Chapter

Mitigation of Environmental Impact of Intensive Animal Farming through Conversion of Animal Wastes to Value-Added Products

Abigail N. Tasaki and Ken Tasaki

Abstract

The environmental impact of concentrated animal farming operations has become serious social issues, with the livestock wastes contaminating waterways and groundwaters and generating greenhouse gas (GHG) emissions that are responsible for more than half the total GHG emissions in agricultural activities in the U.S. These impacts are mostly due to the current practice of spraying manure or manure digestate on croplands. We have recently developed two novel processes not only to mitigate the impacts stemming from the current manure management practice but also to bring in extra revenues to livestock farmers, which should provide an incentive to the farmers, by recovering value-added products from livestock manure or manure digestate. In this review, we discuss the effectiveness of the processes to produce two products: protein hydrolysate feed additives from the manure-digestate solid by one process and renewable ammonia from the manure-digestate liquid by another. One process uses thermal hydrolysis to extract protein from manure-digestate solid at a moderate recovery rate of more than 60%. Another employs acid-base reactions to strip NH_3 from manure-digestate liquid and dissolve the stripped NH_3 gas into the water at a high recovery rate of 90%. By repeating this stripping process, the nitrogen concentration in the water can reach as high as 18%.

Keywords: dairy digestate, greenhouse gas, renewable ammonia, protein hydrolysate, thermal hydrolysis

1. Introduction

According to Food Agriculture Organization, the world's meat consumption in 2020 was estimated to be 328 Mt and is expected to reach 374 Mt by 2030 [1]. As the meat consumption increases worldwide, the environmental impacts of livestock agricultural wastes are becoming serious issues. Insufficient disposal or management of the wastes can not only result in environmental contaminations, but also create human health hazards. Inadequate manure management has been causing

eutrophication in rivers, lakes, and bays through runoff of excess nutrients as well as groundwater contamination by leaching of manure through the soil. As a result, livestock manure alone is responsible for about 100 $TgNy^{-1}$ of the nitrogen (N) input on the earth in 2005, twice as much as the N input of roughly 50 $TgNy^{-1}$ in 1950 [2].

Anaerobic digestion of livestock manure is becoming popular, driven by an incentive to receive the renewable identification number by selling biomethane. An anaerobic digester (AD) generates the digestate as the discharge which is often treated by a solid-liquid separation. The solid is often used as low-valued solid fertilizers, while the liquid is sprayed on croplands. This N-rich liquid needs to be sprayed over a wide area to keep the nitrogen level in the soil at a certain level, currently regulated at the state levels. As the consolidation of livestock operations progresses, the livestock headcount/farm grows rapidly, generating ever more manure per ft^2. As a result, regulations as to how much N can be sprayed per ft^2 of croplands are also being tightened. As a consequence, it is becoming increasingly difficult for livestock farmers to apply manure liquid economically without exceeding the N nutrient required for growing crops in the nearby lands in order to avoid the build-up of excess N in the soil.

The current practice of applying the digestate (to be referred to as digestate) solid and liquid can cause another problem: greenhouse gas (GHG) emissions. Nitrous oxide (N_2O), almost 300 times as potent as the global warming potential of CO_2, is generated by anaerobic denitrifying bacteria in the soil from ammonia after fertilizer applications. More than half (54.8%) of the total GHG emissions from agricultural activities in the U.S. are due to the N_2O emissions from fertilizer applications to the soil. Of those N_2O emissions, 30–50% originate from applications of animal manure, which includes organic nitrogen [3, 4]. Application of liquid manure or digestate to the soil provides the available N and carbon, which in turn promote heterotrophic activity, depleting the oxygen availability in the soil, and thus favor the creation of anaerobic microbes that release N_2O via denitrification [5, 6]. The current manure management practice causes the disruption of the N cycle on Earth. However, there is potentially a considerable opportunity to reduce the N_2O emissions from the soil without spraying manure as is, but instead by applying clean inorganic nitrogen recovered from animal manure without creating anaerobic conditions in the soil. This approach will be the first focus of our report.

Table 1 lists the average concentrations of various nitrogen sources determined by daily samplings by one of the authors over a week from the digestate liquid after a solid-liquid separation on a dairy farm with 5000 heads in a Midwestern state. As **Table 1** indicates, the concentration of NH_4^+ is the highest among nitrogen sources. Since NH_4^+ can be easily converted to NH_3, depending on the temperature and pH, a significant volume of NH_3 can be lost through emissions into the atmosphere during the storage in a lagoon, though some go through the natural transformation of

Nitrogen	Concentration
TN[a]	1900
NH_4^+	1800
NO_3	9
N_{org}[b]	91

[a]*The total nitrogen.*
[b]*The organic nitrogen.*

Table 1.
Nitrogen concentrations in dairy digestate liquid (mg/L).

nitrification or denitrification during the storage as well. Therefore, it is preferable to recover NH_4^+ before storage in a lagoon.

The nutrient runoff or leaching of nutrients in manure into the soil is also a waste of agricultural resources. Livestock manure is an important source of nutrients for crops and grains. For example, the U.S. annual consumption of N for crop production was 13 Mt in 2015 [7]. On the other hand, the estimated N produced from livestock animal manure in 2007 in the U.S. was 6.2 Mt [8]. Almost half the N fertilizer consumption for crop productions could be replaced by N recovered from animal manure. Accordingly, the recycling of N from manure is a key to the efficient utilization of agricultural resources and the protection of the environment.

Stripping/scrubbing processes are common for the NH_3 recovery. There are many NH_3 stripping and scrubbing processes such as AMFER [9], Dorset LGL [10], and BIOCAST Process [11]. AMFER can have a relatively high NH_3 recovery rate of 80%; yet it uses heat for the NH_3 stripping which increases the operation cost. What is common among these ammonia stripping processes is that the initial and operational costs of ammonia recovery using a stripping tower and a scrubber tower are high in general. The CAPEX has been estimated to be up to $17.5 million for 800 m^3 day^{-1} of flow rate with 2500 NH_4-Nmg L^{-1} of the NH_4^+ concentration [9]. Further, to be cost-competitive with other recovery technologies, the NH_4^+ concentration in the influent must be higher than 2000 mg L^{-1} which limits the application of these processes. In addition, when the digestate liquid is used as the feed, it always contains CO_2 which needs to be removed by a CO_2 stripper prior to the NH_3 stripping, since CO_2 is acidic, raising the caustic soda consumption to increase the pH for the NH_3 stripping. Moreover, the NH_4^+ concentration in the recovered solution is determined by the initial concentration of NH_4^+ in the feed which limits how high the N concentration in the recovered solution can go. As we will explain later, our process can produce high N-concentration fertilizers.

Membranes are often used for NH_3 recovery from manure. Membrane filtrations include ultrafiltration, nanofiltration, reverse osmosis (RO), electrodialysis, and membrane distillation [9]. For example, Riaño et al. have developed a gas permeable membrane to recover NH_3 from manure in a lagoon, controlling the pH gradient between inside and outside of the membrane as the mass transfer driving force [12]. Though high total ammonia removal rates, 79–99%, were obtained, the NH_4^+ concentration in the recovered tank was low, below 2% [12]. Furthermore, all membrane technologies have a fundamental problem of membrane fouling, especially treating manure which has a large content of organic matters, a main cause of fouling.

A challenge of recovering ammonia from manure/digestate liquid is removing undesirable materials in manure slurries without serious membrane fouling or high energy costs and then concentrating ammonia without using high-energy processes such as RO. On the other hand, once NH_4^+ in the manure liquid is transferred to the NH_3 gas, the NH_3 recovery becomes less problematic, leaving behind undesirable materials in the liquid phase. We have developed a simple process to recover NH_3 from manure/digestate liquid by applying acid-base reactions for the NH_3 stripping and dissolving the stripped NH_3 gas into the water.

As to the digestate solid, it contains a considerable amount of protein, from 12 to 48 wt.%, depending on the animal and the growth period, according to the report by Pacific Northwest National Laboratories [13]. There is a substantially large volume of protein in livestock manure that could be potentially recovered, as is shown in **Table 2**.

Currently, such a protein is often being wasted. When the digestate solid is sprayed on croplands, protein in manure tends to stay in the soil longer since protein is not available to plants immediately as a nutrient; hence, it can be subject to environmental

	Protein[a] %	Manure[b] kg/day/head	Solid[c] %	Dry manure[d] kg/day/head	Protein[e] kg/y/head	Head counts[f] million	Protein[g] MMt/y
Dairy	18.1	25.8	12.7	3.3	216.7	144	31.2
Cattle	12.1	22.5	11.6	2.6	138.6	987	136.8
Hog	25.1	4.7	9.2	0.4	36.4	654	23.7
Poultry	39.8	0.1	25.2	0.0	2.1	68,566	146.8
Total							338.7

[a]*The protein content in manure on a dry matter basis [13].*
[b]*The weight of manure discharged a day per head of an animal [14].*
[c]*The solid content in manure [14].*
[d]*The weight of dry manure discharged a day per head of an animal [14].*
[e]*The weight of protein discharged a day per head of an animal.*
[f]*The global head counts of each livestock animal [15].*
[g]*The annual weight of protein discharged by livestock animals globally in million.*

Table 2.
The estimated global volume of protein generated by livestock manure.

contamination before the complete breakdown of protein, if not properly treated. From a point of view of biological wastewater treatments, protein belongs to what is called biologically non-degradable organic nitrogen compounds which are difficult to treat by conventional treatment processes [16]. It would be beneficial if the protein is recovered from manure before it causes environmental problems. The recovered protein can be converted to various value-added products. We propose one application using the recovered protein: an antioxidant feed additive. The protein recovery from manure will be the second focus of this review.

A process of protein extraction from manure solid has been patented, using solvent extraction [17]. Their approach applies a high concentration, 1 M, of an alkali to the extraction. Such an approach not only requires separation of the alkali after the extraction, but recycling or disposal of the spent alkali as well. We have developed alternative extraction process, using the thermal hydrolysis process (THP) without the use of any chemical. THP has been applied to the extraction of bioactive compounds from plants successfully [18, 19]. Very few reports have been published on the application of THP for protein recovery from manure/digestate solid in the literature. We will examine the efficacy of the protein recovered from the digestate solid for its antioxidant activity.

Our objective is to discuss the two novel processes to recover value-added products from both digestate solid and liquid not only to mitigate eutrophication and GHG emissions associated with livestock manure, but also to bring in extra revenues from the products, some of which can be a renewable N fertilizer or non-carbon renewable energy, bioammonia, and an antioxidant feed additive.

2. Materials and methods

2.1 Materials

2.1.1 NH_3 recovery

For the NH_3 recovery, we used a formulated dairy digestate liquid sample by preparing an NH_4^+ solution with the NH_4^+ concentration of 2100 mg/L and alkalinity

of 8800 mg/L by mixing NH_4OH and $NaHCO_3$, respectively, with 1 L of distilled water. This was to simplify numerous experiments anticipated to be performed to optimize the recovery conditions. Once optimized, the real digestate liquid will be used for validation of our results. The above concentrations were obtained by analyzing samples of the digestate liquid taken from a centrifuge effluent of the dairy digestate on a dairy farm with 5000 Holstein cows. The chemicals used were ammonium hydroxide (28% NH_3 in H_2O, Sigma Aldrich), sodium bicarbonate ((≧99.7%, Sigma Aldrich), sodium carbonate (≧99.5%, Sigma Aldrich), and sulfuric acid (≧99.99%, Sigma Aldrich), all without further purification.

2.1.2 Protein recovery

The manure digestate solid (DS) sample was collected from the solid separated by a screw separator from the digestate effluent of AD using as the feed the dairy manure from a dairy farm in California Central Valley. The DS sample had a 50% water content. 24.8 g of DS sample was mixed in 1 L of distilled water and treated by THP without any pretreatment. The chemical composition of the dried DS sample is listed in Section 3.

2.2 Methods

2.2.1 NH_3 recovery

Our process design principle is to keep the process as simple as possible: our NH_3 recovery system consists of two columns: the NH_3 stripping by aeration in one column and the NH_3 dissolution into the water in another.

Figure 1 illustrates the experimental setup for the NH_3 recovery. Namely, our process strips the NH_3 gas from manure/digestate liquid by aeration, using a low-cost chemical, specifically a Brønsted base, in Column a and then dissolves the NH_3 gas into an acidic aqueous solution in Column b to produce a highly concentrated N solution.

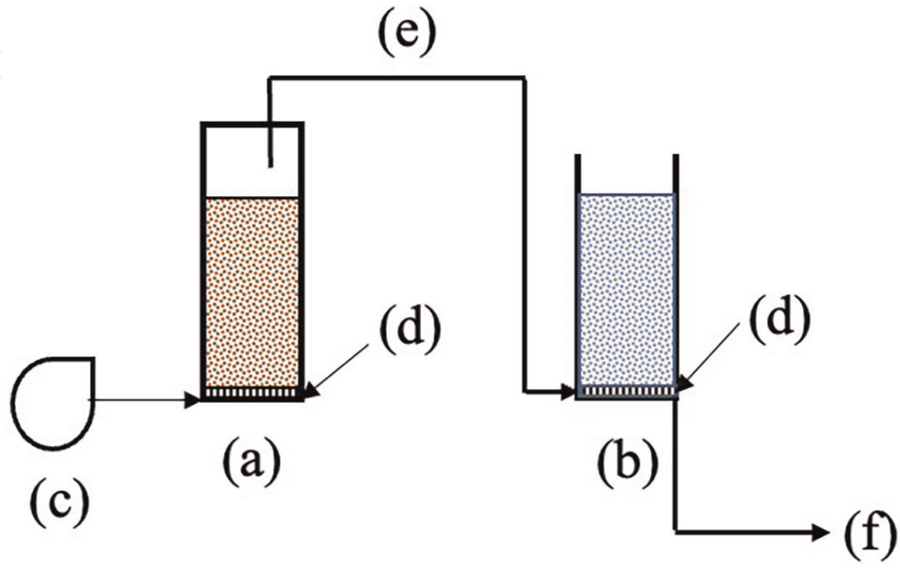

Figure 1.
Experimental setup for the NH_3 recovery: (a) the NH_3 stripping column, (b) the NH_3 recovery column, (c) an air pump, (d) an air diffuser, (e) a pipe, and (f) discharge of the NH_3 solution.

Our process is based on the following reactions for the NH_3 stripping, eq. 1, and the NH_3 dissolving into the water for recovery, eq. 2:

$$NH_4^+ + B^- \rightarrow NH_3 + BH \quad (1)$$

$$NH_3 + AH \rightarrow NH_4^+ + A^- \quad (2)$$

where B^- and AH refer to an anion of a Brønsted base and acid, respectively. There is no membrane and no evaporator involved in our process. Our process can produce highly concentrated N solutions from which liquefied NH_3 can be obtained. Liquefied NH_3 has an energy density twice as much as liquefied H_2 and is receiving increasing attention as the next generation of zero-carbon energy storage or fuel [20].

The formulated sample was first introduced into Column a, the stripping column, while an acid solution was poured into Column b, the recovery column. The acid solution was prepared by mixing 550 g of 99.99% sulfuric acid with 1 L of distilled water. Stone air diffusers were located at the bottom of each column for aeration. Before aeration of the formulated sample, 7 g of Na_2CO_3 was added to Column a which triggered the following reaction:

$$2NH_4^+ + Na_2CO_3 \rightarrow 2Na^{2+} + 2NH_3 + CO_2 + H_2O \quad (3)$$

The stoichiometric amount of Na_2CO_3, given 2100 mg/L of NH_4^+, was 6.18 g. The excess amount of Na_2CO_3 was added to ensure the completion of eq. 3. According to eq. 3, one mole of Na_2CO_3 produces two moles of ammonia, and CO_2 is produced as a by-product. The subsequent aeration stripped ammonia produced by eq. 3. The NH_4^+ concentration was monitored by a UV-vis photo spectrometer (DR 6000 by Hach) over time. The NH_4^+ removal rate, η_{remove}^{NH4}, was defined by the following equation:

$$\eta_{remove}^{NH4} = 100 \times \left\{1 - \left([NH_4^+]_i - [NH_4^+]_f^{strip}\right)/[NH_4^+]_i\right\} \quad (4)$$

where $[NH_4^+]_i$ and $[NH_4^+]_f^{strip}$ refer to the initial and the final NH_4^+ concentration in Column a, respectively.

The NH_3 gas stripped in the stripping column was sent to Column b, along with other gases, N_2, O_2, and CO_2, through a pipe and dissolved into the acid solution through a stone diffuser. When the NH_3 gas contacts the acid solution, the following reaction occurred:

$$2NH_3 + H_2SO_4 \rightarrow (NH_4)_2SO_4 \quad (5)$$

This is an acid-base reaction continuing until all sulfuric acid is consumed. The NH_4^+ concentration in the recovery column was monitored over time. The NH_4^+ recovery rate, $\eta_{recovery}^{NH4}$, was defined by the following equation:

$$\eta_{recovery}^{NH4} = 100 \times \left\{1 - \left([NH_4^+]_i - [NH_4^+]_f^{rec}\right)/[NH_4^+]_i\right\} \quad (6)$$

where $[NH_4^+]_f^{rec}$ represents the final NH_4^+ concentration in Column b.

The above operation was continued by replacing the spent formulated sample in Column a with a new one and adding the same amount of Na_2CO_3 into the Column a after each batch aeration until the NH_4^+ concentration no longer increased in the Column b. No additional H_2SO_4 was added to Column b. The original amount of

H_2SO_4 added to Column b, 550 g, was determined by the maximum solubility of $(NH_4)_2SO_4$ in water, 744 g/L. When all H_2SO_4 was consumed in Column b, the operation was stopped.

2.2.2 Protein recovery

We have developed a two-heating step process for the extraction of protein from DS by THP. The detailed description of the THP treatment followed by ultrafiltration (UF) for the recovery of protein from DS has been described elsewhere [21]. We have utilized a series of instrumental characterizations of the recovered protein hydrolysates (PHs): sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectroscopy, and amino acid analysis (AAA). Then, we evaluated the efficacy of PHs as an antioxidant by the in-vitro oxygen radical absorbance capacity (ORAC) measurements. All methods were described in our previous work [21].

3. Results and discussion

3.1 NH_3 recovery

3.1.1 NH_3 removal and recovery rates

Figure 2 shows the NH_4^+ concentrations in the stripping column as a function of time: (a) without and (b) with Na_2CO_3. When Na_2CO_3 was not used, the concentration decreased about by half and then became a plateau. Since the formulated sample had a pH of 8, NH_3 was stripped in the beginning; hence, the NH_4^+ concentration was reduced. As NH_3 was stripped, H^+ was released by NH_4^+, raising the pH of the formulated sample which slowed down the NH_3 stripping which eventually came to

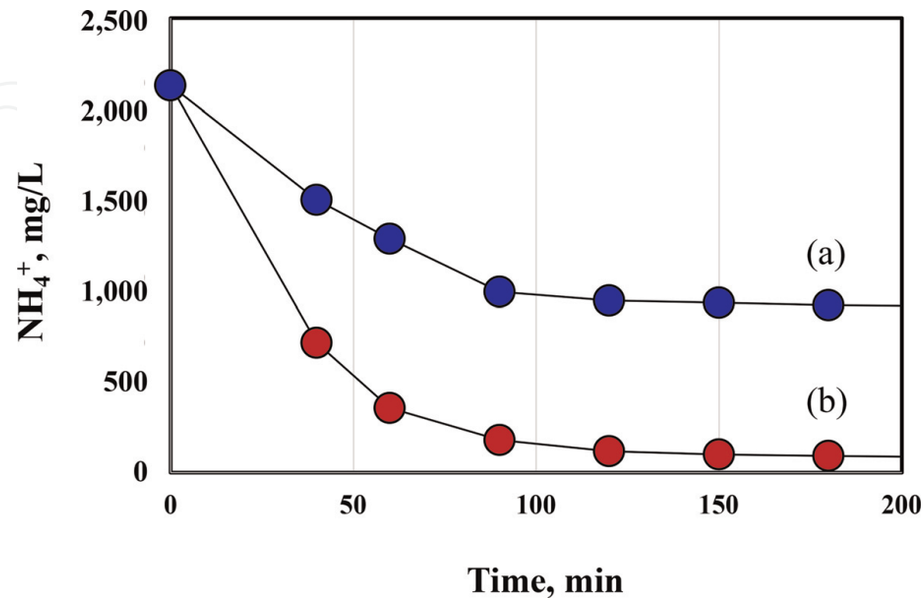

Figure 2.
The NH_4^+ concentrations in the stripping column as a function of time: (a) without Na_2CO_3 and (b) with Na_2CO_3.

an end. When Na_2CO_3 was added, however, the aeration kept stripping NH_3, decreasing the NH_4^+ concentration to nearly zero, driven by eq. 3.

The values of η_{remove}^{NH4} were 96 and 61% with and without Na_2CO_3, respectively. How fast the NH_4^+ concentration decreases depends on the reaction kinetics and the diffusion of the NH_3 gas through the formulated solution to reach air bubbles for the initial NH_4^+ concentration and the volume of the formulated sample.

Figure 3 displays the NH_4^+ concentrations in the recovery column (a) with and (b) without sulfuric acid. A significant difference between the two cases was observed. Without sulfuric acid, the NH_4^+ concentration quickly reached a plateau and did not increase much. CO_2 generated by eq. 3 decreased the pH of water in the recovery column somewhat, dissolving the NH_3 gas into the water to a point; however, the NH_3 dissolution is limited, determined by thermodynamics through pH and the temperature. With sulfuric acid added, the NH_4^+ concentration kept increasing, driven by eq. 5, NH_3 stripped by aeration in the stripping column was mostly recovered in the recovery column. The values of $\eta_{recovery}^{NH4}$ were 90 and 26.9% with and without sulfuric acid, respectively. The efficiency of using chemicals for the NH_3 recovery is clear.

While it took about 2 hours to remove most of NH_4^+ in the stripping column, it took almost 8 hours to dissolve the same amount of NH_4^+ in the recovery column when the volume of water was 1 L for both columns. The reason for the slow process of the NH_3 recovery, relative to the rate of NH_3 removal, is due to the limited amount of the NH_3 gas going into the recovery column available for eq. 3. That is, the flow rate of the NH_3 gas going into the recovery column is smaller than the rate of the NH_3 gas generated in the stripping column through eq. 3. Eq. 3 occurs almost instantly, while eq. 5 only undergoes as the stripped NH_3 gas has a contact with the acidic solution in the recovery column. The rate of eq. 5 is determined by the airflow rate of the air pump, the air flux associated with the air diffusers used, the size of air bubbles, and others. Namely, the faster the rate of the NH_3 gas flow into the recovery column becomes, the more rapidly the dissolving process gets. For example, employing multiple pipes going from the stripping column to the recovery column can speed up the

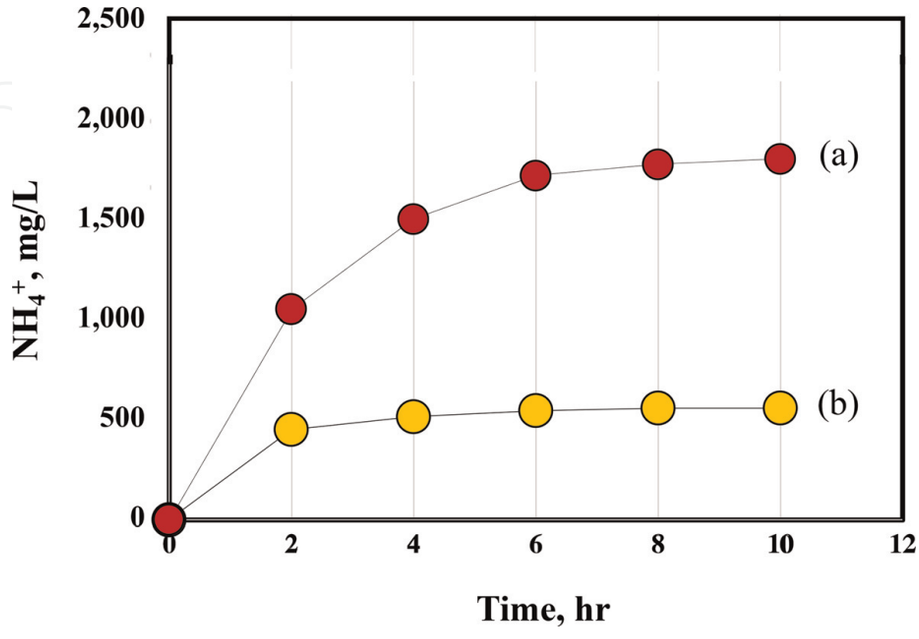

Figure 3.
The NH_4^+ concentrations in the recovery column (a) with and (b) without sulfuric acid.

NH_3 dissolution process. An earlier study has reported that the shape and the behavior of air bubbles also affect the gas solubility in water [22].

As **Figures 2** and **3** show, our process ensures a high recovery of NH_3 from manure digestate liquid by taking advantage of efficient chemical reactions, eqs. 3 and 5. The chemicals used are abundant and affordable. Still, the results shown in **Figures 2** and **3** are simply a proof of concept. The process can be improved by adjusting the kinetic parameters such as the mass transfer and the retention time of the air bubbles inside the columns. It should be noted, however, that eq. 3 produces sodium as a by-product which should be removed before spraying on croplands.

3.1.2 Highly concentrated NH_4 solution

The above operation was repeated by replacing the spent formulated sample a new one in the stripping column and dissolving the stripped NH_3 gas by reaction with sulfuric acid in the recovery column until the NH_4^+ concentration in the recovery column no longer increased. The NH_4^+ concentration in the recovery column is determined by the solubility of the product, $(NH_4)_2SO_4$, which is 744 g/L at ambient temperature. With this concentration, the concentration of NH_4^+ is 19%, a theoretical number. We reached 18% in our experiment. If nitric acid is used, the NH_4^+ concentration would be more than 33%, given the solubility of NH_4NO_3 in water, 1500 g/L.

To demonstrate the liquefaction of the highly concentrated NH_4^+ solution we prepared, we set up an experiment to produce liquefied NH_3. We heated 100 mL of the 18% NH_4^+ solution at 90°C, vaporizing NH_3, and sent the NH_3 gas, through a glass condenser into a metal cylinder half-submerged in iso-propanol which was cooled to −60°C by dry ice. The moisture generated by heating the N solution at 90°C was captured by a desiccant inside the condenser. At −60°C, the NH_3 gas is liquefied, while the other gases N_2 and O_2 stay as gas, being released to the atmosphere. About 50 mL of liquefied NH_3 was collected. The volume of the recovered liquid NH_3 was limited by the volume of the metal cylinder. Hence, no quantitative recovery rate was assessed. Yet, this demonstrates a possibility of NH_3 liquefaction from an 18% NH_4^+ solution. The technology for NH_3 liquefaction and the liquid NH_3 transportation infrastructure already exist. The same experiments should be repeated by using a real DS sample for validation.

Very few studies have been published to report the nitrogen concentration as high as 18% in recovering nitrogen from manure liquid. The high nitrogen concentration was made possible by dissolving the NH_3 gas into the water with a highly soluble acid. The other gases such as N_2, O_2, and CO_2 gases inside the air bubble can interfere with the NH_3 gas dissolving into the water. When the rising velocity of the bubbles is high, the NH_3 gas can be carried away by the other gases which are not water soluble except for CO_2 which dissolves somewhat. Using plastic packing materials inside the recovery column can help slow down the rising velocity.

As to the economic benefit, it is difficult to estimate since there is no market for renewable ammonia at this moment. Still, the price of N fertilizers has been going up significantly, due to the increase in the price of natural gas (NG), and can be unpredictable, given geopolitical reasons such as the economic sanction against Russia, a large exporter of N fertilizers. Using the recycled ammonia can help farmers save money. In addition, many large companies are investing in what is called Green Ammonia which uses water electrolysis followed by the Haber-Bosch process without using NG [23]. It is known that the production of Green Ammonia can cost four to five times as much as the conventional ammonia due to the high cost of water electrolysis [23]. Our process does not use electrolysis, nor does it produce NH_3. It

simply recovers NH_3 from the wastewater. Though a comprehensive cost-benefit comparison is not straightforward for Green Ammonia and our renewable NH_3, it should be clear that recovering NH_3 is much cheaper than producing it, given the extreme chemical stability of water and N_2, both of which are the raw materials for Green Ammonia. Both our renewable NH_3 and Green Ammonia should be qualified as non-fossil-based ammonia, and the demand for such ammonia is expected to grow massively high in the future [24].

3.2 Protein recovery

3.2.1 Composition of DS

Table 3 summarizes the compositions of the original DS sample and the leftover solid after the extraction of protein by THP on a dry matter basis. The condition for THP was the following: heating the DS sample at T_1 = 100°C for 1 hour followed by heating it further at T_2 = 160°C for 1 hour.

Almost 60% of the original protein was extracted by THP. Phosphorous mostly stayed in the leftover solid, while potassium dissolved in the solution after THP.

3.2.2 Protein recovery yield

We analyzed the protein recovery yield, $\eta_{recovery}^{protein}$, defined by the following equation:

$$\eta_{recovery}^{protein} = 100 \times \frac{[W_{hydrolysate}]}{[W_{protein}]} \tag{7}$$

where $[W_{hydrolysate}]$ and $[W_{protein}]$ refer to the weights of the protein hydrolysates (PHs) in the reaction solution after THP and the weight of the protein in the original sample prior to THP, respectively. The THP condition was the following: heating the DS sample at T_1 = 100°C for 1 hour followed by heating it further at T_2 = 160°C for 1 hour. **Table 4** summarizes the recovery yield under this condition, showing a reasonably high yield.

The numbers listed in **Table 4** were determined by AAA. The experiments were performed in triplicate and an error of $\eta_{recovery}^{protein}$ was within 3%. Vanotti et al. did not include the recovery yield in their patent [17].

The protein in manure digestate solid may be embedded in a complex solid matrix or trapped in a web of lignocellulosic components such as cellulose, hemicellulose, and

Solid sample	Protein[b]	P	K	Hemicellulose	Cellulose	Lignin	Others[c]
Before THP	37.2	2.1	1.5	9.3	18.2	30.2	1.5
After THP[d]	14.8	3.5	0.1	12.7	24.9	41.5	2.5

[a] *Dry matter basis.*
[b] *Obtained by multiplying the Kjeldahl nitrogen by 6.25. For the analytical method, refer to our earlier publication [20].*
[c] *Alkali metals such as Na, Ca, and Mg.*
[d] *The leftover solid after THP recovered by filtration by a screen with 90 μm mesh, dried in an oven overnight, and ground by a pestle for analysis.*

Table 3.
Compositions of DS samples before and the leftover solid after THP (wt.%)[a].

$W_{protein}$, g[a]	$W_{hydrolysate}$, g[a]	$\eta_{recovery}^{protein}$, %
9.21	5.55	60.26

[a]*Dry matter basis.*

Table 4.
Protein recovery yields by THP.

lignin. The interactions between the protein and the rest of the components in the solid may be hydrophobic in nature or electrostatic in nature associated with the functional groups of constituent amino acid residues of the protein. It is known that the dielectric constant of water decreases at high temperatures [25]. This creates two unusual characteristics for water: water favoring hydrophobic interactions and obstructing electrostatic interactions [25]. Hence, the first heating step of THP may interfere with the hydrophobic or the electrostatic interactions which may keep the protein trapped inside the solid matrix. It is also known that the pH of water goes down at high temperatures from around 7 [25]. Once the protein is released from the solid phase and dissolved into the solution phase, it should experience an acidic environment created by a low pH which may cause hydrolysis of the extracted protein. Accordingly, the dissolved protein may undergo hydrolysis, yielding short-chain peptides or individual amino acids. Although this is only speculation, the results show that protein can be extracted at a reasonably high yield by the two-step THP and what was extracted from DS was a mixture of oligopeptides and amino acids, as is shown below.

3.2.3 Molecular weight (MW) distributions

Figure 4 displays the SDS-PAGE band images for the PH prepared under the THP condition described above. The PH exhibited very few lines, indicating very few fractions within the range analyzed.

Figure 5 exhibits the MALDI-TOF mass spectra for the PH. The reference peptide, shown at 1046.79 m/z, was added to the sample prior to the measurements for the concentration of PH relative to the reference below the MW of 1000 Da. Contrary to the SDS-PAGE images, there are a number of peaks below 1000 Da. Peptides in this region were low-MW peptides such as oligopeptides or free amino acids. The concentration of the PH in **Figure 5** can be calculated from the peak positions and the intensities of each signal relative to the reference. It was about 3.9 g/L which is about 70% of $W_{hydrolysate}$ in **Table 4**.

Based on the results from SDS-PAGE and MALDI-TOF mass spectroscopy, we conclude that our PH had more low MW fractions than the higher MW fractions. Earlier studies reported low MW peptides exhibited antioxidant activities [26–28].

Next, we will subject our PH to antioxidant activities.

3.2.4 ORAC against the peroxyl and hydroxyl radicals

Figure 6a and **b** show the inhibition of the peroxyl radical attack against fluorescein protein by Trolox and PH, respectively, as a function of the logarithm of the sample concentration, *C*. This assay is important since lipid molecules constituting cell membranes are prone to become peroxyl radicals that attack DNA, protein, and other molecules in a cell [29]. The curve profile shown in **Figure 6b** is very similar to that in **Figure 6a**. In fact, the values of IC_{50} for PH and Trolox were very close: 7.67 and

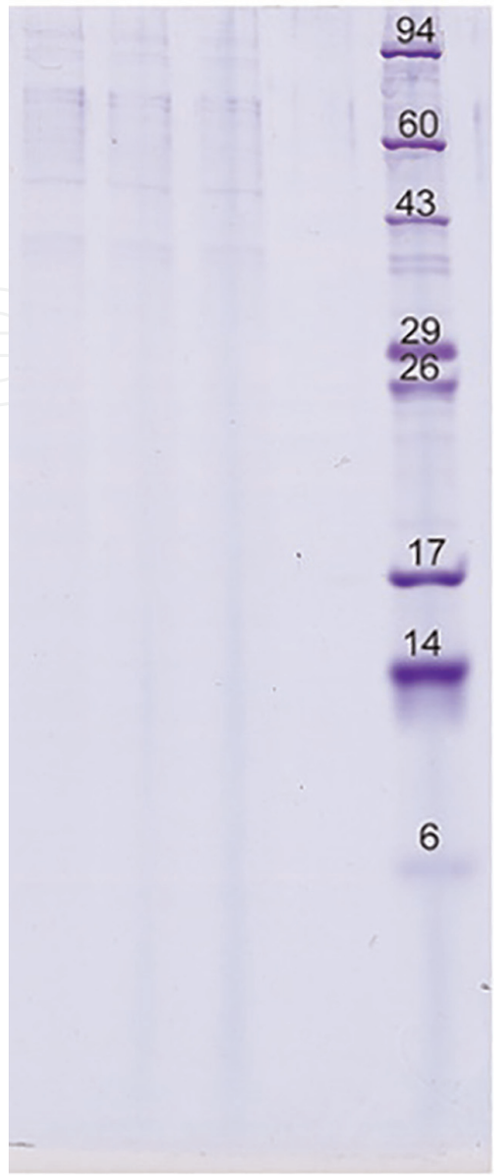

Figure 4.
SDS-PAGE image of PH extracted from DS. The measurements were performed in triplicate. The numbers on the right side are the MW markers in kDa.

8.08 mg/L, respectively. This observation demonstrates that the antioxidant activity of PH was as strong as Trolox against the peroxyl radicals. IC_{50} refers to the concentration of the sample at which the inhibition is 50%. The experimental error for IC_{50} was within 1 mg/L which was estimated over triplicate experiments.

Figure 7a and **b** display the inhibition of the hydroxyl radical attack against fluorescein protein by Trolox and our PH, respectively, as a function of the logarithm of *C*. Hydroxyl radicals are often generated inside a cell in the presence of metal ions such as Fe(II), Cu(I), and Co(II) and attack organic molecules involved in metabolic reaction pathways [30]. We observed a significant difference between the two samples: the inhibition by our PH reached 100% when log *C* was 1.5, while Trolox did not reach 100% inhibition even when log *C* was 2. IC_{50} of 107.6 mg/L for our PH was less than 1/7 of that of Trolox, 741 mg/L. The values of IC_{50} imply that the antioxidant activity of our PH was more than seven times as strong as Trolox. The strong antioxidant activities of our PH are consistent with the previous studies on peptides

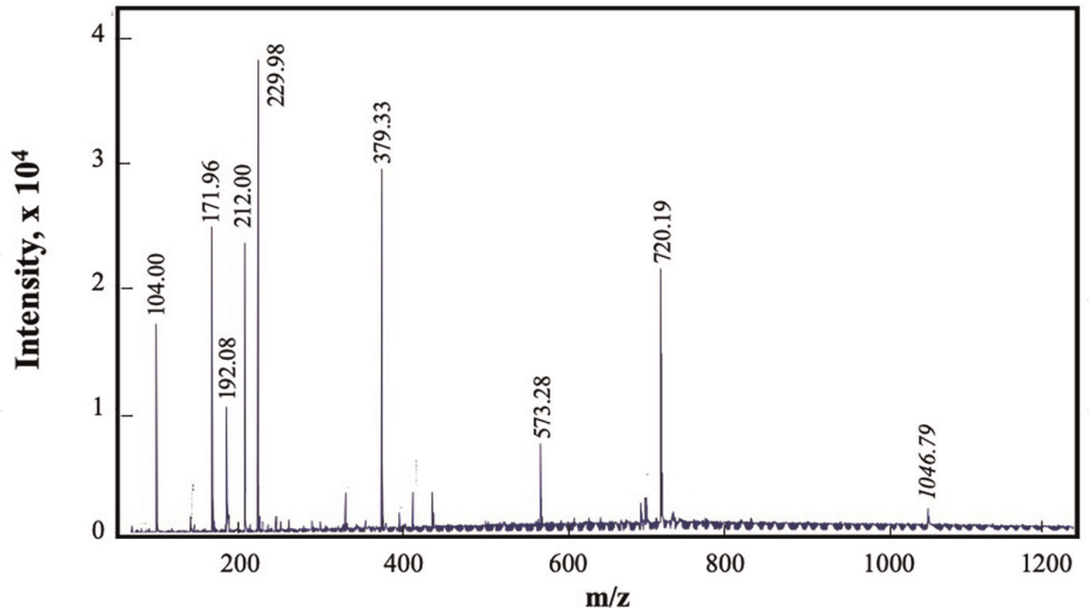

Figure 5.
MALDI-TOF-mass spectrum of PH. A signal for a peptide with a known MW is included as a reference at 1046.79 m/z.

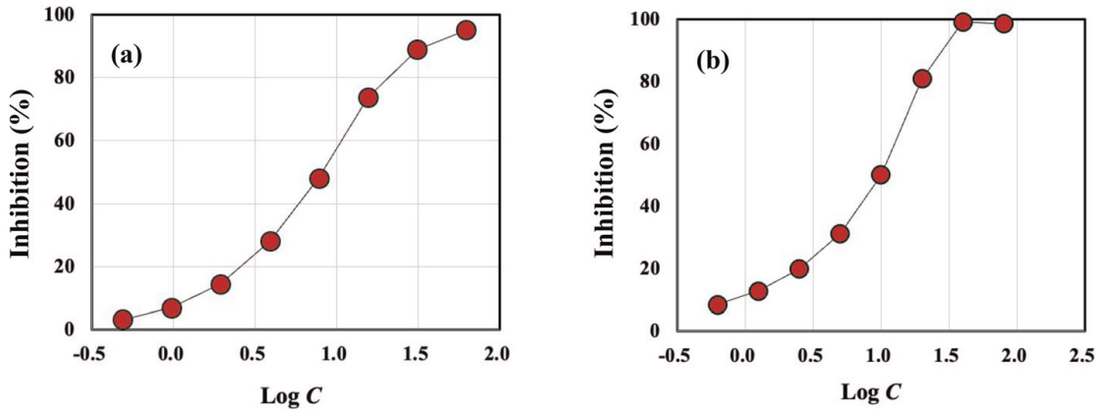

Figure 6.
The inhibition of the peroxyl radical attack against fluorescein protein by (a) Trolox and (b) PH, respectively, as a function of the logarithm of the sample concentration, C.

[26–28, 31–40]. A theoretical study on the antioxidant activity of peptides has been published [41].

The DS sample included some non-protein nitrogen compounds which were not removed from the PH sample prior to the ORAC assay; therefore, their contributions to the inhibition of the radicals cannot be ignored. Our data only demonstrates that the extracted compounds from the DS sample by our THP and recovered by UF with a 150 kDa membrane inhibited both peroxyl and hydroxyl radicals to the extent that the ability to inhibit the former radical was comparable to that of Trolox and the ability to inhibit the latter was seven times stronger than Trolox.

Our assay is an in-vitro test, and the results should be considered preliminary. Further study is warranted to confirm the antioxidant activity of our PH. If confirmed, the recovered protein hydrolysate could be sold as antioxidant feed additives. Currently, a 50% feed-grade vitamin E is sold at ~$13.5/kg [42]. Using the numbers in **Table 2**, the volume of the recovered protein from manure digestate solid generated

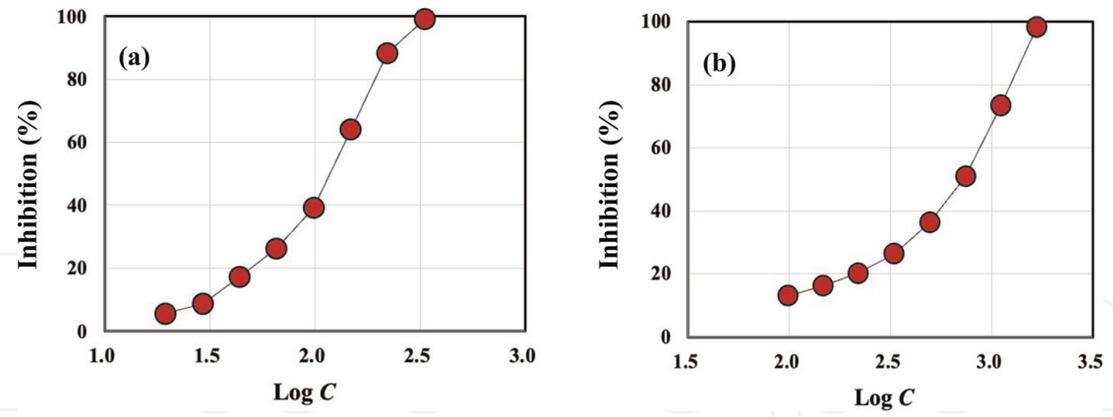

Figure 7.
The inhibition of the hydroxyl radical attack against fluorescein protein by (a) Trolox and (b) PH, respectively, as a function of the logarithm of C.

on a dairy farm would be about 5000 tons/year at the recovery yield of 60%. This would provide an annual revenue of $137 million/year which could potentially overshadow the revenue from selling the milk. It should be noted that this is only a rough estimate under a hypothetical scenario.

The GHG emissions from agricultural activities in the U.S. were 641 Mt of CO_{2eq} in 2019 [43]. Of that volume, 58% was due to the N_2O emission caused by spraying nitrogen fertilizers including manure/digestate liquids through the mechanism mentioned earlier, and 13% was primarily due to the N_2O emissions by manure management mostly from manure storage such as lagoons. N_2O generated by manure management is mainly produced from organic nitrogen, mostly protein, in manure. By recovering protein from manure before manure management through the protein recovery process described in this chapter, we could potentially reduce about 83 Mt of CO_{2eq} emissions, assuming the above 13% was generated by decomposition of protein in manure. 30 to 50% of the N_2O emissions caused by spraying N fertilizers and manure/digestate liquids originate from applications of animal manure which includes organic nitrogen [3, 4]. Hence, practicing a combination of the protein and NH_3 recovery processes described in this chapter could potentially reduce about 109–186 Mt of CO_{2eq} emissions. Altogether, up to 269 Mt of CO_{2eq} emissions could be removed by the combination of the two processes. This number is about 42% of the total GHG emissions from agricultural activities in the U.S. To evaluate these estimates, we assumed 100% protein and NH_3 recovery rates by both recovery processes. Though the actual number will be lower, a significant volume of GHG emissions can be still reduced by the two processes. The potential reduction of eutrophication caused by nitrogen runoff cannot be ignored through our processes.

4. Conclusion

The two processes described here, the NH_3 and the protein recovery process from manure digestate, have shown the high recovery rates. Our processes can be directly applied to the current practice of the solid-liquid separation of manure digestate from which both solid and liquid are otherwise sprayed on lands. The protein recovery process can be applied to the solid, while the NH_3 recovery process can recover NH_3 from the liquid, with the solid and liquid coming from ADs which are rapidly being

adopted by livestock farmers. Using the two processes can significantly limit the leakage of N from the digestate into the environment.

Furthermore, our two processes can produce value-added products including protein-based antioxidant feed additives and concentrated N solutions from which renewable nitrogen fertilizers or non-zero carbon renewable energy source can be recovered. These products can help close the nitrogen loop in the livestock operation that is currently broken, given abundant applications of synthetic fertilizers and frequent use of protein-based feeds. The nitrogen in the recovered protein from manure/digestate solids can go back to animals as feed, while the nitrogen recovered from manure/digestate liquids can grow crops, and the nitrogen in the crops can be recycled back to animals as feed as well. Additionally, our recovered NH_3 is renewable NH_3 produced without fossil fuels and highly energy-intensive processes such as the Haber-Bosch process or water electrolysis.

Intense animal operations (IAO) are expected to grow, given the increasing demand for meats and dairy products worldwide. Accordingly, regulations on manure management will be likely tightened to keep the environmental consequences by IAO under control. Yet, regulations can go only so far as to mitigate the environmental consequences. Our processes add economic incentives to livestock farmers by bringing extra revenue streams which will help livestock farmers, some of whom may be under financial stress due to increasingly higher costs for the operations.

Authors' contribution statements

ANT contributed by preparing samples and conducting THP experiments; KT contributed by the conceptualization of experiments, manuscript preparation, data analysis, and review editing. All authors have read and agreed to the manuscript to be submitted.

Author details

Abigail N. Tasaki[1] and Ken Tasaki[2,3]*

1 South High School, Torrance, CA, United States

2 Tomorrow Water, Anaheim, CA, United States

3 Palos Verdes Research, Redondo Beach, CA, United States

*Address all correspondence to: kt@bkt21.com

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