

Article

Indole Degradation in a Model System and in Poultry Manure by *Acinetobacter* spp.

Tujuba Ayele Tesso ^{1,2}  and Guohua Liu ^{1,*}

¹ The Key Laboratory of Feed Biotechnology of Ministry of Agriculture, National Engineering Research Center of Biological Feed, Feed Research Institute, Chinese Academy of Agricultural Sciences, No. 12, Zhong-guan-cun Nandajie, Haidian, Beijing 100081, China; tuj2001@gmail.com

² Department of Biology, Faculty of Natural Sciences, Mettu University, P. O. Box 318 Mettu, Ethiopia

* Correspondence: liuguohua@caas.cn

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Abstract: Indole degradation in a model system and in poultry manure was studied using an enrichment culture of two *Acinetobacter* species; *Acinetobacter toweneri* NTA1-2A and *Acinetobacter guillouiae* TAT1-6A. Degradation of indole was quantified using reverse phase high performance liquid chromatography (HPLC). The two strains were capable of degrading initial concentrations of indole ranging from 58.58–300 mg/L. The degradation efficiency was 66.36% (NTA1-2A), 94.87% (TAT1-6A), and 96.00% (mix) in 6 days when the initial concentration <300 mg/L. The strains were tested for enzymatic activity using 120 mg/L indole. The enzyme extracts of NTA1-2A and TAT1-6A from culture medium degraded indole completely, and no appreciable change of indole concentration was witnessed in the control group. The NTA1-2A, TAT1-6A, and the mix of strains were also used for in vivo poultry manure fermentation and removed 78.67%, 83.28%, and 83.70% of indole, respectively in 8 d. The strains showed a statistically significant difference ($p < 0.05$) in indole removal efficiency compared with the control, but no significant difference between the two strains and the mix in indole removal capacity. We concluded that *A. toweneri* NTA1-2A and *A. guillouiae* TAT1-6A are promising strains to remove indole and its derivatives to control the notorious odor in poultry and other livestock industries.

Keywords: *Acinetobacter*; biodegradation; indole; fermentation; manure

1. Introduction

Indole is a common metabolite of tryptophan formed in the rumen and colon of monogastric animals [1–4]. It is one of the major recalcitrant odorant compounds in livestock and poultry industries [5] that are considered environmental pollutants [6]. It is produced in animal intestines by tryptophan degradation [1,2]. Indole is also industrially produced for various purposes such as pharmaceuticals, cosmetics, pesticides, disinfectants, agrochemicals, and dyestuffs [7,8], and then released into the environment, where it is often found in waste water, costal lagoons, and sediment. It is a typical nitrogen heterocyclic aromatic pollutant and extensively spread in industrial wastewater [9]. Indole contributes to the unpleasant odor of feces in mammals and birds, and are major pollutants affecting public health and livestock industries [10,11]. It can be absorbed into the blood and hemolyze bovine erythrocytes [12], is toxic to many microorganisms, and has a fairly broad bacteriostatic effect [13].

Previous studies used different physico-chemical and biological methods to remove indole from animal manure and waste water [14,15], however the challenge is still persistent and there is demand for an efficient and eco-friendly means of elimination. Microorganisms play a vital role in the removal of indolic compounds and its derivatives under aerobic and anaerobic conditions [2,3,16–18].

Sakamoto et al. [19] reported that indole was degraded aerobically by a bacterium isolated from tap water. Yin et al. [20] investigated aerobic degradation of indole using *Pseudomonas aeruginosa* Gs isolated from mangrove sediment. Under aerobic conditions, indole metabolism was generally initiated by oxidation of indole followed by heterocyclic ring cleavage [21]. Under anaerobic conditions indole could be metabolized to form methane [22]. Bak and Widdel [1] isolated a sulfate reducing bacterium, *Desulfobacterium indolicum* from marine sediments which used indole as its sole carbon source. Bacterial consortia also degrade indole under anaerobic and denitrifying conditions [23–25]. Gu and Berry [26,27] studied the degradation of indole and 3-methylindole by an indole degrading methanogenic consortium enriched from sewage sludge. Microorganisms either mineralized indolic compounds completely [28] or transformed into other compounds in the presence of an additional carbon source [18,29]. Indole biotransformation has also been studied in different bacterial strains [30,31]. For instance, two phenol-degrading strains, *Pseudomonas* sp. PI1 and *Acinetobacter* sp. PI2 isolated from activated sludge could biosynthesis indigo [32]. Qu et al. [33] reported transformation of indole to indigo using a biphenyl dioxygenase enzyme cloned from *Dyella ginsengisoli* L-4 and heterologously expressed in *Escherichia coli* (DE3). Co-metabolism of indole in glucose and nitrate was also reported in the mold *Aspergillus niger* [2]. Aerobic degradation of organic pollutants using microbes is faster and more efficient compared with anaerobic methods [34].

Several bacteria degrading indole were isolated and studied, but only a few indole-degrading *Acinetobacter* strains were reported. For instance, many *Acinetobacter* have been reported in the degradation of n-alkane and long chain hydrocarbons [35,36], and aromatic compounds such as phenol and chlorophenol [37–40]. Recently indole degradation has been reported in genus *Acinetobacter* by different researchers [41–44]. Even though some work has been done on indole biodegradation by microorganisms from different environments such as marine and mangrove sediments, and others [20,28,45,46], limited work has been done on the biodegradation of indole in the poultry industry.

The strains of *Acinetobacter* used in this experiment therefore degraded indole efficiently under aerobic conditions. In this study, two *Acinetobacter* strains previously isolated from chicken manure and acclimated in 3-methylindole [47] were used to degrade indole, and demonstrated strong ability of indole removal from culture medium and poultry manure. Hence, this study evaluates in vivo and in vitro degradation of indole under different conditions using *Acinetobacter toweneri* NTA1-2A and *Acinetobacter guillouiae* TAT1-6A strains.

2. Materials and Methods

2.1. Source of Microorganisms

Bacterial isolates of *Acinetobacter toweneri* NTA1-2A and *Acinetobacter guillouiae* TAT1-6A, previously isolated from poultry manure were used. The strains have been preserved in the China General Microbiological Culture Collection Center (CGMCC) and the preservation numbers are CGMCC No. 14001 and 14002, respectively.

2.2. Medium Conditions and Bacteria Growth

A minimal salt medium (MSM) containing 150 mL of a solution (per L of distilled water) of 0.80 g K_2HPO_4 , 0.20 g KH_2PO_4 , 0.05 g $CaCl_2$, 0.5 g $MgCl_2$, 0.01 g $FeCl_2$, 1.0 g $(NH_4)_2SO_4$, 5.0 g NaCl, and 1.0 g yeast extract (Difco, Detroit, MI, USA), amended with indole in a 250 mL Erlenmeyer flask was prepared for the enrichment and isolation of aerobic indole degrading bacteria [48]. The pH was adjusted to 6 using 1 mol/L solution of NaOH and HCl before sterilization. Pre-sterilized MSM at 121 °C for 20 min was amended with 58.58 mg/L (0.5 mM). Indole was dissolved with sterilized hot water (50 mL, 40 °C); filtered with 0.22 µm pore-size polytetrafluoroethylene (PTFE) membrane syringe filter and used as the source of carbon and energy for the bacteria during the degradation experiment. The bacterial isolates were grown on agar plates amended with 58.58 mg/L (0.5 mM)

indole and incubated for 36 h. The strains were activated using yeast extract (1g/L) with MSM for 24 h. The enrichment culture was started by transferring 100 µL inoculants to sterile MSM amended with 58.58 mg/L (0.5 mM) of indole and was gradually increased.

2.3. Indole Degradation by NTA1-2A and TAT1-6A

Individual colonies of pure culture from the agar plates were selected for inoculation of pre-sterilized MSM containing 1.0 g/L yeast extract (Difco, Detroit, MI, USA) in Erlenmeyer flasks which were incubated on a shaking incubator operating at 140 rpm and 31 °C for 24 h. To start the biodegradation experiment, 100 µL active cultures was used to inoculate pre-sterilized MSM containing indole, and the initial concentration was increased gradually from 58.58 to 351.45 mg/L (0.5 to 3.0 mM). Each treatment was conducted in triplicate, with both uninoculated flasks and flasks without indole serving as controls.

2.4. Factors Affecting Indole Degradation

Temperature (T) and pH were used as single factors to study the characteristics of the strains NTA1-2A and TAT1-6A. All experiments were carried out under sterile conditions. For temperature, the culture temperature was set to 25, 28, 31, 34 and 37 °C separately with constant pH of 6. For the pH factor, the initial pH was adjusted to 5, 6, 7, 8 and 9, respectively using 1 mol/L HCl or 1 mol/L NaOH solution at 31 °C and 140 rpm shaking speed. The initial concentration of indole remains constant during both factors.

2.5. Enzyme Activity Test for Indole Metabolism

The strains NTA1-2A and TAT1-6A were tested for enzymatic activity of indole degradation. The culture media of the strains were filtered, and the supernatant fluid was used to incubate 120 mg/L of indole for 48 h at 31 °C. Concentration of indole was determined after incubation at 31 °C for 48 h.

2.6. Poultry Manure Fermentation Experiment

Poultry manure was collected from the Chinese Academy of Agricultural Sciences (CAAS) breeding farm and fermented for eight days using the two strains. In this experiment, 4 kg of poultry manure was added to 10 L buckets in triplicate and fermented using NTA1-2A (10^6 colony forming unit/mL) and TAT1-6A (10^7 colony forming unit/mL), which was added to each sample and mixed well. Non-inoculated manure was used as a control for each treatment. Samples were collected before treatment and during the fermentation every 2 d and stored at −21 °C in plastic bags. One gram of each sample was mixed with 3 mL of methanol, heated in a water bath for 20 min at 40 °C and vortex-mixed every 5 min. Finally, the sample was placed in the refrigerator at −21 °C for 15 min to accelerate precipitation speed. The supernatant was carefully collected and prepared for HPLC analysis by centrifuging at 12,000 rpm for 10 min, and filtering onto 0.22 µL pore-sized syringe filter membrane. The samples were stored at 4 °C and analyzed using HPLC for indole concentrations using standard indole (Shanghai Macklin Biochemical Co. Ltd., Shanghai, China).

2.7. Sample Preparation for HPLC Analysis

An Agilent C18 column (Agilent Technologies, Inc., Santa Clara, CA, USA), 250 mm × 4.6 mm, with a particle size of 5 µm was used for high performance liquid chromatography (Shimadzu LC-15C, Kyoto, Japan). The mobile phase contained 1% acetic acid and methanol at a ratio of 50:50 (v:v). The flow rate, injection volume, detection wavelength and column temperature were 0.6 mL/min, 20 µL, 260 nm and 30 °C, respectively. In preparation for HPLC analysis, 2 mL of culture samples were collected and prepared by centrifuging (8000×g) (Eppendorf 5427 R Centrifuge, Eppendorf AG, Hamburg, Germany) and filtering through 0.22 µm pore-size PTFE membrane syringe filters. Quantities of the analytes were examined from a standard curve prepared using standard indole

(Shanghai Macklin Biochemical Co. Ltd., Shanghai, China). The quantity of indole in each sample was determined in mg/kg. The recovered quantity was also calculated as the percentage value with respect to the total concentration of indole added in the medium.

2.8. Statistical Analysis

The results obtained were presented as mean values with standard deviation of triplicate assays. Simple linear regressions for indole standard solutions with different gradient concentrations and the growth of *Acinetobacter* strains in the MSM with indole were calculated using Microsoft Excel 2010. The data were subjected to one-way variance analysis (ANOVA). Statistical Package for the Social Sciences (SPSS) Version 20 (IBM Corp., Armonk, NY, USA) was used to analyze the difference between treatment groups. Post hoc analysis was carried out using least significance difference (LSD) test for comparison of means at $p < 0.05$ significance level. Sigma-plot (Version 12.5) was used to sketch graphs.

3. Results

3.1. Indole Removal Efficiency

Indole degradation efficacy tests of NTA1-2A, TAT1-6A, and a mix of the two strains at different initial concentrations of 58.58, 117.15, 175.73, 234.30, 292.88, 351.45 mg/L were inoculated with 100 μ L of the strains and incubated for 6 days under aerobic conditions. Samples (2 mL) were collected every 2 d to determine indole concentration by HPLC. Indole degradation was performed by the bacterial strains in the culture media amended with different concentrations. The degradation efficiency (%) was 66.36%, 94.87%, and 96.0% respectively in NTA1-2A, TAT1-6A, and a mix of strains within 6 days at 2.5 mM (292.88 mg/L) concentration (Table 1). When indole concentration was 3 mM (351.45 mg/L), the strains degraded only 26.43% (NTA1-2A), 25.29% (TAT1-6A), and 29.0% (mix).

Table 1. Indole degradation ability of NTA1-2A, TAT1-6A, and a mix incubated for 6 days. Values are the mean \pm SD of triplicate assays.

C (mg/L)	Incubation Time (d)	Indole Removal Efficiency (%)			p-Value
		NTA1-2A	TAT1-6A	Mix Strains	
58.58	2	77.72 \pm 0.24 ^a	100 \pm 0.00 ^b	100 \pm 0.00 ^b	0.000
	4	77.87 \pm 0.04 ^a	100 \pm 0.00 ^b	100 \pm 0.00 ^b	0.000
	6	77.86 \pm 0.03 ^a	100 \pm 0.00 ^b	100 \pm 0.00 ^b	0.000
117.15	2	88.64 \pm 0.05 ^b	96.32 \pm 6.37 ^a	98.04 \pm 3.40 ^a	0.069
	4	88.60 \pm 0.04 ^a	100 \pm 0.00 ^b	100 \pm 0.00 ^b	0.000
	6	88.61 \pm 0.14 ^a	100 \pm 0.00 ^b	100 \pm 0.00 ^b	0.000
175.73	2	91.89 \pm 0.10 ^b	92.59 \pm 0.04 ^b	94.67 \pm 2.08 ^a	0.054
	4	91.78 \pm 0.10 ^b	92.61 \pm 0.0 ^b	95.57 \pm 2.45 ^a	0.038
	6	91.42 \pm 0.26 ^a	92.61 \pm 0.0 ^a	95.67 \pm 2.31 ^b	0.012
234.30	2	70.37 \pm 1.87 ^b	91.32 \pm 3.69 ^a	93.00 \pm 2.00 ^a	0.000
	4	70.37 \pm 1.86 ^b	91.32 \pm 3.68 ^a	93.00 \pm 2.00 ^a	0.005
	6	76.88 \pm 3.22 ^b	94.16 \pm 2.44 ^a	95.00 \pm 1.73 ^a	0.000
292.88	2	44.33 \pm 9.19 ^b	94.23 \pm 1.31 ^a	94.67 \pm 1.15 ^a	0.002
	4	59.00 \pm 2.78 ^b	94.31 \pm 2.64 ^a	95.67 \pm 1.53 ^a	0.000
	6	66.36 \pm 25.1 ^a	94.87 \pm 1.06 ^b	96.00 \pm 1.00 ^b	0.038
351.45	2	12.71 \pm 7.99	13.33 \pm 10.25	18.00 \pm 6.93	0.538
	4	17.60 \pm 4.91	18.68 \pm 3.91	22.00 \pm 3.22	0.269
	6	26.43 \pm 3.95	25.29 \pm 5.03	19.00 \pm 3.52	0.482

a,b: Values in row with different letters are significantly different ($p < 0.05$).

3.2. Indole Removal at Different Initial Concentrations

The culture media inoculated with bacterial strains degraded indole under aerobic conditions when the initial concentration was ranging from 0.5–3.0 mM (58.58–351.45 mg/L). There was no degradation when the initial concentration of indole was >300 mg/L (Figures 1–3) and also no bacterial colony formation on the nutrient agar plates. Indole degradation resulting from the mix of two strains was similar with that of TAT1-6A (Figures 2 and 3). No appreciable loss of indole was observed in the sterile controls. Indole degradation and proliferation of bacterial strains were inhibited when indole concentration was >351.45 mg/L owing to the toxicity of the substrate.

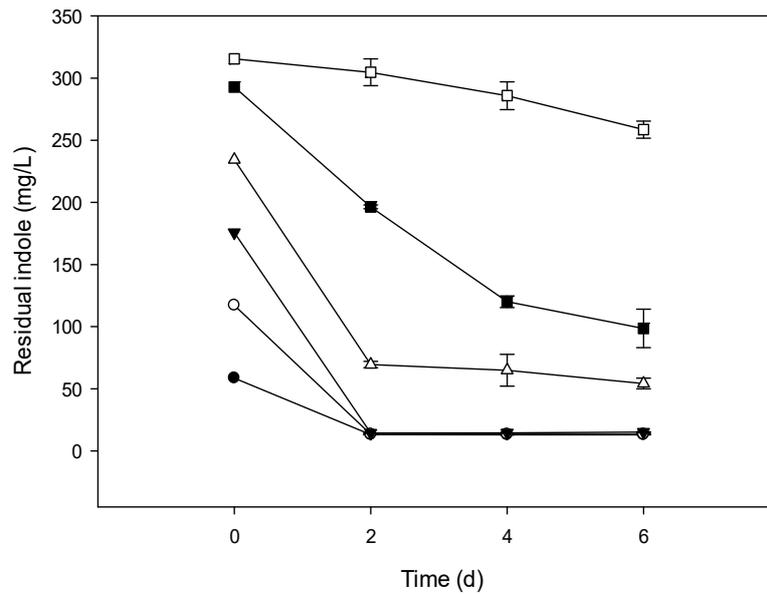


Figure 1. Indole removal capability of NTA1-2A strain at different initial concentrations (mg/L). Symbols: Solid circle, 58.58; open circle, 117.15; solid triangle, 175.73; open triangle, 234.30; solid square, 292.87; open square, 351.45; error bars, mean \pm Standard Error (SE) of three replicates at 31 °C and pH 6.

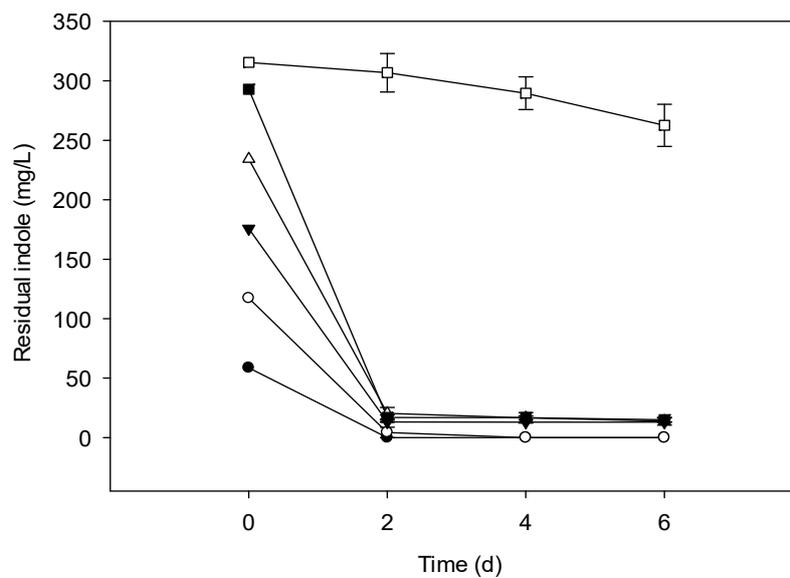


Figure 2. Indole removal capability of TAT1-6A strain at different initial concentrations (mg/L). Symbols: Solid circle, 58.58; open circle, 117.15; solid triangle, 175.73; open triangle, 234.30; solid square, 292.87; open square, 351.45; error bars, mean \pm SE of three replicates at 31 °C and pH 6.

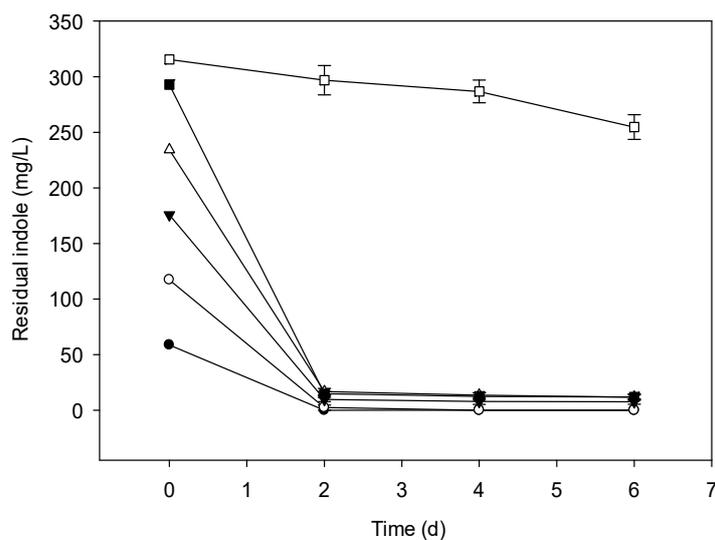


Figure 3. Indole removal capability of a mix of two strains (NTA1-2A and TAT1-6A) at different initial concentrations (mg/L). Symbols: Solid circle, 58.58; open circle, 117.15; solid triangle, 175.73; open triangle, 234.30; solid square, 292.87; open square, 351.45; error bars, mean ± SE of three replicates at 31 °C and pH 6.

3.3. Factors Affecting Indole Degradation

3.3.1. Effect of Temperature and pH

MSM medium amended with 117.15 mg/L (1.0 mM) of indole was inoculated with 100 µL inoculants and incubated for 96 h at temperatures of 25, 28, 31, 34, and 37 °C, and 5, 6, 7, 8, and 9 pH values.

Effect of temperature: The percent degradation efficiency of indole increased as the incubation temperature rose from 25 °C to 31 °C. Peak degradation efficiency was observed at 31 °C and declined as temperature increased to 37 °C (Figure 4).

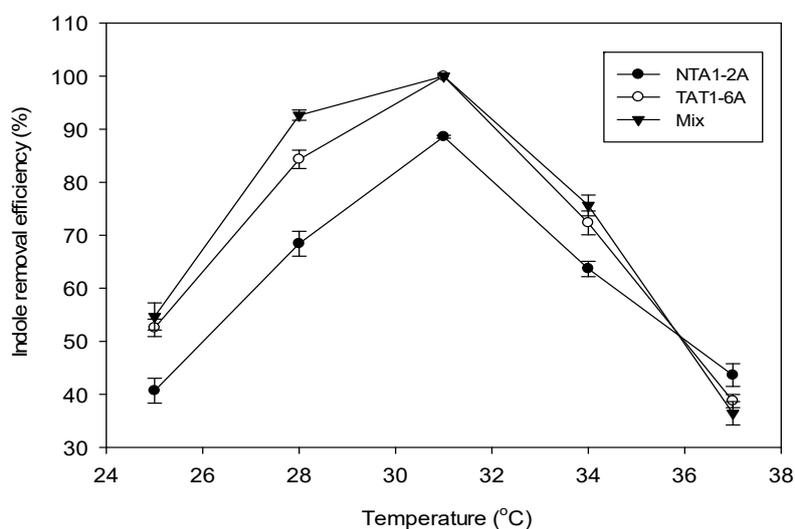


Figure 4. Effect of temperature on indole removal efficiency (%) at pH 6 and 117.15 mg/L (1.0 mM) of indole concentration incubated for 96 h. Error bars: Mean ± SE of three replicates.

Effect of pH: Indole degradation using the two strains was also affected by the pH of the culture media solutions. Both strains and the mix exhibited maximum indole degradation efficiency (%) at pH 6. Strain TAT1-6A and the mix showed increased indole degradation efficiency between pH 6 and 7 (Figure 5).

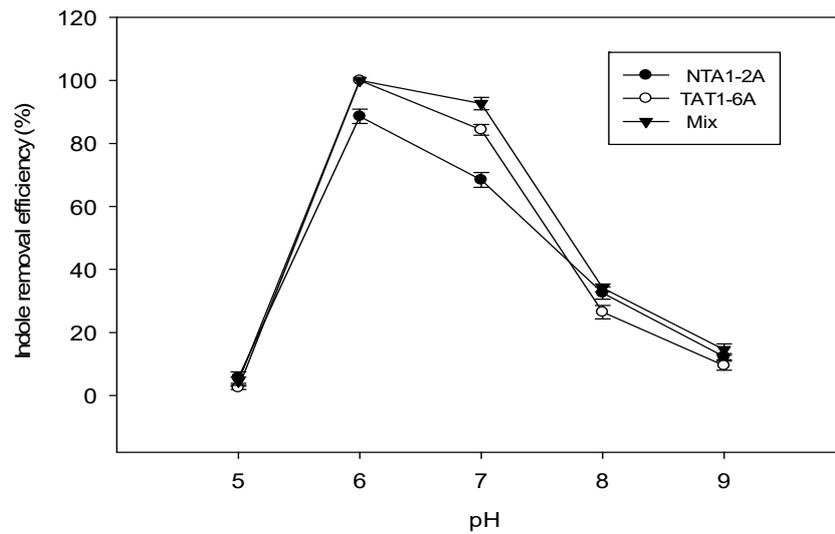


Figure 5. Effect of pH on indole removal efficiency (%) at 31 °C and 117.15 mg/L (1.0 mM) of indole concentration incubated for 96 h. Error bars: Mean ± SE of three replicates.

3.3.2. Effect of Indole on Bacterial Growth

Indolic compounds have an inhibitory effect on the proliferation of microorganisms. In our study, different concentrations of indole were used to demonstrate its effect on the growth of NTA1-2A and TAT1-6A strains (Figure 6).

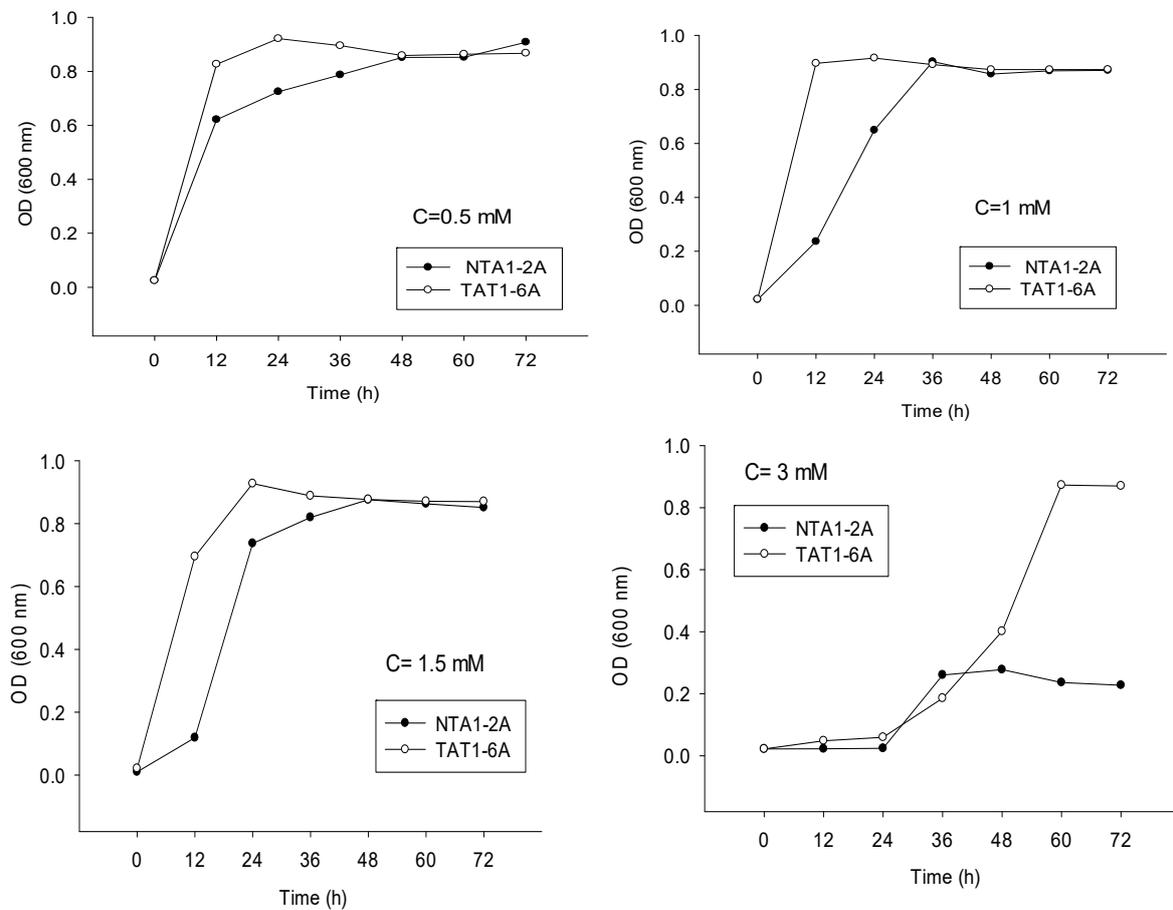


Figure 6. Proliferation of Strains NAT1-2A and TAT1-6A at concentrations (C) of 0.5 mM (58.58 mg/L); 1.0 mM (117.15 mg/L); 1.5 mM (175.73 mg/L) and 3.0 mM (351.45 mg/L) of indole for 72 h.

3.4. Enzymatic Activity of Indole Metabolism

The enzyme activities of crude extracts obtained from cultures of NTA1-2A and TAT1-6A strains were measured. Indole concentration of 120 mg/L was used to test the activity. Supernatant fluid of culture media of both NTA1-2A and TAT1-6A removed 96.34% and 97.71% of indole, respectively (Figure 7) within 48 h. In the control group, there was no appreciable change in indole concentration. The result directly indicated that these strains have the potential to degrade indole.

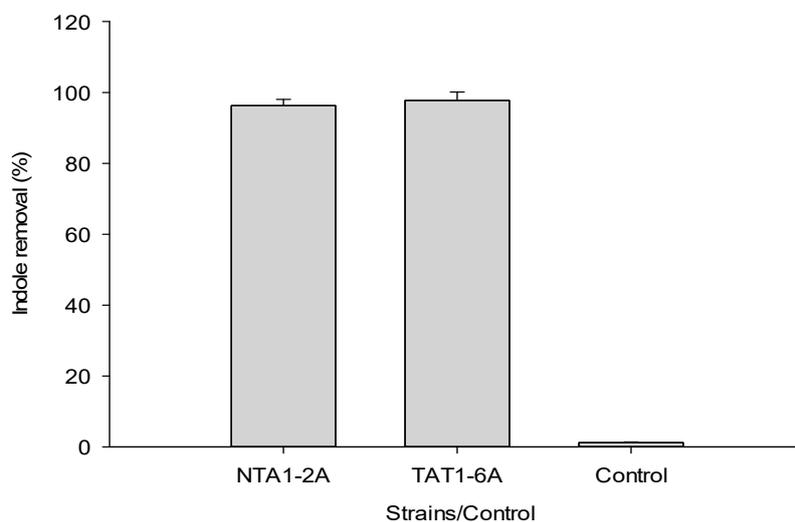


Figure 7. Indole removal test by extract or supernatant fluid obtained from medium cultures of NTA1-2A and TAT1-6A. Error bars: Mean \pm SE of three replicates.

3.5. Indole Removal from Poultry Manure

This experiment was designed to test the practical application and potential of the strains NTA1-2A and TAT1-6A to remove malodorous indolic compounds from poultry manure. The bacterial strains were used to ferment the manure for 8 days. The pretreatment indole content of the manure was determined and used as reference (which was 6.29 ± 0.44 mg/kg of three replicates) to analyze the removal efficiency of the strains. The NTA1-2A, TAT1-6A, and the mix of the strains removal efficacy (Table 2) of indole from the manure was 78.67%, 83.28%, and 83.70%, respectively in 8 days. The strains showed significant removal efficiency ($p < 0.05$) compared with the control group but no significant difference between the two strains and the mix ($p > 0.05$).

Table 2. Indole removal efficiency of NTA1-2A, TAT1-6A and the mix of strains from poultry manure. Data values are mean \pm SD of triplicate assays.

Indole Removal Efficiency from Chicken Manure (%)					
Time (d)	NTA1-2A	TAT1-6A	Mix of Strains	Control	<i>p</i> -Value
2	58.73 ± 5.81 ^{ab}	67.65 ± 4.55 ^a	69.55 ± 1.57 ^a	43.55 ± 6.84 ^b	0.025
4	72.38 ± 3.66 ^b	78.35 ± 2.87 ^{ab}	85.64 ± 1.59 ^a	50.91 ± 5.44 ^c	0.001
6	74.49 ± 2.63 ^a	80.00 ± 2.06 ^a	78.62 ± 3.10 ^a	55.03 ± 4.50 ^b	0.002
8	78.67 ± 1.70 ^a	83.28 ± 1.33 ^a	83.70 ± 1.02 ^a	54.57 ± 2.81 ^b	0.000

a,b,c: Values in row with different letters are significantly different ($p < 0.05$).

4. Discussion

Indole biodegradation was studied in the last two decades using bacteria from different sources; marine sediments [13,20], sewage plant [49,50], and evaluated their degradation potential. For instance, Bak and Widdel [1] studied *Desulfobacterium indolicum* gen. nov., sp. nov. anaerobically degrading

1.5 mM of indole added to sulfate-rich marine mud or sulfate-free sewage digester sludge within one week. Yin et al. [20] reported *Pseudomonas aeruginosa* Gs degrading indole completely in four days when the concentration was 3.0 mM. Recently, *Acinetobacter* strains named NTA1-2A and TAT1-6A obtained from 3-methylindole acclimated samples [47] were used to degrade indole. The strains also utilized indole as their sole carbon source and efficiently removed <300 mg/L indole within six days when cultured in simple mineral salt medium at 31°C and pH 6. *Cupriavidus* strains named IDO and SHE also utilized indole as their sole carbon source and efficiently removed 100 mg/L indole within a day [9,51]. Lin et al. [52] reported that strain *Acinetobacter baumannii* ATCC19606 could detoxify indole by converting it to non-toxic indigo. Sadauskas et al. [41] also investigated *Acinetobacter* sp. O153 which degraded indole via indole-2,3-dihydrodiol, 3-hydroxyindolin-2-one. In another study, *Acinetobacter* sp. JW and *Burkholderia* sp. IDO3 exhibited degradation ability of 100 mg/L indole within 14 and 20 h, respectively [42,43]. Yan et al. [44] reported that *Acinetobacter pittii* L1 can completely remove 400mg/L indole within 48 h. There are also other indole-degrading strains such as *Arthrobacter* sp., *Alcaligenes* sp., *Burkholderia unamae*, *Pseudomonas aeruginosa*, and *Agrobacterium tumefaciens* reported [53–56]. The indole biodegradation results obtained in the current study by strain TAT1-6A is similar with *Pseudomonas aeruginosa* (300 mg/L) reported by Yin et al. [20]. NTA1-2A indole removal ability was low when compared with TAT1-6A, however better than *Pseudomonas* sp. ST-200 (100 mg/L) [28] and *Aspergillus niger* (180mg/L) [2].

Different environmental factors are responsible for the metabolism of indole. Madsen et al. [24] reported that pH, temperature, and concentration of sewage sludge influence indole metabolism under anaerobic conditions. Yin et al. [20] discovered pH and salinity of media solution influences the degradation activity and proliferation of *Pseudomonas aeruginosa* Gs isolated from mangrove sediment. In our experiment, temperature and pH highly affected the degradation of indole (Figures 4 and 5) and our two strains showed better indole removal ability at 28–31 °C and pH 6–7 which is similar with *Acinetobacter* sp. JW [43]. In *Burkholderia* sp. IDO3, Ma et al. [42] reported indole degradation performance maintained under conditions of pH 4.0–9.0 and temperature 25–35 °C which is a broad range compared with NTA1-2A and TAT1-6A strains.

Studies revealed that indole has microbial specific inhibitory effect on their growth [57–59]. Yang et al. [60] reported indole and its derivatives inhibit the growth of microorganisms and biofilm formation. For microorganisms, indole can induce bacterial membrane and oxidant toxicity, prevent cell division by modulating cell membrane potential, inhibit adenosine triphosphate production and protein folding, and cause repairable DNA damage [15,55,61,62]. Due to the toxic nature of indolic compounds to microorganisms, their concentrations were kept low when supplied to culture medium [24,25,48,63,64]. Aromatic compounds are generally lipophilic [28], but it has been observed that *Pseudomonas* sp. ST-200 can grow at a high concentration of indole when an organic solvent–water system consisting of two phases is used to avoid the toxicity effects. In another study, Yin et al. [20] reported that higher concentrations of indole is toxic and inhibits bacterial growth. Similarly, at a concentration >300 mg/L, growth of NTA1-2A and TAT1-6A strains were inhibited and no colony formed on the agar plate. Indole also decreases cell growth of a fungus *Aspergillus niger* [2].

Bacteria play an important role in aerobic and anaerobic fermentation of manure to release nutrients and transform harmful organic compounds. Aerobic fermentation is one of the most effective ways of treating manure harmlessly and resourcefully [65,66]. Animal manure has become a major source of ecological and environmental pollution [67] which is not only harmful for the environment and human health, but also influences the sustainable development of poultry and livestock industries. NTA1-2A and TAT1-6A strains are non-pathogenic, ubiquitous microbes of promising candidates capable of removing 78.67% and 83.28% indole, respectively and 83.70% in a mix from poultry manure in eight days.

Many bacterial strains do have the potential of removing pollutants like indolic compounds and others from the environment under different conditions via the production of intracellular or extracellular enzymes. For example, many non-indole producing bacteria encode enzymes that can

degrade indole and produce indole derivatives [68,69]. Maeng et al. [35] reported n-alkane degradation by *Acinetobacter* sp. attacked by a dioxygenase enzyme. Carr et al. [70] revealed methyl oxidation of 3-methylindole by cytochrome P450 enzymes. Nakai et al. [4] reported supernatant fluids of culture extracted from 3-methylindole degrading isolates showed 3-methylindole degradation activities. In this study, the supernatant fluid extract obtained from the two strains' culture medium completely removed 120 mg/L of indole within 48 h. This is a clear indication that the strains have a promising potential of degrading indolic compounds which are challenging the rapidly expanding intensive poultry and livestock industries.

5. Conclusions

Two *Acinetobacter* strains, *Acinetobacter towneri* NTA1-2A and *Acinetobacter guillouiae* TAT1-6A were tested for in vitro and in vivo indole degradation potential. The strains degraded initial concentrations of indole ranging from 58.58 to 300 mg/L. The degradation rate was >65% in 6 days in both strains when the initial concentration was less than 300 mg/L. The experiment revealed the two strains have the capability of removing indole from poultry manure. We conclude that the outstanding indole removal capacity make the two strains promising candidates to reduce noxious fecal odorants due to indolic compounds in the poultry, livestock industries and other wastewater treatment processes. Future studies should focus on the biochemical and molecular basis of indole degradation by the two strains.

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Conflicts of Interest: The authors declare no conflict of interest.

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