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International Seminar on Reducing Odor and Global warming gases from Livestock Industry

주최 : 농촌진흥청 축산과학원

참여국가 및 기관

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국제공동세미나 일정

시 간	세 부 내 용
13:30-14:00	등 록
14:00-14:05	개회 및 진행(농촌진흥청 축산과학원 축산환경과장)
14:05-14:10	환영사 (농촌진흥청 축산과학원장)
14:10-14:50	무창계사 유래 악취연속 측정을 위한 전자코 활용기술 (호주 퀸즐랜드 농수산성, Dr. JaeHo Sohn)
14:50-15:30	가축분 퇴비화과정에서 아산화질소 저감기술 (일본 축산초지연구소, Dr. YASUYUKI FUKUMOTO)
15:30-16:10	우분과 음식물쓰레기를 활용한 통합 혐기소화 과정에서의 수소생산기술 (일본 축산초지연구소, Dr. HIROSHI YOKOYAMA)
16:10-16:20	휴 식
16:20-17:00	퇴비화과정에서 발생하는 악취제어장치 이용 기술 (일본 축산초지연구소, Dr. YOSHIFUMI HONDA)
17:00-17:40	가축분뇨로부터 발생하는 온실가스 저감 국내 연구동향 및 연구결과 (농촌진흥청 축산과학원 정종원 박사)
17:40-18:00	종합토론
18:00	폐 회


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인 사 말

바쁘신데도 불구하고 오늘 국제공동세미나에 참석해 주신 내 · 외빈 여러분들께 먼저 진심으로 감사의 말씀을 올립니다.

지난 20 여 년간 우리나라 축산업은 기술과 규모면에서 급격한 발전을 해왔고 향 후에도 그 산업적 중요도가 지속적으로 높아질 것으로 예상되고 있습니다. 축산업은 단일품목기준으로 국내 농림생산액 분야에서 가장 큰 비중을 차지함으로써 농촌의 소득증대에 기여함과 동시에 국민의 고급 식량 공급원으로서의 그 중요성이 날로 높아져가고 있습니다.

그러나 최근 들어 산업구조 및 경제형태가 고도화 되어가고, 쾌적한 삶과 환경보존에 대한 국민의 요구가 높아짐에 따라 우리 축산업도 환경친화적 경영체계 구축이라는 과제에 직면하고 있습니다.

특히 악취성분을 비롯한 수질 및 토양오염물질의 제어가 시급히 해결해야 할 현안과제로 대두되고 있고, 범지구적 차원의 기후에 영향을 미치는 온실가스 배출감소 문제 역시 우리 축산업의 안정적 지속기반을 구축하는 중요한 요소가 되었습니다.

이러한 국내 · 외의 시대적 요구에 부응하기 위하여 오늘 이 세미나에 호주와 일본 그리고 한국의 관련 전문가가 그 동안의 연구결과와 향후 추진전략 및 연구방향에 대해 심도 있는 발표를 하게 되어 매우 시의 적절하다 할 수 있습니다.

한국과 호주 그리고 일본은 지금까지 축산악취 및 온실가스 저감기술개발을 위해서 공동연구 양해각서(MOU)를 체결하고 연구원교류 및 기술정보를 공유해 왔으며 양국이 국제공동연구를 수행하여 우수한 악취제어관련 연구결과를 도출해내기도 하였습니다.

향후에도 호주와 일본 그리고 우리나라를 포함한 많은 나라의 우수한 기술들이 상호보완적으로 결합하여 악취저감은 물론 온실가스의 효과적인 제어기술개발 단계까지 발전하여, 쾌적한 자연환경을 조성하는 데에 오늘 이 세미나가 큰 기여를 해줄 수 있기를 기대합니다.

오늘 세미나에서 그 동안의 우수한 연구결과를 발표하여 주시는 한국, 호주, 일본의 연구자들에게 감사드리며, 오늘 이 자리에 참석하여 주신 여러분들께도 다시 한번 깊이 감사드립니다.

2007 년 6 월 27 일

축산과학원장 농학박사 이상진

목 차


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HOW TO IMPLEMENT AN ELECTRONIC NOSE FOR CONTINUOUS ODOUR MONITORING IN A POULTRY SHED

무창계사 유래 악취연속 측정을 위한 전자코 활용기술

손재호 박사

호주 퀸즐랜드 농수산성 선임연구원


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요 약

악취연속측정기술은 계사에서의 복잡한 악취발생기전을 이해할 수 있도록 할뿐만 아니라 악취발생에 의한 지역사회에 대한 영향을 감소시키는 방안을 수립하기 위해서도 필요한 기술이다.

계사에서 악취농도를 연속적으로 측정하기 위한 전자코 기술을 평가하기 위하여 전자코와 악취관련 시료처리 체계가 갖춰진 이동식 실험실이 계사에 설치되었고 육계 사육기간 동안 악취측정 실험시설의 효과를 분석하였다.

실험 결과는 악취농도의 준 연속적 측정이 가능한 전자코의 개발이 가능하다는 것을 보여주었다. 또한 전자코는 계사의 상태가 악취발생에 미치는 영향정도를 파악하는데 이용될 수 있다.


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HOW TO IMPLEMENT AN ELECTRONIC NOSE FOR CONTINUOUS ODOUR MONITORING IN A POULTRY SHED

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ABSTRACT

Continuous odour monitoring technologies are necessary to understand complex odour generating mechanisms within poultry housing as well as to identify strategies to reduce the impact of odour emissions on local communities. To evaluate electronic nose technologies for continuously assessing odour concentration in poultry housing, a mobile laboratory containing an electronic nose and an associated sample delivery system was deployed to a commercial poultry farm and tested over a broiler production cycle. The results demonstrated that it was possible to develop a model to allow an electronic nose to provide a semi-continuous measurement of odour concentrations. The electronic nose was also able to demonstrate the influence of shed conditions on odour emissions.

Key words : Poultry odour, Electronic nose, Continuous odour monitoring, Partial Least Squares

1 INTRODUCTION

Odour issues are a threat to the growth of the poultry industry. The importance of odour issues has escalated due to increasing population densities near poultry farms and the expectation of good air quality by semi-rural communities. Therefore, increasing effort has been made to measure odours in order to improve regulation and development of odour control strategies for poultry farms. These actions aim to reduce negative public attitudes towards poultry farming (GHD, 2003; PAE, 2003; Jiang & Sands, 2000).

Odours from poultry farms are generated predominantly by waste decomposition in the litter. The generation of odour from the litter is influenced by the moisture content and temperature of the litter as well as the amount of manure accumulated in the litter. However, there are few data to corroborate the odour-generating mechanisms in poultry sheds. A more complete data set describing odour emissions from poultry sheds is required.

Current methods for odour assessment rely almost exclusively on dynamic olfactometry. However, olfactometry is limited as an assessment and management tool because of cost (over AUD $400 per sample) and labour requirements (Nimmermark, 2001). Additionally, the non-continuous, discrete nature of odour samples may be suitable for representing steady-state odour emissions, but is not useful for measuring emissions from highly dynamic odour sources. Odour nuisance is often more problematic at night and/or early in the morning when it is impractical to assess odour. Odour dispersion is highly dependent on the wind speed and direction (Gronauer *et al.*, 2003). Consequently, development of a continuous odour monitoring device, independent of the human nose, would be of great benefit.

Recent developments in electronic nose (EN) technology and modern statistical methods including chemometrics and artificial intelligence, provide opportunities to extend the scope of odour measurement. An electronic nose is an instrument consisting of an air sampling apparatus and an array of gas sensors interfaced to a personal computer or an embedded system. A feature that distinguishes an electronic nose from other instruments used for odour measurement is the ability of its sensor array to respond differently to various odours. Each odour may contain hundreds, sometimes thousands, of different Volatile Organic Compounds (VOCs). Classical spectrometry analytical methods such as Gas Chromatography–Mass Spectrometry (GC-MS) are able to identify and measure individual odorous chemical compounds in an odour sample. On the other hand, the electronic nose can react to the "total odour sample" as does a human nose. In the human olfactory sensing system, it is not necessary to separate individual chemicals in the sample as part of an assessment process; the odour is assessed as a whole and the odour is identified using our brain (*i.e.* recognise the odour pattern from memory bank).

Researchers have identified that electronic nose is able to quantify odours in the field and to discriminate between odours from different sources (Gronauer *et al.*, 2003; Steutz & Fenner, 2001). Developments in the statistical techniques required to analyse outputs from electronic nose devices have broadened the scope for electronic nose applications. Bicego *et al.* (2002) identified how an apparent lack of reproducibility of sensors could be compensated using a flexible calibration and recognition tool based on neural networks. Bocorrh *et al.* (2002) postulated that the predictions made with artificial neural networks (ANN) arose from the ability of neural networks to simulate non-linear relationships observed in human perceptions such as taste and odour. Improved statistical methods were used by Guadarrama *et al.* (2002) to discriminate between various VOCs derived from car components.

More recently, Sohn *et al*. (2003, 2004, 2006) and Qu *et al.* (2001) were able to obtain significant relationships between electronic nose output and odour concentration determined by dynamic olfactometry. The former researchers were then able to accurately determine the odour concentration of samples not used to train the electronic nose using an ANN approach. Although errors associated with olfactometry were identified as a constraint to improving the accuracy of odour concentration determination, it was shown that the ANN algorithms used significantly improved the model's ability to predict new samples when compared to alternative linear and non-linear multivariate modelling techniques. Sohn *et al.* (2006) also showed that an electronic nose with a reduced number of sensors could quantify odour concentrations from a specific source. In these approaches, reliable olfactometry data is important because ANNs are trained not only using the sensor outputs of an electronic nose but also the odour concentration results from olfactometry.

The air quality research group in the Department of Primary Industries and Fisheries, Queensland (QDPI&F) has developed and evaluated an electronic nose system. This system includes an array of Metal Oxide Semiconductor (MOS) sensors, which are appropriate for the assessment of odour emissions from intensive livestock industries because of their sensitivity to volatile chemicals found in such odours. The electronic nose is able to provide qualitative information (*i.e.* discriminate between samples from different sources) and predict odour concentrations using a model based on results from olfactometry. This device and associated models were applied to odour derived from a broiler shed.

Two core objectives were investigated in this study. These were to develop an odour prediction model for broiler sheds and to evaluate the continuous odour monitoring capability of electronic nose technology for an "in-shed" application.

2 MATERIALS AND METHODOLOGY

2.1 Experimental program

This study consisted of a laboratory-based investigation and the field-based investigation.

2.1.1 Laboratory-based investigation

Discrete grab samples were collected at a commercial broiler shed in Queensland, Australia during 2005 and 2006. The samples were analysed using the dynamic olfactometry and the electronic nose in QDPI&F. The electronic nose results and olfactometry data were integrated to develop a model to predict odour concentrations.

2.1.2 Field-based investigation

For the field-based investigation, the electronic nose system was deployed in a mobile laboratory stationed alongside the same poultry shed used for collecting discrete odour samples. The electronic nose was used to continuously monitor air quality inside the poultry shed over a complete winter broiler production batch from 09/06/2006 to 24/07/2006.

2.2 Odour sampling site

The samples used for dynamic olfactometry were collected from a broiler shed in Queensland, Australia during the period July 2005 - July 2006. The details of the poultry farm are given in Table 1. A total of 174 discrete grab odour samples were collected from the shed. From June 2006, the electronic nose continuous odour monitoring work was also conducted at the farm using the developed odour prediction model.

Table 1 Details of the poultry farm used for the trial

Farm type	Broiler production
Location	Southeast Queensland
Number of sheds	32 (22 used for broiler production)
Sample shed age	Built 2005
Ventilation (tunnel or natural)	Tunnel
Fan configuration	8 tunnel ventilation fans with circular cowling (cone) and one exhaust fan at the inlet end of the shed (inlet fan)
Fan specifications	48″ (1220 mm) Hired Hand® Mega Flow Fan (45,000 m^3 hr^{-1} @ 0 Pa) fitted with cone. Aluminium shutters and grills
Drinker type	Nipple with cup
Birds per shed	28,000 - 30,000 at start of batch. Harvest 50% of birds around the end of week 5

Litter type	Shavings
Average batch age	51 days
Baffle system	None - roof has shallow pitch
Shed dimensions	14.8 m × 120 m × 2.6 m (W× L× H). 3.1 m to apex
Fan dimensions	Cowling: 1500 mm, Blade: 1200 mm
Shed cleaning products	Prolong (insecticide) and Formaldehyde

2.3 Odour sampling and measurement of grab odour samples

2.3.1 Polyethylene odour sampling duct

A single tunnel ventilation fan on the broiler shed was fitted with a temporary duct (Figure 1) to provide well mixed (homogenous) air samples. Duct dimensions and sampling point were designed to comply with AS 4323.1 (2001a). The duct was constructed from 200 µm polyethylene sheet. The duct was 1.4 m in diameter and 18 m long. Odour samples were collected approximately 13 m from the fan outlet (5 m from exhaust end of duct). It was partially suspended from a tensioned wire that was attached to the top of the duct.

Figure 1 Tunnel ventilated broiler shed showing polyethylene sampling duct attached to an exhaust fan

2.3.2 Ventilation rate measurement

Ventilation fans were locked in or out to maintain a constant airflow during each sampling phase. The shed ventilation rate was calculated by measuring the wind velocity profile inside

the shed using a hot wire anemometer (TSI 8386A Velocicalc™ hot wire anemometer, TSI Ltd, USA) according to the Australian Standard, AS NZS 4323.1: Stationary Source Emission Method 1 (2001b). Thirty two measurement points were used to describe the ventilation rate profile across the shed. These are shown schematically in Figure 2.

Measurements were collected under the last baffle, closest to the fans. A velocity measurement was taken at each sampling point across the sampling traverse/s. Ten averaged measurements were collected at each sampling point using the averaging capabilities of the hot wire anemometer. An average of all measurements across the shed was then used for ventilation rate calculations.

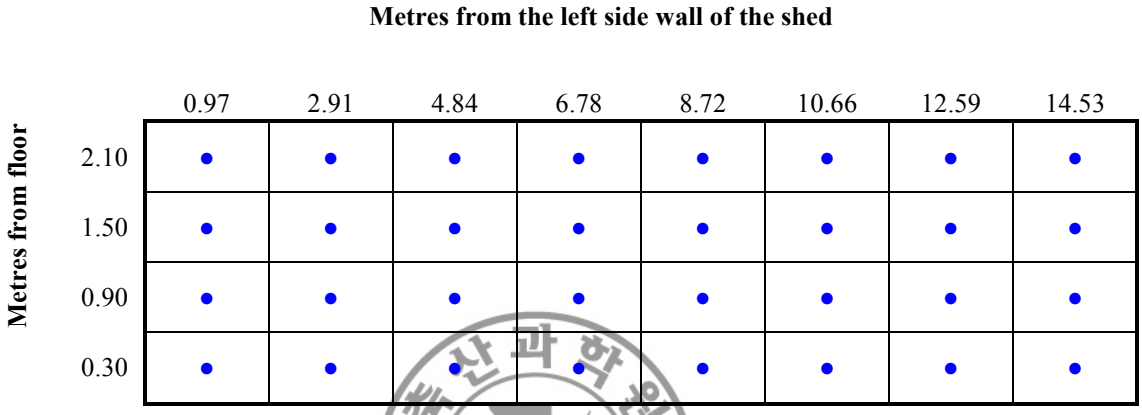

Figure 2 Sampling point positions for ventilation rate measurements according to the Australian Standard 4323.1: Stationary Source Emission Method 1

2.3.3 Sampling probe and sample collection

Samples of the odorous air were drawn from the duct through a stainless probe and polytertrafluoroethylene (PTEE) tubing into 120 L Melinex™ bags (Polyethylene Terephthalate). All components used for sampling were manufactured from stainless steel or PTFE.

An empty sample bag was placed into a rigid 120 L sample drum customised for odour sampling work. One end of a PTFE tube was connected to the sampling probe and the other end attached to a sampling drum fitted with a Melinex™ bag insert. All bags were preconditioned by filling with odorous air from the probe then emptied prior to the sample being collected.

Triplicate samples were collected concurrently. Two samples were used for olfactometry analysis and the other sample was used for electronic nose measurement. Each triplicate sample was collected over a period of approximately ten minutes. The sampling drums were then sealed and transported to the laboratory for analysis by dynamic olfactometry and electronic nose.

All samples was analysed within two to six hours of collection in order to minimise the effect of sample storage. Each bag was used once and discarded after analysis.

2.4 Continuous odour sampling and measurement

2.4.1 Mobile laboratory

An air conditioned, insulated shipping container was used to construct a mobile laboratory where the electronic nose was housed in a temperature controlled, dust-free condition. The mobile laboratory was deployed to the sampling site on 05/06/2006 and was operated over a complete batch for the continuous odour monitoring work.

2.4.2 Air sample delivery system

A system to deliver sample air to the mobile laboratory was built using 110 mm polyvinyl chloride (PVC) stormwater pipe. The length of the air sampling delivery system was 25 m from the sample inlet to the mobile laboratory. The air sampling inlet was located half way across the shed and 10 m back from the exhaust fan end of the shed. The sampling inlet was positioned 1 m above the litter. The sample air was drawn through the duct at a velocity of 6.25 m s^{-1} using an axial type fan. The retention time in the PVC pipe was four seconds. As the retention time was only four seconds, it was assumed that odour emissions from the PVC pipe material were minimal, and that alteration of the shed air composition during the sample transfer would be negligible.

2.4.3 Sub-air sample collection for electronic nose analysis

Air was sub-sampled from the duct and delivered to the electronic nose. Sub-samples were continuously collected using a customised sampling port with four branches and four sampling holes per branch to ensure maximum gas sampling efficiency from the main air sample delivery system. Sixteen sampling points were quadratically spaced acrosss the sampling port. This hole configuration helped to minimise the errors due to any non-uniformity of the air profile inside the duct and thus ensured that samples were representative of bulk air derived from the shed. According to the numerical simulation test carried out by Loubet *et al.* (1999), this type of sampling port showed a theoretical sample recovery efficiency of 100.4 % while a single point sampling port showed a sample recovery efficiency of only 61 %. The sampling port was installed inside the 110 mm PVC pipe.

Dust was removed from the air samples using a custom built PM_{10} dust filter (Type A/E glass fibre 110 mm PALL filter) and then delivered to the electronic nose through the air sample distributor.

2.5 Electronic nose

The electronic nose consisted of 24 different MOS sensors. The sensors were installed in three different types of stainless steel sensing chambers. The results from three different sensing chambers were integrated and analysed together. The details of these sensing chambers are presented in Table 2.

Signals from all sensors were collected at a sample rate of 60 Hz using a DT 800™ data logger (DataTaker®, www.datataker.com) The temperature, relative humidity and sensor responses were monitored and stored using a real-time data logging program developed using Labview 7.1™ (National Instruments, Austin, Texas, USA). Odorous air samples were presented to the sensing chamber of the electronic nose at a flowrate of 500 mL min^{-1}.

A temperature and RH calibration model developed using chemometric approaches (Sohn *et al.*, unpublished) was applied to the raw sensor responses of the electronic nose. The adjusted temperature and RH values of the electronic nose outputs were 25 ^{o}C and 25 %, respectively.

Table 2 Summary of sensing chambers used for the electronic nose

Sensing chamber	Sensor type	Number of sensors	Shape	Internal volume (mL)	Material	Features
Prototype 1	MOS	12	Hexahedron	575.0	Stainless steel	n/a
University of Pisa	MOS	6	Circular cylinder	35.2	Stainless steel	Internal flow distributor
Prototype 2	MOS	6	Circular cylinder	23.5	Stainless steel	Temperature modulation

The schematic of DPI&F's electronic nose and associated sample delivery system is depicted in Figure 3.

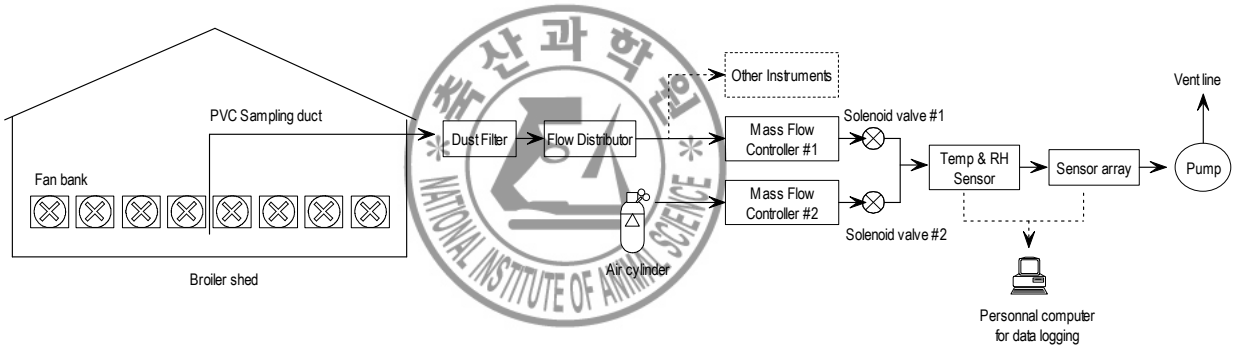

Figure 3 *Electronic nose experimental set-up for continuous monitoring of odours from a broiler shed*

3.6 Olfactometry analysis

Odour concentrations were determined using the eight-panellist, triangular, forced-choice dynamic olfactometer developed by the Department of Primary Industries and Fisheries which has been described previously (Nicholas *et al.*, 1999; Zeller *et al.*, 2002). This olfactometer was constructed to comply with the Australian/New Zealand Standard for Dynamic Olfactometry (AS4323.3) (2001b), hereafter referred to as "the Standard". The conduct of the odour assessment also complied with the Standard.

During a typical odour sample assessment routine, each panellist was first screened with the reference gas (*n*-butanol) to ensure that his or her detection threshold was within the required concentration range of 20–80 ppb (v/v). Thereafter, the odorous sample was diluted and presented to the panellists in one of three ports, while the other two ports emitted clean, odour-free air. The panellists were required to sniff from the ports and determine whether they could detect a difference between the three ports. Each panellist was allowed a maximum of 15 s for this assessment. The panellists indicated via a keypad whether they were certain,

uncertain or guessing that one of the ports was odorous, as well as from which port the odour (if detectable) was emitted.

This process was repeated, doubling the concentration of odorous air of the previous presentation each time, until each panellist had entered a "certain and correct" response for two consecutive presentations. Each panellist's individual threshold estimate ($\overline{Z}_{ITE}$) was then determined by calculating the geometric mean of the dilution at which the panellist did not respond with certainty and correctly and the first of the two dilutions where the panellist did respond with certainty and correctly. A complete dilution series is defined as a round. Three rounds were completed for each sample provided sufficient sample was available.

At the end of the three rounds, the results of the first round were discarded in accordance with the Standard. The results from rounds two and three were then geometrically averaged ($\overline{Z}_{ITE}$). The ratio between Z_{ITE} and $\overline{Z}_{ITE}$ is defined as ΔZ. The calculation of ΔZ is presented in the following equations:

$$if\ Z_{ITE} \geq \overline{Z}_{ITE}, then\ \Delta Z = \frac{Z_{ITE}}{\overline{Z}_{ITE}} \quad \text{(Eqn. 1)}$$

$$if\ Z_{ITE} \leq \overline{Z}_{ITE}, then\ \Delta Z = \frac{\overline{Z}_{ITE}}{Z_{ITE}} \quad \text{(Eqn. 2)}$$

If ΔZ is greater than ± 5 then all $\overline{Z}_{ITE}$ values of the panel member with the largest ΔZ were excluded from the data set. The screening procedure was then repeated, after re-calculation of $\overline{Z}_{ITE}$ for that measurement. If a panel member again did not comply, the results for this panel member (with the largest ΔZ) were omitted. This was repeated until all panel members in the dataset had an acceptable ΔZ value. The last value of $\overline{Z}_{ITE}$ was then defined as the odour concentration and expressed as odour units per cubic metre (OU m^{-3}).

2.7 Development of odour prediction model

2.7.1 Data pre-processing

Raw voltage responses from the electronic nose were converted to sensor resistances for further data analysis. The data derived from the 24 sensors, plus temperature and relative humidity data, were stored in a personal computer (PC) in a binary format. Pre-processing algorithms were then applied to scale and normalise the input data prior to conducting principal component analysis (PCA). One hundred and seventy four data files were available from the grab odour samples. Over five million data points were generated from the continuous odour monitoring trial. The pre-processing work and data analysis was conducted using the SPSS™ statistical package and the Partial Least Squares (PLS) Toolbox 3.5™ for Matlab™.

2.7.2 Outlier handling

Before applying any modelling technique, the identification and removal of outliers (samples significantly different from homologues belonging to the same population) was required. As samples of each category were obtained, in our case, by replicates of the same measurement

procedure, a multivariate normal distribution around an ideal category representative might be expected, with deviations from that point being due to experimental errors. However, large deviations may be generated by random errors either in the sample preparation and measurement, or in the data acquisition and treatment, so that it is not possible to consider all the data as a representative of that category.

PCA was used to display data and detect outliers, by using the Q and T^2 diagnostic tests. Q is defined as the sum of the squares of residual matrix of each sample and indicate how each sample conforms to the PCA model. The T^2 test, known as Hotelling's T^2 statistic, is a measure of the variation in each sample within the PCA model.

Samples identified as outliers (greater than three standard deviations from the mean value, $p < 0.001$) were removed.

2.7.3 Partial least squares

PLS regression is a method for constructing predictive models when many factors exist and are significantly redundant like the results from an electronic nose. PLS regression has been used in various disciplines such as chemistry, economics, medicine, psychology, and pharmaceutical science where predictive linear modelling, especially with a large number of predictors, is necessary. PLS regression has become a standard tool for modelling linear relationships between multivariate measurements in chemometrics.

PLS regression is an extension of the MLR model (*e.g.*, Multiple Regression or General Stepwise Regression). In its simplest form, a linear model specifies the (linear) relationship between a dependent (response) variable *Y*, and a set of predictor variables, the *X's*, so that

$$Y = b_0 + b_1 X_1 + b_2 X_2 + \Lambda + b_p X_p \qquad \text{(Eqn. 3)}$$

In this equation, b_0 is the regression coefficient for the intercept and the b_p values are the regression coefficients (for variables 1 through p) computed from the data.

3 RESULTS AND DISCUSSION

3.1 Odour prediction model

The odour prediction model was applied to predict odour concentrations from sensor output data of the electronic nose. The odour concentrations predicted by the model were then used to investigate the relationship between odour concentrations inside the poultry shed and factors such as climate, bird age, ventilation rates and other variables associated with the broiler production cycle.

The odours emitted from similar odour sources have similar chemical characteristics and should therefore, show similar sensory patterns. The sensory pattern results for 12 sensors to four odour samples are depicted in Figure 4. As shown in Figure 4, the sensory patterns from grab samples are quite similar. In addition, MOS sensor types B, C, E, F, H and J showed greater sensitivity to poultry odours than other sensor types. Therefore, these sensors were the major contributors in the process used to build the odour prediction model.

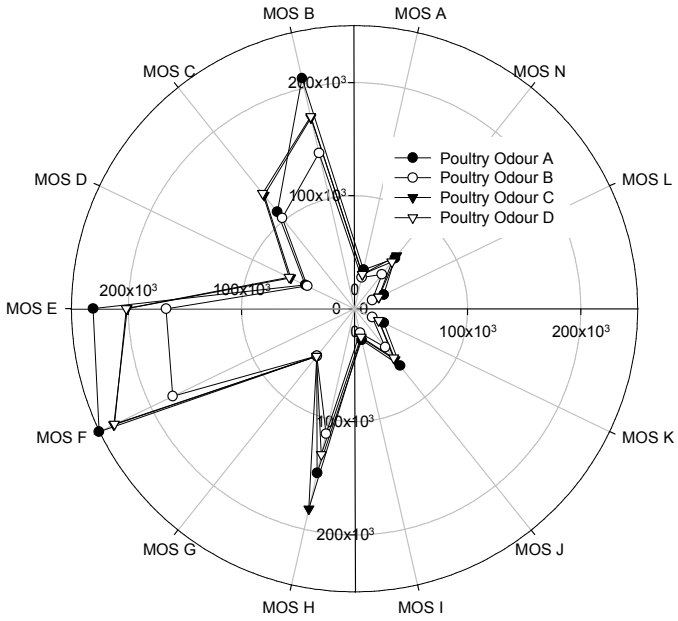

Figure 4 Responses of 12 metal oxide semiconductor sensors to four poultry odour samples

In order to develop an odour prediction model, the odours emitted from an odour source need to be clearly separated from other odours. As presented in Figure 5, the PCA results showed clear separation between poultry odour and the control (clean air from instrument-grade air cylinder). The scores of Principal Component 1, *X-axis* of the graph, show the difference between poultry odour and clean air. The scores of Principal Component 2 plotted along the *Y-axis* of the graph, indicate different odour concentrations of samples.

The results of olfactometry and the electronic nose were used to develop an odour prediction model using PLS. The scatter plot of actual odour concentrations and the predicted PLS model output (scaled into the odour concentrations domain) for the test data is shown in Figure 6. A pre-processing algorithm (*i.e.* autoscale) and outlier handling technique were applied to improve the performance of the model.

As the electronic nose outputs had 26 dimensions (*i.e.* 24 MOS, one temperature and one RH sensor), data compression and dimensionality reduction of the data (*i.e.* 26 dimensions to two or three dimensions) was required. Otherwise, the prediction model tends to 'saturate' and can not predict odour concentrations accurately because of 'over-fitting' or 'under-fitting' issues. During the pre-processing stage of the data analysis, PCA acts as a decorrelator of variables and maximizes the variance within the data and finally, transforms variables into latent variables having less dimensionality.

Three latent variables (*i.e.* compression of the data to three dimensions) resulting from the PCA pre-processing work were used to develop the odour prediction model. The three latent variables capture 76.78 % and 93.87% of the variances of the original electronic nose sensor array outputs and olfactometry data, respectively. Therefore, the three dimensional compressed data was able to adequately represent the original data set having 26 dimensions. The results of the data compression process using PCA are presented in Table 3.

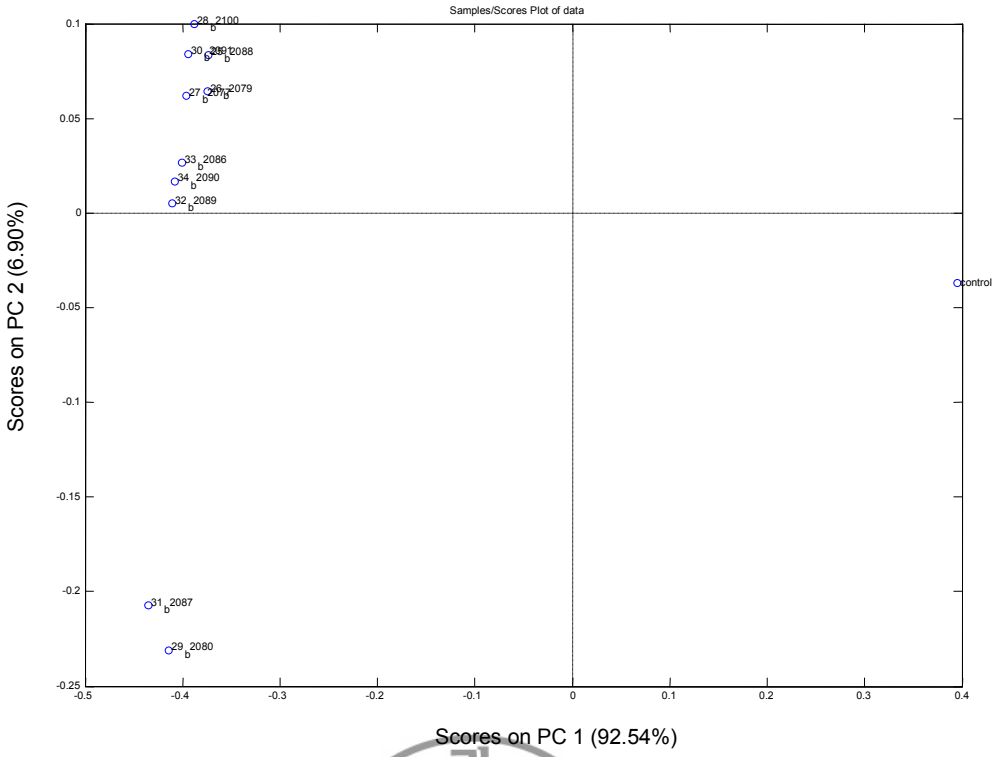

Figure 5 Principal Component Analysis comparison between the odour samples collected from a poultry shed and clean air control

Table 3 Percent variance captured by the odour prediction model

Latent variables	Electronic nose outputs		Olfactometry outputs	
	Latent variables (%)	Cumulated variables (%)	Latent variables (%)	Cumulated variables (%)
1	62.60	62.60	18.46	18.46
2	10.42	73.02	40.39	58.85
3	3.75	**76.78**	35.02	**93.87**

The root-mean-square error of calibration (RMSEC) and the root-mean-square error of cross-validation (RMSECV) are often used to evaluate the performance of a predictive model. The RMSEC is a measure of how well the model fits the calibration data. In contrast, the RMSECV is a measure of a model's ability to predict new samples.

The values for the correlation coefficient (r), the RMSEC and the RMSECV of the model were 0.94, 179.79 and 183.23, respectively. These values implied that the developed model output is expected to have maximum error range of ± 183.23 odour unit (OU m^{-3}) when unknown odour samples are presented to the model.

The predicted odour concentrations obtained by the PLS model are well distributed around the ideal 1:1 straight line as shown in Figure 6. No skewness was observed. In addition, the model explained most of the variances provided in the electronic nose and olfactometry datasets. It can be concluded, therefore that the PLS model is able to predict the odour concentrations from the poultry shed accurately with a high correlation coefficient value.

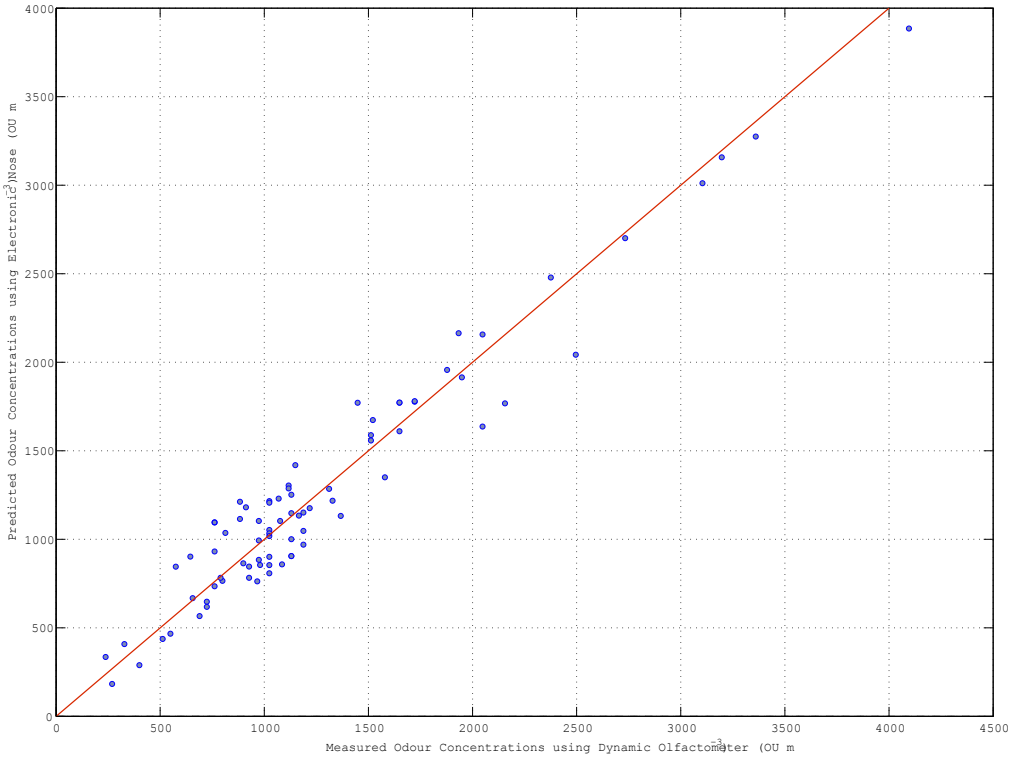

Figure 6 Comparison of predicted odour concentration using the developed Partial Least Squares model and actual odour concentrations measured by olfactometry

The sensor data arising from analysis of four odour samples collected on 24/07/2006, was not used to develop the prediction model. These results were presented to the model to validate the prediction of odour concentrations. The measured odour concentration results were then compared with those predicted by the electronic nose as shown in Figure 7. From the comparison plots, it was observed that the predicted odour concentrations were well correlated with the actual odour concentrations measured by olfactometry. The value for the correlation coefficient (r) of this validation trial was 0.89.

Odour quantification using the PLS technique allows prediction of odour concentrations from the sensor response of the electronic nose with a high level of confidence. However, one must keep in mind that the regression process must only be used for interpolations. In addition, this odour quantification technique needs an adequate number of reliable odour results from dynamic olfactometry to train the model. A rule of thumb suggests that at least 50 olfactometry results are required to develop a reliable prediction model.

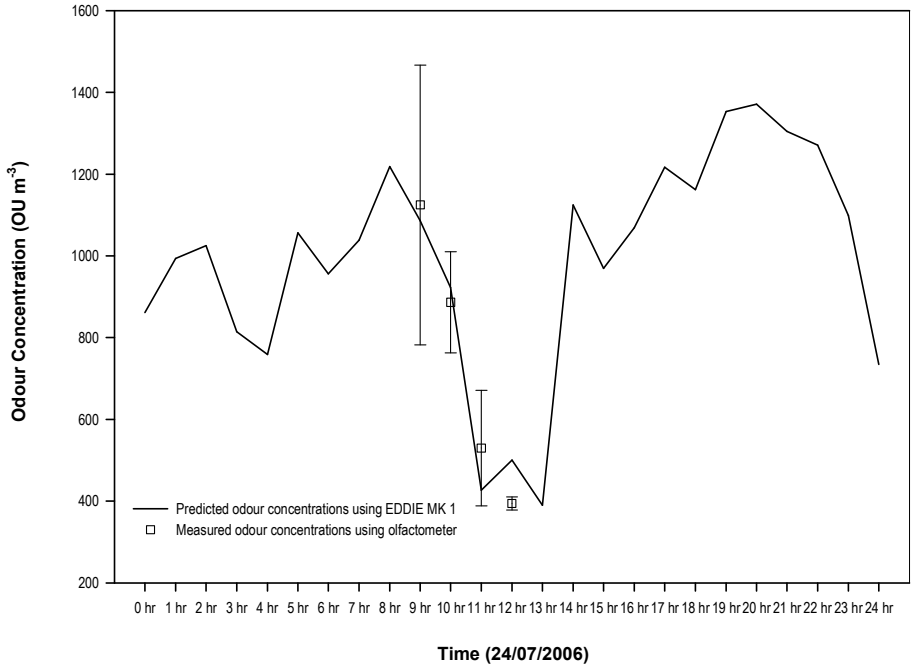

Figure 7 Comparison results of predicted odour concentrations using the electronic nose and the actual odour concentrations measured by olfactometry

3.2 Diurnal variation of the shed air quality

The typical pattern of diurnal variations of the electronic nose responses is shown in Figure 8. The sensor outputs of the electronic nose show a strong relationship to ventilation rate. Electronic nose outputs tend to increase during the night and to decrease during the day as an inverse function of ventilation rate. Increases in ventilation rate reduced the value of the electronic nose outputs (*i.e.* odour concentrations), indicating dilution of odour by the larger volumes of ventilation air. The results were consistent with the prediction of odour concentrations using the Pacific Air & Environment (PAE) model (PAE, 2003). The PAE model shows an inverse relationship with ventilation rate (*i.e.* constant × 1/ventilation rate), giving maximum concentrations under low ventilation conditions (*i.e.* night) and minimum concentrations under high ventilation day time conditions.

Figure 8 provides the following information:

a) As the operation of fan No. 4 was increased from 50 % to 100 % and the inlet fan started to operate continually at around 9:00 am, the predicted odour concentration started to decrease;
b) When fan No. 8 started to work at 95 % operation cycle around 11:00 am, the predicted odour concentration decreased further, reaching a minimum around 4:00 pm;
c) As the ambient temperature dropped during the afternoon, fan No. 8 turned off to reduce heat loss. Consequently, sensor response increased because of reduced dilution; and
d) The operation of fan No. 4 and the inlet fan was decreased to 50 % at 5:00 pm. The predicted odour concentration increased to a more stable value.

It must be stressed, however, that the relationship between odour concentration and ventilation rate is not simple. While ambient temperature is the major driver of ventilation (GHD, 2003), shed ventilation is influenced by a number of interrelated factors, including the

physical location of the temperature sensor, the number and location of operating fans and the extent of opening of side vents. These factors all contribute to the aerodynamic complexities within the shed. The processes whereby odour is generated within the shed are also probably complex. Diet composition, stage of growth and litter characteristics will all probably influence shed odour concentrations. The inter-relationship between odour concentration and ventilation status then determines the odour emission rate.

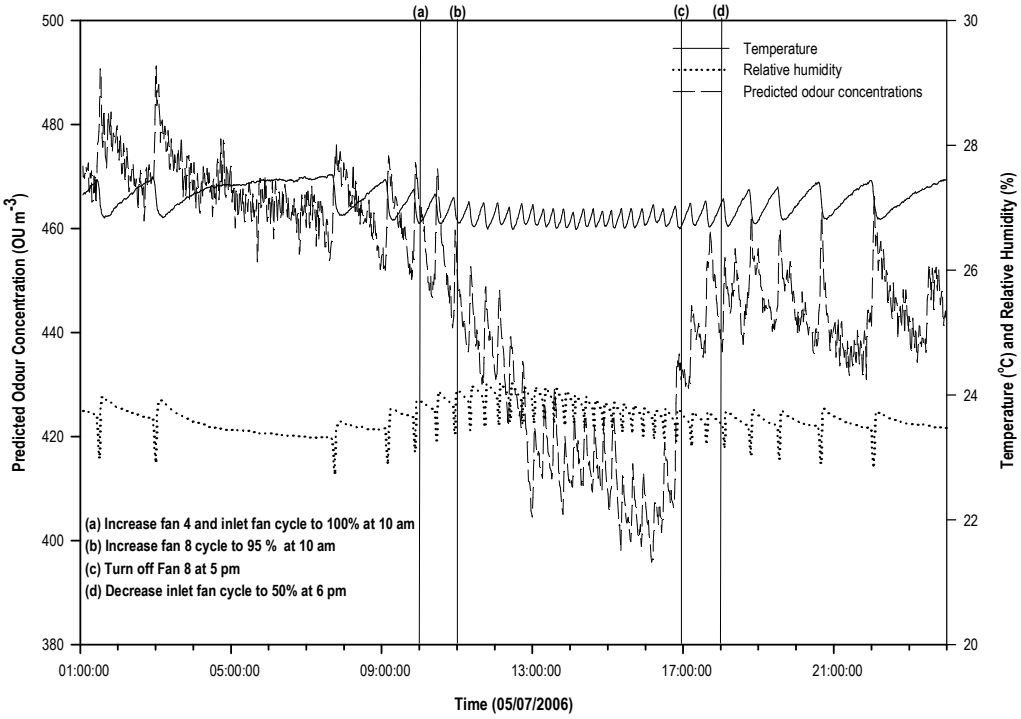

Figure 8 Diurnal variation of the predicted odour concentrations by the electronic nose and the Partial Least Squares model at the broiler shed used for experimentation

3.3 Continuous odour monitoring over a broiler production cycle

To continuously monitor air quality inside the shed, an odour monitoring system is required to detect the events that may influence odour concentrations inside the shed. The electronic nose and associated model provides this capability. The events include the stage of production cycle, bird stocking density and changes in ventilation rate.

The effect of the batch age on shed odour emission can also be observed in Figure 9. Other researchers have reported that the shed odour emission rates show a roughly linear increase until the first harvest at week five – thereafter the odour emission stabilised (Clarkson & Misselbrook, 1991; GHD, 2003).

During the first nine days of the batch, the predicted odour concentrations were about 300 OU m^{-3} due to the minimal daily ventilation rates. The average daily ventilation rates at this stage ranged from 1.2 to 12.1 m^3 s^{-1}. From the 10^{th} day of the batch, the predicted odour concentration results were about 100 OU m^{-3} for a week, and then continuously increased until the 24^{th} day of the batch from 200 OU m^{-3} to 700 OU m^{-3}.

Before the first harvesting at 35 days, the predicted odour concentration results decreased again due to the increase of daily ventilation rate. During this period, the daily ventilation rates were increased from 104.9 to 516.5 $m^3 s^{-1}$. After the harvesting on 08/07/2006, the predicted odour concentrations inside the shed stabilised at about 500 OU m^{-3} and showed fluctuations according to the changes in ventilation rate. The predicted odour concentration results then increased due to three days of rainfall as shown in Figure 9.

Figure 9 illustrates the odour prediction results using the electronic nose and the PLS model during the winter batch. It confirms that the electronic nose can detect events that are closely related to changes in air quality. Around 50 – 60 % of birds are harvested at the end of week 5 of the production cycle. The reduced bird mass and bird volume results in a corresponding reduction in the rate of manure excretion, disturbance of the litter surface and therefore, odour emissions from the shed are reduced. The sensor array response showed a significant decrease over the three days following removal of 50 % of birds.

However, care must be taken when using these values because the odour emission results are based on one specific trial conducted at a single broiler farm. Factors such as season, shed design, litter moisture level, and litter type may affect the odour concentration inside the shed. It was not possible within the scope of the project to generate representative odour emission values using the odour concentrations derived from an olfactometry for all possible conditions inside the shed.

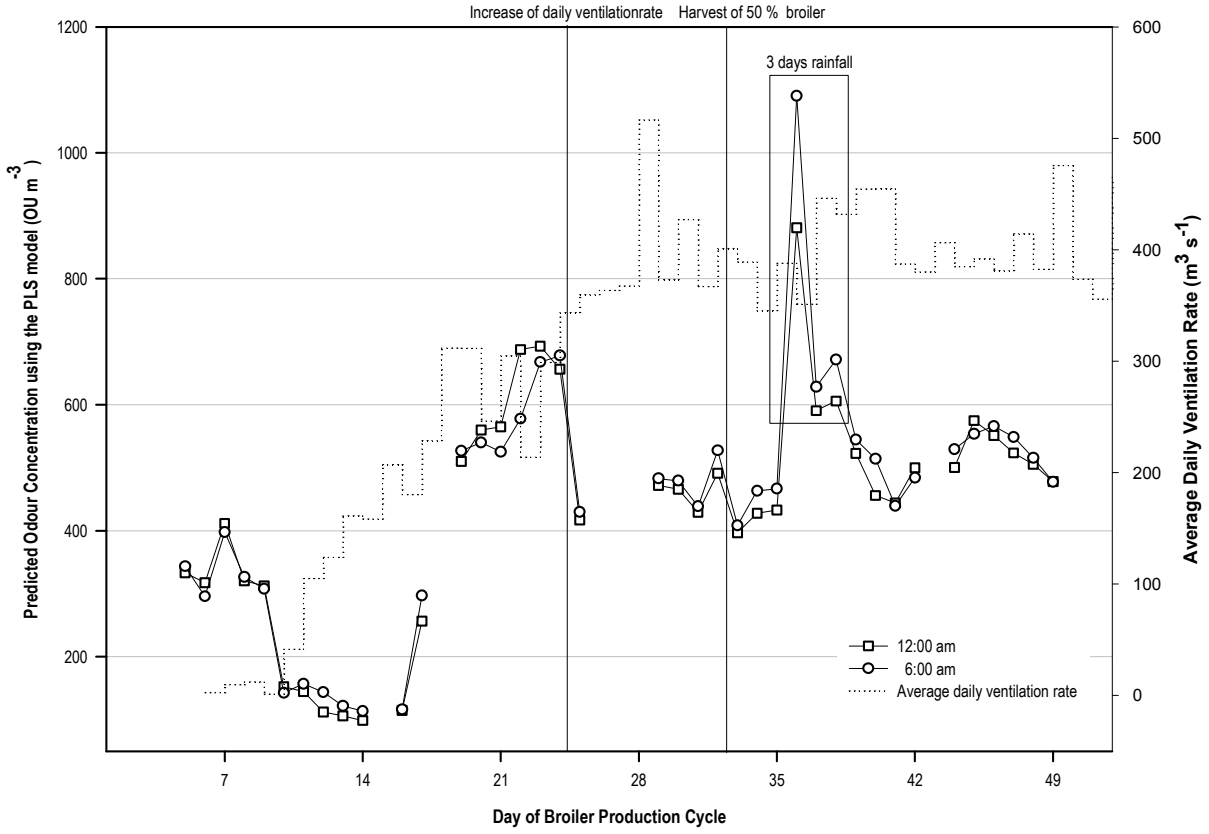

Figure 9 Odour concentrations predicted by the electronic nose during the winter batch at a commercial broiler farm

4 CONCLUSIONS

This study has demonstrated that:

a) It was possible to develop a model to allow an array of sensors to provide a semi-continuous measurement of odour concentrations;
b) The model output appeared creditable and accurate;
c) The model identified specific periods when events altered shed odour concentrations including rainfall and changes in bird stocking density;
d) It appeared possible to use an electronic nose to demonstrate the influence of shed conditions on odour emissions continuously and in real-time.
e) Effective olfactometry assessment requires a large number of odour samples to be collected and analysed. Due to the cost of sample collection and analysis, olfactometry is considered a costly method. These factors make it difficult to evaluate the efficacy of odour management by using olfactometry. Electronic nose can be a cost-effective odour measurement method to assist olfactometry assessment; and
f) For the first time, it has been demonstrated that electronic nose provides accurate, affordable and real-time odour measurement capability.

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Mitigation of N_2O Emission From Animal Waste Composting

가축분 퇴비화 과정에서 아산화질소 저감기술

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요 약

가축폐기물의 처리과정에서 다량의 아산화질소가 발생할 수 있는 가능성이 있다. 퇴비화과정에서 질소의 축적은 아산화질소 방출과 연관관계를 지니고 있다.

돼지분뇨 퇴비화과정에서 질소축적을 억제하기 위한 목적으로 질산화박테리아(NOB)의 식종이 아산화질소의 방출에 미치는 영향에 대해 조사하였다. 부숙된 돈분퇴비(MSC)가 질산화박테리아(NOB) 공급원으로 사용되었다.

고온발효된 돈분퇴비에 부숙된 돈분퇴비(MSC)를 첨가하였다. 부숙된 돈분퇴비(MSC)의 첨가는 질소축적을 완화하고 질산화를 촉진하는 반면에, 부숙된 돈분퇴비(MSC)가 첨가되지 않은 퇴비에서는 독작 질산화박테리아(NOB)의 생장이 암모니아 산화박테리아(AOB)에 비해 상대적으로 지연됨으로 인해서 퇴비단에 질소축적현상이 발생하였다.

퇴비단에서 아질산의 발생은 아산화질소의 발생과 비슷한 형태를 지니게 되기 때문에 부숙된 돈분퇴비(MSC)를 첨가할 경우 아산화질소의 방출은 즉각 중단되었다. 부숙된 돈분퇴비(MSC)의 첨가여부에 의한 퇴비단으로부터 아산화질소의 방출율은 각각 17.5 와 88.5 g N_2O-N kg^{-1} T-N(개시시)이었다(아산화질소 방출율 감소비율은 80%임).

질산화 균체의 구성비율개선은 가축분뇨 퇴비화시 아산화질소의 방출을 효과적으로 감소시킬 수 있을 것이다.


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Mitigation of N_2O Emission From Animal Waste Composting

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ABSTRACT

Livestock waste treatment holds a large nitrous oxide (N_2O) potential with global emission. In the composting process, accumulation of nitrite correlates with the N_2O emission. In the present study, inoculation of nitrite-oxidizing bacteria (NOB) during composting of swine feces was conducted to inhibit nitrite accumulation, and its effect on N_2O emission was evaluated. Mature swine-feces compost (MSC) was used as the source of NOB (NOB content: 10^6 MPN g^{-1}). The MSC was added into the composting swine feces after the thermophilic fermentation. The addition of MSC prevented nitrite accumulation, promoting oxidation to nitrate, whereas the nitrite accumulation occurred in the material which MSC was not added as the result of the delayed growth of indigenous NOB compared with that of ammonia-oxidizing bacteria (AOB). The patterns of nitrite in the material agreed with that of N_2O emissions; therefore, the emission of N_2O ceased rapidly when the MSC was added. Emission rates of N_2O from the composting material with or without MSC addition were 17.5 and 88.5 g N_2O-N kg^{-1} T-N (initial), respectively (decreasing rate of N_2O emission was 80%). Improving composition of nitrifying communities not to cause the nitrite accumulation would be effective to reduce N_2O emission from composting of animal waste.

INTRODUCTION

Suitable handling and/or treatment of livestock excretions is important not to cause the serious pollution problems such as water pollution and offensive odor evolution [10]. Composting is a traditional treatment method changing odoriferous and unsanitary livestock waste to odorless, sanitary, and marketable organic fertilizer [9]. However, composting of organic wastes has also caused the emissions of environmental harmful gases including nitrous oxide (N_2O) [4, 6].

N_2O is a strong greenhouse gas [12] and contributes to stratospheric ozone depletion [3]. Global increases in N_2O concentration are primarily due to agriculture, and it was estimated that 65% of anthropogenic N_2O is generated in the livestock sector, mostly from manure [5]. Because the number of domestic animals continues increasing in the world, mitigation of N_2O emission from livestock production systems is important to prevent global warming acceleration.

Nitrification (the oxidation of ammonia nitrogen to nitrate nitrogen by ammonia-oxidizing bacteria (AOB) and nitrite-oxidizing bacteria (NOB)) is a necessary prerequisite for the N_2O emission from stored livestock manures. In composting of livestock waste, nitrification usually starts after the thermophilic composting fermentation because nitrifiers can not be active in the temperature over 40 ^oC [15]. When nitrification starts in the composting process,

however, nitrite tends to be accumulated because of incomplete nitrification. A good correlation between N_2O emission and nitrite accumulation has been observed in organic waste composting [7, 11]. Therefore, there is a possibility that N_2O emission can be reduced by preventing nitrite accumulation in the material during the composting process.

In the present study, inoculation of NOB was conducted during composting of swine feces to prevent nitrite accumulation, and its effect on N_2O emission was evaluated.

MATERIALS & METHODS

Fresh swine feces were collected from the pigpen, and mixed with sawdust to amend the property of swine feces suitable for aerobic fermentation. Thirteen kilogram of the mixture (swine feces + sawdust) was piled into the laboratory-scale composting apparatus which was a stainless steel, airtight box (inside volume of the box, 58 L) with two ports for inlet and outlet air [8]. Continuous ventilation of headspace inside the reactor was conducted by an air pump at a fixed rate of 10 L min^{-1}. N_2O concentration in the inlet and outlet air was continuously measured by an infrared photoacoustic detector (IPD, multi gas monitor type 1312, Innova, Copenhagen, Denmark). The emission rate of N_2O was computed from the amount of ventilation and the concentration differences of N_2O between the inlet and outlet air samples [16].

Emission rate of N_2O (mg t-min^{-1}) = {conc. of outlet air (mg m^{-3}) – conc. of inlet air (mg m^{-3})} × measurement interval (t/60) × ventilation rate (m^3 $hour^{-1}$)

Mature swine-feces compost (MSC) aged over one year was used as the source of NOB. This MSC contained NOB at 1.7×10^6 MPN g^{-1} WM. The MSC was added into the composting material at an additional rate of 10% (w/w) after the thermophilic phase of composting to avoid decreasing the number of nitrifiers added at high temperature. Physicochemical parameters (moisture content, pH, Kjeldahl-N, ammonium-N, nitrite-N, nitrate-N and biochemical oxygen demand (BOD)) and population sizes of nitrifiers were analyzed about the compost materials which were sampled at the time of pile turning [1, 2, 13, 14, 17].

RESULTS

Temperature & BOD

After the start of composting, the temperature rose approximately 60 ^oC, and rerising of temperature was observed after the turning at the first and second week. The temperature never exceeded 30 ^oC after the turning at the third week and dropped closer to the ambient temperature. BOD concentration was decreased dynamically from 22 to 2% during the first 3 weeks. From these results, we determined that the thermophilic phase of this composting was restricted to the first 3 weeks, and MSC was added after the turning at the third week.

Nitrifiers and inorganic nitrogen compounds

At the start and during the thermophilic phase, there were very few nitrifiers in the composting material. The first increase of nitrifiers was confirmed at the second week, and it was solely AOB. After the addition of MSC, the NOB population remained high (approximately 10^5 - 10^7 MPN g^{-1} DM) until the end of the experiment. On the other hand, a long lag in proliferation of indigenous NOB was observed in the control (without MSC

addition), and it was in the eighth week that NOB was first observed to exceed the detection limit. The number of NOB in the control increased gradually, and it took a long time to recover the community of nitrifiers capable for complete nitrification (Fig 1).

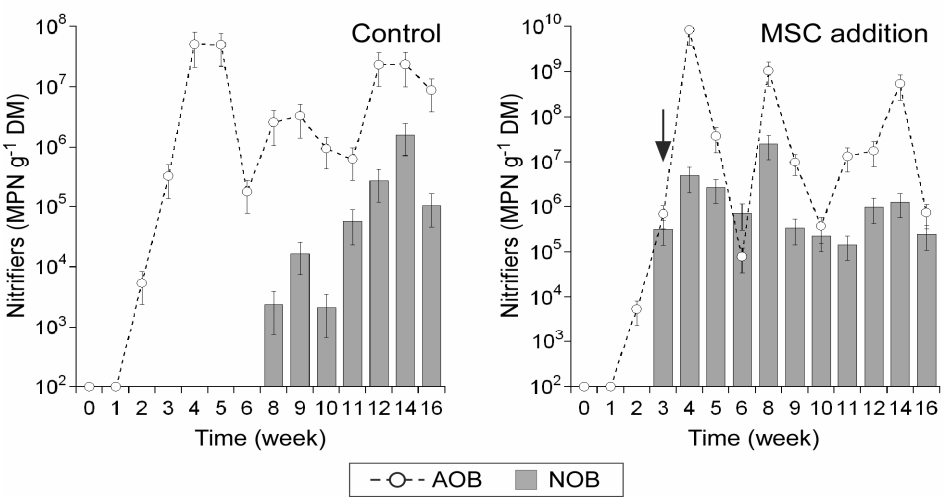

Fig 1. Changes in MPN of ammonia-oxidizing bacteria (AOB) & nitrite-oxidizing bacteria (NOB) in compost material during swine feces composting. Arrow indicates addition of MSC. Error bars indicate 95% confidence limits. (adapted from Fukumoto et al., 2006)

In the initial material, ammonium accounted for most of the inorganic N, and it began to decline after the peak formation at first week. Instead of ammonium decline, nitrite and nitrate started to increase. In the control, nitrite formed a peak at the fourth week and then declined gradually until the 14th week, while it was not detected at the fifth week in the treatment of MSC addition. These patterns of nitrite seemed to be reflected by the changes in the population of nitrifiers. In the treatment of MSC addition, nitrate was quickly increased after the MSC addition, and reached to considerable higher level than the control (Fig 2).

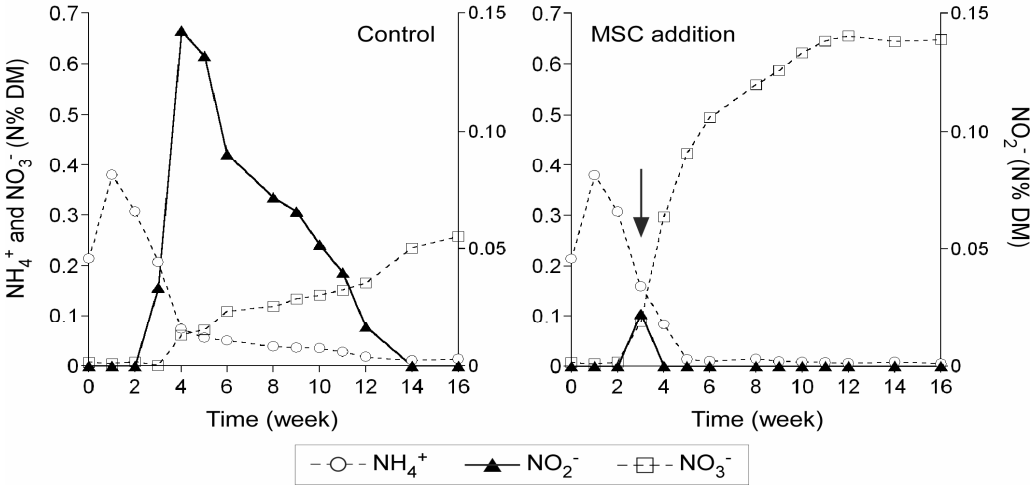

Fig 2. Changes in the concentration of inorganic nitrogen compounds during swine feces composting. Arrow indicates MSC addition. (adapted from Fukumoto et al., 2006)

N_2O emission

During the first 3 weeks, N_2O emissions were changed at low level in the both control and treatment of MSC addition. The emission began to increase between second and third weeks, and then formed the highest peak after the turning at the third week. In the control, the emission was declining gradually, however it kept the N_2O concentration that is clearly higher than the background level for a long time. In contrast, N_2O emission ceased within 1 week after the peak in the treatment of MSC addition. These patterns seemed to reflect the changes in the nitrite concentration in composting material (Fig 3).

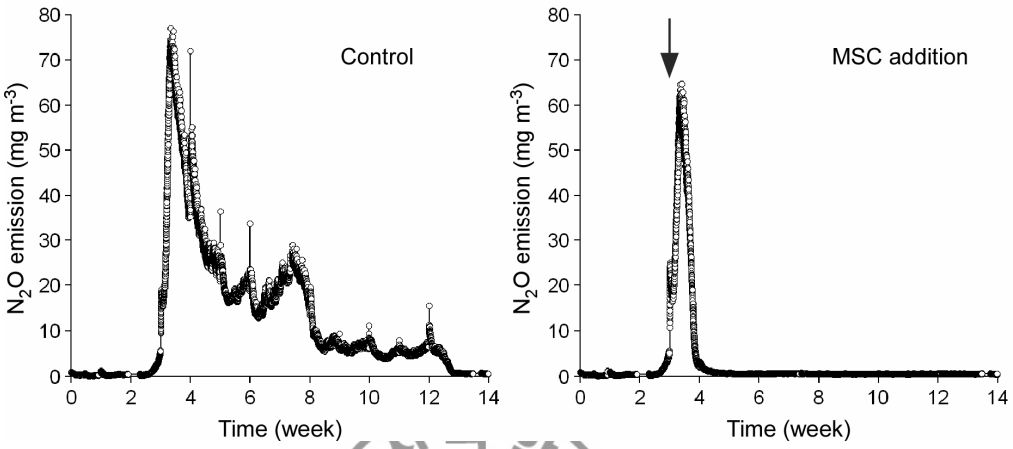

Fig 3. Patterns of N_2O emissions during swine feces composting. Arrow indicates MSC addition. (adapted from Fukumoto et al., 2006)

Total amounts of N_2O emission in the control and treatment of MSC addition were 10.4 and 2.0 g N_2O-N, respectively. The emission rates of N_2O based on the T-N in the initial swine feces in the control and treatment of MSC addition were 88.5 and 17.5 g N_2O-N kg^{-1} T-N (initial), respectively. Decreasing rate of N_2O emission from the control to treatment of MSC addition was calculated as 80%; therefore, it was found that the addition of MSC which contained adequate number of NOB capable for complete nitrification was effective to reduce N_2O emission from composting of swine feces.

CONCLUSIONS

Accumulation of nitrite, which was derived from a lag in proliferation of indigenous NOB, seemed to be a cause of significant N_2O emission during swine feces composting. Addition of MSC which contained adequate number of NOB capable for complete nitrification was effective to prevent nitrite accumulation and reduce N_2O emission. Present study was conducted using the laboratory-scale composting apparatus in a controlled laboratory condition. To establish the composting method with low N_2O emission, further study considering the more complex circumstances observed in actual treatment must be necessary.

This paper is revised version of the paper I published in Environmental Science & Technology, 40(21), p6787-6791 (2006).

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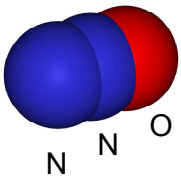
家畜排泄物の堆肥化処理におけるN_2Oの発生制御

Mitigation of N_2O emission from animal waste composting



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Pollution Control Research Team, National Institute of Livestock & Grassland Science, Japan

なぜ家畜排泄物からのN_2Oを制御するのか？

Necessity for control of N_2O emission from animal excretions

- 世界的に増加し続ける家畜頭羽数

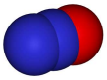
  1.8 billion head (1950) → 4.4 billion head (2002)

  The world domestic animal head count continues increasing in a ratio to exceed population growth.

- 排泄物の量も膨大→N_2Oの発生量も多い

  人為的なN_2O排出量の65%は畜産活動（主に排泄物）に由来する (FAO, 2006. Livestock's long shadow, pp102-123)

  Livestock activities contribute almost two-thirds of all anthropogenic N_2O emissions, and 75-80 percent of agricultural emissions.



堆肥化からのN_2O発生

N_2O emission from animal waste composting

- 硝化が家畜排泄物からのN_2O発生の前提

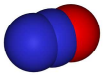
  Nitrification is necessary prerequisite for N_2O emission from stored manures.

- 堆肥化では硝化は高温発酵終了後に開始

  Nitrification starts after the thermophlic fermentation in composting because nitrifiers can not be active in the temperature over 40 °C.

- N_2Oは堆肥化の中期~後期に発生極大が生じる

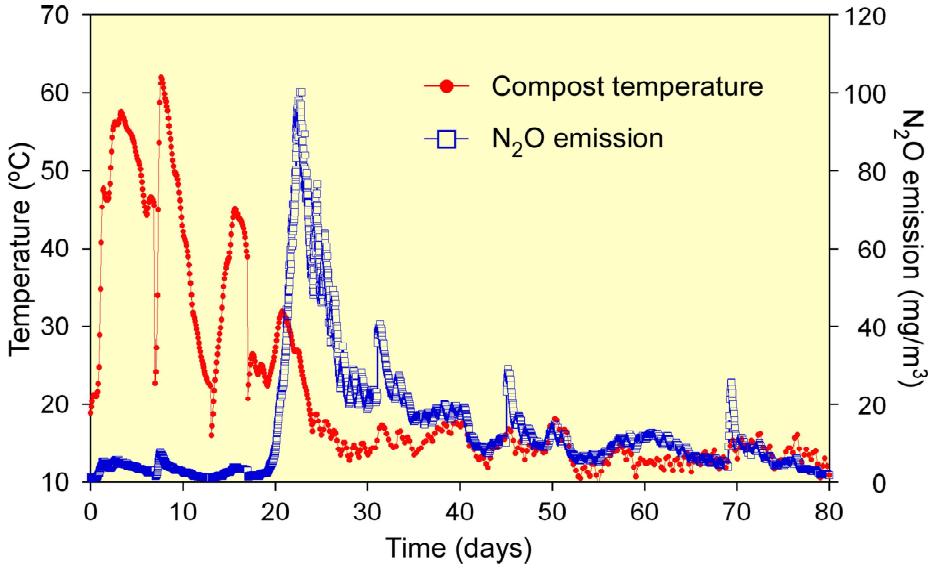
  Most of N_2O is released during the later period of composting.



3

堆肥の発酵温度とN_2O発生

Temperature profile of composting material and N_2O emission during swine feces composting



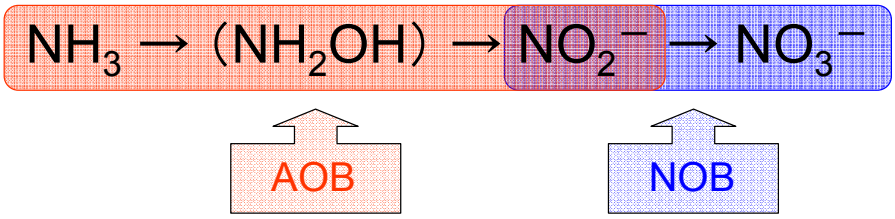
4

N_2O発生に関わる要因①：硝化細菌

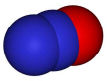
Factor of N_2O emission in composting : Nitrifying bacterium

- アンモニア酸化細菌と亜硝酸酸化細菌

Ammonia-oxidizing bacteria (AOB) & Nitrite-oxidizing bacteria (NOB)

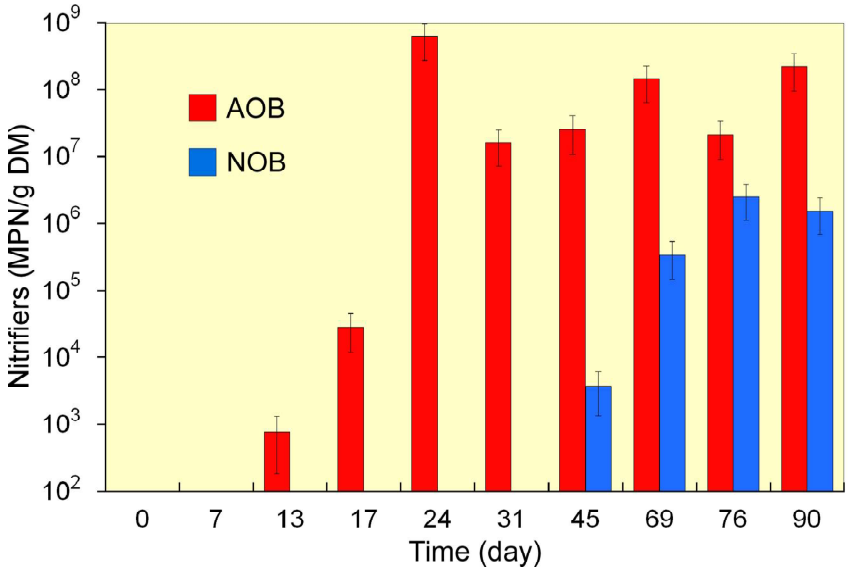


NH_3 is oxidized to NO_3^- by the collaboration of two kinds of microbes. Between these two kinds of microbes, there are differences in characteristics, e.g., proliferation time, sensitivity to toxicities.



堆肥化における硝化細菌数の推移

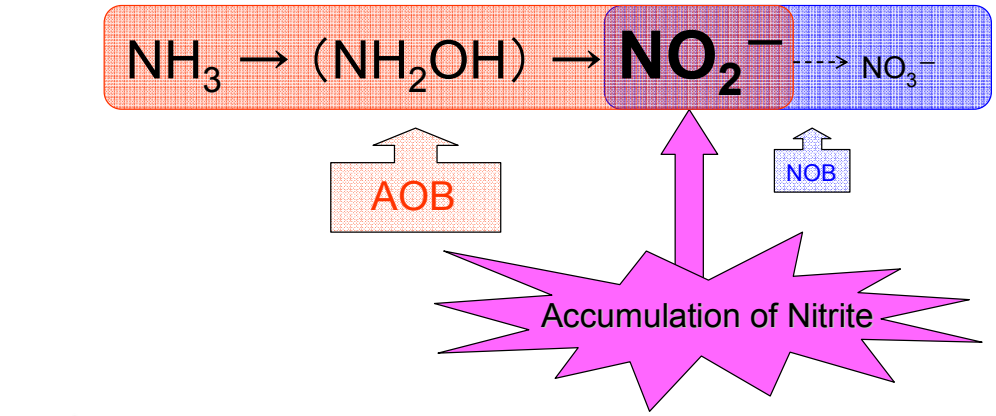
Changes in the population sizes of nitrifiers during swine feces composting



N_2O発生に関わる要因②：亜硝酸態窒素

Factor of N_2O emission in composting : Nitrite nitrogen

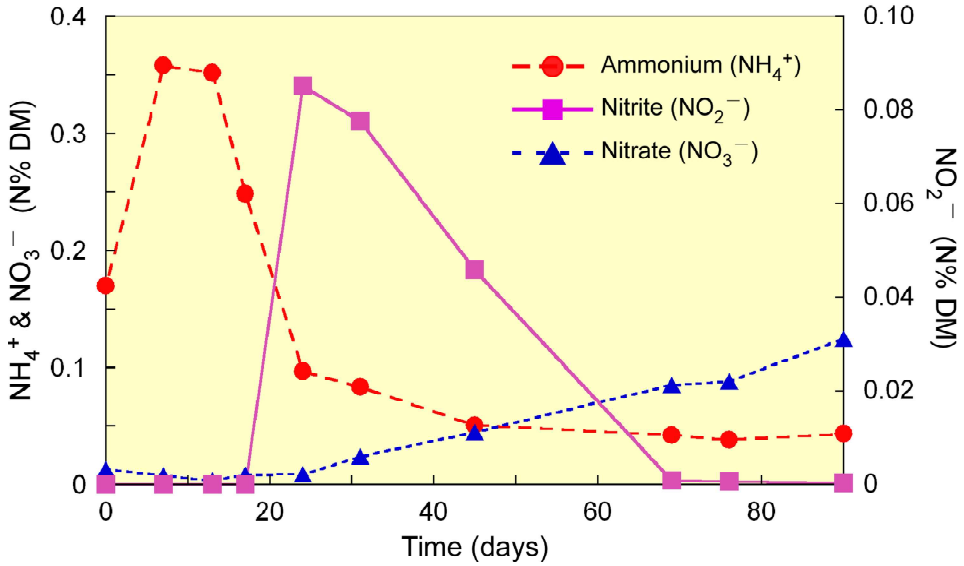
- 亜硝酸酸化細菌の増殖遅延により亜硝酸態窒素の蓄積が生じる



7

堆肥化における無機態窒素成分の推移

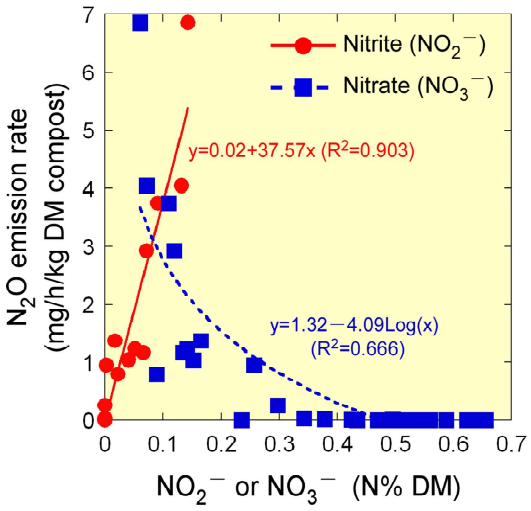
Changes in the inorganic nitrogen compounds during swine feces composting



8

N_2O発生速度と亜硝酸・硝酸態窒素濃度との関係

Relationship between N_2O emission rate and nitrite/nitrate concentrations during swine feces composting



- 亜硝酸との正の相関・硝酸との負の相関

  N_2O has a positive relation to NO_2^- and negative to NO_3^-

亜硝酸→硝酸の酸化促進によるN_2O発生抑制の可能性

Possibility for mitigation of N_2O emission by prompt oxidization of nitrite to nitrate

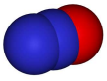
9

亜硝酸酸化細菌の添加によるN_2O発生制御Control of N_2O emission by addition of nitrite-oxidizing bacteria (NOB) during swine feces composting

目的（Purpose）

亜硝酸酸化細菌（NOB）を添加した場合に、堆肥化過程にある原料中の亜硝酸態窒素濃度の推移およびN_2O発生に及ぼす影響について、豚ふん堆肥化処理のケースで検証する。

The purpose of this experiment was to evaluate the effect of NOB inoculation on nitrite oxidization and N_2O emission during swine feces composting.



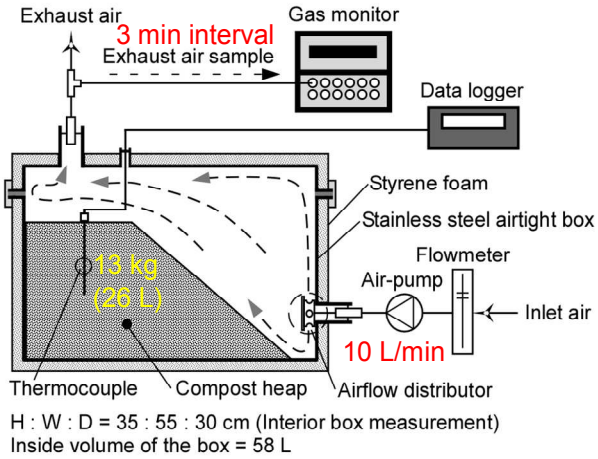
10

材料・方法
Materials & Methods

- 原料：新鮮豚ふん＋オガクズ（水分調整用）
  Materials for composting: Fresh swine feces mixed with sawdust
- NOB供給源：完熟豚ふん堆肥
  Source of NOB: Mature swine-feces compost (MSC)
- 添加時期：高温発酵終了直後
  Time of NOB addition: Immediately after a thermophilic composting
- 試験区：対照区 vs NOB添加区（10%完熟堆肥）
  Experimental treatment: Control vs NOB-added (10% mature compost)

11

堆肥化試験装置
Laboratory scale composting apparatus



Gas emission (mg/ *t* min) = (conc. of outlet air (mg/m^3)−conc. of inlet air (mg/m^3))
×measurement interval (*t*/60) ×ventilation rate (m^3/hour)

12

初発時原料（豚ふん＋オガ）と完熟豚ふん堆肥性状

Properties of initial swine feces mixed with sawdust and

Properties of initial swine feces mixed with sawdust								
Moisture content, %	pH	N content, N% DM				BOD, % DM	*Nitrifiers, MPN/g DM	
		TN	NH_4^+	NO_2^-	NO_3^-		AOB	NOB
61.9	7.5	2.36	0.21	0.00	0.01	21.6	$<10^2$	$<10^2$

Properties of mature swine-feces compost (MSC)							
Moisture content, %	pH	N content, N% DM			BOD, % DM	*Nitrifiers, MPN/g DM	
		TN	NH_4^+ & NO_2^-	NO_3^-		AOB	NOB
57.5	6.9	3.25	0.00	0.65	0.84	2.8×10^7	4.0×10^6

*Detection limit of MPN: $<10^2$ cells/g DM

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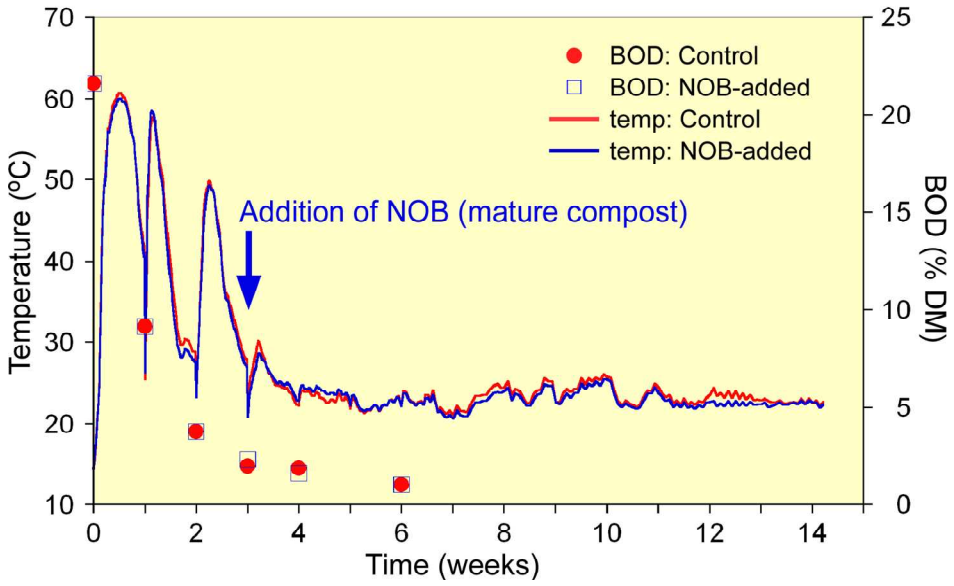
堆肥発酵におけるNOB添加時点

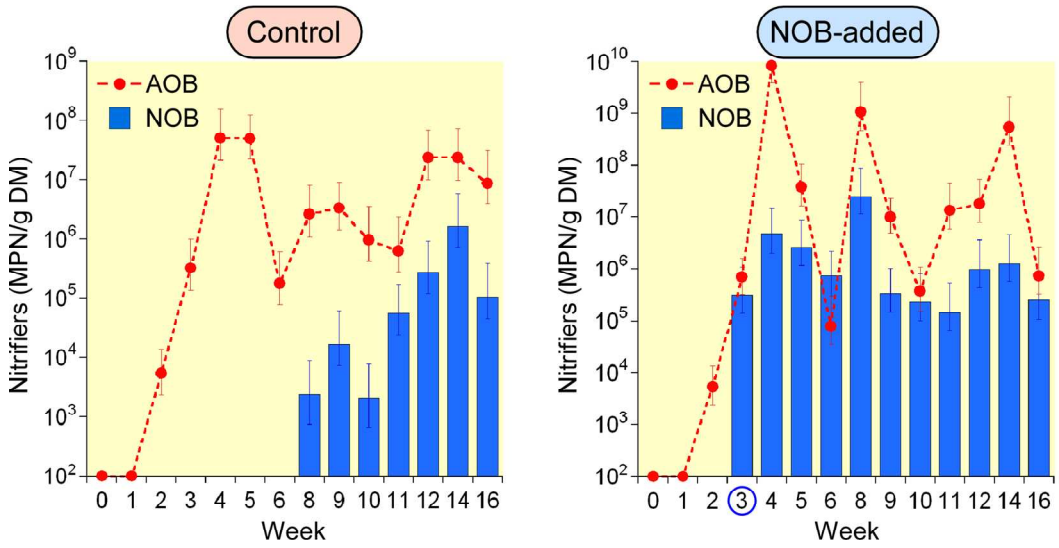
Point in time of NOB addition in composting fermentation



15

硝化細菌数の推移（NOB添加試験）

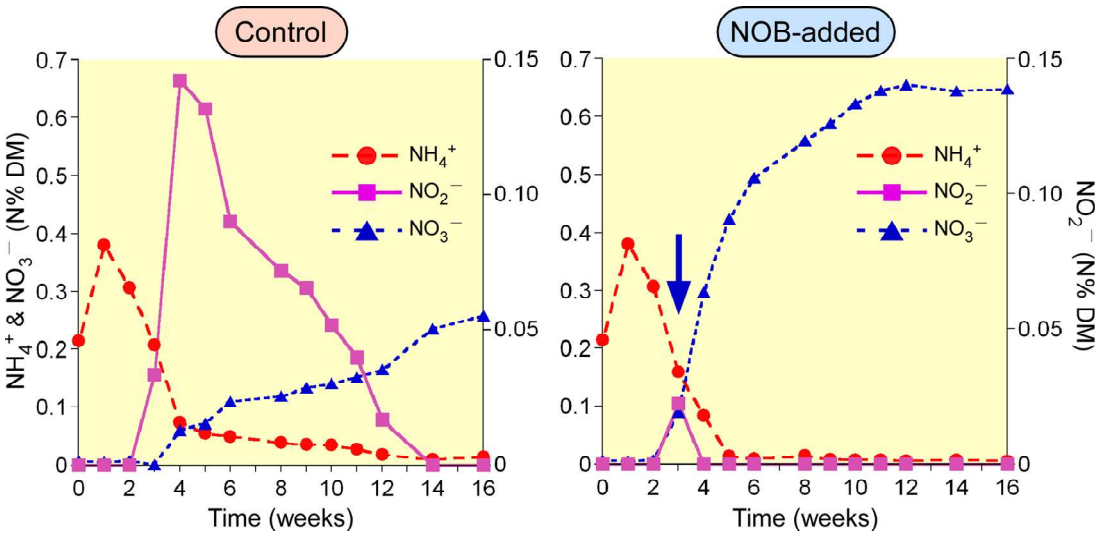
Changes in the population sizes of nitrifiers
in the test of composting with NOB addition



16

無機態窒素成分の推移（NOB添加試験）

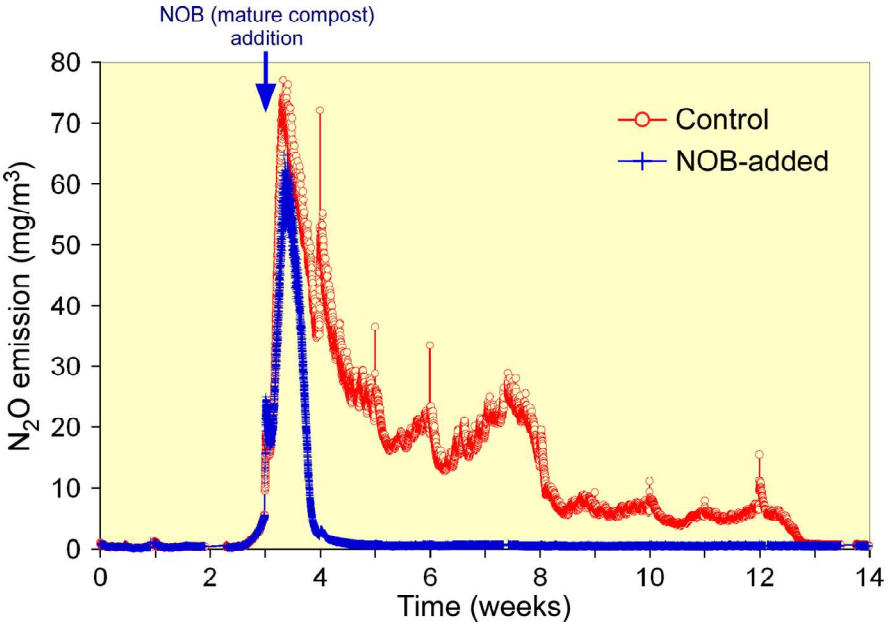
Changes in the inorganic nitrogen compounds
in the test of composting with NOB addition



17

N_2O発生パターン（NOB添加試験）

Patterns of N_2O emission in the test of composting with NOB addition



18

N_2O総発生量（NOB添加試験）

Total N_2O emission in the test of composting with NOB addition

Total N_2O emission and its emission rate based on total nitrogen (TN) in the initial swine feces mixed with sawdust		
Experimental treatment	Total emission, g N_2O-N	Emission rate, g N_2O-N/kg $TN_{initial}$
Control	10.4	88.5
NOB-added	2.0	17.5

NOB inoculation reduced the N_2O emission by 80%

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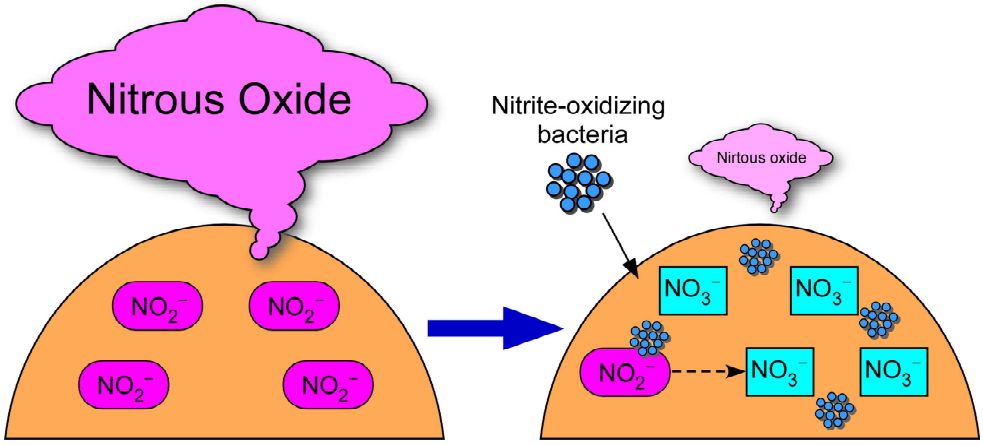
堆肥化からのN_2O発生抑制には、迅速な完全硝化の回復によるNO_2^-蓄積期間の短縮が有効



For reduction of N_2O emission during animal waste composting, it is effective to shorten the duration of nitrite accumulation by quick recovery of complete nitrification.

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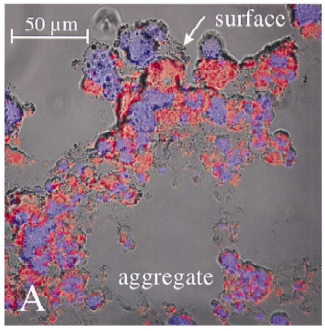
今後の研究展開
Forthcoming (future) study・・・

- N_2O削減効果の高度化に関する検討
  Examination for reduction of N_2O emission with higher efficiency
- 大規模・実処理施設での検証
  Evaluation in actual composting



Blue: Nitrosospira spp. (AOB)
Red: Nitrospira like bacteria (NOB)
Schramm, et al. (1998) Appl. Environ. Microbiol.

21

Hydrogen Production from Cow Manure and a Mixture of the Manure with an Artificial Food Waste

우분과 음식물쓰레기를 활용한 통합 혐기소화 과정에서의 수소 생산기술

히로시 요코야마 박사

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우분과 음식물쓰레기를 활용한 통합 혐기소화 과정에서의 수소 생산기술

요 약

슬러리에 자연적으로 존재하는 미생물총을 이용하여 37℃부터 85℃의 온도 범위대에서 실험규모의 배양시험을 통해 젖소분뇨슬러리로부터 수소를 생산하는 연구가 수행되었다.

박테리아의 식종없이 슬러리의 단순배양에 의해 수소를 생산하였다. 60℃와 75℃의 발효온도대에서 슬러리로부터 수소를 생산효과를 시험하였다(각 각 392 와 248 ㎖ H_2/ L Slurry 의 결과가 얻어짐).

16S rDNA 분석에 의해 60℃와 75℃의 발효온도대에서 배양된 슬러리중의 박테리아를 분석한 결과 각 각의 슬러리에서 *Clostridium thermocellum* 과 *Cladanaerobacter subterraneus* 가 발견되었다.

60℃에서의 총 용해물의 양이 75℃의 경우보다 많았는데, 이는 60℃에서의 수소발효과정에서의 유출물이 그 후속단계의 메탄발효에 더 적절한 기질로 이용될 수 있었기 때문인 것으로 판단된다.

슬러리와 인공음식물로 이용된 maltose 의 혼합물을 재료로 하여 60℃에서의 수소발효 후에 37℃에서의 메탄발효를 거치는 2 단계 처리시험을 수행하였다.

메탄발효만 일어나는 한 단계 처리에 비해 2 단계 처리에서의 메탄 생산량이 월등히 높았다. 이 결과는 한 단계 처리과정에서는 maltose 가 빠른 속도로 분해되는 과정에서 pH 를 낮아지게 하는 관계로 메탄 생산량이 낮아지게 된다.

그러므로 60℃에서의 수소발효는 에너지 획득기술뿐만 아니라 메탄발효 전처리 과정으로도 유용하게 이용되어질 수 있다.


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Hydrogen Production from Cow Manure and a Mixture of the Manure with an Artificial Food Waste

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Abstract

Hydrogen production from a slurry of dairy-cow manure was examined by batch cultures in a temperature range from 37 to 85˚C, using microflora naturally present within the slurry. Without the addition of seed bacteria, hydrogen was produced by simply incubating the slurry. Two peaks of fermentation temperatures for hydrogen production from the slurry were observed at 60 and 75˚C (392 and 248 ml H_2/L-slurry, respectively). Bacteria related to *Clostridium thermocellum* and *Caldanaerobacter subterraneus* were detected in the slurries cultured at 60 and 75˚C, respectively, by a 16S rDNA analysis. Total amount of soluble by-product production at 60˚C was higher than that at 75˚C, suggesting that the effluent from the hydrogen fermentation at 60˚C was a suitable feedstock for the following methane fermentation. Two-step treatments of a mixture of the slurry with maltose, as an artificial food waste, were conducted by the hydrogen fermentation at 60˚C followed by methane fermentation at 37˚C. Amount of methane production by the two-step treatments was significantly higher that that by a one-step treatment (only methane fermentation). Rapid decomposition of maltose caused a pH drop in the one-step treatment, resulting in its low amount of methane production. Therefore, the hydrogen fermentation at 60˚C would be useful as an energy recovery technique as well as a pre-treatment for methane fermentation.

Introduction

Hydrogen production from organic wastes is interested as a sustainable bioenergy resource. Animal waste contains organic matter available for biogas production, and also contains various microbes including hydrogen-producing bacteria, methanogens, and cellulose-degrading bacteria. Therefore, anaerobic fermentation of animal wastes does not require the addition of seed bacteria for biogas production, and this feature distinguishes animal waste from other organic wastes.

Currently, two-step treatments (hydrogen fermentation followed by methane fermentation) have been proposed (1, 2). As compared to the one-step treatment, the hydrogen/methane fermentation is predicted to increase the efficiency of energy recovery. In addition, the pre-treatment by hydrogen fermentation is expected to facilitate methane fermentation. However, many properties of hydrogen production from animal wastes remain unclear. In this paper, our current studies with regard to the hydrogen production from cow manure have been described.

Materials and Methods

A slurry (13.4g VS/L, pH 8.2) of dairy cow manure was prepared by mixing the feces and urine from Holstein cows, and was fermented at the indicated temperatures. The hydrogen, methane, and VFAs were analyzed with gas chromatography, as described (3). Bacterial population was analyzed by denaturing gradient gel electrophoresis (DGGE), and the

nucleotide sequences of DGGE bands were determined as described (3). In two-step treatments, maltose monohydrate (4.5g) was added into the slurry (600 mL), and the mixture was fermented at 60˚C for 4 days. Then, the pH of the slurry was adjusted to 6.5 with 1N NaOH. Methanogenic granules (100 mL) from an UASB plant of NILGS was added into the fermented slurry, and the mixture was further fermented at 37˚C for 10 days. In a one-step treatment, the hydrogen fermentation at 60˚C was omitted.

Results and Discussion

1. Hydrogen production from a slurry of cow manure

To examine the effect of the fermentation temperature on the hydrogen production, a slurry of cow manure was cultured by batch experiments, at 37, 50, 55, 60, 67, 75, and 85˚C. Total amounts of evolved hydrogen were strongly dependent on the incubation temperature (Fig. 1). Interestingly, two peaks of fermentation temperatures for hydrogen production from the slurry were observed, at 60 and 75˚C (392 and 248 ml H_2/l slurry, respectively). Methane was produced from the slurries at 37 and 50˚C, but it was not detected over 50˚C, at least for 14 days (data not shown). This result suggested that hydrogen consumption by methanogens in the slurry caused the low amounts of hydrogen production from the slurries at 37 and 50˚C. These results show that hydrogen can be produced from cow manure quite simply, by only incubating the slurry at 60 or 75˚C, without addition of seed bacteria.

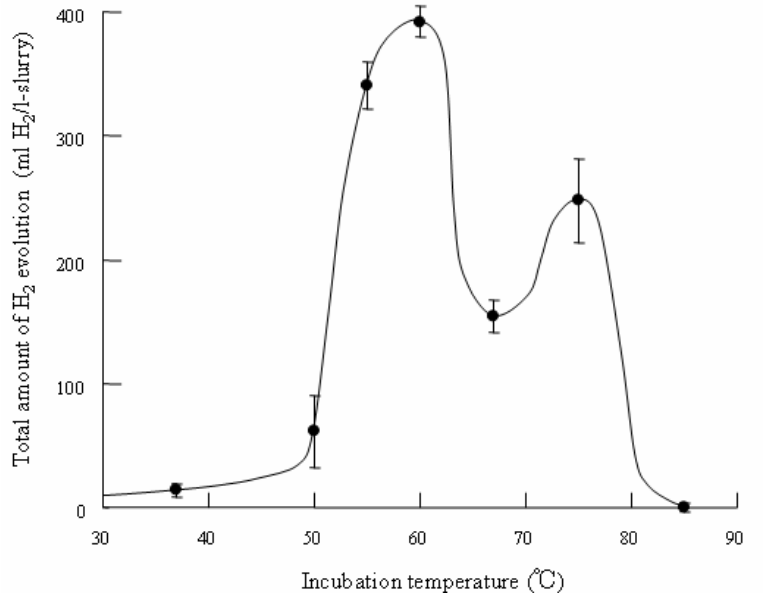

Fig. 1. Hydrogen production from cow manure at various fermentation temperatures

Analyses of the hydrogen-producing bacteria in the slurry are important for understanding the microbial mechanism of hydrogen production from the slurry. Therefore, the bacterial population in the slurries cultured at 60 and 75˚C were analyzed by DGGE. Several specific bands for the slurries after fermentation were detected, and their nucleotide sequences were

determined. The determined sequences were compared with the sequences in GenBank by a BLAST search. (Table 1).

Table 1. Sequence similarity of the DGGE bands

Band No.	fermentation temp. (^{0}C)	Closest relatives	Similarity (%)	Accession No.
B1	60	*Clostridium stercorarium*	93	AJ310082
B2	60	*Clostridium thermocellum*	96	AJ410280
C1	75	*Caldanaerobacter subterraneus*	100	AY216597

Bands B1 and B2, specific for the 60˚C fermentation, showed similarities to hydrogen-producing moderate thermophiles, *Clostridium stercorarium* and *Clostridium thermocellum*, respectively (4). Band C1, specific for the 75˚C fermentation, showed 100% similarity to a hydrogen-producing extreme thermophile, *Caldanaerobacter subterraneus* (5). The presence of hydrogen-producing thermophiles in the cow manure is of quite interest. The cow manure would be useful as seed sludge for hydrogen fermentation.

Profiles of soluble by-products in the fermentation at 60 and 75˚C were analyzed. Acetate was predominantly produced at 60 and 75˚C (Fig. 2). Total amounts of produced VFAs at 60˚C was significantly higher than that at 75˚C. Generally, the amount of produced VFAs was frequently correlated with the solubilization of suspended solids in animal manures. Therefore, the 60˚C fermentation would be more effective as a solubilization treatment rather than the 75˚C fermentation.

2. Two-step treatments of a mixture of cow manure with an artificial food waste

In a mixed treatment of animal manure and food waste, the organic load tends to be high, as compared to that in a treatment of only animal manure, and the high organic load potentially leads to a system failure of methane fermentation. In this case, hydrogen fermentation is predicted to be effective as a pre-treatment for methane fermentation. Therefore, two-step treatments of a mixture of cow manure with an artificial food waste were examined by the hydrogen fermentation at 60˚C, followed by mesophilic methane fermentation. Maltose is an easily biodegradable organic matter, and is abundantly contained in food waste. Thus, maltose was selected as an artificial food waste. Total amount of methane production by the two-step treatments was significantly higher than that by a one-step treatment, only methane fermentation (Figs. 3 and 4). In the one-step treatment, the pH of the mixture dropped to 6.0, because of a rapid decomposition of maltose. Most methanogens are inactive below a pH of 6.5, and therefore, the pH drop led to a system failure of the one-step treatment. In the two-step treatments, maltose was converted to VFAs by the hydrogen fermentation, and then, the pH of the mixture was adjusted to be neutral before the second treatment, methane

fermentation. Therefore, the pH did not drop in the methane fermentation in the two-step treatments. These results show that the hydrogen fermentation at 60˚C would be useful as an energy recovery technique as well as a pre-treatment for methane fermentation.

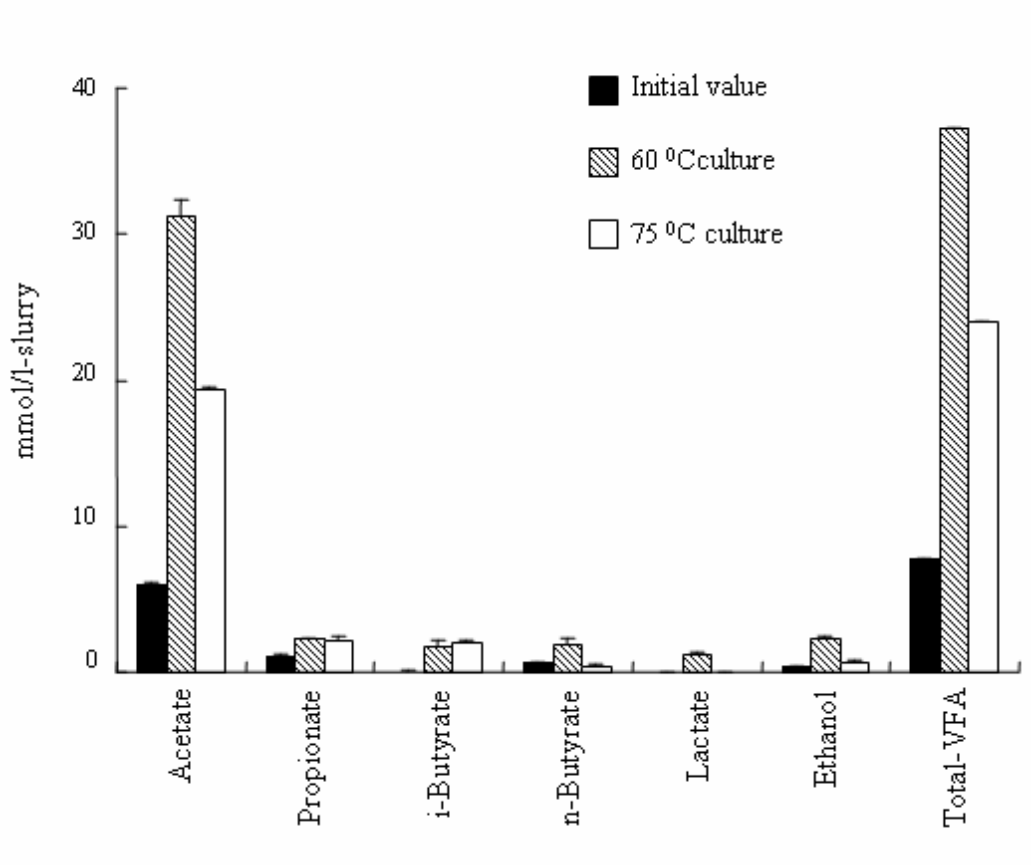

Fig.. 2. Soluble by-product profiles in the fermented cow manure

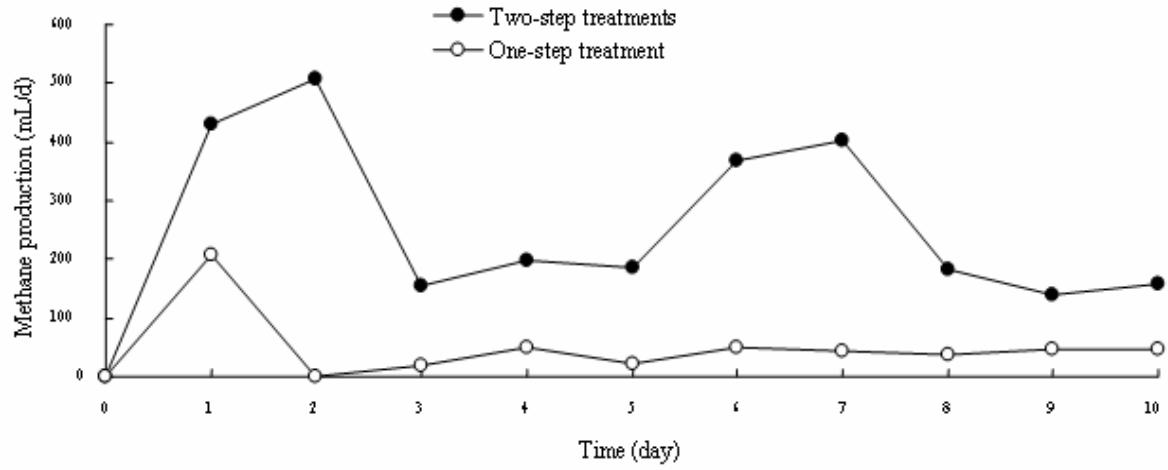

Fig. 3. Time courses of methane production from the mixture of cow manure with the artificial food waste. Methane production by the one- and two-step treatments in the methane fermentation is shown.

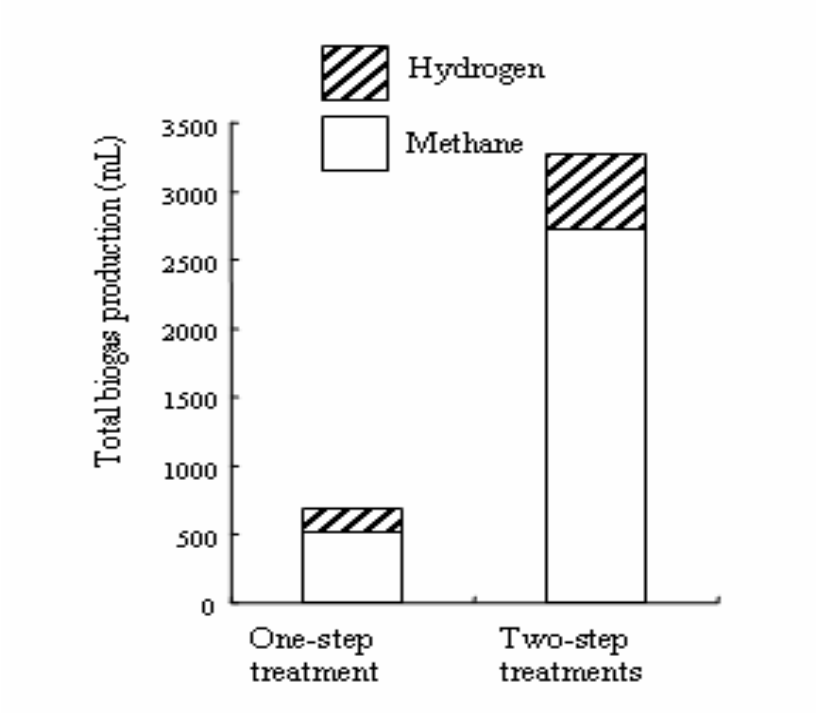

Fig. 4. Total amounts of hydrogen and methane production from the mixture of cow manure and the artificial food waste

Acknowledgments

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A part of this study has been published in Ref. (3).

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NLRI, Suwon, Korea, June 26, 2007

Hydrogen Production from Cow Manure and a Mixture of the Manure with an Artificial Food Waste

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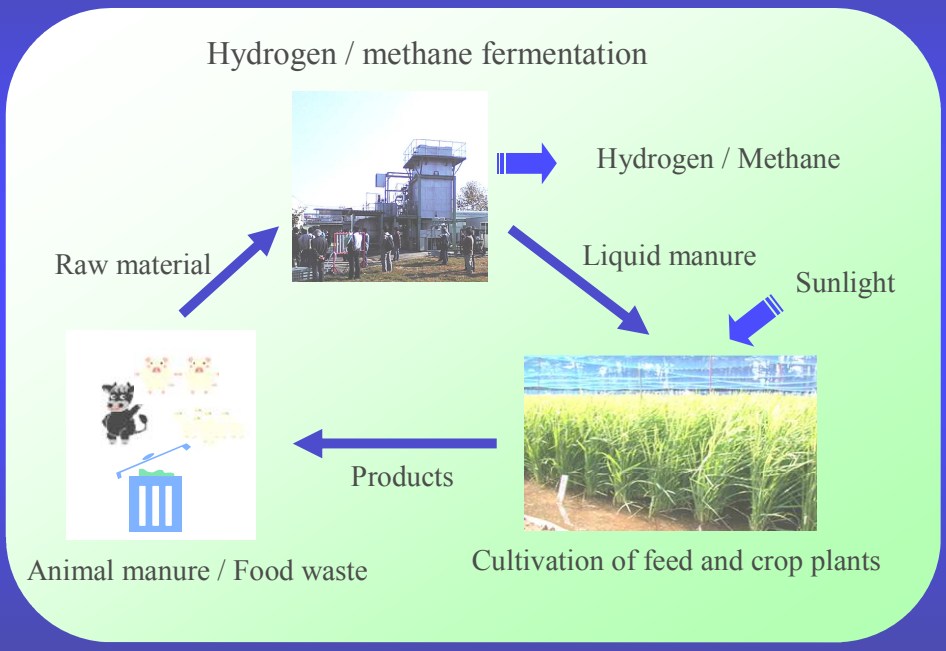
Resource recycling of animal manure and food waste



- *Methane fermentation*

  Cellulose → glucose → VFAs + CO_2 + H_2 → CH_4 + CO_2

- *Hydrogen fermentation*

  Cellulose → glucose → VFAs + CO_2 + H_2 ~~→~~ CH_4 + CO_2

- *Hydrogen-producing bacteria*

  Clostridium sp., Enteribactor sp., etc.

  Spore formation, heat resistance, high growth rate, wide optimum pH and temperature ranges

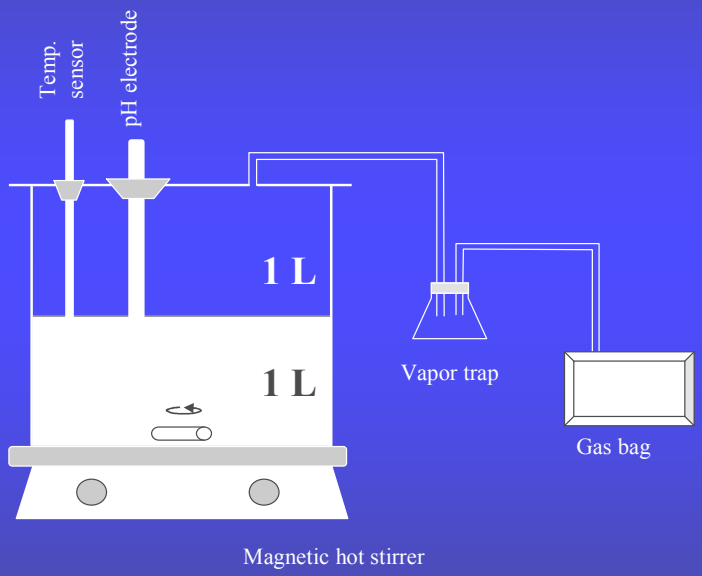
- *Hydrogen-consuming bacteria*

  Methanogen, Homoacetogen, Sulfate-reducing bacteria, etc.
  H_2 + CO_2 → Acetate, dG < 0

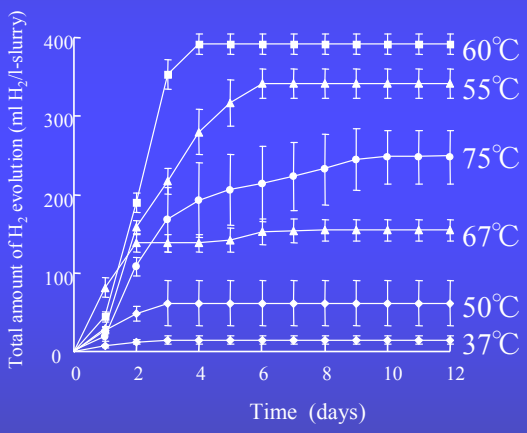
Composition of a slurry of dairy-cow manure

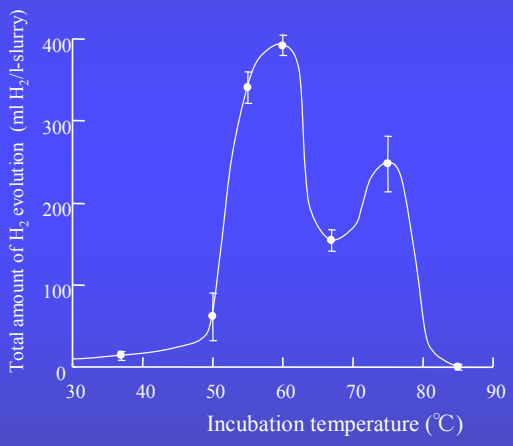
Total solids	17.7 g/L
Volatile solids	13.4 g/L
Total carbon	6.7 g/L
Total nitrogen	0.47 g/L
pH	8.2 (at 25^0C)

Schematic representation of the bioreactor

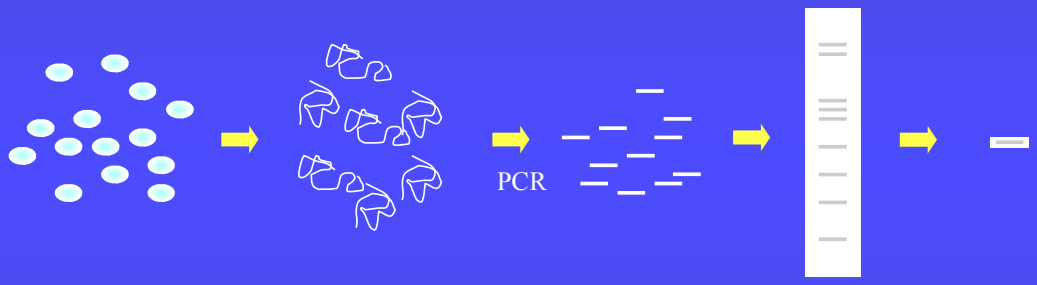


Temperature dependency of fermentative hydrogen production from the slurry

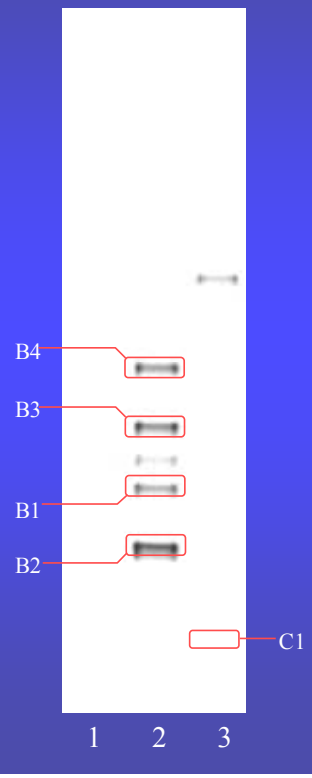




Denaturing Gradient Gel Electrophoresis (DGGE) analysis



DGGE profiles of the microflora in the slurries

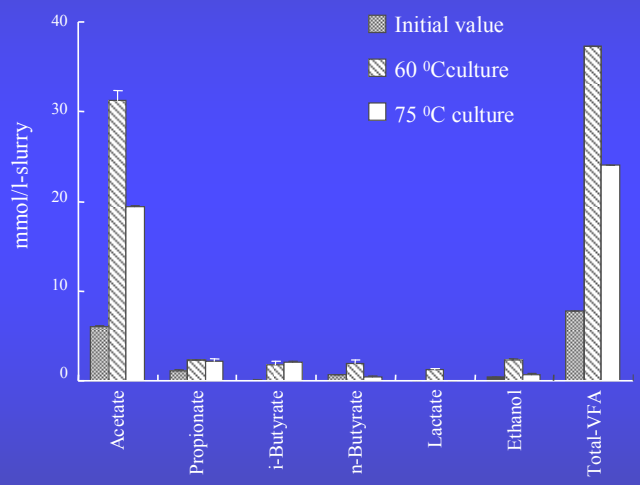


Lane 1 the slurry before culture
Lane 2 the slurry cultured at 60^0C
Lane 2 the slurry cultured at 75^0C

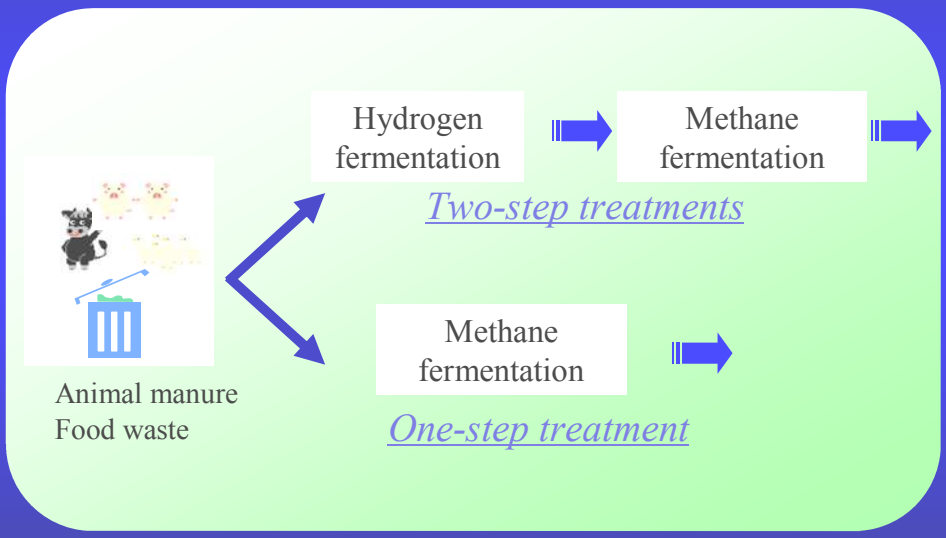
Sequence similarity of the bands from the DGGE analysis

Band No.	Closest relatives	Similarity (%)	Isolation source
B1	Uncultured bacterium clone F3_824-07	99	anaerobic microflora enriched fro m compost
	Clostridium stercorarium	93	compost
B2	Uncultured bacterium clone EBR-02E-0596	100	enrichment culture from methano genic sludge
	Clostridium thermocellum	96	
B3	Uncultured bacterium clone MBNA07	96	thermophilic anaerobic reactor
B4	Uncultured bacterium clone 9G	99	compost
	Bacteroides xylanolyticus	96	cattle manure
C1	*Caldanaerobacter subterraneus*	100	oilfield water, geothermal hot stre am, hot spring

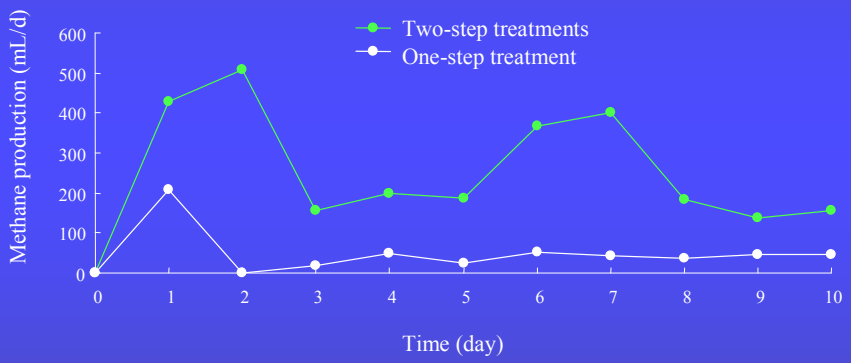
Profiles of soluble by-products in the slurries



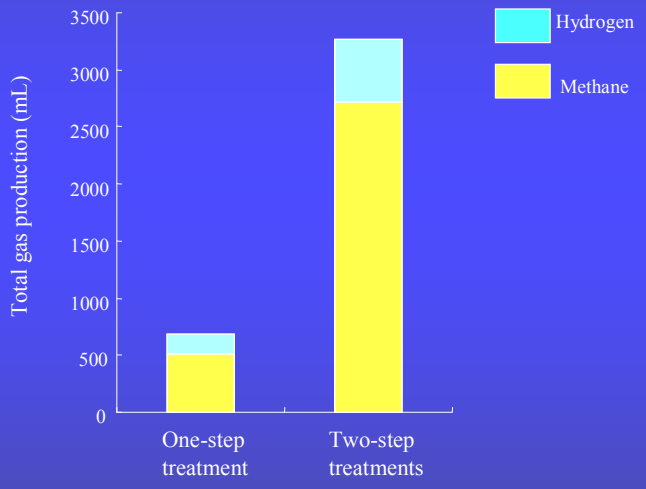
Two-step treatments of a mixture of cow manure and an artificial food waste



Methane production from the mixture of cow manure and an artificial food waste by two-step treatments



Total biogas production from the mixture of cow manure and an artificial food waste



Summary

1, Hydrogen can be produced from cow manure quite simply, by only incubating the slurry at 60 and 75 °C.

2, Hydrogen-producing bacteria, with similarities to *C. thermocellum* and to *C. subterraneus*, are involved in the hydrogen production at 60 and 75 °C, respectively.

3, Cow manure is useful as seed sludge for hydrogen fermentation.

4, Two-step treatments of a mixture of cow manure with soluble organic matter produced much more methane rather than one-step treatment.

5, The hydrogen fermentation at 60°C is useful as a pre-treatment for methane fermentation.