



Effectiveness of Indigenous Micro-Organism Treatment of Deep Litter Floor in Pig House Foul Odour Control

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Abstract: Pig (*Sus scrofa domesticus*) production amongst smallholder farmers is constrained by bad odour arising from pig houses. The objective of this study was to investigate the effectiveness of Indigenous Microorganisms (IMO) treatment of deep litter floor in controlling bad odour emission from pig houses of IMO treated and untreated deep litter floor. Twenty four three-month old pigs (Large White x Landrace) were raised on deep litter floor; one floor type treated with IMO solution and control not treated. Significant ($LSD \leq 0.05$) differences were observed in bad odour intensity of extremely strong, very strong, strong, distinct, weak, and very weak at a distance of 0 to 40 metres from pig houses of IMO treated and untreated deep litter floor; with high intensity of bad odour (16.9%) arising from untreated deep litter floor houses than IMO treated litter floor (0.4%). Therefore, deep litter floor treatment with IMO solution does not eliminate bad odour from pig house but rather reduces the intensity of foul odour to a level which is environmentally friendly and acceptable to the people living around the piggery. Further research should be conducted to determine IMO lifespan in vitro of their natural habitat for efficient storage, packaging and frequency of its application on different organic bedding materials.

Keywords: Indigenous micro-organisms, pig, deep litter, foul smell.

INTRODUCTION

In sub-Saharan Africa, over 80% of the pigs (*Sus scrofa domesticus*) are kept traditionally under smallholder farming system (Natalie, 2015). Pig production and marketing have the potential to improve the livelihoods of smallholder pig farmers given its better production efficiency per unit area of land, good feed conversion efficiency, high prolificacy; rapid growth rate compared to other livestock thus generating income within a short period of time and ensuring food security (Muhanguzi *et al.*, 2012). Pig production in Uganda is still low compared to other livestock especially in the Northern region which is the second last in the country with only 11% of pig production (Tatwangire, 2014). However, as consumption of other livestock products such as beef is reducing, that of pork is increasing at rate of 3.4 kg/person/year making pork to be ranked fourth in terms of per capita meat consumption and the

demand is still increasing amongst urban and peri-urban population (Tatwangire, 2013).

Despite the growing demand for pork, pig production amongst smallholder farmers is still constraint by offensive odour originating from piggery unit (Chapin *et al.*, 1998). The strength and offensiveness of the bad odour generated depends on the quantity and composition of the feed being digested, anaerobic microbial decomposition and incomplete microbial decomposition of manure which produces a complex of ammonia (NH_3), volatile sulphur (H_2S , SO_2) and large volume of volatile organic compounds (Kim *et al.*, 2014). Several ill effects are associated with high exposures to ammonia and Hydrogen sulphide (H_2S) amongst which include; nausea, coughing, headaches, dizziness, nasal complaints, eye irritation and skin malformations, development of severe pulmonary illnesses, impairment of lung function, cardiovascular health and premature death (Simonton and Spears, 2007). Unpleasant odour originating from piggery units significantly cause more tension, anger and fatigue to residents who lived near pig farmers for an average of 5 years than residents not exposed to piggery foul odour at home resulting to low income and decreased livelihood of

communities engaged in swine production thereby, imposing a great threat to food security in a long run (Schiffman, 1995).

Management of foul odour arising from piggery still remains a big challenge to most smallholder farmers although some less effective efforts such as planting fast growing trees to absorb the bad odour have been made to mitigate the challenge (Chastain, 1999). Daily application of indigenous micro-organism (IMO) solution on deep litter floors of pig houses has been suggested to reduce foul odour in piggery units (Ndyomugenyi and Kyasimire, 2015). But, the effectiveness of IMO in foul odour control has not been ascertained therefore, the objective of this study was to assess foul odour in IMO treated deep litter floor of pig house.

MATERIALS AND METHODS

Study area

The study was carried out in Omoro and Gulu districts, Northern Uganda. Omoro district is located between longitudes 30° 21' East to longitude 32° East and latitude 2° North to latitude 4° North. The district occupies 1,724.5 km², comprising of six sub-counties (Lalogi, Odek, Bobi, Koro, Lakwana and Ongako) and bordered by Gulu in the North, Pader in the East, Oyam in the South and Lamwo in the West. The average rainfall received is 1,500 mm per annum with a monthly average varying between 14 mm in January and 230 mm in August. The wet season normally extends to October with the highest rainfall in May, August and October. The dry season is from November through March. The average maximum temperature is 30°C with a minimum of 18°C (Ikwap *et al.*, 2014). Conversely, Gulu district is located in Northern Uganda between longitudes 30° 32' East and latitudes 2° 4' north. It is bordered by Amuru District in the West, Lamwo District in the North East, Pader District in the East, Lira District in the South East, Oyam District in the South and Nwoya District in the South West. The total land area of Gulu district is 3,449.08 sq. km (1.44 % of the Uganda land size) and 96.9 sq. km (0.8 %) is open water. The district comprises six sub-counties (Awac, Paicho, Patiko, Unyama, Bungatira and Palaro) and one municipality with four divisions (Bar-dege, Laroo, Layibi and Pece). Altitude ranges from 1000 to 1200 meters above sea level with average rainfall of 1500 mm per annum (Nabukenya *et al.*, 2014).

Study design and treatments

Three experimental units (deep litter floor units with IMO treatment) and control units (deep litter floor units without IMO treatment) were used in this study. Twenty four-three month old crosses of Large White and Landrace pigs of mixed sex were purchased from the existing pig farmers within the two districts; a completely randomised block design (CRBD) was used in the study. The distribution of pigs into the six piggery units was done at random. Laying of the bedding materials in all the study units was done systematically in layers on one metre deep hole bare

soil ground measuring 3x3; with charcoal as the first layer, followed by sizable tree shoots, maize stalks, dry red soil mixed with lime and wood shavings. Application of IMO solution in all the experimental units was done on a daily basis.

Pigs in all the units were fed on growers' mash using restricted feeding method; the mash consisted of maize bran (74 kg), fishmeal (5 kg), soybean meal (10 kg), sunflower cake (5 kg), lake shells (4 kg), bone ash (1 kg), salt (0.5 kg) and premix (0.5 kg). Generally, the feed contained energy (2971 kcal kg⁻¹), crude protein (13.6%), lysine (0.957%), methionine + cysteine (0.685%), fats (5.1%), crude fibre (6.3%), calcium (1.97%) and phosphorus (0.74%).

Effectiveness of molasses and maize bran in multiplication of IMO under different temperatures, pH and humidity

The Indigenous Microorganisms (IMO) was collected using boiled rice and, multiplied using molasses and maize bran as growth media to produce IMO solution (Reddy, 2011). Samples of IMO multiplied by molasses and maize bran medium were cultured on 54 differential media in petri dishes under different treatment conditions of temperature, humidity and pH. Twenty seven (27) treatments for molasses and the other 27 for maize bran were randomized; different treatments for temperatures (T₁, T₂ and T₃) were repeated three times at a fixed pH and humidity for precision. Aerobic Indigenous Microbial population were determined microscopically using Petroff- Hauser counting chamber under different temperature conditions (Stuart, 2005). Average population of aerobic microorganism were calculated by getting the mean temperature and the mean different microbial populations. The same procedure was used for determining the pH and humidity suitable for the multiplication of IMO.

Relationship between ambient temperature, humidity, pH and odour intensity in deep litter floor piggery unit

Digital thermometer for measuring temperature (°C), digital hygrometer for measuring relative humidity (%) and pH meters for measuring the acidic or alkaline condition were hanged in all piggery units, both experimental and control at a height of 50cm from the deep litter floor level. The foul odour temperature, humidity and pH were recorded three times a day (morning, afternoon and evening) after every two weeks for three months (90 days). Random sampling method was used to select 120 people who participated in the study to evaluate bad odour intensity arising from the piggery units, 20 farmers for each unit. The foul odour intensity was examined using parametric sensory measurement (Brattoli *et al.*, 2011). Participants were interviewed using a structured questionnaire for foul odour intensity at various

distance (metres) and time (hour) interval (morning, afternoon and evening) for five days.

DATA ANALYSIS

All data obtained were analysed using Genstat version 16 and a One-way ANOVA was generated. Significant means were separated by Least Significant Difference (LSD) Tests at 5 % significant level ($LSD \leq 0.05$).

RESULTS

Effectiveness of molasses and maize bran in IMO multiplication

Microorganism species isolated from indigenous microorganism solution were gram positive and facultative anaerobes, moulds and yeast isolated were entirely aerobic microorganism (Table 1).

Table 1: Microorganisms isolated from indigenous microorganism solution

Microbes	Identification	Characteristics	Description
Bacteria	Enterobacteriaceae	Grain stain	Gram Negative
		Microscopic appearance	Rods
		Oxygen relationship	Facultative anaerobic bacteria
		Oxidase test	Negative
		Spores	Non-spore forming
	Lactobacillus species	Grain stain	Gram – positive
		Microscopic appearance	Rods in palisades
		Oxygen relationship	Anaerobic bacteria
		Oxidase test	Negative
		Spores	Non – spore forming
	Bacillus species	Grain stain	Gram – Positive
		Microscopic appearance	Rods in chain
		Oxygen relationship	Facultative anaerobic bacteria
		Oxidase test	Negative
		Spores	Spore forming
	Staphylococcus	Grain stain	Gram – Positive
		Microscopic appearance	Cocci in grape – like clusters
		Oxygen relationship	Facultative anaerobic bacteria
		Oxidase test	Positive
		Spores	Non-spore forming
Yeast and Moulds	Aspergillus niger	Stipes color	Slightly brown
		Surface	Smooth walled
		Vesicle serration	Biseriate large size
		Metula covering	Entirely
		Shape	Glucose
	Aspergillus flavus	Stipes color	Pale brown roughened
		Surface	Spherical
		Vesicle serration	Biseriate
		Metula covering	$\frac{1}{2}$ to $\frac{3}{4}$
		Shape	Glucose ellipsoid

Multiplication of IMO at constant pH and Humidity under varying temperature using maize bran and molasses as growth media

At a temperature of 37°C, pH of 7 and Humidity of 40% in pure maize bran as microorganism growth medium, 413×10^2 cfu/gm total viable cells were counted and 510×10^2 cfu/gm total viable cells with molasses medium (Table 2).

Table 2: Multiplication of IMO at constant pH and Humidity under varying temperature using maize bran and molasses as media

Sample	Treatment conditions			Total viable count $\times 10^2$ (cfu)/gm
	Temperature (°C)	pH – Value	Humidity %	
Solution IMO Pure Maize Bran	22.0	7.0	40	172
	37.0	7.0	40	413

		45	7.0	40	280
Solution	IMO	22.0	7.0	40	220
Dilute Molasses		37.0	7.0	40	510
		45	7.0	40	355

Multiplication of IMO at constant Temperature and Humidity under varying pH using maize bran and molasses as media

At pH of 8.5, temperature of 37°C and 40% humidity, the growth of the microorganisms was 460x10²cfu/gm in maize bran and 595x10²cfu/gm in molasses (Table 3).

Table 3: Multiplication of IMO at constant Temperature and Humidity under varying pH using maize bran and molasses as media

Sample	Treatment conditions			Total viable count x10 ² (cfu)/gm
	Temperature (°C)	pH – Value	Humidity	
Solution IMO Pure Maize	37.0	5.5	40	145
	37.0	7.0	40	415
	37.0	8.5	40	460
Solution IMO Dilute Molasses	37.0	5.5	40	200
	37.0	7.0	40	510
	37.0	8.5	40	595

Multiplication of IMO at constant Temperature and pH under varying Humidity for using maize bran and molasses as media

At a humidity of 60%, temperature of 37 °C and pH 7, there were 530 x10² cfu/gm total viable cell count in maize bran and 580 x10²cfu/gm in molasses as growth medium (Table 4).

Table 4: Multiplication of IMO at constant Temperature and pH under varying Humidity for using maize bran and molasses as media

Sample	Treatment conditions			Total viable count x10 ² (cfu)/gm
	Temperature (°C)	pH - Value	Humidity	
Solution IMO Pure Maize Bran	37.0	7.0	20	310
	37.0	7.0	40	405
	37.0	7.0	60	530
Solution IMO Dilute Molasses	37.0	7.0	20	450
	37.0	7.0	40	520
	37.0	7.0	60	580

Effectiveness of maize bran and molasses in multiplication of IMO

At pH of 7, humidity 40% and temperature 37°C, significant ($LSD \leq 0.05$) difference was observed in IMO multiplication using molasses and maize bran as media (Table 5).

Table 5: Effectiveness of maize bran and molasses in IMO multiplication

Media	Mean viable counts x 10 ² (cfu/gm)	P-value	LSD (0.05)
Maize bran	288	0.001	0.031
Molasses	360		

Effect of temperatures, humidity and pH on foul odour intensity

In deep litter floor pig house without IMO treatment, temperature, pH and humidity affected foul odour intensity especially in the morning at lower temperature of 23°C and high humidity of 62% (Table 6). In IMO treated deep litter floor, majority of the participants did not detect any foul odour in the house throughout the day at various temperatures, pH and humidity.

Table 6: Effect of temperature, pH and humidity on foul odour intensity in deep litter floor pig house treated with IMO in comparison with deep litter floor without IMO treatment

Deep litter floor without IMO treatment				
Odour Intensity	Environmental condition	Odour detection at different environmental conditions (Mean % of participants)		
		Morning	Afternoon	Evening

	Temperature	22.8	28.1	27.6
	pH	7.70	7.80	7.80
	Humidity	61.8	50.5	51.5
Extremely strong		7.30	3.30	4.30
Very Strong		33.7	23.7	20.3
Strong		30.3	31.3	32.3
Distinct		16.0	21.0	19.7
Weak		12.0	16.0	19.3
Very Weak		0.700	4.70	4.00
No Smell		0.000	0.000	0.000
Total		100	100	100
Deep litter floor treated with IMO				
		Morning	Afternoon	Evening
	Temperature	21.9	28.8	27.4
	pH	8.03	7.96	8.07
	Humidity	70.7	50.8	53.9
Extremely strong		0.000	0.000	0.000
Very Strong		0.000	0.000	0.000
Strong		0.000	0.000	0.000
Distinct		0.000	0.000	0.000
Weak		4.30	9.30	9.70
Very Weak		30.0	23.0	25.7
No Smell		65.7	67.7	64.7
Total		100	100	100

Effect of temperature, pH and humidity on foul odour intensity in deep litter floor pig house

Temperature, pH and humidity greatly influenced foul odour intensity on deep litter floor pig house treated with IMO and deep litter floor without IMO treatment (Table 7). Extremely strong, very strong, strong, distinct weak, very weak and no smell significantly ($LSD \leq 0.05$) differed in deep litter floor pig house treated with IMO and deep litter floor without IMO treatment. However, no significant ($LSD \geq 0.05$) different was observed in no smell between the two floors. Foul odour intensity of extremely strong and very strong were generally higher on deep litter floor pig house without IMO treatment than deep litter floor treated with IMO. In contrast, foul odour intensity of strong; distinct, weak and very weak were higher on deep litter floor pig house treated with IMO than deep litter floor pig house without IMO treatment.

Table 7: Effect of temperature, pH and humidity on odour intensity (%) in IMO treated and untreated deep litter floor pig house

Odour intensity	IMO treated and untreated deep litter		P-value	LSD (0.05)
Extremely strong	Litter floor with IMO	6.67 ^a	0.010	0.012
	Litter floor without IMO	14.7 ^b		
Very strong	Litter floor with IMO	47.3 ^a	0.040	0.027
	Litter floor without IMO	67.3 ^b		
Strong	Litter floor with IMO	62.7 ^a	0.042	0.029
	Litter floor without IMO	60.7 ^b		
Distinct	Litter floor with IMO	42.0 ^a	0.021	0.010
	Litter floor without IMO	30.7 ^b		
Weak	Litter floor with IMO	41.3 ^a	0.029	0.013
	Litter floor without IMO	25.7 ^b		
Very weak	Litter floor with IMO	32.3 ^a	0.028	0.020
	Litter floor without IMO	31.3 ^b		
No smell	Litter floor with IMO	67.67	0.071	0.064
	Litter floor without IMO	65.67		

Means within a row with different superscripts differ significantly ($LSD < 0.05$)

Relationship between human residence and foul odour detection from IMO treated and untreated deep litter floor pig house

Generally, all participants detected foul odour in both IMO treated and untreated deep litter floor pig house (Table 8). At a distance of 0, 10, 20, 30 and 40 meters, foul odour detection differed significantly ($LSD \leq 0.05$) between IMO treated and untreated deep litter floor with higher intensity of foul odour in deep litter floor without IMO treatment compared to IMO treated deep litter floor.

Table 8: Relationship between human residence and foul odour detection from IMO treated and untreated deep litter floor pig house

Distance (meters)	IMO treated and untreated deep litter		P-value	LSD (0.05)
0.000	Litter floor with IMO	0.000 ^a	0.010	0.020
	Litter floor without IMO	3.170 ^b		
10.0	Litter floor with IMO	0.000 ^a	0.021	0.025
	Litter floor without IMO	12.700 ^b		
20.0	Litter floor with IMO	0.000 ^a	0.018	0.014
	Litter floor without IMO	19.500 ^b		
30.0	Litter floor with IMO	0.000 ^a	0.013	0.011
	Litter floor without IMO	17.500 ^b		
40.0	Litter floor with IMO	2.170 ^a	0.011	0.010
	Litter floor without IMO	31.700 ^b		

Means with different superscripts differ significantly ($LSD < 0.05$)

DISCUSSION

Effectiveness of maize bran and molasses in multiplication of Indigenous Microorganisms

Molasses growth medium being more effective in multiplication of indigenous microorganisms suggests that, molasses were richer in nutrient contents than maize bran (Table 5). These variations could be due to the difference in fermentable sugar contents in the two growths medium where the microorganisms derived their nutrients for growth and metabolism, this might have had an effect in the multiplication of the microorganisms. This suggestion concurs with Walker and Stewart (2016) who reported that, the main fermentable sugars (2.8%) in maize were glucose, maltose and maltotriose whereas those in sugar molasses (25%) were sucrose, fructose and dextrose. The common microorganisms isolated from indigenous microorganism solution were gram positive and facultative anaerobes except *Aspergillus Niger*, *Aspergillus flavours* which were obligate aerobes as they derive their energy by aerobic respiration (Table 1). This suggests that facultative anaerobes were capable of growing in oxygen deprived condition and deriving their energy from fermentation but, if oxygen is available, they can derive their energy through aerobic respiration as well Anyanwu *et al.* (2015). The micro-organisms growth rate using molasses as a medium was lowest (200×10^2 cfu/gm) in acidic pH of 5.5 and highest (595×10^2 cfu/gm) in alkaline pH of 8.5 which implies that, they best multiply under alkaline pH (Table 3). This variation could be due to the fact that, acidic pH inhibits the micro-organism's growth enzymes whereas alkaline pH activates it. This suggestion concurs with Lacoma (2018) who reported that, most microorganisms are neutrophils organisms that prefer a neutral pH level in the medium or environment in which they multiply.

However, at a optimal temperature of 37° C and relative humidity of 60% using molasses as a suitable growth medium compared to maize bran, higher microbial colonies of (595×10^2 cfu/gm) and

580×10^2 cfu/gm were respectively identified (Table 3 and 4). This implies that at temperature and relative humidity below or above the optimal, the free flow of water in and out of the microorganism's cells to exchange materials and for metabolic processes was retarded. High temperatures could have also contributed to the structural cell components denaturation and inactivation of heat-sensitive enzymes meanwhile the low temperature partially disrupted the metabolic regulations. This suggestion agrees with Rajan (2017) and Farkas (2007) who reported that, at low temperatures, microorganism growth reduced reaction rates for the individual enzymes in the organism became much slower, and reduced fluidity of the cytoplasmic membrane that disrupted the metabolic regulations and interfered with transport mechanisms. In addition, Ndobeni (2017) also reported that, temperature heavily affected the bio-processes of microorganisms especially the rate of cell reactions, nature of metabolism, nutritional requirements and biomass composition.

Environmental conditions and foul odour intensity

Disparity in Temperature, pH and humidity of the piggery surrounding greatly affected the relationship between foul odour intensity and human residence in IMO treated and untreated deep litter floor pig house (Table 7 and 8). Low temperature of 22.8° C and high relative humidity of 61.8% increased the intensity of foul odour around the piggery environment especially in deep litter floor pig house without IMO treatment; the increase could be due to inadequate microbial decomposition of pig waste. This suggestion concurs with Kim *et al.* (2014) who reported that, undigested carbohydrate and proteins that passed through the gastrointestinal and urinary tract undergo anaerobic microbial decomposition to produce odorous compounds. In addition, Ranadheera *et al.*, (2017) and Zhang *et al.* (2002) also reported that, during cold weather, relative humidity above 70% low temperature created heavy atmosphere, moist surfaces, increased foul odour generation and retention within pig house

surrounding as stale air was trapped under an inversion layer that restricted dispersion. Furthermore, Le (2006) reported that pH above 7.7 reduced hydrogen sulphides but enhanced ammonia released that aided the generation of stale odour under different environmental condition of temperature and humidity. However, our study disagrees with Le (2005) who reported that, foul odour dispersion increased with increased in temperature.

In IMO treated deep litter floor, high temperature of 28 °C and low relative humidity of 50.5% reduced the foul odour intensity within and around the piggery, the reduction came probably as much of the foul odour were dispersed in the surrounding environment at higher temperature. Another reason could be due to adequate decomposition of pig manure by the IMO, absorption of the emitted or dispersed foul odour by the vegetation cover in the piggery surrounding and distance of human residence away from the piggery units since the frequency of foul odour detection decreased with increased in distance away from the pig house. The surrounding vegetation could have lowered foul odour intensity through intercepting the odorous compounds and diluting ground level air with upper air streams. This suggestion concurs with Anyanwu et al. (2015) who reported that, indigenous microorganisms were capable of accelerating rapid decomposition of organic materials and converting complex organic compound mainly ammonia and hydrogen sulphides into simple odourless gases. In addition, Rahman and Borhan (2012) reported that, deposition of odorous gases on plant surfaces was disrupted by aerodynamically rough surfaces of plant's leaves and branches which lowered odorous gases emission by 60%.

CONCLUSION

Deep litter floor treatment with IMO solution does not eliminate bad odour from pig house but rather reduces the intensity of foul odour to a level which is environmentally friendly and acceptable to the people living around the piggery. Further research should be conducted to determine IMO lifespan in a controlled environment for efficient storage, packaging and frequency of its application on different organic bedding materials.

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Conflicts of Interest

The authors declare no conflicts of interest concerning the publication of this paper.

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