteases specific (keratinases) (Steinert 1993, Wang & Parsons 1997, Letourneau et al. 1998, Onifade et al. 1998). Many species of soil bacteria is able to degrade the keratin (Kaul & Sumbali 1997). The *Bacillus* spp. was identified as the most productive feather-degrading bacteria in soil (Kao & Lai, 1995). The most study focus on *Bacillus*, especially on *Bacillus licheniformis*. *Bacillus licheniformis* PWD-1 was isolated by Williams et al. (1990) from aerobic extract of poultry slaughter waste. Wang & Shih (1999) examined the keratinase productivity of *Bacillus licheniformis*; while other researchers used it for intensify  $\beta$ -keratin degradation (Lin et al., 1995). In Hungary, Kovács et al. (2002), Perei et al. (2004) and Bálint et al. (2005) examined *Bacillus licheniformis*. They isolated *Bacillus licheniformis* KK1 from nature which produce extracellular protease and can degrade well the feather because of enzymatic ability.

Previously, feather powder was produced which was used for animal feedstuff in Hungary. The modified constitutional law (1576/2007/EK decree, 1774/2002/EK, European Parliament) disabled the feather utilization as feedstuff and dispose to the

landfill, so innovative developments and methods are needed to reach the objectives of alternative poultry feather utilization. The high protein content of poultry feather makes it an excellent raw material for biogas production. Dried feather contains 91% proteins and has 0.2 methane potential ( $\text{m}^3 \text{kg}^{-1} \text{VS}_{\text{added}}$ ) and 0.05 methane potential ( $\text{m}^3 \text{kg}^{-1}$  wet weight) (Salminen & Rintala 2002).

This paper shows the effect of enzymatic pre-treatment of chicken feather and the anaerobic digestion of pre-treated feather. Limited information is available about direct application of feather waste as raw material by biogas production and methane potential of feather waste. In this report, we describe the most effective treatment ratios. The effect of treatments was expressed in the percentage of the controls. Feather waste recycling with anaerobic digestion provides an environmentally friendly way of utilization.

## **2. Material and methods**

### *2.1. Preparation and analysing of chicken feather*

Slaughterhouse poultry feathers were collected from a local slaughterhouse industry in the northeast region of Hungary. Slaughterhouse chicken feathers were autoclaved at 100°C, 30 min (whole feather) and pre-treated with keratinolytic bacteria or followed autoclaving was dried at 80°C for 10 hours and was chopped into 2-5 mm sieve (feather

powder) with stainless steel homogenizer (WARING®, Snijders) and was used by C- and N-content analysing. The sterilization of wet poultry feather from slaughterhouse was completed by a Raypa type autoclave (on 1.5-2.5 atm pressure). The dry matter (DM), organic matter (oDM) content and ash content were determined according to the standard methods (APHA, 1998). Carbon and nitrogen content of chicken feather were analysed by Elementar VARIO EL universal analyser.

## 2.2. Bacterial strain and medium

*Bacillus licheniformis* KK1 strain was isolated and identified by (Kovács et al. 2002, Perei et al. 2004). The *Bacillus licheniformis* KK1 is an aerobic, endo-spore forming bacteria strain with neutral pH and 42°C optimum (Bálint et al. 2005). This bacteria strain produces extracellular keratinolytic enzyme. LB medium was applied to sustain the bacteria which contain: (g l<sup>-1</sup>): peptone, 10; yeast extract, 5; and NaCl, 5 (Miller 1972). The pH setting of LB liquid medium was determined to pH 7.5 with Tris-HCl. Hanna HI2550 multifunctional pH/ORP/temperature/EC/TDS/NaCl device was used to pH setting (Measuring limit: 0-14 pH±0.01; -20-+120°C±0.4). Chemicals used for mediums and buffers were obtained from Sigma-Aldrich Co. (USA) and VWR International (USA).

### *2.3. Pre-treatment of the Chicken Feather*

Following the autoclaving, processes of incubation (42°C) were completed by 45 l water bath (Memmert). Feather pre-hydrolysing was carried out for 5 days with 1:2 (w v-1) feather: water ratio (0.67 kg whole feather: 1.33 l deionized water) in four 3 litre glass bottles triplicate. The agitation was effected with air pump and controlled by flow meter (1.5 l min<sup>-1</sup> air flow). The pH optimum (7.2) was occurred with 7.5 phosphate buffer solution. Cell number of bacteria was 4,55x10<sup>6</sup> cell ml<sup>-1</sup> by inoculating (10 ml) except of the control experiment. Cell counting was occurred in Burker chamber with Alpha BIO-3CCD light microscope.

### *2.4. Determination of the protein concentration and chemical oxygen demands*

Culture was centrifuged daily at 10000 rev min<sup>-1</sup> for 20 min. The uncontaminated, cell-free supernatant fluid was used for the measurement of soluble protein by spectrophotometer (Sacomán Athelie Junior) with Bradford (1976) method. 1 absorbance was considered as 1 mg ml<sup>-1</sup> protein using bovine serum albumin as standard. Keratin degradation rate (%) was calculated from protein content of raw chicken feather and protein concentration of liquid culture filtrate. After centrifuge the soluble chemical oxygen demands (sCOD) was determined in the pre-hydrolysed chicken feather culture with PF-10 compact photometer (Macherey-Nagel). 2 ml liquid

sample was reacted with 0–15,000 mg sCOD I-1 test solution and the absorbance was measured at 620 nm after 148°C, 2h destruction. Calculation of solubilisation degree(%) was based on method described by Forgács et al. (2013).

### *2.5. Batch digestion experiments*

The anaerobic degradation was examined in the Biodegradation Laboratory of the Institute, where the fermentation areas were 4 stainless steel digester (the volume was 6 l per each) in Incubators. The inoculum (Liquid digestate) was obtained from a large Hungarian Agricultural Biogas Plant that operating mesophilic and thermophilic condition. The batch experiments were carried out at mesophilic conditions (38°C) 30 day. Mesophilic liquid digestate (2.2 kg), corn silage (0.2 kg), cattle slurry (2.6 kg) and pre-treated feather (0, 5, 10 and 20%) was added to the batch digesters (Fig. 1). All experimental setups were performed in triplicates.



**Figure 1.** Batch anaerobic digestion with pre-treated chicken feather

Quantity parameters of raw materials were measured before setting up experiments, and after that was calculated a weighted average of DM% and oDM% by each the experiments (Table 1). The highest DM and oDM% was calculated by the control experiment without pre-hydrolysed feather.

**Table 1.** Average DM and oDM% by different experiments

Digester number	1	2	3	4
Pre-hydrolysed feather%	0	5	10	20
DM%	3,9±0,5	3,7±0,6	3,6±0,4	3,4±0,5
oDM% in DM%	3,1±0,4	2,8±0,3	2,5±0,2	2,3±0,2

Controlled thermometer probes (Pt100) and ventilators to ensure the optimal conditions in the incubator. The bioreactor system with was controlled by ACE SCADA software running on Linux platforms which granted pre-programmed measurement and points of intervention for pH, temperature, CH<sub>4</sub>, CO<sub>2</sub>, O<sub>2</sub>. The produced biogas flows through a safety gas-washer bottle and a cryogenic inventory. Following this the produced biogas was switched, so the producing line continues through a doubled valve-system or went directly to the output pipe. The gas-washer bottle was utilized to remove the organic acid while the cryogenic instalment to remove water (Tamás et al. 2012). The content of the gas mixture was monitored with custom created gas-analyser (CH<sub>4</sub>, CO<sub>2</sub> and O<sub>2</sub>) and with MX42A gas-analyser (H<sub>2</sub>S, NH<sub>3</sub>) basis of absorbance measurement. The data transfer was achieved with serial RS232 port.

## *2.6. Statistical analysis*

In case of reproduction Student's t-test, independent sample t-test and variance analysis with Tukey's test were applied to examine significant differences between the control and different treatments by 5% significant level. The effect of treatments was expressed in the percentage of the controls.

### 3. Results and discussion

#### 3.1. Pre-treatment of the Chicken Feather

Quantity parameters of poultry feather were measured before experiments. Table 2 shows the results, means and standard deviations (SD).

**Table 2.** Quality parameters of chicken feather

Number of samples	Carbon-content (%)	Nitrogen-content (%)	DM%	oDM%
1.	50.59	14.31	38.63	97.56
2.	53.66	14.605	39.67	97.76
3.	50.58	14.81	38.82	93.14
4.	-	12.63	39.4	89.34
<i>Mean±SD</i>	<i>51.61±1.78</i>	<i>14.09±0.995</i>	<i>39.13±0.49</i>	<i>93.35±4.11</i>

The protein content of examined feather sample (nitrogen content\*6.25) on average was  $88.1\pm 6.22\%$  which was similar to results of Papadopoulos (1985) experiments (85-99%) and lower than Xia et al. (2012) ( $92\%\pm 0.48$ ) and Salminen & Rintala (2002) (91%) results. Comparable oDM% (99.2%) was detected in raw feather by Xia et al. (2012) like our results. Monitoring of growth of bacteria cells was also examined by all of the soluble protein concentration measuring with Burkler chamber (Table 3).

**Table 3.** Changes of bacterial cell number during feather biodegradation process

<b>Bacterial cell number (<math>\times 10^6</math> cell ml<sup>-1</sup>)</b>	1. day	2. day	3. day	4. day	5. day
Infected experiments	4.55 $\pm$ 0.00	11.2 $\pm$ 0.58	23.7 $\pm$ 1.11	5232 $\pm$ 1.33	70.8 $\pm$ 4.62

The calibration curve was determined with known absorbance protein (Bovine serum albumin solution with 1 mg m<sup>-1</sup> protein content) for measurement of protein concentration according to Bradford method. The dissolved protein concentration could be known by intersection reading, in our case the value was 0.19 mg ml<sup>-1</sup>. After calculation of total soluble protein concentration, the efficiency of feather pre-hydrolysis is comparable to the control experiment. The protein concentration of liquid culture sample after pre-hydrolysis is shown in the Table 4 and the calculated keratin degradation rate (%) shown in the Table 5.

**Table 4.** Protein concentration changes (mg ml<sup>-1</sup>)

<b>Experiments and differences</b>	<b>Protein concentration changes (mg ml<sup>-1</sup>)</b>				
	1. day	2. day	3. day	4. day	5. day
Infected experiments	0.012 $\pm$ 0.001	0.09 $\pm$ 0.014	0.16 $\pm$ 0.018	0.24 $\pm$ 0.031	0.36 $\pm$ 0.014
Control	0.00 $\pm$ 0.00	0.00 $\pm$ 0.00	0.008 $\pm$ 0.002	0.013 $\pm$ 0.003	0.026 $\pm$ 0.012

Differences%	1.2	9.0	16.0	22.7	33.4
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**Table 5.** Keratin degradation rate (%)

Experiments and differences	Keratin degradation rate (%)				
	1. day	2. day	3. day	4. day	5. day
Infected experiments	0.91±0.052	6.79±2.14	12.08±0.96	18.12±1.66	27.17±0.75
Control	0.00±0.00	0.00±0.00	0.60±0.10	0.98±0.16	1.96±0.64
Differences%	0.91	6.79	11.48	17.14	25.21

After applying Student's t-test and variance analysis with Tukey's test a significant difference (SD 0.1%) was detected between the protein concentration values and keratin degradation ratios of infected and control experiments.

The sCOD of infected experiments was in average  $106.15 \pm 3.18$  g l<sup>-1</sup> and in case of control experiments was in average  $6.8 \pm 0.56$  g l<sup>-1</sup>. Solubilisation degree (%) was  $4.53 \pm 0.37$  by control experiments and in case of incubated experiments was  $70.77 \pm 2.12$  therefore 66.23% difference was determined compared the control experiments. Difference between control and biodegraded chicken feather shows the Fig. 2.



**Figure 2.** Control (left) and biodegraded chicken feather (right)

### 3.2. Batch digestion experiments

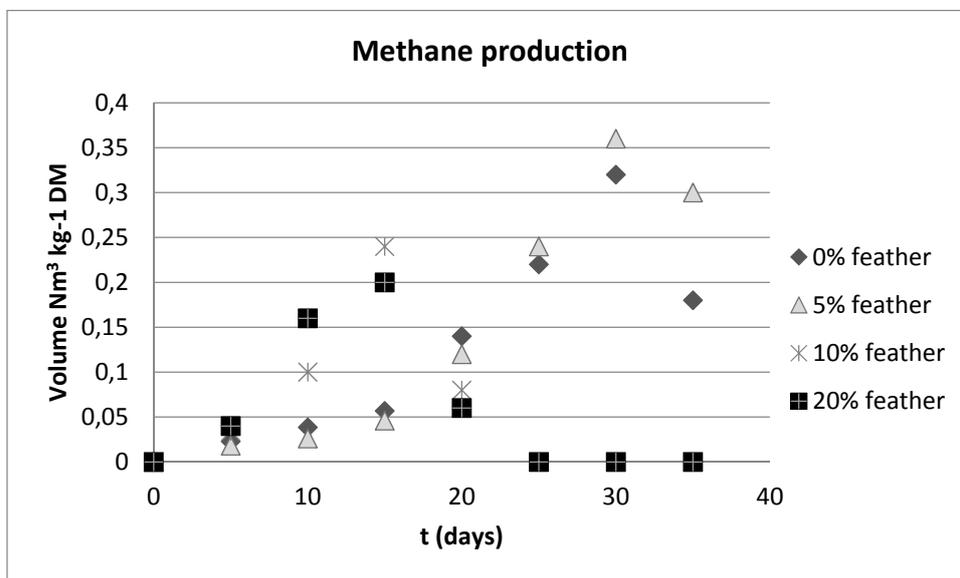
The biogas raw materials were analysed before anaerobic degradation process (Table 6). Under these parameters were calculated the optimal DM, oDM content (%) and C/N ratio of the digesters. C- and N-content of biodegraded feather were also calculated.

**Table 6.** Quality parameters of biogas raw materials

Raw materials	Corn silage	Cattle slurry	Liquid digestate	Pre-treated feather
DM%	26.0±2.73	3.6±0.52	2.8±0.91	19.94±1.36
oDM %	93.0±3.35	82.7±3.89	72.4±4.02	96.4±2.31
C:N ratio	27.6	13,0	18.0	1.47
C-content	45.8±0.87	40.4±2.40	47.3±2.07	15.09
N-content	1.7±0.29	3.1±1.21	2.6±0.25	10.26

Biogas production of biodegraded chicken feather:

Upon the results of the experiments it can be stated the mixture rate of the raw material that contains both cattle slurry and poultry feather determined the biogas production significantly (Fig. 3).



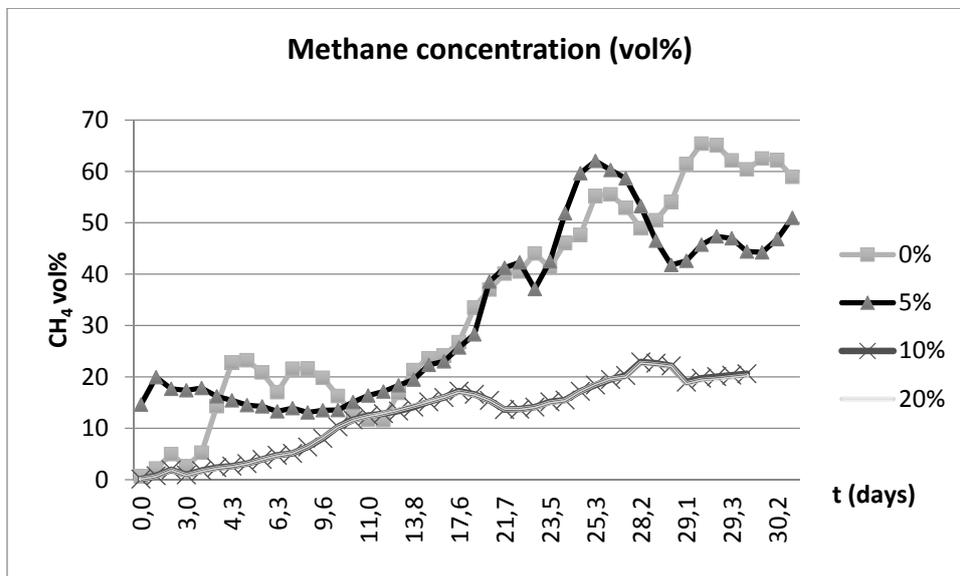
**Figure 3.** Methane production during co-digestion of different pre-treated feather ratio

Under mesophilic conditions the mixture rates of 5% result in a favourable production, the amount of the produced biogas (Nm³ day<sup>-1</sup>) exceeded the values of the production

at mixture rates of 10 and 20% by far (50%). The 5% mixture result the highest methane yield, the maximal value was  $0.36 \pm 0.13 \text{ Nm}^3 \text{ kg}^{-1} \text{ DM}$ , second was the control experiment ( $0.32 \pm 0.13 \text{ Nm}^3 \text{ kg}^{-1} \text{ DM}$ ) after 30 days, therefore did not show any significant differences between control and 5% experiments. Forgács et al. (2011) reported  $0.35 \text{ Nm}^3 \text{ kg}^{-1} \text{ VS}$  methane production of feather after biological pre-treatment with *B. licheniformis* ATCC 53757 strain. Anaerobic digestion process of 10 and 20% mixture was stopped after 20 days despite the initial high biogas yields. Under methane yields two groups could be selected. Control, 5% experiments and 10, 20% experiments showed significant differences.

#### Biogas quality analysis:

The biogas quality in case of the poultry feather mixture rate of 5 showed better results and differed significantly from the rates of 10 and 20%. In case of treatments with a feather mixture rate of 5 methane concentrations around 60% stayed stable (Fig. 4).



**Figure 4.** Methane concentration (vol%) production during co-digestion of different pre-treated feather ratio

The amount of  $H_2S$  – that has a corrosive effect and causes bad smell – was significantly increased in case of a feather mixture rate of more than 10% (10 and 20%) at the beginning of the fermentation and it affected the methane production negatively. In case of the mixture rates of 5 the hydrogen sulphide concentrations of the produced biogas – in contrast to the higher mixture rates – were more favourable and showed a significant difference in the first phase of the production. The production of the hydrogen sulphide reached its maximal value (200 ppm) already on the 9<sup>th</sup> day in case of the 10% treatment.

Regarding the ammonia content of the biogas it can be stated that the produced amount was significantly high in the first stage (250 ppm) because the most of the easily degradable nitrogen. After that this value decreased as the not so easily degradable forms were degraded. This process was more balanced. In the first stage of the ammonia production a significant difference could be revealed between the following groups: 0, 5% and 10, 20% treatments. In the much more balanced ammonia-producing final stage three groups could be differed: 0% treatment build the first, and 5% was the second, while the 10 and 20% were the third group. These treatments showed significant differences.

#### **4. Conclusion**

After applying Student's t-test and variance analysis with Tukey's test a significant difference (SD 0.1%) was detected between the protein concentration values and keratin degradation ratios (%) of infected and control experiments. Solubilisation degree (%) - calculated from sCOD - shows 66.23% difference compared the control experiments. 5% pre-treated feather ratio result in a favourable biogas production, the amount of the produced biogas ( $\text{Nm}^3 \text{ day}^{-1}$ ) exceeded the values of the production at mixture rates of 10 and 20% by far (50%). The 5% mixture result the highest methane yield, the maximal value was  $0.36 \pm 0.13 \text{ Nm}^3 \text{ kg}^{-1} \text{ DM}$ , second was the control experiment

( $0.32 \pm 0.13 \text{ Nm}^3 \text{ kg}^{-1} \text{ DM}$ ) after 30 days. Anaerobic digestion process of 10 and 20% mixture was stopped after 20 days despite the initial high biogas yields. Control, 5% experiments and 10, 20% experiments showed significant differences. Due to the amount of produced hydrogen sulphide the critical mixing ratio of feather proved to be 10% in laboratory environment. The production of the hydrogen sulphide reached its maximal value (200 ppm) already on the 9<sup>th</sup> day in case of the 10% treatment. Three treatments group could be selected in case of the highest ammonia concentrations (control and 5% and 10, 20%) which were detected after 20 days.

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