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Efficacy of Chitosan Derived from Tahong (*Perna viridis*) Shells in Treating Swine Biogas Digester Effluent

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Abstract. Chitosan, a natural linear biopolyaminosaccharide, is a non-toxic and environmentally friendly coagulant derived through the deacetylation of chitin, a major component of Tahong (*Perna viridis*) shells. This study evaluates the efficacy of chitosan in modifying the physicochemical properties of treated swine biogas digester effluent under varying chitosan dosages (8 g/L and 10 g/L) and agitation speeds (30 rpm and 50 rpm). The extracted chitosan powder exhibited high deacetylation, quantified at 74.65%. The untreated swine biogas digester effluent displayed the following properties: pH 7.34, COD 704 mg/L, BOD 338 mg/L, phosphate 81.54 mg/L, nitrate 138.75 mg/L, TDS 2027 mg/L, and TSS 2355 mg/L. Optimal treatment conditions were observed at a dosage of 8 g/L and an agitation speed of 30 rpm, yielding effluent properties of pH 8.30, COD 1499.00 mg/L, BOD 348.33 mg/L, phosphate 2.28 mg/L, nitrate 64.93 mg/L, TDS 2950.67 mg/L, and TSS 25.33 mg/L. Comparative analysis against the Department of Environment and Natural Resources (DENR) Class C water standards revealed significant improvements in BOD, phosphate, and nitrate levels, with p-values of 0.000. Conversely, the differences in pH and TSS were not statistically significant, with p-values of 0.141 and 0.199, respectively.

Keywords: Chitin, Chitosan, Coagulant, Effluent, *Perna Viridis* Shells, Wastewater Treatment.

INTRODUCTION

Today, one of the growing commercial sectors in the Philippines is the swine raising. In fact, it is the second leading contributor to the Philippine agriculture despite being almost exclusively without government subsidy which means a greater income and more employment opportunities (Lapuz, 2009). However, alongside its feasibility, environmental issues have been a concern, particularly with the waste it produces, like manure, wastewater, and dead animals. The waste generated by swine farms that are not properly managed and treated is attributed to environmental degradation and pollution. Indiscriminate disposal of large amounts of swine waste and untreated wastewater into the creeks, rivers, and other bodies of water pollutes surface waters.

Swine wastewater is characterized by high organic matter content such as chemical oxygen demand (COD) and biochemical oxygen demand (BOD), and nutrients

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(nitrogen and phosphorus content). Phosphorus (P) and nitrogen (N) are the major elements present in swine wastewater which are involved in the degradation of the environment. The phosphorus causes eutrophication when carried by surface runoff to receiving bodies of water. Nitrogen may threaten public health when it is converted to nitrate which is associated with the occurrence of methemoglobinemia and the possible formation of carcinogenic nitrosamines and nitrosamides (Ferreira, de Lucas Jr.,and do Amaral, 2003).

The current trend in biogas production focuses on utilizing waste generated by swine farms, processed through biogas digesters, to produce renewable energy. However, these digesters also generate effluent containing organic matter and nutrients. The effluent from the digester can be used to irrigate farmlands (Cheng and Liu, 2002). However, it is more economical if the water can be recovered for reuse in the operation of the swine farm, especially if the farm owner has no land to irrigate the effluent discharge.

Generally, swine wastewater can be treated in several ways like using organisms such as algae and bacteria or by engineered treatment. The problem with the use of organisms is that they are difficult to handle. In case of engineered treatment, sophisticated equipment and processes are being utilized. An alternative way of treating swine wastewater is through the use of coagulants. Some of the coagulants that are being used in swine wastewater treatment are aluminium sulfate ($Al_2(SO_4)_3$) or alum, ferric chloride ($FeCl_3$), ferric sulfate ($Fe_2(SO_4)_3$), ferrous sulfate ($FeSO_4$), poly aluminium chloride (PAC), and ferrous chloride ($FeCl_2$). Though, many researchers have stated that chemical coagulants have negative effects on people's health and on the environment because of their toxic and carcinogenic characteristics (MohdZin et. al., 2012). Most of these coagulants are hazardous, corrosive and expensive.

The negative effects of the existing coagulants call for an alternative coagulant that is cost-effective, nontoxic, and environmentally friendly. Hence, chitosan is a compound that can act as a coagulant and satisfy the desired properties. Chitosan, a natural linear biopolyaminosaccharide, that can be obtained by deacetylation of chitin, is the primary component of protective cuticles of crustaceans like crabs, shrimps, lobsters, shellfishes, and some fungi such as aspergillus and mucor. It can be used for the removal of particulate and dissolved contaminants. It can also serve as an alternative for aluminum salts and synthetic polyelectrolytes used in water treatment to avoid the health effects from residual aluminum (III) and synthetic polymers and to produce biodegradable sludge.

Philippine green mussel (*Perna viridis*), which is also known as Asian green mussel and locally known Tahong is found to be rich in chitosan (Sinardi, et. al., 2013). It is a bivalve belonging to the family Mytilidae which is widely distributed in the Asia-Pacific region. These mussels are harvested for food in the Philippines and have provided a staple diet for Filipinos. Once the meats are consumed, the shell wastes that have been generated are usually dumped to the landfills, thrown back to the water systems, or are incinerated (Musico, 2007). These shells are dumped due to the foul odor it generates which attracts vectors of diseases such rodents and insects (Solidum, 2010).

The study aimed to determine the efficacy of chitosan derived from Tahong (*Perna viridis*) shells in treating swine wastewater. Specifically, it sought to attain the following objectives: To characterize the extracted chitosan from Tahong (*Perna viridis*) shells based on the degree of deacetylation (DDA); to determine the properties of the untreated swine biogas digester effluent in terms of the pH, chemical oxygen demand (COD), biochemical oxygen demand (BOD), phosphate content, nitrate content, total dissolved solids (TDS), and total suspended solids (TSS); to investigate the effect of varying the dosage (8g/L and 10g/L) and agitation speed (30rpm and 50rpm) in the aforementioned properties of treated swine biogas digester effluent; to evaluate if there is a significant difference on the above-mentioned properties of the swine biogas digester effluent before and after treatment and to compare the properties of the treated swine biogas digester effluent with the Philippine Class C standard water of Department of Environment and Natural Resources (DENR).

MATERIALS AND METHODS

Collection of Raw Materials

Two (2) kilograms of Tahong shells were used to extract chitosan from Batangas City Public Market. The effluent was collected in the Agricultural Training Institute-International Training Center on Pig Husbandry (ATI-ITCPH), Lipa City, Batangas. Bulk particles in swine biogas digester effluent were removed using a plastic strainer. Thirty liters of swine biogas digester effluent were collected for the study.

Extraction of Chitosan

Chitosan extraction was carried out through the following steps: pre-treatment, demineralization, deproteinization, purification of extracted chitin, and deacetylation. The obtained shells were thoroughly washed with water and dried in an oven to constant weight at a temperature of 35°C. Then, 1 kg of the sample was taken out of the 2 kg for demineralization. Dried shells were crushed and soaked in 0.68 M HCl (1:10 w/v) at ambient temperature (approximately 29°C) for 6 hours, after which it was washed in the acid until no bubbles were seen and no color change was observed. The sample was then thoroughly washed with distilled water until a neutral pH was obtained (using a pH meter to measure the pH of the water used in rinsing the demineralized sample). Then, the demineralized shell was dried to constant weight at 35°C.

The next step was deproteinization, where the dried mussel shells were weighed and soaked in a 0.62 M NaOH solution (1:10 w/w) at ambient temperature (approximately 28°C) for 16 hours. The shells were washed thoroughly with distilled water until a neutral pH was obtained. The chitin was dried to constant weight, ground, and screened with a 150 µm sieve. The weight was then recorded.

The extracted chitin was purified by undergoing demineralization and deproteinization processes again. This time, the demineralization was done by treating the extracted chitin with 1.2 M HCl in a ratio of 15 mL to 1 g of chitin. The mixture was thoroughly mixed for 2 hours to dissolve the remaining minerals. It was filtered and washed free of acid to prevent the acid-alkali reaction in the next stage. The chitin was dried at 30°C and was then weighed. A deproteinization step. This time, deproteinization was carried out using 1 M NaOH in a ratio of 15 mL NaOH to 1 g of chitin. The mixture was thoroughly mixed for 2 hours to dissolve any unwanted proteins. The supernatant liquid was decanted, and the pH of the residue was adjusted to pH 8 using distilled water. It was then dried at 30°C and weighed.

The final step in the extraction of chitosan was the deacetylation step. Using a heating mantle, the chitin obtained from the purification process was deacetylated in 25 M NaOH (1:10 w/w) for 20 hours at 75°C. After deacetylation, the chitosan was washed thoroughly with water, followed by distilled water. The resulting chitosan was then dried to constant weight at 45°C and prepared for characterization. Chitosan extraction was carried out following the procedure described by Abdulkarim (2014), while chitin purification was performed based on the method outlined by Delgado and Dimatulac (2005). Figure 1 presents the schematic diagram illustrating the process of producing chitosan powder from tahong shells.

Preparation of Chitosan Solution

The obtained chitosan powder (248.4 g) was accurately weighed into a glass beaker, mixed with 4.968 L of 1% acetic acid solution (v/v), and kept aside for about one hour to dissolve to obtain 50mg in 1 mL of chitosan (w/v).

Preparation of Swine Biogas Digester Effluent

This study prepared the swine biogas digester effluent by removing the bulk particles using a plastic strainer.

Effluent Treatment with Extracted Chitosan

The water treatment shown in Figure 2 was carried out via agitation using an agitator and magnetic stirrer, followed by sedimentation and filtration at the Chemical Engineering Analytical Laboratory, Batangas State University, Main Campus II. All tests were carried out with 2.3 L samples in a clean container. The prepared chitosan solution was agitated and poured into the effluent swine biogas digester. The coagulation was carried out by first strongly mixing the swine wastewater with the chitosan solution at 250

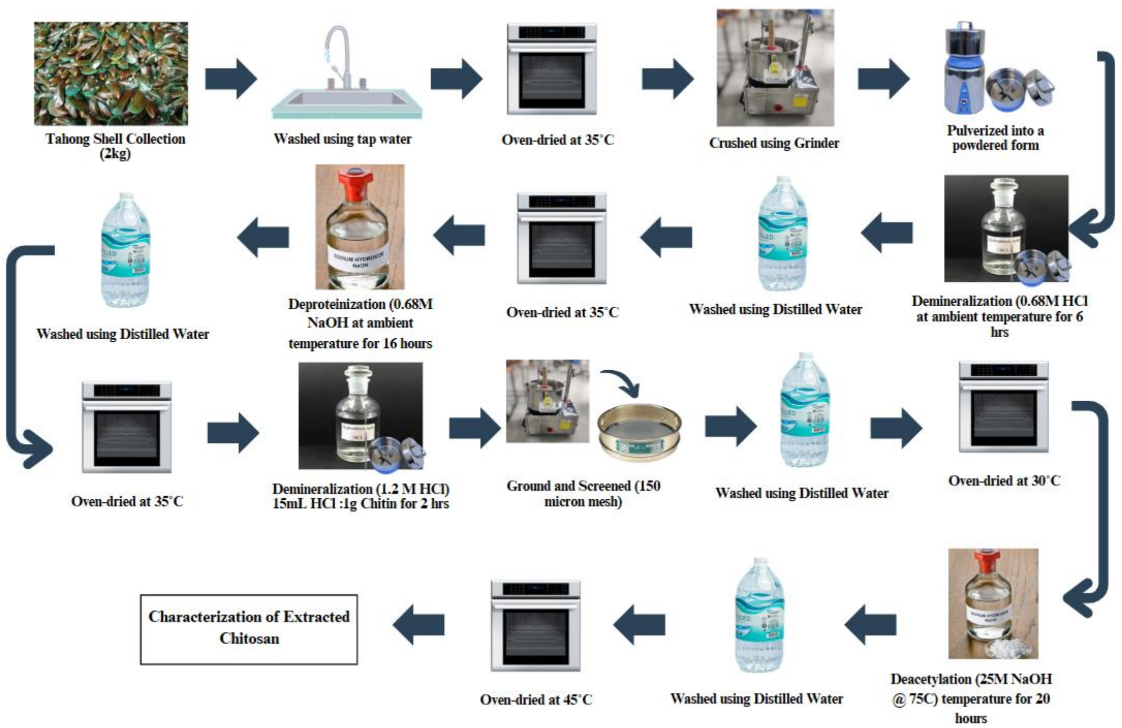

Fig. 1: Process Diagram for the Production of Chitosan Powder

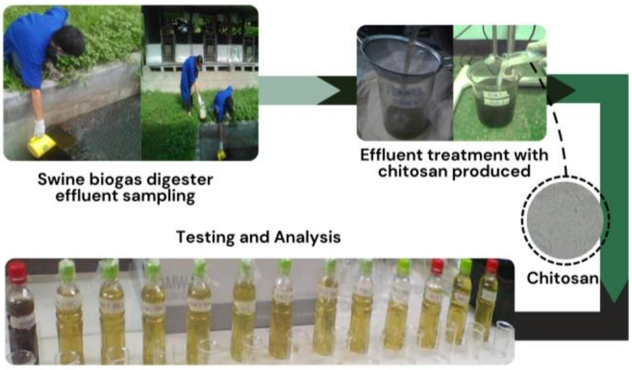

Fig. 2: Water treatment process using chitosan

rpm for 1 minute, followed by slow agitation for 20 minutes. For this study, researchers varied chitosan dosage (8 g/L and 10 g/L) and slow agitation speed (30 rpm and 50 rpm). The optimum condition was concluded after all the tests were done and evaluated.

Testing and Analysis

The degree of deacetylation was determined through Fourier Transform Infrared (FTIR) Spectroscopy, which was conducted at the University of Santo Tomas (UST), Manila.

The properties of swine wastewater, such as pH, TDS, and TSS, were performed at the Chemical Engineering Analytical Laboratory, Batangas State University, Main Campus II. The ASTM standard for filterable and non-filterable matter in water will be used for TDS and TSS. The properties of swine wastewater, such as Chemical Oxygen Demand (COD), Biochemical Oxygen Demand (BOD), phosphate content, and nitrate content, were tested and determined at the Lipa Quality Control Center before and after treatment.

Paired t-test and two-way analysis of variance (ANOVA) were used to test the validity of the hypotheses. A paired t-test was used to statistically determine if there was a significant difference between the properties of the swine biogas digester effluent before and after treatment. A two-way analysis of variance was employed since dosage and slow agitation speed were varied simultaneously. The average value of water properties obtained after the extraction of chitosan treatment was taken as the true value in computing the p-value.

RESULTS AND DISCUSSION

Degree of Deacetylation (DDA)

The Degree of Deacetylation of the extracted chitosan derived from Tahong (*Perna viridis*) shells was 74.6518%, calculated using the absorbance ratio (amine I band) to that of the hydroxyl group. The data used for the calculation was determined using the Fourier Transform Infrared (FTIR) Spectroscopy as illustrated in Figure 3. This result shows higher chitosan conversion compared to the study of Dimatulac and Delgado (2005) on chitosan derived from crab shells with a DDA value of 68.70%.

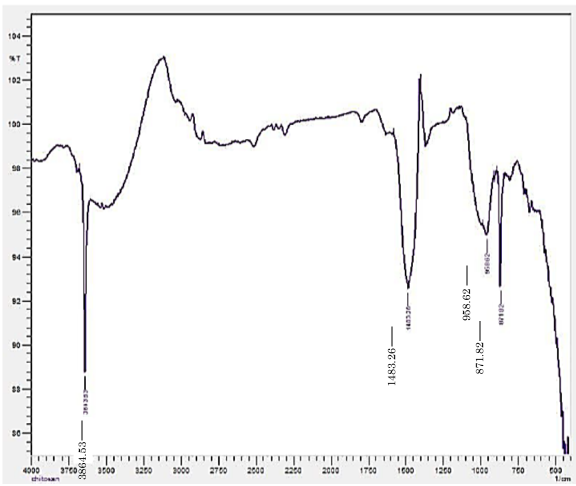

Fig. 3: FTIR results of chitosan

Properties of Untreated Swine Biogas Digester Effluent

The collected swine biogas digester effluent was first analyzed to obtain physicochemical properties such as pH, COD, BOD, phosphate content, nitrate content, TDS, and TSS before treatment. Table 1 presents the properties of untreated swine biogas digester effluent.

Table 1. Properties of untreated swine biogas digester effluent

Properties	Result
pH	7.34
COD (mg/L)	704.00
BOD (mg/L)	338.00
Phosphate (mg/L)	81.54
Nitrate (mg/L)	138.75
TDS (mg/L)	2027.00
TSS (mg/L)	2355.00

Table 2. pH values of swine biogas digester effluent upon varying dosage and agitation speed

Dosage (g/L)	Agitation Speed (rpm)	Untreated pH	Treated pH
8	30	7.34	8.30
	50	7.34	8.38
10	30	7.34	8.63
	50	7.34	8.73

A pH of 7.34 indicates that the effluent is neutral to slightly basic. A value of 704 mg/L for COD indicates that the effluent has a high COD and is highly polluted. The same goes for the BOD, which has a 338 mg/L value. The effluent has high nutrient content, as indicated by its high phosphate and nitrate content, which are 81.54 mg/L and 138.75 mg/L, respectively. The TDS and TSS are indications of the solid content of the effluent. The effluent has a very high solid content based on its values, 2027 mg/L and 2355 mg/L, respectively.

The untreated swine biogas digester effluent in this study, specifically the pH, is almost the same as the effluent studied by Tokhun, N. (2010), with a pH value of 7.4+0.05. On the other hand, the COD, BOD, TDS, and TSS were higher compared to the study of Tokhun, N. (2010) with values of 67+4.00 mg/L, 698+2.65 mg/L, 1,050+7.00 mg/L, and 1,200+624 mg/L respectively. This result shows that the swine biogas digester effluent used in this study has a very high potential of polluting the waterways when discharged without prior treatment.

Properties of Treated Swine Biogas Digester Effluent upon Varying Dosage and Agitation Speed

Each effluent sample was treated with chitosan solution using simultaneous variation of two parameters, such as dosage and agitation speed. The effect of each parameter on the properties of the swine biogas digester effluent was investigated by comparing each possible combination of parameter variables from a common perspective. The following tables present the properties of the swine biogas digester effluent upon varying dosages and agitation speeds.

Table 2 below shows that increasing dosage and agitation speed increases the pH value. For 8 g/L dosage with an agitation speed of 30 rpm and 50 rpm, there was an increase from 7.34 to 8.30 and 8.38, respectively. For 10 g/L dosage with an agitation speed of 30 rpm and 50 rpm, there was an increase from 7.34 to 8.63 and 8.73, respectively. Higher dosage and agitation speed showed a relatively high pH value among the varied parameters. The basic nature of chitosan is attributed to the increased pH of the swine biogas digester effluent upon increasing the chitosan dosage. Also, faster agitation speed enhances the mixing and reaction efficiency, allowing the chitosan to interact more thoroughly with the effluent, thereby increasing the pH.

Table 3 shows that increasing dosage and agitation speed increases COD. An 8 g/L dosage with an agitation speed of 30 rpm and 50 rpm increased from 704 mg/L to 1499.00 mg/L and 1767.67 mg/L, respectively. For 10 g/L dosage with an agitation speed of 30 rpm and 50 rpm, there was an increase from 704 mg/L to 1771.67 mg/L and 1590.00 mg/L, respectively. This result can be attributed to the organic nature of chitosan, which is composed of glucosamine and N-acetylglucosamine units, which increases the overall organic load of effluent (Zaman *et al.*, 2022)

Table 3. COD of swine biogas digester effluent upon varying dosage and agitation speed

Dosage (g/L)	Agitation Speed (rpm)	Untreated COD (mg/L)	Treated COD (mg/L)
8	30	704	1499.00
	50	704	1767.67
10	30	704	1771.67
	50	704	1590.00

Table 4. BOD of swine biogas digester effluent upon varying dosage and agitation speed

Dosage (g/L)	Agitation Speed (rpm)	Untreated BOD (mg/L)	Treated BOD (mg/L)
8	30	338	348.33
	50	338	342.33
10	30	338	362.00
	50	338	354.00

Table 4 shows that increasing dosage and agitation speed increases the BOD. For 8 g/L dosage with an agitation speed of 30 rpm and 50 rpm, there was an increase from 338 mg/L to 348.33 mg/L and 342.33 mg/L, respectively. For 10 g/L dosage with an agitation speed of 30 rpm and 50 rpm, there was an increase from 338 mg/L to 362.00 mg/L and 354.00 mg/L, respectively. The result of this study is comparable to the result of the study of Pontius, F. (2016), which showed that the pH of the wastewater impacts the COD and BOD of wastewater when chitosan is used as a coagulant. It increases the COD and BOD values at higher pH levels, which happened in this study because of a pH of 7.34 for the untreated swine biogas digester effluent. The study by Pontius, F. (2016) found that the optimum wastewater pH for chitosan treatment is 5.5.

Table 5 shows that increasing both dosage and agitation speed decreases the phosphate content. For 8 g/L dosage with an agitation speed of 30 rpm and 50 rpm, there was a decrease from 81.54 mg/L to 2.28 mg/L and 2.91 mg/L, respectively. For 10 g/L dosage with an agitation speed of 30 rpm and 50 rpm, there was a decrease from 81.54 mg/L to 2.77 mg/L and 2.59 mg/L, respectively.

Table 5. Phosphate content of swine biogas digester effluent upon varying dosage and agitation speed

Dosage (g/L)	Agitation Speed (rpm)	Untreated Phosphate Content (mg/L)	Treated Phosphate Content (mg/L)
8	30	81.54	2.28
	50	81.54	2.91
10	30	81.54	2.77
	50	81.54	2.59

Table 6 shows that increasing both dosage and agitation speed decreases the nitrate content. For 8 g/L dosage with an agitation speed of 30 rpm and 50 rpm, there was a decrease from 138.75 mg/L to 64.93 mg/L and 89.60 mg/L, respectively. For 10 g/L dosage with an agitation speed of 30 rpm and 50 rpm, there was a decrease from 138.75 mg/L to 82.13 mg/L and 84.28 mg/L, respectively.

Table 6. Nitrate content of swine biogas digester effluent upon varying dosage and agitation speed

Dosage (g/L)	Agitation Speed (rpm)	Untreated Nitrate Content (mg/L)	Treated Nitrate Content (mg/L)
8	30	138.75	64.93
	50	138.75	89.60
10	30	138.75	82.13
	50	138.75	84.28

Table 7. TDS of swine biogas digester effluent upon varying dosage and agitation speed

Dosage (g/L)	Agitation Speed (rpm)	Untreated TDS (mg/L)	Treated TDS (mg/L)
8	30	2027	2950.67
	50	2027	2575.33
10	30	2027	2910.00
	50	2027	2899.00

Table 8. TSS of swine biogas digester effluent upon varying dosage and agitation speed

Dosage (g/L)	Agitation Speed (rpm)	Untreated TSS (mg/L)	Treated TSS (mg/L)
8	30	2355	25.33
	50	2355	18.00
10	30	2355	13.00
	50	2355	13.67

Table 7 shows that increasing both dosage and agitation speed increases the TDS. An 8 g/L dosage with an agitation speed of 30 and 50 rpm increased from 2027 to 2950.67 and 2575.33, respectively. For 10 g/L dosage with an agitation speed of 30 rpm and 50 rpm, there was an increase from 2027 to 2910.00 and 2899.00, respectively. The increase in the value of TDS is due to the remaining chitosan in the effluent. This result is comparable to the study of Mehta *et al.* (2004), which showed increased dissolved solids in treating wastewater from the pulp and paper industry.

Table 8 shows that increasing both dosage and agitation speed decreases the TSS. For 8 g/L dosage with an agitation speed of 30 rpm and 50 rpm, there was a decrease from 2355 mg/L to 25.33 mg/L and 18.00 mg/L, respectively. For 10 g/L dosage with an agitation speed of 30 rpm and 50 rpm, there was a decrease from 2355 mg/L to 13.00 mg/L and 13.67 mg/L, respectively.

Table 9. Effects of varying parameters in the properties of swine biogas digester effluent

Properties		p-value	Decision H_o	Verbal Interpretation
pH	D	0.087	Accept	Not Significant
	AS	0.606	Accept	Not Significant
	D*AS	0.941	Accept	Not Significant
COD	D	0.576	Accept	Not Significant
	AS	0.542	Accept	Not Significant
	D*AS	0.017	Do not Accept	Significant
BOD	D	0.439	Accept	Not significant
	AS	0.178	Accept	Not significant
	D*AS	0.910	Accept	Not significant
Phosphate	D	0.379	Accept	Not significant
	AS	0.741	Accept	Not significant
	D*AS	0.142	Accept	Not significant
Nitrate	D	0.009	Do not Accept	Significant
	AS	0.00005	Do not Accept	Significant
	D*AS	0.0002	Do not Accept	Significant
TDS	D	0.39	Accept	Not significant
	AS	0.252	Accept	Not significant
	D*AS	0.278	Accept	Not significant
TSS	D	0.294	Accept	Not significant
	AS	0.023	Do not Accept	Significant
	D*AS	0.215	Accept	Not significant

D= Dosage AS= Agitation Speed

The chitosan effectively works as a coagulant in treating wastewater, specifically reducing COD, BOD, TDS, and TTS through charge neutralization. As stated by Rockson-

Itiveh *et al.* (2023), chitosan is a cationic polymer, which means it has a positive charge, while most particles in wastewater are negatively charged. When chitosan is added, it neutralizes these charges, reducing the repulsive force between particles and allowing them to come together and form larger aggregates or flocs. Furthermore, it has a high affinity for various pollutants due to its molecular structure. This allows organic compounds, heavy metals, and other contaminants to be adsorbed, effectively reducing COD and BOD. In addition, this process also captures dissolved and suspended solids, contributing to the reduction of TDS and TSS (Coleman *et al.*, 2024).

Two-way ANOVA was performed and presented in the table below to statistically determine the significance of varying the parameters above.

Table 9 shows the effect of varying dosages and agitation speeds on the properties of the swine biogas digester effluent. Varying dosage and agitation speed in the treatments have no significant difference in the pH of swine biogas digester effluent. As the dosage increased, there was no significant difference in the pH value. Moreover, as the agitation speed increased, there was no significant difference in the pH value. Although there were noticeable changes in the pH upon treatment of the swine biogas digester effluent with varying parameters, the changes were considered statistically insignificant.

As the dosage increased, there was no significant difference in the COD of swine biogas digester effluent. As the dosage increased, there was no significant difference in the COD. Moreover, as the agitation speed increased, there was no significant difference in the COD. On the other hand, simultaneous variation of dosage and agitation speed causes a significant difference in the COD of swine biogas digester effluent. There was also no significant difference in the BOD of swine biogas digester effluent upon varying the parameters. As the dosage increased, there was no significant difference in the BOD. Moreover, as the agitation speed increased, there was no significant difference in the BOD. The same applies to COD. However, there were noticeable changes in the BOD upon treatment of the swine biogas digester effluent with chitosan; the changes were considered statistically insignificant.

The phosphate content of the swine biogas digester effluent also did not significantly differ upon varying dosage and agitation speed. Increasing the dosage and agitation speed did not significantly affect the phosphate content. Upon treatment of the swine biogas digester effluent with chitosan, noticeable phosphate content changes were considered statistically insignificant.

Variable parameters caused a significant difference in the nitrate content of swine biogas digester effluent. As the dosage increased, there was also a significant difference. Likewise, there was also a significant difference as the agitation speed increased. As the dosage and agitation speed increased, there was also a significant increase in the reduction of the nitrate content of the swine biogas digester effluent.

When the dosage was increased, the TDS of the swine biogas digester effluent was not significantly different. Likewise, increasing the agitation speed also did not significantly change the TDS. Although the parameters varied the TDS of the swine biogas digester effluent, the changes were statistically insignificant when the parameters were varied.

The table also shows that the TSS of swine biogas digester effluent is not significant in the treatment upon varying dosage while being significantly different upon varying agitation speed. As the dosage increased, there was no significant difference. Meanwhile, as the agitation speed increased, there was a significant difference, thus significantly reducing the TSS of the swine biogas digester effluent. Increasing the agitation speed also increases the intimate contact between the chitosan and effluent, making the suspended solids coagulate more effectively.

Considering factors such as dosage, agitation speed, and the interaction of dosage agitation speed, there was a significant effect on the nitrate content of the properties of the treated swine biogas digester effluent, which became the basis for choosing the optimum condition. Also, only the agitation speed was considered for TSS since it was the only factor that showed a significant effect.

Table 10. Difference on the properties of swine biogas digester effluent before and after treatment upon varying dosage and agitation speed

Properties	Un-treated	8 g/L		10 g/L	
		30 rpm	50 rpm	30 rpm	50 rpm
pH	7.34	8.30	8.38	8.63	8.73
COD (mg/L)	704	1499	1767	1771	1590
BOD (mg/L)	338	348	342	362	354
Phosphate (mg/L)	81.54	2.28	2.91	2.77	2.59
Nitrate (mg/L)	138.75	64.93	89.60	83.13	84.28
TDS (mg/L)	2027	2950	2575	2910	2899
TSS (mg/L)	2355	25.33	18.00	13.00	13.67

As shown in Table 10, the variation that had the highest reduction for the nitrate content of the swine biogas digester effluent was at 8 g/L dosage and 30 rpm agitation speed. On the other hand, the highest reduction in TSS was attained with an agitation speed of 30 rpm. Also, treatment using 8 g/L dosage and 30 rpm agitation speed attained the highest reduction for phosphate content.

Difference on the Properties of Swine Biogas Digester Effluent before and after Treatment

Table 11 presents the statistical difference in the properties of swine biogas digester effluent before and after chitosan treatment. A paired t-test was used to analyze the data.

Table 11. Difference on the properties of swine biogas digester effluent before and after treatment

Properties	Un-treated	8g/L 30rpm	p-value	Verbal Inter-pretation
pH	7.34	8.30	0.001	Significant
COD (mg/L)	704	1499	0.024	Significant
BOD (mg/L)	338	348	0.508	Not Significant
Phosphate (mg/L)	81.54	2.28	0.000011	Significant
Nitrate (mg/L)	138.75	64.93	0.001	Significant
TDS (mg/L)	2027	2950	0.052	Not Significant
TSS (mg/L)	2355	25.33	0.000	Significant

The optimum condition for the treatment of swine biogas digester effluent using chitosan caused a significant change in pH value along with COD, phosphate content, nitrate content, and TDS having a p-value of 0.001, 0.024, 0.000011, 0.001 and 0.000 respectively. The change in pH was statistically significant, which caused a 13.08% increase, while COD was doubled upon treatment. As per the nutrient content of the effluent, the phosphate and nitrate content decreased by 97.21% and 53.2%, respectively. The change in the TSS showed a high significance causing a reduction of 98.92%.

The change in the pH before and after the treatment of the swine biogas digester effluent can be attributed to the basic nature of the chitosan. On the other hand, an increase in COD and BOD after treatment is comparable to the study of Pontius (2016). The pH of swine biogas digester effluent must first be lowered by adding acid to attain the optimum pH of 5.5 before treatment with chitosan to reduce the COD and BOD. However, this study had a higher TSS removal of 98.92% compared to the study of Singh *et al.* (2006), which utilized alum in the treatment of liquid swine manure, and the study of Garcia and Moreno (2008), which utilized chitosan in the separation of solid-liquid dairy manure having a TSS removal of 85% and 95% respectively. This means that the chitosan is very efficient in removing suspended solids.

Difference on the Properties of Treated Swine Biogas Digester Effluent and DENR Class C Standard

Table 12 presents the statistical difference in treated swine biogas digester effluent properties and the DENR quality standard for Class C water. The data were analyzed using an independent t-test.

Table 12. Difference on the properties of treated swine biogas digester effluent and DENR Class C standard

Properties	Class C	Treated Effluent	p-value	Verbal Interpre-tation
pH	6.5-8.5	8.30	0.141	Not Significant
BOD (mg/L)	7-10	348.33	0.000	Significant
Phosphate (mg/L)	0-0.4	2.28	0.000	Significant
Nitrate (mg/L)	0-10	64.93	0.000	Significant
TSS (mg/L)	0-30	25.33	0.199	Not Significant

As shown in Table 12, the properties of swine biogas digester effluent, such as BOD, phosphate, and nitrate content, all have p-values of 0.000, thus having a significant difference with Class C standard water of the Department of Environment and Natural Resources (DENR). On the other hand, pH and TSS with p-values of 0.141 and 0.199, respectively, have no significant difference with that of Class C standard water of the Department of Environment and Natural Resources (DENR).

Among the properties being monitored by the DENR for Class C standard water, only the pH and TSS, with 8.30 and 25.33 mg/L values, passed the standard. Although there was a large reduction in the value of nitrate and phosphate of swine biogas digester effluent upon treatment with chitosan, values still did not meet the Class C standard. Moreover, lower nutrient content in swine biogas digester effluent can lessen the risk of eutrophication when discharged to bodies of water. On the other hand, a high BOD value can lower the dissolved oxygen in the receiving bodies of water upon discharge, which may cause the death of the organisms in the bodies of water

CONCLUSIONS

The following conclusions were made after conducting the whole experiment and analyzing the data statistically. The chitosan powder possesses a high degree of deacetylation, indicating a good coagulating capability for treating swine biogas digester effluent. The effluent's properties exceed the wastewater quality standard except for pH. Upon varying dosage and agitation speed, there are noticeable changes in the properties of the treated swine biogas digester effluent. The optimum dosage and agitation speeds are 8g/L and 30 rpm, respectively. There is a significant difference in the properties of the swine biogas digester effluent before and after treatment with chitosan. The change in pH was statistically significant, which caused a 13.08% increase. COD was doubled upon after treatment. As per the nutrient content of the effluent, the phosphate and nitrate decreased to 97.21 and 53.2%, respectively. The change in the TSS showed a high significance, which caused a reduction of 98.92%. The produced chitosan has proved to be effective in removing suspended solids and nutrients such as phosphate and nitrate. Upon comparing the properties of the treated effluent to the Class C Standard of DENR, only the pH and TSS met the standard.

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