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Biochemical Methane Potential of Winter Wheat (*Triticum aestivum* L.): Influence of Growth Stage and Storage Practice.

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Abstract

The effect of growth stage at harvest and of storage practice on the biochemical methane potential (BMP) of winter wheat was investigated using batch-fed stirred mesophilic digesters. The wheat used was a single variety sown at the same time (autumn) and harvested at 3 different stages in its growth: medium milk (A); soft dough (B) and Caryopsis (C). Wheats (A and B) were ensiled whilst the later harvested material (C) with a higher dry matter content was treated by the alkalage process. The BMP values expressed on the basis of volatile solids (VS) were 0.360±0.030, 0.346±0.006 and 0.311±0.016 l CH_4 g^{-1} VS_{added} for A, B and C respectively. A simple first order kinetic model gave only a poor fit to the experimental data but a close match was obtained using a modified first order pseudo-parallel model which explains the methane production curve on the basis of differences in biodegradability of the plant material.

Keywords: biochemical methane potential, energy crops, growth stage, storage practice, kinetic model.

1. Introduction

Increasing interest in renewable energy production and in reduction of the greenhouse gas emissions associated with fossil fuels has made anaerobic digestion of plant biomass an attractive option. The Biochemical Methane Potential (BMP) test has been used to evaluate the energy yield from a number of different crop species including maize, triticale, winter rye and sunflowers (Amon et al., 2007). This parameter gives a useful preliminary assessment of a crop biomass, although the information obtained must then be considered within a whole systems energy analysis that includes biomass yield and associated energy inputs to provide a valid prediction of the net energy gain (Salter and Banks, 2009). There is no single recognised procedure for carrying out the BMP test itself, although recommendations have been made by an international working group (Angelidaki et al., 2009); even so the test results are influenced

by different factors, including the substrate to inoculum ratio and the mixing regime, which can affect both methane yield and production kinetics (Chynoweth et al., 1993; Hashimoto, 1989).

Different varieties of wheat are classified as either spring wheat (sown in the spring, and harvested the same year) or winter wheat (sown in the autumn and harvested the following year). Depending on the purpose for which it is grown, the crop can be harvested at different growth stages e.g. medium milk, soft dough, high dry matter.

For crop biomass that is harvested as a green 'whole crop' for forage, rather than dry as grain and straw, the two main methods of preservation involve the production of silage or alkalage. Ensiling is the most common method, and has been utilised for centuries. In this process, a combination of anaerobic conditions and acidity protects the forage from the proliferation of deleterious bacteria and fungi (Yang et al., 2001). The required anaerobic fermentation may be carried out by indigenous microorganisms or induced by inoculation of the material with a starter culture. The use of additives may also improve the silage quality. Alkalage is a more recent treatment which uses ammonia gas as the preserving agent by increasing the alkalinity. This process is commonly applied to winter wheat and other crops such as barley, triticale and spring wheat. Alkalage preservation is applied to forage materials with a high dry matter (DM) content of between 65-85 %, and allows extension of the harvest period by up to 30 days. This treatment maintains a high protein content, which is beneficial for animal feed.

The BMP test allows determination of the methane yield of an organic matter substrate by anaerobic digestion in specific conditions and media. BMP tests were initially developed in the seventies (Owen et al., 1979) and since then have been modified in various ways for different purposes (ASTM, 1992; Chen and Hashimoto, 1996; Chynoweth et al., 1993; Gunaseelan, 2004; Isci and Demirer, 2007). The aim of the current work was to evaluate how harvest growth stage and storage practice influence the composition of winter wheat (*Triticum aestivum* L.) and its overall BMP, the biogas composition and the reaction kinetics. The importance of the influence of growth stage at harvest is described in previous studies on other energy crops and crop residues (e.g. Lehtomaki et al., 2008; Pouech et al., 1998).

2. Materials and Methods

2.1. Equipment.

The BMP test used twelve 1.5-litre working volume digesters continuously stirred at 40 rpm and maintained in a thermostatic water bath at a temperature of 35±2 ºC. Biogas was collected by displacement of a 75 % saturated solution of sodium chloride acidified to pH 2 in calibrated glass cylinders. Similar techniques to minimise solubilisation of gases have been used previously by Borja and Banks and Telles *et al*. (Borja and Banks, 1995; Telles et al., 2002). Vapour pressure and salt solution density were taken into account in correction of gas volumes to standard temperature and pressure (101.325 kPa, 0 ^{o}C).

2.2. Substrate.

The samples of winter wheat used in the experiments are referred to as A, B and C respectively. The wheats were all grown commercially as part of a main crop harvest. Wheat A was harvested at the medium milk growth stage (moisture content 63.4 %), wheat B about two weeks later at the soft dough stage (moisture content 47.5 %), and wheat C at the caryopsis hard growth stage (moisture content 16.5 %). Wheats A and B were ensiled after

collection using Pioneer Hybrids 11A44 high dry matter Buchnerii innoculant according to the supplier's instructions and wheat C was given an alkalage treatment with Dugdales Home'n Dry ammonia release additive again according to the supplier's instructions. In both cases the samples were taken from within the bulk of the material at a freshly-cut face of a full-size commercial silage or alkalage clamp. After removal from the farm, samples for analysis were kept at 4 ^{o}C to preserve the original characteristics of the substrates. The wheat samples used in the BMP test were milled to a particle size of 0.5-1.5 cm to ensure homogeneity and good inoculum-substrate transfer (Hills and Nakano, 1984).

2.3. Inoculum.

The inoculum used was from an anaerobic digester receiving municipal wastewater biosolids (Millbrook, Southampton, UK). Before use the inoculum was sieved through a 1 mm mesh to remove large particles and grit. After sieving it had a pH of 8.1, total solids (TS) of 39.8 g kg^{-1} and volatile solids (VS) of 25.7 g kg^{-1}. In the test an inoculum:substrate ratio of 2:1 was used based on volatile solids content (VS), as recommended by Chynoweth *et al.* (1993).

2.4. Experimental Procedure.

For each of the wheats tested, 3 digesters were set up and run against 3 controls without substrate addition. No positive control was used as it has been shown that inoculum of this type can successfully degrade lignocellulosic substrates (Banks and Humphreys, 1998; Siegert and Banks, 2005; Qu et al., 2009; Jensen et al, 2009). Each digester received 1400 g of the sieved inoculum, which was then left for 24 hours at 35 ^{o}C to reduce the residual readily available organic matter. The amount of wheat needed to give the required inoculum:substrate ratio was then added to each test digester, with 1.4 ml of a trace elements solution. The composition of the trace elements solution was: $FeCl_2 \cdot 4H_2O$: 2000 mg l^{-1}, $CoCl_2 \cdot 6H_2O$: 2000 mg l^{-1}, $MnCl_2 \cdot 4\ H_2O$: 500 mg l^{-1}, $CuCl_2 \cdot 2\ H_2O$: 38 mg l^{-1}, $ZnCl_2$: 50 mg l^{-1}, H_3BO_3: 50 mg $l^{-1,}$ $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$: 50 mg l^{-1}, $Na_2SeO_3 \cdot 5H_2O$: 194 mg l^{-1}, $AlCl_3 \cdot 6H_2O$: 90 mg l^{-1}, $NiCl_2 \cdot 6H_2O$: 50 mg l^{-1}, EDTA: 1000 mg l^{-1} and resarzurine: 200 mg l^{-1} (Gonzalez-Gil et al., 2001).

The digesters were sealed and allowed to run for a period of 96 days until there was no difference between the daily gas production in the test and control digesters. Samples for gas composition analysis were taken from the collection cylinders each time they were refilled, at intervals of not more than 5 days to minimise the loss of methane by dissolution (Walker et al, 2009).

2.5. Chemical Analyses.

Total solids (TS), volatile solids (VS), total Kjeldahl nitrogen (TKN), ammonia, and pH were measured on fresh samples according to Standard Methods (APHA, 2005). Further characterisation was carried out on samples prepared by air drying to constant weight and then milling to a particle size $\leq$0.5 mm in a micro hammer mill (Glen Creston Ltd, Standmore Mill, England). Fibre composition was measured on the air-dried sample following the method given by Kitcherside *et al*. (Kitcherside et al., 2000) using a Foss Analytical FibreCap^{TM} 2021/2023 system. The method allowed quantification of Neutral Detergent Fibre (NDF), Acid Detergent Fibre (ADF) and Acid Detergent Lignin (ADL). Air-dried material was also used for Elemental Analysis (C, H, N) following the manufacturer's recommended procedure (model Flash EA 1112, Thermo Finnigan, Hemel Hempsted, UK) using L-Aspartic Acid, Atropine and Nicotinamide as standards. Phosphorus was measured using the ascorbic acid spectrophotometry method of APHA

(4500-PE), with measurements taken at 880 nm using a Cecil 3000 Series Spectrophotometer (Cecil Instruments, Cambridge, UK).
An acid extract of the air-dried material was then prepared with nitric acid using microwave digestion (Microwave Accelerated Reaction System, Model MARS X^R, XP-1500 Plus, CEM Corporation). The extract was filtered and diluted to 50 ml with deionised water (Milli-Q Gradient, Millipore, Watford, UK). The nitric acid extract was used for determination of Cd, Cr, Cu, K, Ni, Pb, and Zn using a flame atomic absorption spectrometer according to manufacturer's procedures (Spectr AA-200, Varian, USA) with samples measured against a dilution of appropriate stock standards (Sigma Aldrich Co, Gillingham, UK; Fisher Scientific, Loughborough, UK). Biogas composition was analysed using a Varian CP 3800 gas chromatograph with a gas sampling loop with argon as the carrier gas at a flow of 50 ml min^{-1}. The GC was fitted with a Hayesep C column and a molecular sieve 13 x (80-100 mesh) operating at a temperature of 50 ^oC. The GC was calibrated using a standard gas containing 35% CO_2 and 65% CH_4 (BOC, Guildford, UK). Samples of digestate for volatile fatty acid (VFA) analysis were prepared by centrifuging at 13000 rpm for 10 min (model 5417C, Eppendorf, Hamburg, Germany) and acidifying the centrifugate to 10% (v/v) with formic acid. VFA in the centrifugate was measured by gas chromatography (model GC-2010, Shimazdu, Tokyo, Japan), using a flame ionization detector and an FFAP capillary column (SGE Europe Ltd, UK) with helium as the carrier gas at a flow of 190.8 ml min^{-1}. The GC oven temperature increased from 60 to 210 ^oC in 15 min, with a final hold time of 3 min. The temperatures of injector and detector were 200 and 250 ^oC, respectively.

3. Results and discussion

3.1. Influence of growth stage and storage practice in the BMP test

As can be seen in Table 1, initial characterisation showed the properties of the winter wheat were influenced by the growth stage at harvest and the method of storage. The ensiled wheats A and B were acidic due to the lactic fermentation, whereas wheat C was basic reflecting the ammonia treatment. The total Kjeldahl nitrogen (TKN) of the wheat silages A and B was around 6.4-7.0 g N kg^{-1} while wheat C had a higher value of 17 g N kg^{-1} because the alkalage treatment supplied a major input of N. Wheat C consequently had a lower Carbon/Nitrogen ratio (C/N) of 17.2 compared to 20.2 and 22.7 for wheat A and B respectively. This difference between ratios could influence the methane yield. The optimum C/N ratio for anaerobic digestion has been found to be 25 (Hills, 1979) or between 20-30 (Speece, 1987). The phosphorus content of 0.74-0.84 mg g^{-1} for wheats A and B was lower than the 1.77 mg g^{-1} in wheat C. The heavy metals composition for wheats A, B and C were measured and the two regarded as critical in terms of toxicity (Commission regulation (EC) No1881/2006) are high within an acceptable range except for wheat C. This regulation gives values of Cd = 0.2 mg/kg and Pb = 0.2 mg/kg on a wet weight basis.

The test and control digesters were run for a total of 96 days and at the end of this time the digestate was analysed. The volatile fatty acid concentration in all digestates was low (170-260 mg l^{-1}) indicating that intermediate metabolites had been depleted by the end of the test. Figure 1 shows the cumulative biogas production of the test digesters and controls. The initiation of gas production in the BMP test was very rapid, without any apparent lag phase.

Over the 96 days of the test the methane potential from wheat A, B and C was 6.56±0.58, 6.25±0.10 and 5.60±0.28 L respectively after the methane production of the control had been subtracted.

The composition of the biogas was monitored throughout the test and the results over the first 25 days are shown in Figure 2. The concentration of methane at the start of the test was low in all the digesters (50-60%). After this the control digester showed a uniform methane concentration of around 70% for the duration of the experiment. The 3 test digesters, however, all showed a fall in methane concentration (37.4-46.1 % for wheat A, 35.7-46.5 % for wheat B and 44.6-50.3 % for wheat C) very early in the test (0.9 to 1.1 days). This was probably as a result of the rapid fermentation of readily utilisable substrate resulting in a small build-up in acid products and partial inhibition of the methanogens, which then responded after a short lag. Consequently, the methane concentration in these digesters rose to 65% by day 2 and then fell again temporarily on day 3 to a value of around 58%, before making a recovery by day 4. Over the duration of the trial the methane content in the biogas increased in all the digesters containing wheat, but this was most apparent in the alkalage treated, wheat C, where the final methane concentration in the biogas was around 75%.
The rise in methane concentration during the test might be attributed to a change in the source of carbon being converted, from readily available soluble sugars to longer-chain polymeric materials including proteins and some oils which stoichiometrically give rise to a higher percentage of methane. The overall higher methane composition of the alkalage biogas could be attributed to the higher protein concentration as indicated by the TKN.

The methane production attributable to the substrate organic matter was 0.360±0.030, 0.346±0.006 and 0.311±0.016 l CH_4 g^{-1} VS added for wheat A, B and C respectively. These values were obtained by subtracting the average methane production in control reactors from those in the test reactors.

These values are fairly typical of BMP values for whole crop forage materials, which generally have a high cellulose content, some of which is protected in complex fibre structures including lignin, which may limit the proportion of carbon available for conversion to methane. The two early harvested and ensiled wheats (A and B) showed a similar BMP value which was higher than the alkalage treated (wheat C). The concentration of lignin in wheat C (5.3 %) was, however, lower than in wheat A (8.4%) and B (8.7 %) indicating that other factors are also involved. These differences are an inherent property of the material tested, but may not reflect the total energy available per hectare, for example if additional time for growth leads to an increase in the total biomass yield. There are also losses in total energy potential due to the ensiling process, which may differ from those associated with alkalage treatments (Neuriter et al., 2005).

The methane yield was similar to that of other ensiled whole crop materials tested for their energy potential through anaerobic digestion. Values of 0.390 and 0.300 l CH_4 g^{-1} VS_{added} for maize and sunflower have been reported in the literature (Amon et al., 2007). The values for wheat obtained in this study were higher than the values obtained by Amon *et al.* (Amon et al., 2007), who reported BMP values for winter wheat at milk stage, dough stage and at maturity of 0.254, 0.228 and 0.245 l CH_4 g^{-1} VS respectively for Capo variety and 0.244, 0.243 and 0.251 l CH_4 g^{-1} VS for Levendis variety; but lower than those of Pouech *et al.* (Pouech et al., 1998) who gave values of 0.42, 0.39 and 0.38 l CH_4 g^{-1} VS_{added} for wheat at the flowering, milky and dough stages.

3.2. Kinetic study

Researchers have often attempted to relate the kinetics of the BMP test to the degradability and gas production of the material in a continuous or semi-continuous anaerobic digestion system. This can only be done on a fairly qualitative basis as the rate of reaction in a BMP

test is dependent on the nature of the test and the characteristics of the inoculum, including its degree of acclimatisation and the ratio of inoculum to substrate at the start of the test. Consideration of the kinetics can however provide some insight into the potential behaviour of the material in a full-scale system.

To determine the kinetic constants, the specific methane production was modelled using two sets of assumptions: simple first-order degradation (Model 1), and a pseudo-parallel first-order model (Model 2). For model 1 the methane production is given by

$$Y = Y_{max} (1 - e^{-kt}) \quad [1]$$

where Y is the cumulative methane yield at time t, Y_{max} is the ultimate methane yield and k is the first order rate constant.

Rao et al. (2002) suggested that when using solid organic materials as the substrate, it may be better to consider that the gas production curve corresponds to the rapid breakdown of readily degradable components followed by a much slower degradation of the fibrous proportion of the material. The methane production is therefore governed by two rate constants k_1 and k_2 rather than by a single constant:

$$Y = Y_{max} (1 - Pe^{-k_1 t} - (1-P) e^{-k_2 t}) \quad [2]$$

where Y is the cumulative methane yield at time t, Y_{max} is the ultimate methane yield, k_1 is the first order rate constant for the proportion of readily degradable material, k_2 is the first order rate constant for the proportion of less readily degradable material, and P is the proportion of readily degradable material

The values obtained for these parameters are shown in Table 2. For wheat A the proportion of readily degradable material is slightly higher, as indicated by a larger value of P. The value of k_1 for wheats A and B is greater than for wheat C. Figure 3 shows the adjustment of these models to the experimental data. It is clear that the pseudo-parallel kinetic model provides a much better fit, reflecting the heterogeneous molecular composition of the feedstock with the presence of both simple readily degradable components and complex polymeric materials.

From the above results it can be seen that wheat has a high energy potential. It is also know to have a high biomass yield per hectare, which could make it an attractive energy crop either alone or in rotation with other crop species. The economics of this are dependent on a number of factors: a case study for Ireland carried out by Murphy and Power (2009) showed the feasibility depended on the market price for wheat grain and on liquid fuel prices. Each project therefore needs independent assessment taking into account local subsidies for energy production and the long-term security of markets.

4. Conclusions

Wheat A and B showed BMP values which were typical of ensiled forage crops. Wheat C, alkalage, showed a lower BMP but had a higher methane concentration in the biogas produced that may reflect the higher protein content. These BMP values do not necessarily indicate the overall net energy yield/hectare of land, as the DM of the crop changes in the field and VS are also lost during preservation. The results indicate that wheat has a readily fermentable fraction that leads to a rapid production of biogas and a less available fraction

that converts to methane more slowly. A first order model gave a poor representation of the BMP test kinetic, whereas a pseudo-parallel approach gave a very close fit to the data.

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Table 1. Characteristics of the winter wheat A, B and C

Parameters[a]	wheat A	wheat B	wheat C
pH (1:10) (weight:volume)	4.25±0.02	4.08±0.02	8.28±0.02
TKN (g N kg^{-1} wheat)	6.4±0.2	7.0±0.1	17±1.0
Ammonia (g N kg^{-1} wheat)	1.3±0.0	1.8±0.2	5.6±0.1
Fibre (%) (H+C+L)	37.8±0.3	31.7±0.5	34.7±0.1
Fibre (%) (C+L)	22.5±0.2	18.6±0.5	19.3±0.3
Fibre (%) (L)	8.4±0.6	8.7±0.2	5.3±0.2
TS (g kg^{-1} wheat)	363±2	525±1	835±2
MS (g kg^{-1} wheat)	16±1	12±0	24±2
VS (g kg^{-1} wheat)	347±1	513±1	811±4
Carbon (% of dry matter)	38.4±1.1	40.8±1.8	39.5±1.7
Nitrogen (% of dry matter)	1.9±0.0	1.8±0.1	2.3±0.0
Hydrogen (% of dry matter)	7.7±0.2	8.0±0.3	7.8±0.2
Phosphorus (g kg^{-1} wheat)	0.74±0.04	0.84±0.05	1.77±0.04
Cd (µg g^{-1} wheat)	0.38±0.26	0.14±0.01	0.92±0.14
Cr (µg g^{-1} wheat)	98.77±0.28	63.19±3.04	35.16±0.25
Cu (µg g^{-1} wheat)	35.80±0.15	9.25±0.04	59.13±0.55
K (µg g^{-1} wheat)	12707.59±0.13	9951.06±0.64	7180.16±1.09
Ni (µg g^{-1} wheat)	33.67±0.97	24.15±0.07	15.93±0.68
Pb (µg g^{-1} wheat)	2.09±1.94	-	3.41±2.44
Zn (µg g^{-1} wheat)	155.55±0.30	95.73±0.12	252.30±0.61

[a]TKN: Total Kjeldahl Nitrogen, H: hemicellulose, C: cellulose and L: lignin. TS: Total Solids, MS: Mineral Solids and VS: Volatile solids. pH, TKN, Ammonia, TS, MS, VS and phosphorous were analyzed on a wet weight basis. The other parameters are expressed on a dry weight basis.

Table 2. Methane yields, constants and R^2 values obtained from models

	Wheat A	Wheat B	Wheat C
Y_{max} (l CH_4 g^{-1}VS)	0.360	0.346	0.311
k (d^{-1})	0.21	0.20	0.20
R^2 (model 1)	0.9601	0.9632	0.9762
P	0.67	0.64	0.63
k_1 (d^{-1})	0.78	0.83	0.67
k_2 (d^{-1})	0.07	0.077	0.08
R^2 (model 2)	0.9987	0.9987	0.9990

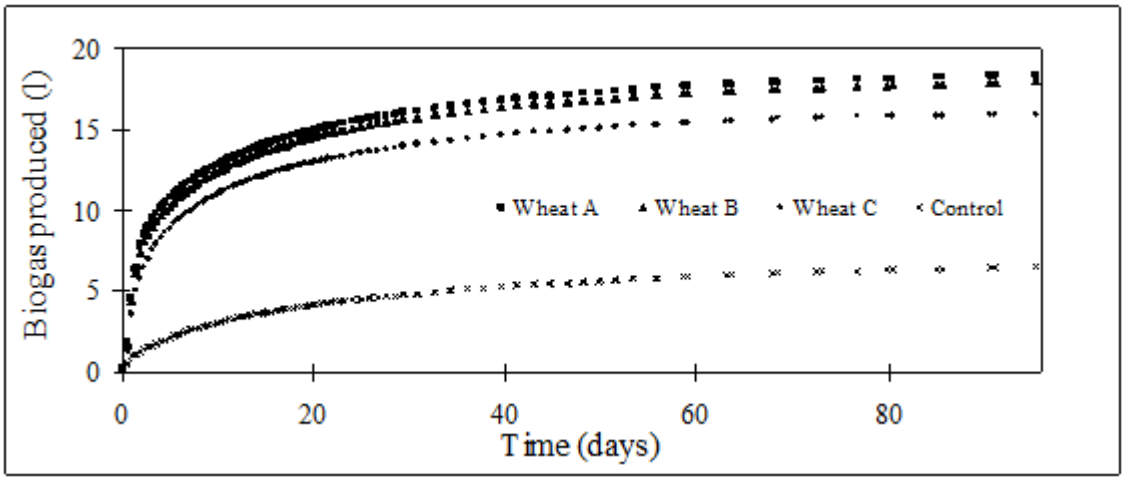

Figure 1. Evolution of the biogas production

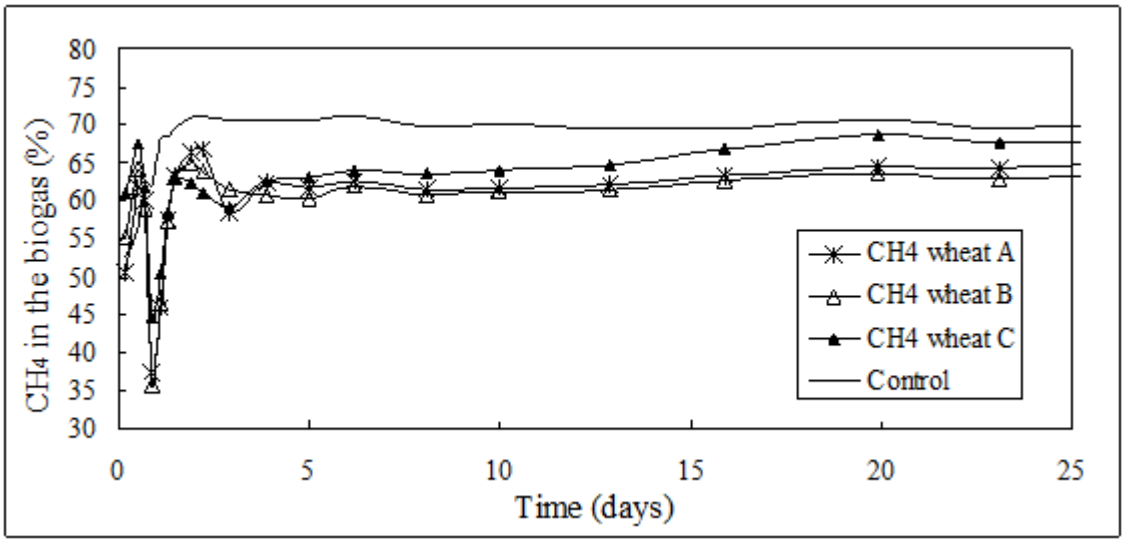

Figure 2. Variation of the methane concentration into the biogas during the first 25 days

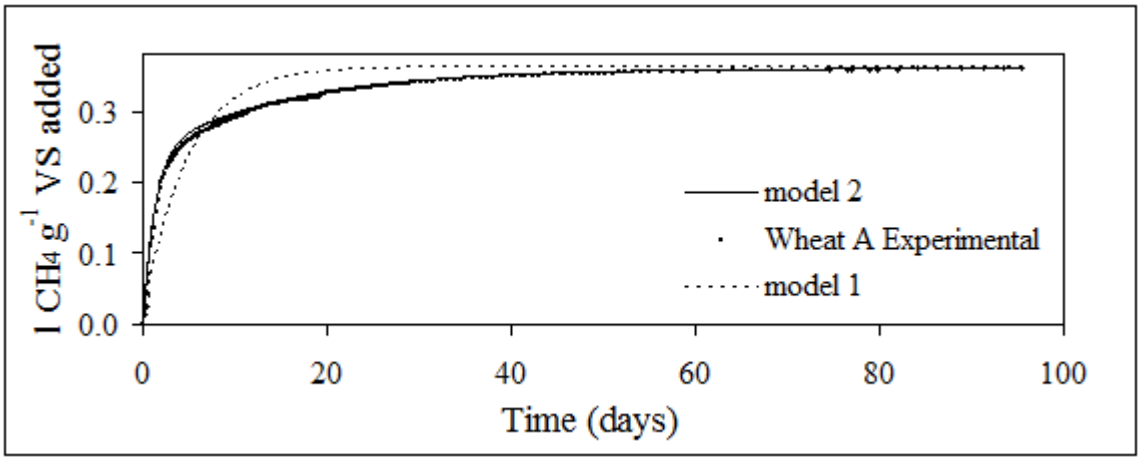

Figure 3. First order model, modified first order model and experimental data for wheat A, B and C